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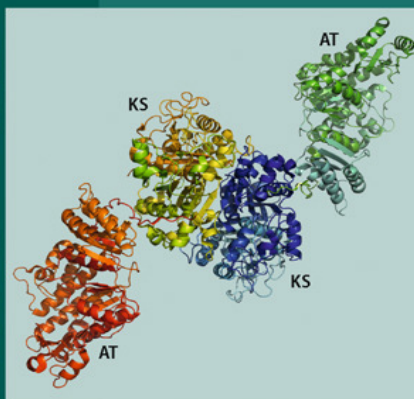
A. D. Kinghorn · H. Falk · J. Kobayashi

Authors

S. A. Begum, M. Sahai, and A. B. Ray

E. Gössinger

M. Luzhetska, J. Härle, and A. Bechthold



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of Organic Natural Products

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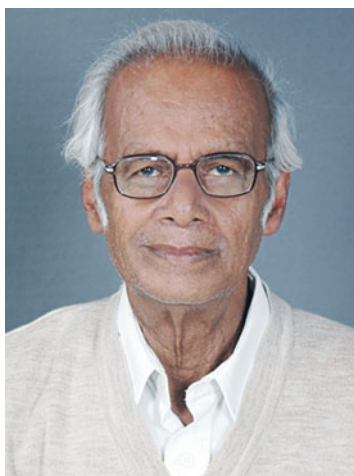
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After having received his Master's degree in Applied Chemistry (Pharmaceutical Chemistry) from Calcutta University in 1955, **Anil B. Ray** spent brief periods in the School of Tropical Medicine, Calcutta, as a Junior Research Fellow and in the Directorate of Health Services, Govt. of West Bengal, India, as an Inspector of Drugs. In 1961, he joined the laboratory of the late Professor (Mrs.) Asima Chatterjee, D.Sc., F.N.A, and worked on the isolation and structure elucidation of complex indole alkaloids for his doctoral thesis and received his Ph.D. in 1965. He then joined the Research group of the late Professor Jack L. Beal, of the College of Pharmacy, Ohio State University, Columbus, Ohio, U.S.A., and worked as a Research Associate for three years on the isolation, structure determination, and synthesis of bis-benzylisoquinoline alkaloids. He also worked on the structure determination of spermidine alkaloids and codonocarpienes. After his return to India, he joined the Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, in 1971 as a Reader and subsequently became Professor. He worked on the chemistry and biological activity of different groups of plant constituents and has a total of 147 publications including several review articles in standard refereed journals. He visited Japan and USSR under a bilateral exchange program with INSA. After superannuation, he worked as an Emeritus Scientist, CSIR, until the age of 65.



Edda Gössinger was born in Asparn/Zaya, Austria, in 1942. She completed her studies in chemistry at the University of Vienna with a Ph.D. thesis on the isolation and structure determination of natural products. She then went as a postdoctoral fellow to ETH/Zürich, where she assisted in the partial synthesis of a steroid alkaloid and later on was allowed to work on a total synthesis of her own design. With this synthesis, she moved to NIH in Bethesda, MD, where she continued to work on total syntheses of natural products. She concluded her stay in the United States by getting acquainted with high-pressure chemistry at the State University of New York at Stony Brook. Back in Austria, she joined the Institute of Organic Chemistry at the University of Vienna. There she received the *venia legendi* in 1982, which enabled her to lecture about planning of synthesis and diverse themes about natural products chemistry. Her research interests were mainly with the total synthesis of natural products with excursions into chemical methodology and mechanistic aspects. She has retired recently.



Marta Luzhetska was born in L'viv, Ukraine, in 1980. She studied genetics and biotechnology at Ivan Franko National University, L'viv, and in 2007 obtained her Ph.D. in the genetics of *Streptomyces* at Polytechnic National University, L'viv, under the supervision of Professor Novikov. During 2003–2004, she was a DAAD fellow of Professor Bechthold at the Albert-Ludwigs-Universität, Freiburg. In 2007, she started her work as a postdoctoral researcher in the laboratory of professor Bechthold. Her major interest is investigation of secondary metabolites biosynthetic pathways in *Actinomycetes*.



In 2008, **Johannes Härle** completed his biochemistry studies in the Eberhard-Karls-Universität Tübingen. During his study, he was employed as a research assistant in the laboratory of Prof. L. Heide in the Department of Pharmaceutical Biology, where he also submitted a diploma thesis by “Investigating the regulation of the novobiocin biosynthetic gene cluster from *Streptomyces spheroides*”. In the context of his study, he carried out an internship in the laboratory of Prof. B. Moore in Scripps Institution of Oceanography, San Diego, USA. Currently, he is working as a PhD student in the laboratory of Prof. A. Bechthold at the Institute of Pharmaceutical Science in the Department of Pharmaceutical Biology and Biotechnology, Albert-Ludwigs-Universität Freiburg, Germany, where he is focusing on protein engineering of glycosyltransferases.



Prof. Dr. **Andreas Bechthold** commenced his studies in pharmacy at the University of Bonn in 1983, graduating in 1988. He then completed his Ph.D. at Bonn in 1991 in Pharmaceutical Biology. From 1992 to 1993, he carried out postdoctoral research at the University of Washington in Seattle, Washington, USA, as a DFG Fellow, and followed this with postdoctoral studies at the University of Kyoto, Japan, as a JSPS Fellow. He has since held positions at the University of Tübingen (1994–1998) and professorships at the Universities of Kiel (2000–2001) and Freiburg (since 2001), winning the ‘Phoenix Pharmazie Wissenschaftspreis’ prize in 2006. His research interests are novel natural products by genetic engineering, glycosyltransferases as important tools for drug design, and the development of novel technologies for *Streptomyces* genetics. In total, he has published 105 articles, including several reviews and book articles. He has visited Japan, USA, and Ukraine under bilateral exchange programs.

Non-conventional Lignans: Coumarinolignans, Flavonolignans, and Stilbenolignans

Sajeli A. Begum, Mahendra Sahai, and Anil B. Ray

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1. Introduction

Lignans, by convention, are a group of natural products that are formed by linking two phenylpropanoid units (C_6C_3 units) by oxidative coupling. Most importantly, in a lignan, two (C_6C_3 units) are bound through the central carbon of their side chains, *i.e.* the 8 and 8' positions (1, 2). The occurrence of C_6C_3 -dimers, linked at sites other than the 8–8' positions, is also known and these compounds have been termed neolignans (3, 4). As these two groups of compounds have close structural as well as biosynthetic relationships, they are often associated together and incorporated under the general term “lignan” (5). The diverse structural categorization of true lignans and of a few neolignans is presented in Fig. 1. Through the years, several review articles or books covering different facets of lignans, including their chemistry (6, 7), biogenesis (8), synthesis (9), and biological activities (10) have been published.

Enduring research for the investigation of secondary metabolites of plants has evidenced some compounds that are biogenetically related to true lignans or neolignans but bear some features not discerned in conventional lignans. These compounds or groups of compounds have been termed as “non-conventional lignans”, and include coumarinolignans, flavonolignans, and stilbenolignans. The non-conventional lignans, like the conventional ones, have two C_6C_3 units linked together but have additional structural features to place them also under the category of coumarins, flavonoids, or stilbenes. The basic skeletons of these non-conventional lignans are shown in Fig. 2. Some authors have also used the term “xanthonolignans” for xanthenes linked with a phenylpropanoid unit, but on the

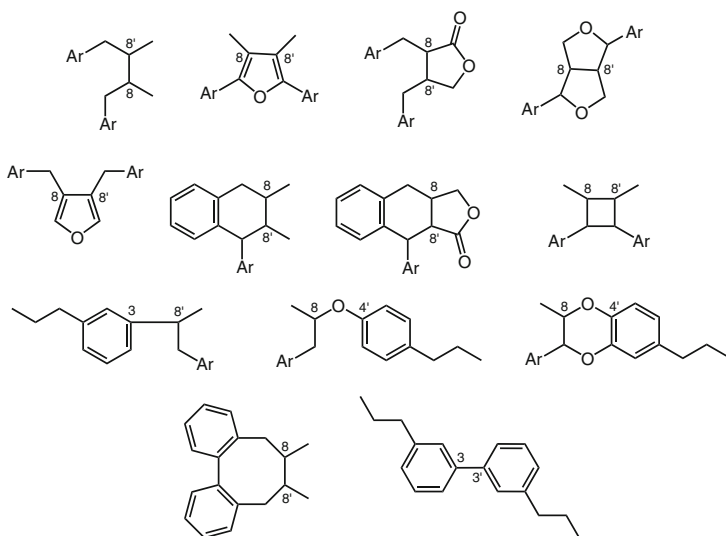
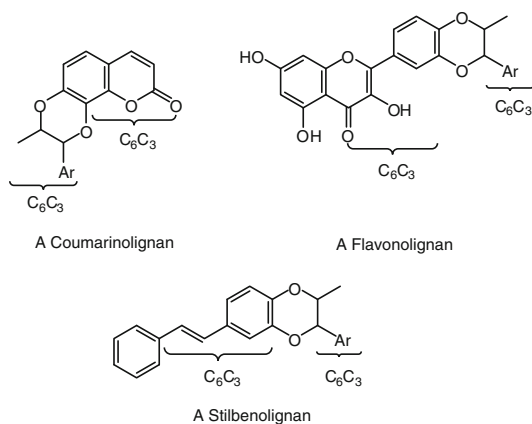


Fig. 1. Structural patterns of some lignans and neolignans

Fig. 2. Basic skeletons of “non-conventional” lignans

basis of the known biogenesis of xanthenes (11), which do not incorporate any phenylpropanoid unit, we prefer to exclude them from our discussion. Also excluded are lignoflavonoids, in which a phenylpropanoid unit is linked to the “A” ring of a flavonoid, because it is well known that this ring is derived from a malonate rather than a cinnamate precursor (12). Several review articles (13, 14) on these groups of lignans have appeared in literature but these are rather fragmentary in nature and do not provide an overall picture of non-conventional lignans. Our endeavor herein is to present a complete picture of these groups of compounds, highlighting some recent findings.

2. Coumarinolignans

The first non-conventional lignan was a flavonolignan, silybin (1), isolated from the fruits of *Silybum marianum* Gaertn. (15). However, in view of the fact that coumarinolignans are closest to true lignans by being C_6C_3 dimers, this group of compounds will be discussed first. Coumarins, known to be biosynthesized by cyclization of cinnamic acid (16), a C_6C_3 unit, give rise to coumarinolignans by appropriate union with a second C_6C_3 unit.

2.1. Occurrence of Coumarinolignans

Coumarinolignans are not restricted to any particular plant family or genus and, in fact, are rather widely distributed. Some 44 plant species belonging to 19 different plant families have so far been known to produce this group of phytochemicals. The various sources known so far to yield coumarinolignans are listed in Table 1.

Table 1. Sources of coumarinolignans

Plant family	Plant species (references)
Aceraceae	1a. <i>Acer nikoense</i> (17, 18) 1b. <i>Acer okamotoanum</i> (19)
Asclepiadaceae	2a. <i>Biondia hemsleyana</i> (20) 2b. <i>Hemidesma indicus</i> (21, 22) 2c. <i>Mondia whiteii</i> (23) 2d. <i>Stelmocrypton khasianum</i> (24)
Bombacaceae	3a. <i>Ochroma lagopus</i> (25)
Burseraceae	4a. <i>Protium heptaphyllum</i> (26) 4b. <i>Protium opacum</i> (27) 4c. <i>Protium unifoliolatum</i> (28)
Capparaceae	5a. <i>Cleome viscosa</i> (29–33)
Chenopodiaceae	6a. <i>Salsola laricifolia</i> (34) 6b. <i>Salsola tetrandra</i> (35)
Ericaceae	7a. <i>Rhododendron collettianum</i> (36)
Euphorbiaceae	8a. <i>Aleurites fordii</i> (37) 8b. <i>Aleurites moluccana</i> (38) 8c. <i>Antidesma pentandrum</i> var. <i>barbatum</i> (39) 8d. <i>Euphorbia esula</i> (40) 8e. <i>Euphorbia lunulata</i> (40) 8f. <i>Mallotus apelta</i> (41) 8g. <i>Jatropha glandulifera</i> (42, 43) 8h. <i>Jatropha gossypifolia</i> (44–47)
Hippocastanaceae	9a. <i>Aesculus turbinata</i> (48)
Malvaceae	10a. <i>Hibiscus syriacus</i> (49)
Meliaceae	11a. <i>Carapa guianensis</i> (50)
Ranunculaceae	12a. <i>Coptis japonica</i> var. <i>dissecta</i> (51)
Rutaceae	13a. <i>Zanthoxylum avicennae</i> (52)
Sapindaceae	14a. <i>Diatenopteryx sorbifolia</i> (53) 14b. <i>Dodonea viscosa</i> (54, 55) 14c. <i>Matayba arborescens</i> (56)
Simaroubaceae	15a. <i>Brucea javanica</i> (57–59) 15b. <i>Castela texana</i> (60) 15c. <i>Hannoa klaineana</i> (61) 15d. <i>Simaba multiflora</i> (56, 62) 15e. <i>Soulamea soulameoides</i> (56)
Solanaceae	16a. <i>Hyoscyamus niger</i> (63)
Thymelaeaceae	17a. <i>Aquilaria agallocha</i> (64) 17b. <i>Daphne mezereum</i> (65) 17c. <i>Daphne gnidium</i> (66) 17d. <i>Daphne oleoides</i> (67) 17e. <i>Daphne tangutica</i> (68)
Tiliaceae	18a. <i>Grewia bilamellata</i> (69)
Verbenaceae	19a. <i>Duranta repens</i> (70)

Table 2 presents a list of coumarinolignans arranged alphabetically together with their structure numbers, sources and references. The colored photographs of two coumarinolignan-bearing plants, *Cleome viscosa* and *Daphne oleoides* are presented in Figs. 3 and 4.

Table 2. Coumarinolignans and their plant sources

Compound (see Chart 1)	Plant Source(s) (see Table 1)	References
Aleuritin (2)	8a	(37)
Antidesmanin A (3)	8c	(39)
Antidesmanin B (4)	8c	(39)
Antidesmanin C (5)	8c	(39)
Antidesmanin D (6)	8c	(39)
Aquillochin (7) = Cleomiscosin C	1a, 1b, 5a, 7a, 8f, 10a, 12a, 14b, 15b, 17a	(31, 64)
5-Chloropropacin (8)	2c	(23)
Cleomiscosin A (9) = Cleosandrin	1a, 1b, 2a, 2d, 3a, 5a, 7a, 8f, 8h, 9a, 10a, 12a, 14a-14c, 15a-15e, 16a, 19a	(29-31, 56, 71, 72)
Cleomiscosin B (10)	5a, 6a, 9a, 11a, 15c, 16a	(30, 31, 72)
Cleomiscosin D (11)	5a, 6a, 6b, 10a, 13a, 18a	(32)
Daphneticin (12)	17b-17e	(67, 68)
Daphneticin glycoside (13)	18d	(67)
5'-Demethyl aquillochin (14)	8f	(41)
Durantin B (15)	19a	(70)
Durantin C (16)	19a	(70)
8'-Epi-cleomiscosin A (17)	7a	(36)
Grewin (18)	18a	(69)
Hemidesmin 1 (19)	2b	(22)
Hemidesmin 2 (20)	2b	(22)
Hemidesminin (21)	2b	(21)
Hyosgerin (22)	16a	(63)
Jatrocin A (23)	8h	(44)
Jatrocin B (24) = 5'-Methoxypropacin	2c, 8h	(44, 49)
Jatrorin A (25)	8h	(45)
Jatrorin B (26)	8h	(45)
Maoyancaosu (27)	8d, 8e	(40)
5-Methoxypropacin (28)	4c	(28)
4'-O-Methyl-cleomiscosin D (29)	13a	(52)
Moluccanin (30)	8b	(38)
Propacin (31)	2c, 4b	(27, 72)
Propacin isomer (32)	8g	(42)
Venkatasin (33) = Durantin A	8h, 16a, 19a	(46, 63, 70)

2.2. Classification of Coumarinolignans

All coumarinolignans known to date bear a 1,4-dioxane bridge and this group of natural products may be broadly classified into linearly fused and angularly fused coumarinolignans. Again, angular fusion between a coumarin moiety and a phenylpropanoid unit may take place in two different ways: by fusion at the 5 and 6 positions or at the 7 and 8 positions of the coumarin nucleus. Further, fusion of a phenylpropanoid with two *ortho*-hydroxy groups may take place in two different ways giving rise to regioisomers. In order to avoid this complexity, it is deemed proper to classify these substances by pointing out the mode of linkage of the phenylpropanoid unit with the



Fig. 3. *Cleome viscosa*



Fig. 4. *Daphne oleoides*

coumarin nucleus. Thus, naturally occurring coumarinolignans may be classified into six groups (A–F), having the linkages as shown below.

A: 8-O-8'/7-O-7'; **B:** 8-O-7'/7-O-8'; **C:** 7-O-8'/6-O-7';
D: 7-O-7'/6-O-8'; **E:** 6-O-8'/5-O-7'; and **F:** 6-O-7'/5-O-8'.

2.3. Structure Elucidation of Coumarinolignans

With the advent of modern spectroscopic methods, structure determination of natural products largely depends upon careful interpretation of various spectroscopic data of the compounds and of their appropriate derivatives, and the coumarinolignans are no exceptions. Different spectroscopic methods used in the structure clarification of coumarinolignans are discussed in the following paragraphs.

2.3.1. Ultraviolet Absorption Spectra and Infrared Spectra

A coumarinolignan has two distinct chromophores: a substituted coumarin and a substituted benzene nucleus. As these two chromophores are not conjugated there is only a summation effect and most coumarinolignans exhibit an absorption maximum in the region of 315–325 nm with shoulders around 288 and 230–235 nm. Compounds exhibiting such ultraviolet spectroscopic features with a C₁₈ skeleton, derivable by subtraction of the number of carbons due to methoxy or other carbon-containing substituents from the number of carbons in the molecular formula, are likely to possess a coumarinolignan structure. This can be verified by appropriate interpretation of other spectroscopic data. In the IR spectrum, the lactone carbonyl of a coumarin nucleus exhibits strong absorption bands at 1,715, 1,620, and 1,580 cm⁻¹. The phenolic hydroxy groups absorb around 3,500 cm⁻¹.

2.3.2. ¹H NMR Spectra

A coumarinolignan skeleton can be recognized easily through the detailed analysis of its ¹H NMR spectroscopic data (73). The coumarin C-3 and C-4 protons appear as a pair of doublets generally at δ 6.33 and 7.95 ppm ($J \sim 9.5$ Hz). Antidesmanins (3–6), which are substituted at C-3 of the coumarin nucleus, exhibit a singlet for the C-4 proton at δ 7.5 (39). The C-5 and C-6 protons generally appear around δ 7.25 and 7.02 (AB pattern, $J \sim 9.0$ Hz) as doublets [e.g. daphneticin (12)]. Most of the reported coumarinolignans are substituted at C-6, either with a methoxy group [e.g. the cleomiscosins (9–11), propacin (31), hyosgerin (22)] or a hydroxy group [e.g. jatrocinn A (23) and the jatrocinins (25, 26)]. The C-7' benzylic protons appear as an AB system between δ 4.97 and 5.00 ppm ($J \sim 8.0$ Hz). The chemical shift and splitting pattern of H-8' are largely dictated by the substituent at C-9' (–CH₃, –CH₂OH, –CH₂OAc) and the signal for H-8' generally appears between 4.30 and 4.50 ppm either as a ddd or a multiplet. A large coupling constant ($J \sim 8.0$ Hz) between the two oxymethine protons H-7' and H-8' indicates a *trans* orientation of these two substituents. A nuclear Overhauser effect (NOE) correlation between H-7' and H₂-9' further supports the

Table 3. ^1H NMR spectroscopic data (δ/ppm (J/Hz)) of compounds **9**, **12**, **19**, and **33**

Proton(s)	Cleomiscosin A (9) ^a	Daphneticin (12) ^a	Hemidesmin 1 (19) ^a	Venkatasin (33) ^b
H-3	6.33 d (9.5)	6.36 d (10.0)	6.29 d (9.5)	6.33 d (9.5)
H-4	7.95 d (9.5)	8.01 d (10.0)	7.91 d (9.5)	7.61 d (9.5)
H-5	6.90 s	7.25 d (9.0)	6.86 s	6.54 s
H-6	–	7.01 d (9.0)	–	–
H-2'	7.02 d (1.8)	6.81 s	6.67 s	6.87 d (1.9)
H-5'	6.82 d (8.1)	–	–	6.95 d (8.1)
H-6'	6.89 dd (8.1, 1.8)	6.81 s	6.67 s	6.94 dd (8.1, 1.9)
H-7'	4.98 d (7.9)	5.10 d (7.5)	4.90 d (7.7)	4.99 d (7.9)
H-8'	4.31 ddd (7.9, 3.5)	4.34 m	4.30 m	4.38 ddd (7.6, 4.5, 3.1)
H-9'	3.40 ddd (11.3, 4.3, 3.5)	3.59 m	3.60 m	4.06 dd (12.1, 4.4)
	3.67 ddd (11.3, 4.3, 2.2)			4.35 dd (12.1, 3.0)
OMe-6	3.78 s	–	8.55 s (OH)	3.91 s
OMe-3'	3.77 s	3.81 s	3.73 s	3.89 s
OMe-5'	–	3.81 s	3.73 s	–
OCOMe-9'	–	–	–	2.08 s

^aIn DMSO- d_6 ^bIn CDCl₃

trans relationship of H-7' and H-8'. The chemical shifts of the remaining aromatic protons differ depending on the oxygen substituents in the phenyl ring. The chemical shifts of different hydrogens of some coumarinolignans are shown in Tables 3 and 4.

2.3.3. ^{13}C NMR Spectra

In their ^{13}C NMR spectra, the regioisomeric coumarinolignans show almost similar chemical shifts with slight differences in the shielding of C-7', C-8', and C-9', since the mode of fusion of the two C_6C_3 units differs among these compounds. The carbon resonances of some coumarinolignans are presented in Tables 5 and 6.

2.3.4. Additional NMR Observations

An intriguing problem in the structure elaboration of coumarinolignans is the determination of the exact mode of linkage of the phenylpropanoid unit to the coumarin nucleus or, in other words, to decide which of two possible regioisomers the compound in question may be. This difficulty arises because no significant differences in conventional ^1H - and ^{13}C NMR spectroscopic data are expected between two isomers under consideration and hydrogenolysis of the benzyl ether linkage has so far been unsuccessful. One of the earliest NMR methods used to

Table 4. ^1H NMR spectroscopic data (δ/ppm (J/Hz)) of compounds **2**, **4**, **18**, and **30**

Proton(s)	Aleuritin (2) ^a	Antidesmanin B (4) ^b	Grewin (18) ^c	Moluccanin (30) ^a
H-3	6.13 d (9.5)	–	6.36 d (9.5)	6.36 d (9.0)
H-4	7.92 d (9.5)	7.49 s	7.64 d (9.6)	7.99 d (9.0)
H-5	–	6.47 s	7.18 s	7.36 s
H-8	6.66 s	–	7.08 s	7.04 s
H-12	–	6.19 dd (17.8, 10.4)	–	–
H-13a	–	5.09 d (17.8)	–	–
H-13b	–	5.10 d (10.4)	–	–
H-14	–	1.49 s	–	–
H-15	–	1.49 s	–	–
H-2'	6.72 s	6.84 br s	7.01 d (1.9)	6.83 s
H-5'	–	6.87 br s	–	–
H-6'	6.72 s	6.87 br s	7.35 d (1.9)	6.83 s
H-7'	5.02 d (7.0)	4.94 d (6.3)	5.50 d (8.0)	5.11 d (8.0)
H-8'	4.28 m	4.06 d (6.3)	4.46 m	4.35 m
H-9'	4.11 dd (12.1, 4.3) 4.42 dd (12.1, 3.3)	–	4.21 br d (12.3) 3.98 br d (12.3)	3.60 m
H-10'	–	5.78 dd (17.8, 10.4)	–	–
H-11'a	–	4.87 d (17.8)	–	–
H-11'b	–	4.88 d (10.4)	–	–
H-12'	–	0.96 s	–	–
H-13'	–	1.19 s	–	–
OH-4'	–	5.72 s	–	–
OMe-6	–	3.84 s	–	–
OMe-7	3.98 s	–	–	–
OMe-3'	3.93 s	3.86 s	3.79 s	3.85 s
OMe-5'	3.93 s	–	–	3.85 s

^aIn DMSO- d_6 ^bIn CHCl_3 ^cIn $\text{C}_5\text{D}_5\text{N}$

settle this issue for the coumarinolignans was heteronuclear decoupling, which was used successfully in the structure determination of cleomiscosin A (**9**) (**30**). Irradiation at the H-7' signal of cleomiscosin A diacetate (**9b**) sharpened the carbon signal of C-7, and that of H-8' the resonance of C-8, indicating a 7-O-7'/8-O-8' linkage in **9**. This method was also used in the structure elaboration of aquillochin (**7**) (**31**).

Arnoldi et al. however, observed a systematic difference in the chemical shift values of C-7 and C-8 between two coumarinolignan regioisomers and proposed a simple empirical rule to assign the correct structures (**72**). The selective INEPT (SINEPT) NMR technique, which is independent of the assignments of C-7 and C-8, may also be employed for characterization of regioisomers, even in the case of the availability of only one isomer (**56**). This low-power, single frequency, selective decoupling technique has been utilized successfully to distinguish the regioisomers A and B, as shown in Fig. 5.

Irradiation at a certain hydrogen frequency has been observed to simplify the carbon signal at its β -position due to decoupling. Thus, in isomer **A**, the same carbon signal is simplified by irradiation of the aromatic hydrogen as well as of the benzylic hydrogen attached to the dioxane bridge. In isomer **B**, however, two

Fig. 5. Differentiation of regioisomers by selective decoupling

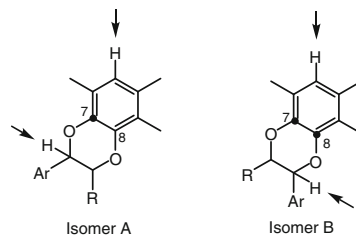


Table 5. ^{13}C NMR spectroscopic data (δ/ppm) of compounds **9**, **10**, **12**, **31**, and **33**

Carbon(s)	Cleomiscosin A (9) ^a	Cleomiscosin B (10) ^a	Daphneticin (12) ^a	Propacin (31) ^b	Venkatasin (33) ^b
C-2	160.2	160.2	160.4	160.8	160.1
C-3	113.4	113.4	113.6	114.0	114.2
C-4	144.9	144.4	144.3	145.8	144.0
C-5	101.0	101.1	119.8	99.9	100.7
C-6	145.4	145.4	113.2	143.7	145.7
C-7	137.2	137.1	147.6	137.6	137.1
C-8	131.8	132.1	138.4	132.3	132.0
C-9	138.2	138.3	149.2	138.7	140.1
C-10	111.4	111.3	113.6	111.5	111.9
C-1'	126.8	126.9	126.4	127.7	130.9
C-2'	112.2	112.1	106.3	109.7	111.9
C-3'	147.8	147.9	149.2	146.9	151.1
C-4'	147.5	147.5	132.2	146.9	148.6
C-5'	115.5	115.6	149.2	114.6	116.3
C-6'	120.9	121.0	106.3	121.2	121.8
C-7'	78.0	78.5	77.8	81.4	77.3
C-8'	76.4	76.2	79.9	74.3	76.6
C-9'	60.0	60.1	60.7	17.1	62.5
-OCOMe	–	–	–	–	176.2
-OCOMe	–	–	–	–	23.2
OMe-6	55.9	55.9	–	56.0	56.4
OMe-3'	56.0	56.1	56.4	56.0	56.8
OMe-4'	–	–	–	–	–
OMe-5'	–	–	56.4	–	–

^aIn DMSO-*d*₆

^bIn CDCl₃

different carbon signals are simplified by irradiation of the aromatic and benzylic hydrogens. This method has been utilized in the structure determination of cleomiscosin A (**9**) (56). *Lin* and *Cordell* have also shown the value of the SINEPT pulse program in the structure elucidation of coumarinolignans (74). Observation of the enhancements induced in the spectra through the irradiation of H-5 and H-7' or H-6 and H-8' provides the unambiguous structure determination of the two isomers. Utilizing this method, the structure of daphneticin was revised (68) as **12** and the structures of propacin (**31**) and aquillochin (**7**) were established (73).

Table 6. ^{13}C NMR spectroscopic data (δ/ppm) of compounds **2a**, **4**, **18**, and **30a**

Carbon(s)	Aleuritin acetate (2a) ^a	Antidesmanin B (4) ^a	Grewin (18) ^b	Moluccanin diacetate (30a) ^a
C-2	161.2	159.7	161.5	160.9
C-3	112.3	132.6	114.7	114.5
C-4	137.6	138.1	144.1	142.7
C-5	139.5	99.8	115.3	114.3
C-6	128.9	145.4	142.1	139.9
C-7	152.3	136.0	148.6	146.7
C-8	93.3	132.0	105.4	105.0
C-9	149.6	137.9	150.1	149.3
C-10	103.3	111.9	113.8	113.2
C-11	–	40.5	–	–
C-12	–	145.6	–	–
C-13	–	112.1	–	–
C-14	–	26.0	–	–
C-15	–	26.0	–	–
C-1'	133.0	128.8	127.6	132.9
C-2'	104.0	110.9	104.2	103.7
C-3'	152.7	146.6	150.2	152.1
C-4'	129.1	146.5	137.5	129.5
C-5'	152.7	114.4	148.9	152.1
C-6'	104.0	121.8	110.7	103.7
C-7'	77.4	77.3	78.6	–
C-8'	75.2	82.6	80.2	77.2
C-9'	62.6	40.9	61.9	62.3
C-10'	–	142.7	–	–
C-11'	–	112.7	–	–
C-12'	–	25.1	–	–
C-13'	–	23.1	–	–
–OCOMe	170.2	–	–	170.1
–OCOMe	168.4	–	–	168.2
–OCOMe	20.4	–	–	20.5
–OCOMe	20.6	–	–	20.3
OMe-6	–	56.0	–	56.1
OMe-7	56.6	–	–	–
OMe-3'	56.3	56.3	56.7	56.1
OMe-5'	56.3	–	–	–

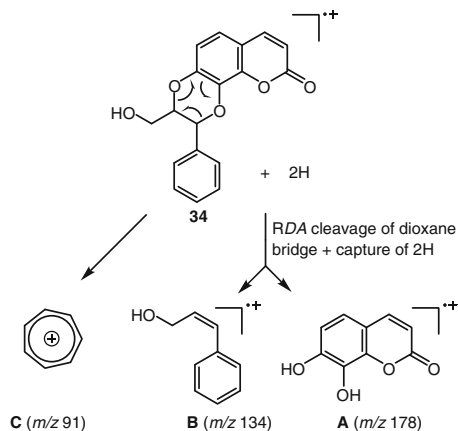
^aIn CDCl₃^bIn C₅D₅N

2.3.5. Mass Spectra

Useful information about the structure of a coumarinolignan can be secured from its electron-impact mass spectrum (EIMS). The most important fragmentation of a typical coumarinolignan, **34**, is a retro-*Diels-Alder* (RDA) cleavage of the dioxane bridge regardless of the sites of linkage. This fragmentation gives rise to a substituted coumarin ion **A** and a typical phenylpropanoid ion **B** as shown in Fig. 6.

The substitution pattern of these two C₆C₃ units may be readily determined from the major fragment peaks observed in the mass spectrum. Thus, daphneticin (**12**),

Fig. 6. The mass fragmentation pattern of a typical coumarinolignan **34**



with no additional substituent in the coumarin moiety, shows a peak at m/z 178 (**A**), which is incidentally the base peak (68). In turn, cleomiscosins **A** (**9**) and **B** (**10**), which bear a methoxy group at the C-6 position of the coumarin moiety, exhibit this coumarin ion peak at m/z 208 (ion **A**) (31). Antidesmanin **C** (**5**), bearing a methoxy group at C-6 and an isopentenyl side chain at C-3, shows this peak at m/z 276 (39). The phenylpropanoid unit with a hydroxy group at the C-9' position and without a substituent in the aromatic ring exhibits a peak at m/z 134 (ion **B**).

However, the absence of an aromatic substituent is uncommon for this type of compound and this peak is shifted by 76 mass units and is discernible at m/z 210 in the mass spectra of aquillochin (**7**), cleomiscosin **D** (**11**), daphneticin (**12**), and moluccanin (**30**), all of which bear two methoxy groups (+60) and a hydroxy (+16) group in the aromatic ring (31, 32, 38). The genesis of the peak at m/z 137 in **9** may occur through benzylic cleavage (ion **C**). The characteristic mass spectrometric fragment ions (**A**, **B**, and **C**) of some coumarinolignans are shown in Table 7.

2.3.6. Optical Activity

Most of the coumarinolignans reported so far have been found to be racemic and thus optically inactive [*e.g.* cleomiscosins **A–D** (**7**, **9–11**), antidesmanins **A–C** (**3–5**), durantins **A–C** (**33**, **15**, **16**), grewin (**18**), hemidesminin (**21**)]. However, some optically active coumarinolignans are also known, including hyosgerin (**22**) (63), 8'-*epi*-cleomiscosin **A** (**17**) (3), the propacin isomer **32** (42), and 4'-*O*-methylcleomiscosin **D** (**29**) (52). The specific rotation values of these coumarinolignans are shown in Table 8.

Interestingly, both enantiomers of 5'-methoxypropacin (**24**) are known and these were isolated from two different plant sources: the dextrorotatory compound was

Table 7. Mass spectrometric data of some coumarinolignans

Coumarinolignan	M ⁺ (<i>m/z</i>)	A ⁺ (<i>m/z</i>)	B ⁺ (<i>m/z</i>)	C ⁺ (<i>m/z</i>)
Antidesmanin A (3)	468	276	194	167
Antidesmanin B (4)	492	276	204	137
5-Chloropropacin (8)	404	242	164	137
Cleomiscosin A (9)	386	208	180	137
Cleomiscosin C (7)	416	208	210	167
Daphneticin (12)	386	178	210	167
Hemidesmin 1 (19)	416	194	224	181
Hemidesmin 2 (20)	386	208	180	137
Hemidesminin (21)	426	178	249	181
Moluccanin (30)	386	178	210	157
Propacin (31)	370	208	164	137
Venkatasin (33)	428	208	222	137

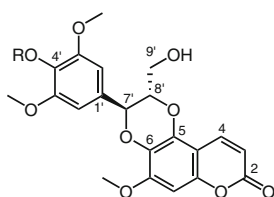
Table 8. Specific rotations of some coumarinolignans

Compound	Specific rotation [α] _D ²⁰ /cm ² g ⁻¹
8'- <i>epi</i> -Cleomiscosin A (17)	+15.5 (<i>c</i> 0.18, C ₅ D ₅ N)
Hyosgerin (22)	-65.4 (<i>c</i> 0.38, CHCl ₃)
4'- <i>O</i> -Methyl-cleomiscosin D (29)	-15.2 (<i>c</i> 0.11, CDCl ₃)
Propacin isomer 32	-56

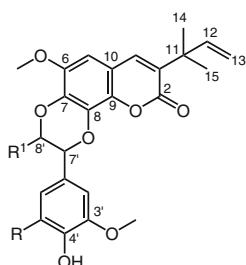
isolated from *Jatropha gossypifolia* (**44**), and the levorotatory one from *Hibiscus syriacus* (**49**). Some apparent anomalies in optical properties of certain coumarinolignans were noticed but these were resolved by appropriate experimental studies. Hyosgerin (**22**), isolated from the seeds of *Hyoscyamus niger*, was optically active and identified as cleomiscosin B 9'-acetate, but cleomiscosin B (**10**), isolated from this same plant species as well as from other sources, was racemic. This rather unusual finding was confirmed to be true by ¹H NMR spectroscopic analysis of the MTPA [4',9'-bis-(*S*)- α -methoxy- α -(trifluoromethyl)phenylacetic acid] esters of natural cleomiscosin B (**10**) and derived cleomiscosin B from **22** by deacetylation with K₂CO₃ in MeOH/THF. While the ¹H NMR spectrum of the MTPA ester of natural **10** showed two sets of signals in a ratio of 1:1, ascertaining its racemic nature, the MTPA ester of cleomiscosin B, derived from **22**, showed only one set of ¹H NMR signals, confirming the enantiomeric nature of hyosgerin (**63**). It is speculated that enzymatic acylation of racemic cleomiscosin B is stereospecific, giving rise to only one particular optical isomer. An almost similar observation was made with venkatasin (**33**), the 9'-*O*-acetyl derivative of cleomiscosin A, isolated from *Hyoscyamus niger* (**63**). While **33** (= durantin A), purified from *Jatropha gossypifolia* (**46**) and *Duranta repens* (**70**), was reported to be racemic, the form from *H. niger* was optically active and ¹H NMR spectroscopic analysis of its bis-MTPA ester showed it to be a mixture of (+)- and (-)-**33**, with a preponderance of the former.

2.4. Chemistry of Coumarinolignans

In this section, the structure determination and synthesis of some important coumarinolignans will be discussed.

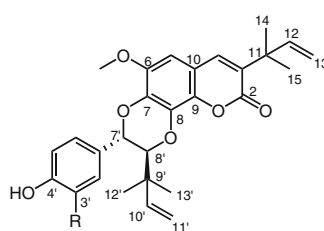


2 R = H (aleuritin)
2a R = Ac (aleuritin diacetate)



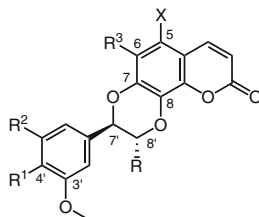
3 R = OCH₃, R¹ = CH₃ (antidesmanin A)

5 R = H, R₁ = C(CH₃)₂CH=CH₂ (antidesmanin C)



4 R = OCH₃ (antidesmanin B)

6 R = OH (antidesmanin D)



7 R = CH₂OH, R¹ = OH, R² = R³ = OCH₃, X = H (aquillochin)

8 R = CH₃, R¹ = OH, R² = H, R³ = OCH₃, X = Cl (5-chloropropacin)

9 R = CH₂OH, R¹ = OH, R² = H, R³ = OCH₃, X = H (cleomiscosin A)

12 R = CH₂OH, R¹ = H, R² = OCH₃, R³ = X = H (daphneticin)

14 R = CH₂OH, R¹ = R² = OH, R³ = OCH₃, X = H (5'-demethylaquillochin)

15 R = CH₂OAc, R¹ = OCH₃, R² = H, R³ = OCH₃, X = H (durantintin B)

16 R = CH₂OAc, R¹ = OH, R² = OCH₃, R³ = OCH₃, X = H (durantintin C)

23 R = CH₃, R¹ = OH, R² = H, R³ = OH, X = H (jatrocinn A)

24 R = CH₃, R¹ = OH, R² = R³ = OCH₃, X = H (jatrocinn B)

25 R = CH₂OH, R¹ = OH, R² = H, R³ = OH, X = H (jatorrin A)

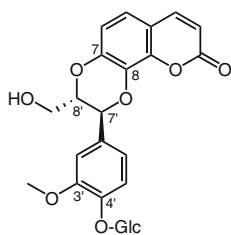
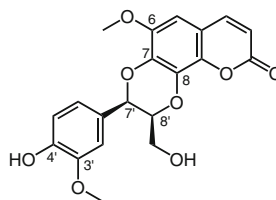
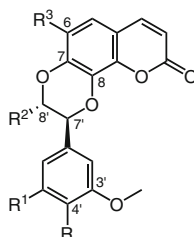
26 R = CH₂OH, R¹ = OCH₃, R² = H, R³ = OH, X = H (jatorrin B)

28 R = CH₃, R¹ = OH, R² = H, R³ = OCH₃, X = OCH₃ (5-methoxypropacin)

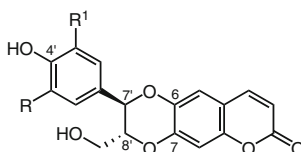
31 R = CH₃, R¹ = OH, R² = H, R³ = OCH₃, X = H (propacin)

33 R = CH₂OAc, R¹ = OH, R² = H, R³ = OCH₃, X = H (venkatasin)

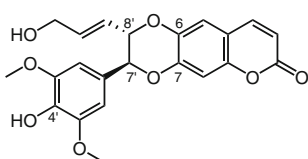
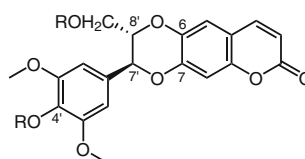
Chart 1. Structure formulas of coumarinolignans

**13** (daphneticin glucoside)**17** (8'-epi-cleomiscosin A)

- 10** R = OH, R¹ = H, R² = CH₂OH, R³ = OCH₃ (cleomiscosin B)
11 R = OH, R¹ = OCH₃, R² = CH₂OH, R³ = OCH₃ (cleomiscosin D)
19 R = OCH₃, R¹ = OCH₃, R² = CH₂OH, R³ = OH (hemidesmin 1)
20 R = H, R¹ = OH, R² = CH₂OH, R³ = OCH₃ (hemidesmin 2)
22 R = OH, R¹ = H, R² = CH₂OAc, R³ = OCH₃ (hyosgerin)
29 R = OCH₃, R¹ = OCH₃, R² = CH₂OH, R³ = OCH₃ (methyl cleomiscosin D)
32 R = OH, R¹ = H, R² = CH₃, R³ = OCH₃ (propacin isomer)



- 18** R = OCH₃, R¹ = OH (grewin)
27 R = OH, R¹ = H (maoyancaosu)

**21** (hemidesminin)

- 30** R = H (moluccanin)
30a R = Ac (moluccanin diacetate)

Chart 1. (continued)

2.4.1. Cleomiscosins

Cleomiscosins A (**9**) and B (**10**), the first regioisomeric pair of coumarinolignans, were isolated from the seeds of *Cleome viscosa* (**29**, **30**). Cleomiscosin A (**9**), C₂₀H₁₈O₈ (M⁺ *m/z* 386.0992), mp 247°C, [α]_D ± 0°cm² g⁻¹, was recognized as possessing a coumarin nucleus from its UV (λ_{max} 327, 288 nm) and IR (ν_{max} 1,710,

1,610 cm^{-1}) spectroscopic data and from the characteristic doublets ($J = 9.5$ Hz) at δ 6.39 and 7.70 ppm in its ^1H NMR spectrum. The coumarin moiety together with two aromatic methoxy groups (δ 3.70 and 3.78 ppm) accounted for 11 of 20 carbons of the molecule, and the remaining nine carbons were considered to be due to a phenylpropanoid unit (C_6C_3), from its ^{13}C NMR spectrum (Table 5), which indicated 12 aromatic carbons in the molecule. Cleomiscosin A is phenolic and thus it provided the monomethyl ether **9a**, $\text{C}_{21}\text{H}_{20}\text{O}_8$, mp 214°C , on methylation with diazomethane, and the diacetate **9b**, $\text{C}_{24}\text{H}_{22}\text{O}_{10}$, mp 175°C , on acetylation with $\text{Ac}_2\text{O}/\text{Et}_3\text{N}$, indicating the presence of a phenolic and an alcoholic hydroxy group. Of the eight oxygen atoms of the molecule, two are present as methoxy groups, one as a phenol, one as an alcohol, and two as part of the coumarin nucleus. The remaining two inert oxygen atoms were considered to constitute oxide linkages. Based on this assumption and an examination of the ^{13}C NMR spectrum of cleomiscosin A, which showed in addition to signals for a coumarin nucleus, signals for six additional aromatic carbons, two CH-O, and one $\text{CH}_2\text{-O}$ moieties, the partial structure of cleomiscosin A was postulated as shown in Fig. 7.

The number of unsaturated sites of cleomiscosin A was calculated $[(\text{C}+1) - \text{H}/2]$ as 12, but this partial structure has only 11 unsaturated sites, and therefore an additional ring by participation of the oxide functions was reasonable. Cleomiscosin A was thus considered to be a coumarin linked with a phenylpropanoid unit through a dioxane bridge. As mentioned previously, such compounds are known to undergo RDA cleavage on electron impact in a mass spectrometer (15) to give rise to characteristic fragment ions for an olefin (**F**) and a substituted catechol (**E**), as shown in Fig. 8.

The electron-impact mass spectrum of cleomiscosin A showed prominent fragment peaks at m/z 208 and 180, corresponding to ions **E'** and **F'**, which is explained in Fig. 9.

The position of the methoxy group in the coumarin nucleus of cleomiscosin A was concluded to be at C-6, from the recognition of one lone aromatic hydrogen appearing as a singlet at δ 6.55 ppm in the ^1H NMR spectrum of cleomiscosin diacetate (**9b**) as the coumarin C-5 hydrogen. This assignment was made from the observed NOE between the coumarin H-4 signal at δ 7.60 ppm and the H-5 resonance, as well as that discerned between this H-5 and the $-\text{OCH}_3$ signal at δ

Fig. 7. The partial structure of cleomiscosin A (**9**)

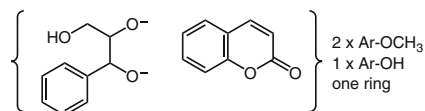


Fig. 8. RDA cleavage of dioxane bridge **D**

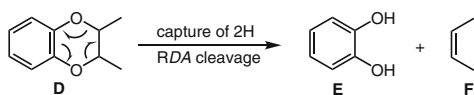
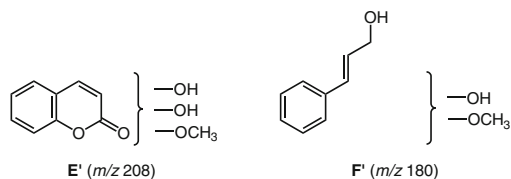
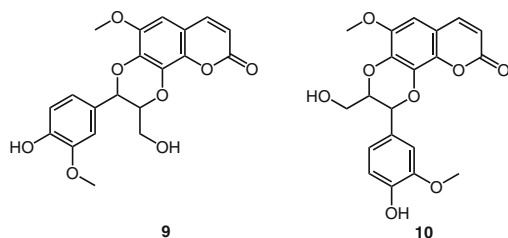


Fig. 9. EIMS fragment ions of cleomiscosin A (**9**)**Fig. 10.** Alternative regioisomeric structures of cleomiscosin A

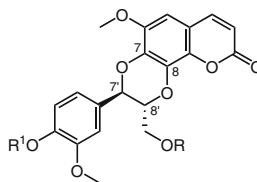
3.88 ppm. The position of the methoxy group in the coumarin nucleus was later confirmed by isolation of fraxetin (**35**) and 6,7,8-trihydroxycoumarin after heating **9** with pyridine hydrochloride at 210°C for 5 min (**32**). The isolation of **35** from cleomiscosin A not only clarified the position of the methoxy group in the coumarin nucleus, but also confirmed that the dioxane bridge involved the coumarin 7 and 8 positions.

The substitution pattern of the aromatic nucleus of the phenylpropanoid moiety was clarified from the isolation of veratric acid by permanganate oxidation of cleomiscosin A methyl ether (**9a**) and a careful examination of the ABX pattern of aromatic hydrogens in **9b**. In the spectrum, the X proton appeared as a doublet at a low field (δ 7.08 ppm, $J = 8.5$ Hz) and the near-equivalent protons appeared as a doublet ($J = 2$ Hz) and a double doublet ($J = 8.5, 2$ Hz) at δ 6.99 ppm, signifying that the phenol acetate group is symmetrically disposed to the AB protons, which, in other words, suggests that the acetoxy group is at the 4' position. Based on these findings, the structure of cleomiscosin A was narrowed down to alternative structures **9** and **10** (**31**) as given in Fig. 10.

A choice between the two structures was possible by a heteronuclear decoupling NMR experiment. Irradiation of H-7' (δ 5.03 ppm) sharpened the signal for C-7 (δ 136.9 ppm) and that of H-8' (δ 4.4 ppm) had a similar effect on the signal for C-8 (δ 131.7 ppm), indicating that cleomiscosin A is a coumarinolignan having 7-O-7' and 8-O-8' linkages. The coupling constants between H-7' and H-8' were found to be 8 Hz, thus indicating that they were of *trans* configuration (**56**). In view of the fact that cleomiscosin A is optically inactive, this isolate was concluded to be racemic. The formula **9** shows the relative stereostructure of cleomiscosin A (see Fig. 11), which was later confirmed by its synthesis (**75**).

The isomeric compound cleomiscosin B (**10**), C₂₀H₁₈O₈ (M⁺ m/z 386.1014), mp 274°C, $[\alpha]_D \pm 0^\circ \text{cm}^2 \text{g}^{-1}$, showed a striking resemblance to **9** in all its spectroscopic

Fig. 11. Relative stereostructure of cleomiscosin A (**9**)



- 9** R = R¹ = H (cleomiscosin A)
9a R = H, R¹ = Me (cleomiscosin A methyl ether)
9b R = R¹ = Ac (cleomiscosin A diacetate)

properties, indicating a close structural similarity, which was confirmed from ¹H- and ¹³C NMR spectroscopic comparison of their diacetate and ethyl ether monoacetate derivatives (31). In view of these similarities, **10** was logically assumed to be the positional isomer of **9**, which was further verified by the ¹³C-¹H spin couplings between the C-8 signal at δ 132.1 ppm and the H-7' signal at δ 5.04 ppm, and between the C-7 signal at δ 136.3 ppm and the H-8' signal at δ 4.40 ppm in the diacetate derivative (31).

Cleomiscosin C (**7**), C₂₁H₂₀O₉ (M⁺ *m/z* 416), mp 255°C, [α]_D ±0°cm² g⁻¹, showed virtually identical UV and IR spectroscopic data to those of **9** and **10**. The ¹H NMR spectrum of cleomiscosin C exhibited signals for a 6,7,8-trisubstituted coumarin nucleus, a phenylpropanoid moiety, and three methoxy groups [δ 3.79 (s, 3H) and 3.77 (s, 6H) ppm] instead of two such groups as in its congeners. Consistent with this observation, cleomiscosin C showed in its mass spectrum the molecular ion peak at *m/z* 416 and a phenylpropanoid ion at *m/z* 210 (**B**), which are 30 mass units higher than the corresponding peaks for **9** and **10** (Table 7). The extra methoxy group in cleomiscosin C was thus proved to be in its phenylpropanoid unit. While a symmetrical substitution pattern of the aromatic ring of the phenylpropanoid unit was apparent from the ¹H NMR spectrum, the ¹³C NMR resonances of cleomiscosin C diacetate were found to be in good agreement with those of cleomiscosin A diacetate (**9b**) rather than for cleomiscosin B diacetate, especially in the chemical shifts of C-7', C-8', and C-9'. Thus, cleomiscosin C was proved to have the same structural framework (8-O-8'/7-O-7') as cleomiscosin A and hence was formulated as **7**. The coumarinolignan, aquillochin, isolated from *Aquilaria agallocha* (64) was also identified as cleomiscosin C (**7**) by direct comparison (31).

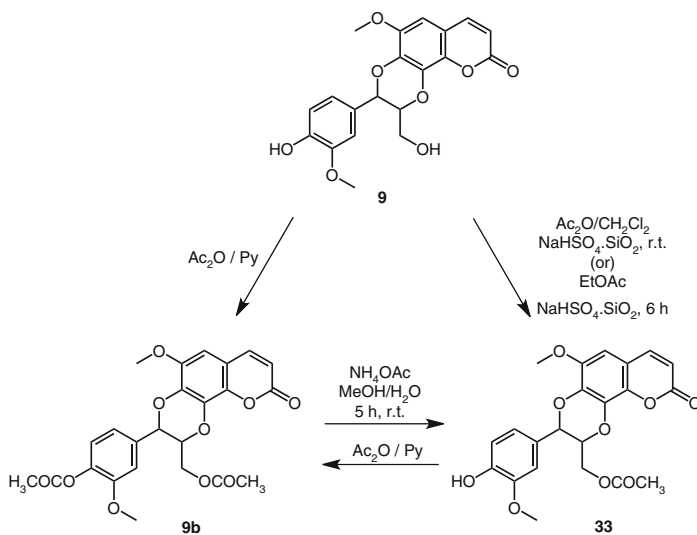
The regioisomer of **7** was also isolated and named cleomiscosin D (**11**). The spectroscopic data (¹H and ¹³C NMR) of **11**, C₂₁H₂₀O₉ (M⁺ *m/z* 416), mp 243–246°C, showed a close resemblance to those of **10**, but an additional methoxy group was evident in the phenylpropanoid ring, like in **7** (32). (7'*S*,8'*S*)-4'-O-Methylcleomiscosin D (**29**) has been isolated from *Zanthoxylum avicennae* (52).

2.4.2. Venkatasin and Hyosgerin

Venkatasin (**33**) and hyosgerin (**22**) constitute the first natural regioisomeric pair of acylated coumarinolignans, with both having the molecular formula C₂₂H₂₀O₉.

The ^1H and ^{13}C NMR data of these compounds (Table 3 and 5) are markedly similar and found to resemble those of **9** and **10**, with the exception of also exhibiting signals for an acetyl group. The acetoxy group was positioned at C-9' in both compounds **33** and **22** on comparing the ^1H NMR chemical shifts of $\text{H}_{2-9'}$ (δ 4.07 (dd, $J = 13.2, 5.5$ Hz) and 4.38 (dd, $J = 13.2, 3.1$ Hz) ppm) with those for the same protons of **9** and **10** (Table 3). The acetylated product of **33** showed identity with cleomiscosin A diacetate (**9b**) from its physical and spectroscopic properties, thus establishing it as 9'-*O*-acetyl-cleomiscosin A (**33**) (46), which was confirmed by the regioselective acetylation of the hydroxy group at C-9' of **9** with acetic anhydride in the presence of NaHSO_4 , with silica gel acting as a heterogeneous catalyst (76). Compound **33** can be synthesized directly from **9b** utilizing ammonium acetate catalyst (77), as shown in Scheme 1. This compound, **33**, was also isolated from *Duranta repens* and reported under the name durantin A (70).

The optically active hyosgerin (**22**) ($[\alpha]_{\text{D}} -65.4^\circ \text{cm}^2 \text{g}^{-1}$), isolated from the seeds of *Hyoscyamus niger* (63), showed a striking resemblance to cleomiscosin B 9'-acetate in all its spectroscopic properties and a logical assumption of their identity was verified by deacetylation of hyosgerin with K_2CO_3 in MeOH/THF to cleomiscosin B (**10**). As all cleomiscosin derivatives are known to be racemic, it was considered of interest to determine whether **10**, derived from **22**, is optically active. To resolve this problem, **10** isolated from *H. niger*, and that derived from **22** were converted to their MTPA esters and their ^1H NMR spectra were compared. While the natural cleomiscosin B MTPA ester showed two sets of ^1H NMR signals in a ratio of 1:1, ascertaining its racemic nature, the MTPA ester of the deacetylated product of **22** ($[\alpha]_{\text{D}} -50^\circ \text{cm}^2 \text{g}^{-1}$) showed only one set of ^1H NMR signals, thus confirming the enantiomeric nature of hyosgerin (**22**) (63).



Scheme 1. Interconversion of some cleomiscosin derivatives

2.4.3. Antidesmanins

The antidesmanins, isolated from the roots of Formosan *Antidesma pentandrum* var. *barbatum*, are a series of coumarinolignans having an unusual 1,1-dimethylallyl substituent, and are rather rare in Nature (39).

Antidesmanin A (**3**), C₂₆H₂₈O₈ (M⁺ *m/z* 468.1779), was obtained as a colorless oil. The presence of a coumarin moiety was revealed from its UV [328, 235 (sh), 220 nm], IR (1,710, 1,615, 1,580 cm⁻¹), and ¹³C NMR spectroscopic (δ 159.5 ppm) data. The ¹H NMR spectrum confirmed the presence of a coumarinolignan skeleton. However, the absence of the characteristic doublets for the coumarin H-3 and H-4 in the ¹H NMR spectrum of **3** and the presence of a singlet at δ 7.50 ppm (H-4) suggested that H-3 is substituted. This substituent at C-3 was considered to be a 1,1-dimethylallyl group [δ 1.49 (6H s, 2 x *quat*-Me), δ 5.09 (1H d, *J* = 17.4 Hz), δ 5.10 (1H d, *J* = 10.6 Hz), and δ 6.12 (1H dd, *J* = 17.4, 10.6 Hz, CH=CH₂) ppm], which is consistent with coumarin ion **A** at *m/z* 276 in the mass spectrum (Table 7). The phenylpropanoid unit of **3** was identified as 4-hydroxy-3,5-dimethoxyphenylpropane with oxide functions at the 7' and 8' positions from its ¹H NMR data. Their assignments were based on COSY and NOESY NMR experiments (39). The two oxide functions are involved as ether linkages to C-7 and C-8 of the coumarin moiety. While the 8-O-7'/7-O-8' linkages were confirmed through HMBC correlations, the coupling constant (7.6 Hz) between the two oxymethine protons disclosed their *trans*-relationship (39).

Antidesmanin B (**4**) and antidesmanin C (**5**) are regioisomers having the molecular formula, C₂₉H₃₂O₇ (M⁺ *m/z* 492.2177). The UV and IR spectra were found to be similar to those of antidesmanin A (**3**) but the ¹H NMR spectrum showed signals for an additional 1,1-dimethylallyl unit in place of CH₃ at 9' and a 4-hydroxy-3-methoxyphenyl group in place of a 4-hydroxy-3,5-dimethoxyphenyl group (Table 3). Through HMBC correlations, the two ether linkages of **4** were identified as 8-O-8'/7-O-7'. Based on 1D and 2D NMR analysis, the dioxane bridge of antidesmanin C (**5**) was proved to have 8-O-7'/7-O-8' linkages (39).

2.4.4. Grewin

Grewin (**18**), C₁₉H₁₆O₈ [(M+Na)⁺ *m/z* 395.0814], is one of the four linearly fused coumarinolignans known so far (69). The recognition of **18** as a coumarin derivative from UV and IR data was confirmed from its ¹H NMR spectrum, which showed characteristic doublets for coumarin protons (δ 7.64 (*J* = 9.6 Hz, H-4) and 6.36 (*J* = 9.6 Hz, H-3) ppm) and two singlets in the aromatic region (δ 7.08 (H-8) and 7.18 (H-5) ppm] for a 6,7-dioxygenated coumarin moiety. The appearance of two aromatic hydrogens as *meta*-coupled signals [δ 7.35 (d, *J* = 1.9 Hz, H-6') and 7.01 (d, *J* = 1.9 Hz, H-2') ppm] and a methoxy singlet (δ 3.79 ppm) indicated an unsymmetrical 3'-methoxy-4',5'-dioxyphenyl group, extended with a three-carbon sequence [δ 5.50 (d, *J* = 8.0 Hz, H-7'), 4.46 (m, H-8'), 3.98 and 4.21 (bd each, *J* = 12.3 Hz, H₂-9') ppm]. The presence of a phenylpropanoid unit in **18** was also

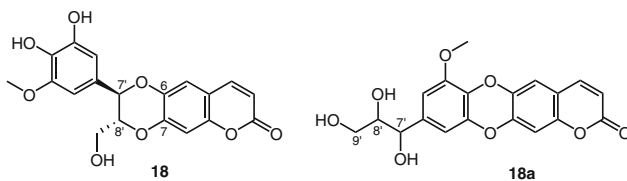


Fig. 12. Possible structures for grewin

confirmed from the ^{13}C (Table 6), ^1H - ^1H COSY, and HMBC NMR spectroscopic data. On the basis of these data and calculation of the double-bond equivalence, grewin was postulated to be formed by linear fusion of a 6,7-dioxygenated coumarin moiety with a C_6C_3 unit through a 1,4-dioxane bridge, with two possible structures, **18** and **18a**, advanced for this coumarinolignan, as shown in Fig. 12.

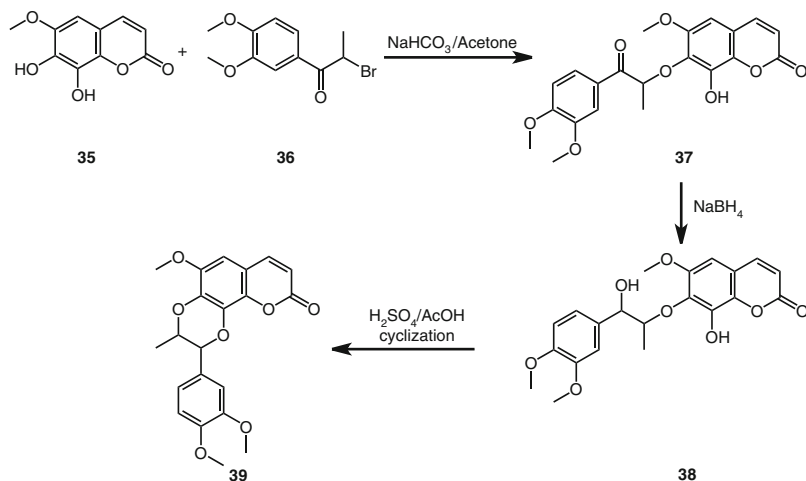
A comparison of the observed ^1H and ^{13}C NMR spectroscopic data with those of the predicted values for structure **18a** ruled out the possibility of this structure for grewin, with the structure **18** assigned to this substance. A large coupling constant (8 Hz) showed a *trans* relationship between H-7' and H-8'. Structure **18** for grewin was confirmed through selected HMBC spectroscopic correlations (69).

2.4.5. Synthesis of Coumarinolignans

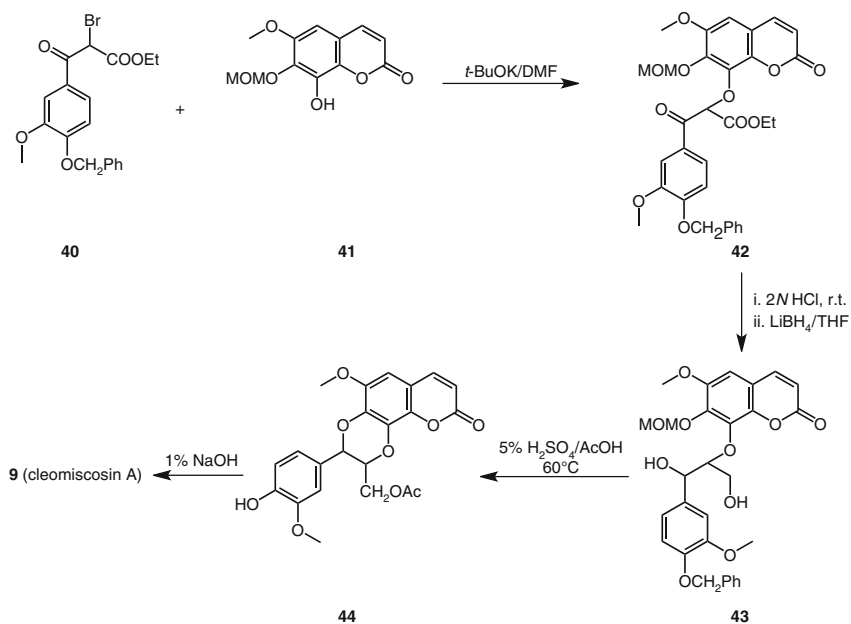
Corroborative evidence for the structures of various coumarinolignans, deduced almost exclusively from spectroscopic evidence, has been provided by their appropriate synthesis. The synthesis of this group of natural products has been achieved mainly through oxidation (chemical or enzymatic) of appropriate coumarin and phenylpropanoid derivatives, although other methods are also known.

The first synthesis of a coumarinolignan derivative was achieved by *Parthasarathy* and *Pardhasaradhi* (43), who prepared the methyl ether of the propacin isomer **39**, through the condensation of fraxetin (**35**) with α -bromo-propioveratrone (**36**), followed by reduction and cyclization (Scheme 2). The product obtained showed identity with the authentic methyl ether of the natural propacin isomer, isolated from *Jatropha glandulifera* (42).

A starting material for the synthesis of **9** is the 7-methoxymethyl ether of fraxetin (**41**), which was prepared by different workers using alternative methods. Tanaka *et al.* found it convenient to prepare it from fraxetin (**35**) by dropwise addition of a solution of chloromethyl methyl ether to a solution of **35** in a suspension of sodium hydride in THF. The reaction of this fraxetin derivative **41** with ethyl 2-bromo-3-(4-benzyloxy-3-methoxyphenyl)-3-oxopropionate (**40**) yielded an ether (**42**), which on lithium borohydride reduction yielded a mixture of diastereomers with *threo*- and *erythro*-diols, **43**. Cyclization of this compound with sulphuric acid (5%) afforded cleomiscosin A monoacetate (**44**), which gave cleomiscosin A (**9**) on mild alkaline



Scheme 2. Outline of the synthesis of the methyl ether of propacin isomer (39)



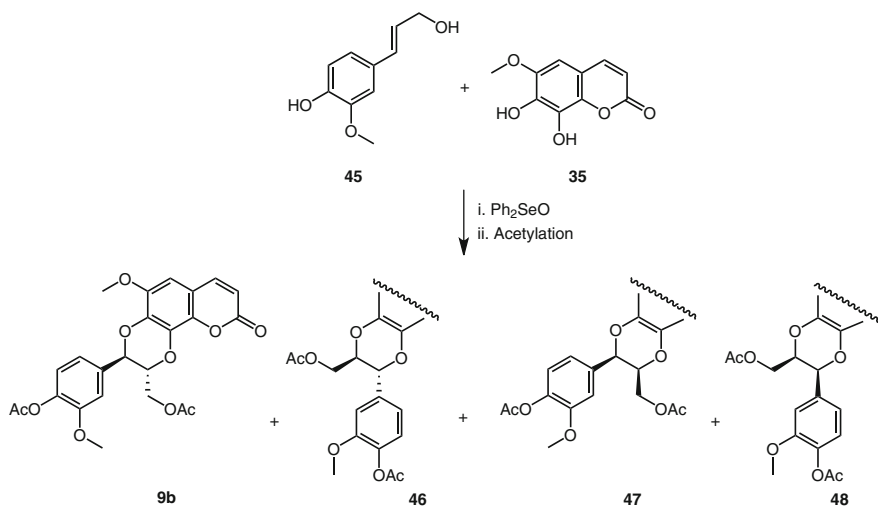
Scheme 3. Synthesis of cleomiscosin A (9)

hydrolysis (75). The synthesis steps for 9 are summarized in Scheme 3. Tanaka and his colleagues also achieved the syntheses of cleomiscosin B (10) (78), daphneticin (12) (79), a propacin isomer (32) (80), and cleomiscosins C (7) and D (11) (81).

A biomimetic method was developed by *Arnoldi* and coworkers to synthesize propacin (**31**). Oxidation of an equimolar mixture of **35** and isoeugenol in toluene/methanol with Ag_2O at room temperature provided 30% of **31**. A similar reaction has also been attempted with coniferyl alcohol (**45**) to synthesize **9** and **10** (**72**).

Lin and *Cordell* devised a method for the synthesis of coumarinolignans using chemical and enzymatic approaches (**73**). Chemical oxidizing agents, like ferric chloride, silver oxide, and dichlorodicyanoquinone (DDQ), and enzymes, like horseradish peroxidase, tyrosinase, and chloroperoxidase were used for oxidative coupling of *ortho*-dihydroxycoumarin derivatives. Included were fraxetin, daphnetin, and esculetin with phenylpropenoids like isoeugenol, coniferyl alcohol, and sinapyl alcohol. Silver oxide was found to be superior to oxidizing agents like FeCl_3 and DDQ. Enzymatic oxidation processes afforded a single regioisomer of the resultant coumarinolignans in higher yields than by chemical oxidation methods (**73**).

Use of Ph_2SeO was soon recognized to be a more satisfactory method for such oxidations (**82**). Oxidation of **35** and **45** in methanol and benzene using Ph_2SeO at room temperature followed by acetylation yielded cleomiscosin A diacetate (**9b**) as the major product, along with three isomers, cleomiscosin B diacetate (**46**), *cis*-cleomiscosin A diacetate (**47**), and *cis*-cleomiscosin B diacetate (**48**) as minor products. Similarly, oxidative coupling of fraxetin and daphnetin with sinapyl alcohol followed by acetylation yielded aquillochin diacetate and daphneticin diacetate, respectively. In the presence of Ph_2SeO , hydroxy groups in 7,8-dihydroxycoumarins would oxidize rapidly to generate the corresponding *ortho*-quinones, in which the oxygen atom at the C-8 position is immediately attacked by the double bond (C-8') of phenylpropenes to afford the coumarinolignans. Among the methods discussed so far, this procedure has shown high regio- and stereo-selectivity in the synthesis of naturally occurring coumarinolignans. This is shown in Scheme 4.



Scheme 4. Regio- and stereo-selective synthesis of coumarinolignans using Ph_2SeO

16% yield (83). Recently, Ramesh *et al.* have reported the synthesis of venkatasin (33) (yield 95%) directly from cleomiscosin A diacetate (9b), utilizing ammonium acetate as the catalyst for the selective deprotection of aromatic acetates (77).

2.5. Biological Activity of Coumarinolignans

Whenever a new compound is isolated from a plant, it is customary to explore its biological activity to potentially enrich our medical armamentarium in terms of new drugs, and coumarinolignans have been no exception. While coumarinolignans have shown many interesting biological activities, the majority of these refer to their cytotoxic and hepatoprotective potential. The different activities shown by this group of compounds are summarized in Table 9.

Daphneticin (12), isolated from *Daphne tangutica*, showed cytotoxic activity against *Walker* carcinosarcoma ascites cells (68) but was inactive against KB cells (68). In vitro studies showed cleomiscosin A (9), isolated from the chloroform extract of *Brucea javanica*, to be active against the murine P-388 lymphocytic leukemia cell line ($ED_{50} = 0.4 \mu\text{g ml}^{-1}$), but inactive against the KB test system (57). Luyengi *et al.* reported the lack of activity of the same compound when evaluated against HL-60 human promyelocytic leukemia cells (59).

Antidesmanins A (3), B (4) and C (5), isolated from the roots of *Antidesma pentadrum*, were evaluated for their in vitro effects on the growth of three human

Table 9. Biological activities of coumarinolignans

Coumarinolignan(s)	Biological activity ^a	References
Antidesmanins A (3), B (4), and C (5)	Cytotoxic against MCF-7 (breast) and SF-268 (CNS) cancer cell lines	(39)
Cleomiscosin A (9)	Cytotoxic against P-388 (lymphocytic leukemia) cell line	(57)
Cleomiscosins A (9), B (10), and C (7) (Cliv 92)	Liver protective properties	(31)
Cleomiscosins A (9), B (10), and C (7)	Immunomodulatory activity (in vivo)	(84, 85)
Cleomiscosins A (9) and C (7)	Anti-HIV, vasorelaxant effect, lipid peroxidation inhibitory activity	(18, 19, 41)
Cleomiscosin D (11)	Anti-inflammatory activity	(52)
Daphneticin (12)	Antibacterial activity, cytotoxic against <i>Walker</i> carcinosarcoma ascites cells	(66, 68)
5'-Demethyl-aquilochin (14)	Anti- HIV activity	(41)
Durantins A (33), B (15), and C (16)	Phosphodiesterase inhibitory effect	(70)
8'- <i>epi</i> -Cleomiscosin A (17)	Tyrosinase inhibitory activity	(36)
Grewin (18)	Antimalarial activity	(69)
Jatrocin B (25)	Lipid peroxidation inhibitory activity	(49)
(7'S,8'S)-4'- <i>O</i> -Methylcleomiscosin D (29)	Anti-inflammatory activity	(52)

^aIn vitro activity unless otherwise stated

cancer cell lines: MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS) by Chen *et al.* (39). These compounds exhibited marginal cytotoxicity against the MCF-7 and SF-268 cancer cell lines, but, however, they were found to be ineffective against NCI-H460 cells.

Ray and coworkers (31) studied the liver protective properties of cleomiscosins A (9), B (10) and C (7), isolated from *Cleome viscosa* seeds, and found significant activity against D-galactosamine-induced cytotoxicity in primary cultured rat hepatocytes, with 10 being the most potent of these substances. Chattopadhyay *et al.* (33) have patented a hepatoprotective pharmaceutical preparation (Cliv-92), composed of a mixture of three coumarinolignans isolated from the seeds of *C. viscosa*, in the optimized ratio of 3:5:2.

Cleomiscosin C (7) and 5'-methoxypropacin (24) from *Hibiscus syriacus* (with the latter compound also named jatrocinn B, after being purified from *Jatropha gossypifolia*), showed lipid peroxidation inhibitory activity, with IC_{50} values of 0.7 and 1.4 $\mu\text{g ml}^{-1}$ comparable to vitamin E (IC_{50} 0.8 $\mu\text{g ml}^{-1}$), when tested using a rat liver microsomal model (49).

Jin and collaborators reported the antioxidant activity of cleomiscosins A (9) and C (7), isolated from the leaves and twigs of *Acer okamotoatum* (19). Compound 7 inhibited LDL oxidation mediated by either catalytic copper ions or free radicals generated with 2,2'-azobis(2-amidinopropane) dihydrochloride, in a dose-dependent manner. By means of electrophoretic analysis it was also observed that 7 protected apolipoprotein B-100 against Cu^{2+} fragmentation. Also, fluorescence analysis clearly indicated that both 7 and 9 protect against the oxidative modification of apoB-100, induced by either Cu^{2+} or HOCl. Compounds 7 and 9 could, therefore, be beneficial in preventing LDL oxidation in atherosclerotic lesions.

Cleomiscosins A (9), B (10), and C (7), isolated from the seeds of *Cleome viscosa*, were shown to have immunomodulatory activity *in vivo* by Bawankule *et al.* (84). These coumarinolignans, at a dose of 10 mg kg^{-1} body weight, enhanced the body immune function by significantly increasing the white blood cell count and hemagglutination antibody titer responses, by reducing the delayed-type hypersensitivity response towards rabbit red blood cells. The same group, led by Chattopadhyay, filed a patent on an immunomodulatory pharmaceutical composition, comprised of cleomiscosins isolated from the seeds of *C. viscosa* (85).

Daphneticin (12), isolated from the leaves of *Daphne gnidium*, has been reported to possess moderate antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* (66). Cheng and Chen demonstrated that the coumarinolignans, aquillochin (7), cleomiscosin A (9), and 5'-demethyl aquillochin (14) were active against HIV (41).

Iqbal *et al.* studied the phosphodiesterase inhibitory effect of durantins A (33), B (15), and C (16), isolated from *Duranta repens* (70). Compounds 16 and 33, along with compound 9, showed moderate to strong inhibitory activity against snake venom phosphodiesterase 1, using cysteine and EDTA as positive controls, while 15 showed weak activity.

8'-*epi*-Cleomiscosin A (**17**) was found to be a more potent tyrosinase inhibitor (IC_{50} 1.33 μM) than the standard tyrosinase inhibitors, kojic acid (IC_{50} 16.67 μM) and L-mimosine (IC_{50} 3.68 μM). This coumarinolignan thus appears to be a potential candidate for the treatment of hyperpigmentation associated with the high production of melanocytes (**36**).

The antimalarial activity of the linear coumarinolignan, grewin (**18**), was evaluated on cultures of *Plasmodium falciparum* clones D6 and W2 and it displayed weak antimalarial activity with no discernible cytotoxicity (**69**). Cleomiscosin A (**9**) and aquillochin (**7**), isolated as major constituents from the heartwood of *Acer nikoense*, exhibited moderately potent vasorelaxant effects in the rat aorta using *nor*-epinephrine-stimulated and high K^+ -depolarized preparations (**18**). (7'*S*,8'*S*)-4'-*O*-Methyl-cleomiscosin D (**29**) and cleomiscosin D (**11**), isolated from *Zanthoxylum avicennae*, were found to inhibit superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochlasin B (**52**).

No major toxicity study of the coumarinolignans has appeared in scientific literature to date.

2.6. Biogenesis of Coumarinolignans

The biogenesis in plants of coumarins, flavonoids, and stilbene derivatives is well documented (**16**, **86**) and it is known that these are all derived, fully or in part, from a phenylpropanoid (C_6C_3) unit. However, it is still a subject of speculation as to how these molecules connect with another C_6C_3 unit to elaborate these "non-conventional" lignans. The characteristic feature of all coumarinolignans is a dioxane bridge that links the coumarin moiety with a phenylpropanoid unit, and this necessitates the presence of an *ortho*-dihydroxy group in the aromatic ring of the coumarin moiety. It is believed that a coumarin with an *ortho*-dihydroxy group, through one-electron oxidation, affords a radical (**35a**), which may combine with the radical produced from a phenylpropanoid unit like coniferyl alcohol (**45a**) to form an adduct (**59**). This adduct then cyclizes by attack of the phenol nucleophile on the quinone methide structure provided by coniferyl alcohol and produces a mixture of two *trans* diastereomers of the coumarinolignan, **9c** and **9d**, because of the non-stereospecificity of the radical coupling. This is clarified from the probable biogenetic steps for elaboration of cleomiscosins A (**9**) and B (**10**), as shown in Fig. 13. The antidesmanins (**3–5**) bear a 1,1-dimethylallyl substituent in place of a methyl or hydroxymethyl group at the C-9' position. It is conjectured that the initial product formed by radical coupling of coniferyl alcohol or its analog with an *ortho*-dihydroxy coumarin generates a vinylogous β -hydroxyketone (**60**), which undergoes deformylation and subsequent alkylation by a nucleophilic attack on dimethyl allyl pyrophosphate (**62**). The essential steps are shown in Fig. 14.

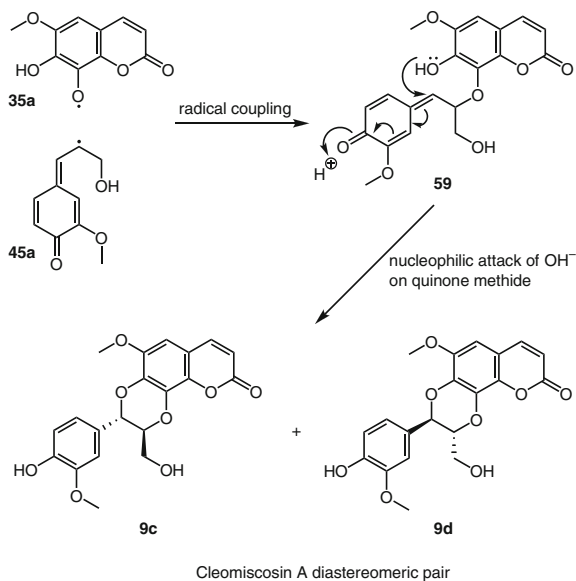


Fig. 13. Biogenesis of cleomiscosin A (9)

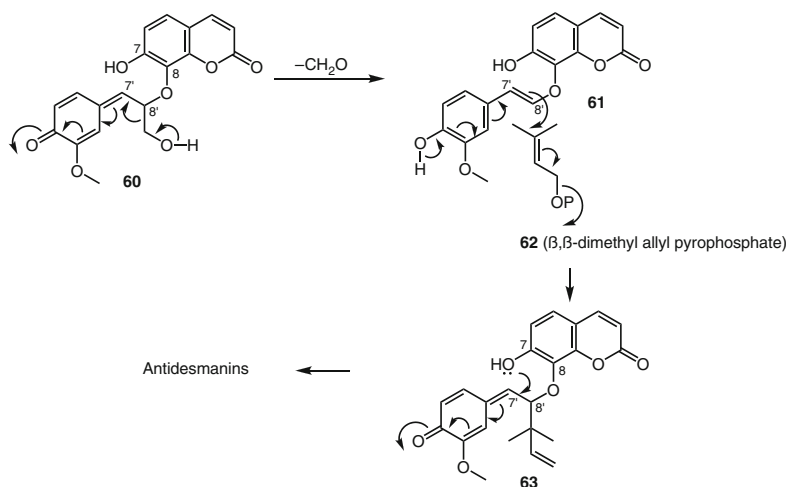


Fig. 14. Biogenesis proposal for the antidesmanins

3. Flavonolignans

Flavonolignans are the most extensively studied group of non-conventional lignans, particularly because of their diverse pharmacological activities. “Milk thistle” (*Silybum marianum*), an ancient therapy for acute and chronic liver diseases, is the first known source of this group of compounds (15). Flavonolignans, isolated

from the fruits of milk thistle, have been characterized, evaluated for their medicinal properties, and commercialized in the pharmaceutical market as “Silymarin” (87). The structural novelty and interesting biological activities elicited by flavonolignans has led to a thorough chemical investigation on different plant parts of *S. marianum*, and numerous compounds having similar structural features have been isolated. Several review articles on flavonolignans (13, 88, 89, 90) and their synthetic derivatives (91) have appeared since the isolation of silybin (1).

3.1. Occurrence of Flavonolignans

The distribution of flavonolignans is fairly widespread and these have so far been isolated from plants belonging to seven different plant families. *Silybum marianum* of the family Asteraceae is the richest source of this group of compounds found to date and it has so far yielded 17 flavonolignans. *Hydnocarpus wightiana* has also contributed in a major way as a source of flavonolignans. The various plant sources so far known to produce this group of “non-conventional” lignans are listed in Table 10, and the image of *Sasa veitchii* is shown in Fig. 15. Table 11 presents a list of flavonolignans arranged alphabetically together with their structure numbers, sources, and references.

3.2. Features of Flavonolignans and Their Classification

Flavonolignans are not merely products formed by oxidative coupling of a phenylpropanoid unit with a typical flavone, since this term embraces all such products derived from other flavonoid types, viz. flavonols, chalcones, catechins, and others. However, plant constituents like rhodiolinin (103) (125) and the iryantherins (104) (126–129), in which a phenylpropanoid unit is linked with the A ring of the flavone nucleus, would be more appropriately termed “lignoflavonoids” (126) rather than

Table 10. Sources of flavonolignans

Plant family	Plant species
1. Asteraceae	1a. <i>Onopordon corymbosum</i> (109)
	1b. <i>Silybum marianum</i> (15, 92–105)
	1c. <i>Silybum marianum</i> subsp. <i>anatolicum</i> (106)
2. Berberidaceae	2a. <i>Berberis fremontii</i> (107)
3. Chenopodiaceae	3a. <i>Salsola collina</i> (108)
4. Flacourtiaceae	4a. <i>Hydnocarpus wightiana</i> (110–113)
5. Fabaceae	5a. <i>Cassia absus</i> (116)
	5b. <i>Hymenaea palustris</i> (117)
6. Poaceae	6a. <i>Aegilops ovata</i> (114)
	6b. <i>Avena sativa</i> (118)
	6c. <i>Hyparrhenia hirta</i> (119)
	6d. <i>Sasa veitchii</i> (115)
7. Scrophulariaceae	7a. <i>Verbascum sinaiticum</i> (120)
	7b. <i>Gratiola officinalis</i> (121, 122)



Fig. 15. *Sasa veitchii*

“flavonolignans” (127–129). These compounds neither fulfill the classical definition of lignans or neolignans nor our definition of “non-conventional” lignans, because the “A rings” of the flavonoid components are not a part of the C_6C_3 units (86) (see Fig. 16).

Unlike coumarinolignans, of which all bear a dioxane bridge, the linkages of two phenylpropanoid units in the flavonolignans are rather varied in form. No doubt there are compounds with a typical dioxane bridge linking the two C_6C_3 units, but flavonolignans with furan, cyclohexane, and simple ether linkages are also known. This diversity originates mainly from the fact that besides a typical ether linkage, union of two phenylpropanoid units in flavonolignans takes place

Table 11. Flavonolignans and their plant sources

Compound (see Chart 2)	Plant source(s) (see Table 10)	References
Aegicin (64)	6a	(114)
Compound 2 from <i>Avena sativa</i> (65)	6a	(118)
Dehydrosilybin (66)	1b	(94)
2,3-Dehydrosilychristin (67)	1b	(96)
Hydnocarpin (68)	1a, 4a, 5a, 7a	(110, 113, 116, 120)
Hydnocarpin D (69)	5b	(117)
Hydnowitzin (70)	4a	(113)
Isohydnocarpin (71)	4a, 5a	(111, 113, 116, 123)
Isolignoside (72)	7b	(121, 122)
Isosilybin A (73)	1b, 1c	(100, 102, 124)
Isosilybin B (74)	1b, 1c	(102, 124)
Isosilychristin (75)	1b	(102)
Lignoside (76)	7b	(121, 122)
5'-Methoxyhydnocarpin (77)	2a, 4a	(112, 113)
5''-Methoxyhydnocarpin (78)	1a	(109)
5'-Methoxyhydnocarpin D (79)	5b	(107)
Neohydnocarpin (80)	5a	(113)
Neosilyhermin A (81)	1b	(101)
Neosilyhermin B (82)	1b	(101)
Palstatin (83)	5b	(117)
Salcolin A (84)	3a, 6b–6d	(108, 115, 118, 119)
Salcolin A glycoside (85)	6c	(119)
Salcolin B (86)	3a, 6b–6d	(108, 115, 118, 119)
Salcolin B glycoside (87)	6c	(119)
Silandrin (88)	1b	(99)
Silybin A (89)	1b, 1c	(14, 102, 124)
Silybin B (90)	1b, 1c	(102, 124)
Silychristin A (91)	1b, 1c	(97, 98, 102, 104)
Silychristin B (92)	1b	(104)
Silydianin (93)	1b, 1c	(92, 102)
Silyhermin (94)	1b	(101)
Silymonin (95)	1b	(99)
Sinaiticin (96)	7a	(120)
Tricin 4'- <i>O</i> -[<i>erythro</i> - β -guaiacyl-(9''- <i>O</i> -acetyl)-glyceryl] ether (97)	6d	(115)
Tricin 4'- <i>O</i> -[<i>threo</i> - β -guaiacyl-(9''- <i>O</i> -acetyl)-glyceryl] ether (98)	6d	(115)
Tricin 4'- <i>O</i> -[<i>erythro</i> - β -guaiacyl-(7''- <i>O</i> -methyl-9''- <i>O</i> -acetyl)-glyceryl] ether (99)	6d	(115)
Tricin 4'- <i>O</i> -[<i>threo</i> - β -guaiacyl-(7''- <i>O</i> -methyl-9''- <i>O</i> -acetyl)-glyceryl] ether (100)	6d	(115)
Tricin 4'- <i>O</i> -[<i>erythro</i> - β -guaiacyl-(9''- <i>O</i> - <i>p</i> -coumaroyl)-glyceryl] ether (101)	6d	(115)
Tricin 4'- <i>O</i> -[<i>threo</i> - β -guaiacyl-(9''- <i>O</i> - <i>p</i> -coumaroyl)-glyceryl] ether (102)	6d	(115)

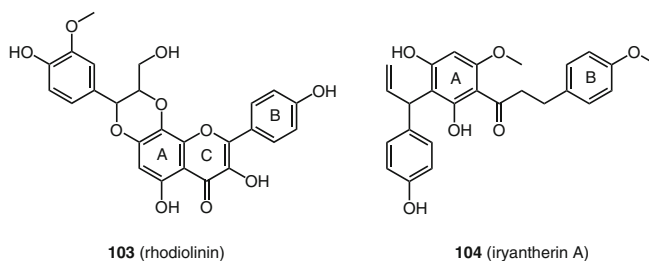


Fig. 16. Examples of “lignoflavonoids”

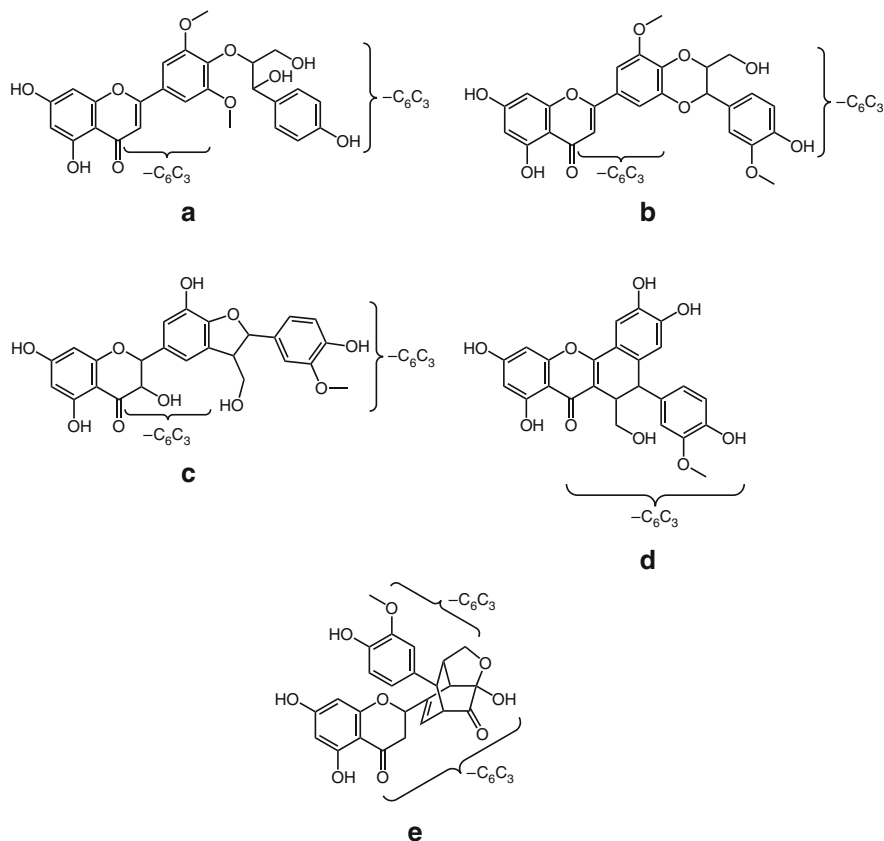


Fig. 17. Classification of flavonolignans: simple ether (a), dioxane bridge (b), furan bridge (c), cyclohexane bridge (d), and tricyclic ketone (e)

through carbon-carbon linkages, which lead to furan and cyclohexane rings and complex tricyclic ketones. It is interesting to note that in almost all the flavonolignans so far reported, the C_6C_3 units linking the flavonoids are coniferyl alcohol or its analogs. The different modes of fusion of two C_6C_3 units in flavonolignans are shown in Fig. 17. Hence, flavonolignans can be broadly classified into five groups (A-E), as shown in Fig. 17.

3.3. Structure Elucidation of Flavonolignans

In the early years after the discovery of silybin (**1**), the structure determination of this compound class was mainly achieved through extensive chemical conversions and degradative reactions (15, 95), which often led to structures that later needed revision. Analysis of comprehensive spectroscopic data along with classical chemical reactions and synthesis approaches has led to the unequivocal determination of the absolute structures of flavonolignans (102, 135). The complete structure elucidation of flavonolignans is summarized below.

3.3.1. Ultraviolet Absorption Spectra and Infrared Spectra

Generally, flavones and flavonols exhibit high intensity peaks in the regions 320–380 nm (Band I) and 240–270 nm (Band II) in the UV spectrum. Flavonones absorb more strongly at 270–290 nm (Band II) with a slight low intensity inflection in the 320–330 nm region. Substitutions (hydroxy or methoxy group) in either of the “A” or “B” rings produce bathochromic shifts (130). The UV spectroscopic data of some flavonolignans are detailed in Table 12. In the IR spectrum, the chromone carbonyl exhibits a strong absorption band around 1,660 cm^{-1} and the aromatic ether linkage around 1,160 cm^{-1} . The phenolic hydroxy group shows an absorption around 3,500 cm^{-1} .

3.3.2. ^1H NMR Spectra

The flavonoid skeleton present in flavonolignans can be readily identified from their ^1H NMR spectra. Silybin (**1**) and its congeners, practically all of which bear a 3,5,7-trihydroxy-3',4'-dioxxygenated dihydroflavone moiety, exhibit signals for

Table 12. Ultraviolet absorption spectroscopic data of some flavonolignans

Compound	$\lambda_{\text{max}}/\text{nm}$ ($\log \epsilon$) ^a
Aegicin (64)	244, 269, 299, 314, 330
Compound 2 from <i>A. sativa</i> (65)	271, 365
2,3-Dehydrosilybin (66)	285 (4.4), 360 (4.0)
Hydnocarpin (68)	270, 335 sh
Isosilybin A (73)	212 (5.4), 230 (5.3), 288 (5.1)
Neosilyhermin A (81)	288 (4.0), 322 sh
Palstatin (83)	269 (4.0), 341 (4.1)
Salcolin A (87)	271, 285 sh, 305 sh, 335
Silybins A (89)	217 (5.2), 230 (5.2), 288 (5.1)
Silychristin A (91)	288 (4.3), 322 sh (3.7)
Silydianin (93)	216 (5.4), 231 sh (5.4), 289 (5.3)

^aLog ϵ data included where available

Table 13. ^1H NMR data (δ/ppm (J/Hz)) of compounds **73**, **75**, **89**, **91a**, and **93** in $\text{DMSO}-d_6$

Proton(s)	Isosilybin A (73)	Isosilychristin (75)	Silybin A (89)	Silychristin (91a)	Silydianin (93)
H-2	5.08 d (11.2)	5.16 d (11.7)	5.06 d (11.3)	4.99 d (11.4)	4.84 d (10.4)
H-3	4.57 d (11.2)	4.63 dd (11.6, 5.5)	4.60 d (11.3)	4.51 dd (11.3, 4.8)	4.42 dd (10.4, 5.2)
H-6	5.89 d (1.6)	5.92 d (1.9)	5.89 d (1.8)	5.90 d (1.9)	5.90 d (1.4)
H-8	5.85 d (1.6)	5.87 d (2.0)	5.84 d (1.8)	5.85 d (2.0)	5.88 d (1.8)
H-2'	7.08 d (1.3)	–	7.07 d (1.4)	6.83 br s	3.43 m
H-5'	6.92 d (8.2)	6.74 d (8.1)	6.96 d (8.3)	–	3.17 dd
H-6'	6.99 br s	6.95 d (8.4)	7.01 d (1.6)	6.87 br s	6.01 d (6.6)
H-2''	6.98 dd (8.1, 1.4)	6.86 d (1.8)	6.99 dd (6.1, 1.5)	6.96 d (1.7)	6.74 d (1.3)
H-5''	6.79 d (8.0)	6.70 d (8.1)	6.79 d (8.1)	6.76 d (8.1)	6.62 d (8.1)
H-6''	6.84 dd (9.2, 1.5)	6.76 dd (8.1, 1.8)	6.85 dd (8.1, 1.4)	6.78 dd (8.9, 2.2)	6.54 dd (8.1, 1.3)
H-7''	4.90 d (7.8)	5.57 m	4.89 d (7.9)	5.46 d (6.9)	3.29 m
H-8''	4.15 m	3.67 dd (13.2, 6.3)	4.16 m	3.47 dd (12.5, 6.4)	2.71 m
H-9''	3.52 d (10.6) 3.33 dd (12.3, 4.5)	3.76 m 3.44 m	3.52 d (10.6) 3.33 d (7.6)	3.72 m 3.63 dd (10.6, 6.8)	4.11 dd (7.9, 3.0)
OH-5	11.83 s	11.93 s	11.89 s	11.92 s	10.57 s
OMe-3''	3.77 s	3.69 s	3.76 s	3.76 s	3.72 s

Table 14. ^1H NMR data (δ/ppm (J/Hz)) of compounds **64**, **65**, **68**, **84**, and **96** in $\text{DMSO}-d_6$

Proton(s)	Aegicin (64)	Compound 2 (65)	Hydnocarpin (68)	Salcolin A (84) ^a	Sinaiticin (96)
H-3	7.0 s	–	6.87 s	6.93 s	6.81 s
H-6	6.18 d (2.2)	6.67 s	6.21 d (2.1)	6.73 d (1.6)	6.14 d (1.4)
H-8	6.51 d (2.0)	6.83 s	6.52 d (2.1)	6.81 d (1.6)	6.43 d (1.8)
H-2'	7.27 s	–	7.66 d (2.2)	7.29 s	7.63 d (1.9)
H-3'	–	–	–	–	–
H-5'	–	–	7.08 d (8.7)	–	7.06 d (8.6)
H-6'	7.27 s	7.66 s	7.59 dd (8.5, 2.2)	7.29 s	7.57 dd (8.6, 1.9)
H-2''	7.16 d (8.0)	7.24 s	7.03 d (1.8)	7.52 s	7.29 d (8.4)
H-3''	6.65 d (8.0)	–	–	–	6.83 d (8.4)
H-5''	6.65 d (8.0)	7.01 d (7.3)	6.83 d (8.1)	7.24 d (8.1)	6.83 d (8.4)
H-6''	7.16 d (8.0)	6.86 d (7.3)	6.91 dd (8.1, 1.8)	7.35 d (8.1)	7.29 d (8.4)
H-7''	4.82 br s	5.70 s	5.02 d (8.0)	5.65 d (4.8)	5.04 d (7.8)
H-8''	4.2 m	4.38 s	4.27 m	5.03 q (5.2, 3.7)	4.21 m
H-9''	3.4 br m	3.84 s 4.37 s	3.38 dd (11.4, 3.7) 3.59 dd (11.4, 1.7)	4.30 dd (12.3, 3.4) 4.59 dd (12.4, 5.1)	3.36 dd (11.3, 4.0) 3.60 d (11.3)
OMe-3'	3.85 s	3.91 s	–	3.82 s	–
OMe-5'	3.85 s	3.84 s	–	3.82 s	–
OMe-3''	–	3.57 s	3.80 s	3.75 s	–

^aIn pyridine- d_5

two *meta*-coupled aromatic AB systems at δ 5.90 and 5.85 ($J \sim 1.8$ Hz) ppm, a singlet for a chelated hydroxy group at the C-5 position at δ 11.8–12.0 ppm, two doublets of an AB system at δ 5.06 ($J \sim 11.0$ Hz) and 4.60 ($J \sim 11.0$ Hz) ppm, along with the aromatic signals of an ABX pattern at δ 6.95–7.10 ppm (103). Hydnocarpin-type flavonolignans with a flavone nucleus show a singlet at δ 6.87 ppm for H-3 (123). In turn, silyhermin (94), which is a flavanone, exhibits double doublet signals for the protons at the same position at δ 2.80 ($J \sim 17.0, 3.0$ Hz, H-3a) ppm, δ 3.11 ($J \sim 17.0, 13.0$ Hz, H-3b) ppm, and δ 5.56 ($J \sim 13.0, 3.0$ Hz, H-2) ppm (101). The substitution in the B ring at C-3' and C-4' can be recognized from the appearance of signals of an ABX pattern in the aromatic region. The oxymethine and oxymethylene protons of the propanol unit in compounds with a dioxane bridge can be detected readily from their characteristic coupling constants: H-7'' generally appears between δ 4.70–4.90 ppm as a doublet ($J = 8.0$ Hz), H-8'' as a doublet of double doublets around $\delta \sim 4.1$ ppm, and the geminally and vicinally coupled H₂-9'' between $\delta \sim 3.50$ and 3.75 ppm (102). The H-7'' and H-8'' protons, in the case of benzofurano-flavonolignans, show signals at δ 5.46 (d) ppm and 3.47 (dd system) ppm. Tricyclic ketone-type flavonolignans, e.g. silydianin (93) show H-7'' and H-8'' signals at about δ 3.29 (m) and 2.71 (m) ppm. The ¹H NMR chemical shifts of the different protons of some flavonolignans are given in Tables 13 and 14.

3.3.3. ¹³C NMR Spectra

The ¹³C NMR spectrum is a useful tool in the structure elucidation of flavonolignans, particularly in distinguishing compounds with different modes of fusion. In the case of typical dioxane-bridged flavonolignans, the C-7'' and C-8'' signals appear at δ 75.9 ppm and δ 78.1 ppm [e.g. silybin A (89)]. While the furan-bridged flavonolignans exhibit C-7'' and C-8'' signals at δ 87.0 ppm and δ 53.3 ppm [e.g. silychristin A (91)], the cyclohexane-bridged compounds display these signals at δ 37.4 ppm and δ 42.8 ppm, respectively [e.g. compound 2 (65)]. The tricyclic ketone group of flavonolignans, e.g. silydianin (93), shows a distinction in the chemical shift values (δ 95.2 and 200.4 ppm) for C-3' and C-4' when compared to other compound classes. The different ¹³C NMR resonances of some flavonolignans are presented in Table 15.

3.3.4. Mass Spectra

Like coumarinolignans, the dioxane bridge of flavonolignans undergoes mass spectrometric RDA cleavage to give rise to a substituted flavonoid-quinone ion (H) and a typical phenylpropanoid ion (I), as shown in Fig. 18.

The exact flavonoid nucleus and the substituted phenylpropanoid unit present in a flavonolignan molecule can be predicted from these fragment ions. The fragment ion at m/z 302 (H), observed in the silybin derivatives, indicates that these are

Table 15. ^{13}C NMR spectroscopic data (δ/ppm) of compounds **65**, **68**, **84**, **89**, **91**, and **93** in $\text{DMSO-}d_6$

Carbon	Compound 2 (65)	Hydnocarpin (68)	Salcolin A (84)	Silybin A (89)	Silychristin A (91)	Silydianin (93)
C-2	160.0	164.2	164.2	82.6	83.2	80.1
C-3	112.2	103.8	105.9	71.4	71.7	69.3
C-4	181.5	181.6	182.7	197.6	197.5	194.9
C-5	163.4	157.3	163.6	163.3	163.3	161.8
C-6	100.2	98.5	100.3	96.1	96.1	94.7
C-7	166.0	161.4	166.5	167.1	167.2	165.4
C-8	95.2	93.9	95.2	95.1	95.0	93.5
C-9	158.2	162.8	158.9	162.5	162.5	160.5
C-10	105.4	103.8	105.3	100.4	100.3	98.7
C-1'	118.5	123.6	127.2	130.1	129.9	138.0
C-2'	129.1	114.6	105.2	116.5	115.6	47.1
C-3'	148.0	143.6	154.3	143.3	146.3	95.2
C-4'	146.2	147.2	140.9	143.7	140.7	200.4
C-5'	149.4	116.7	154.3	116.3	129.0	51.8
C-6'	104.4	119.3	105.2	121.3	115.3	122.5
C-1''	136.3	127.0	134.5	127.5	132.4	131.4
C-2''	112.9	110.9	112.0	111.8	110.4	110.9
C-3''	148.9	147.7	148.7	147.6	147.5	145.6
C-4''	147.0	147.0	147.7	147.0	147.0	143.5
C-5''	116.5	115.4	116.1	115.3	115.3	113.4
C-6''	120.9	120.6	120.6	120.5	118.7	118.8
C-7''	37.4	78.1	74.1	75.9	87.0	42.4
C-8''	42.8	76.4	88.2	78.1	53.3	44.5
C-9''	62.7	59.6	61.8	60.2	63.5	71.2
OMe-3', 5'	60.5, 56.6	–	56.6	–	–	–
OMe-3''	56.0	55.8	56.0	55.7	55.6	53.9

**Fig. 18.** RDA cleavage of dioxane bridge in flavonolignans

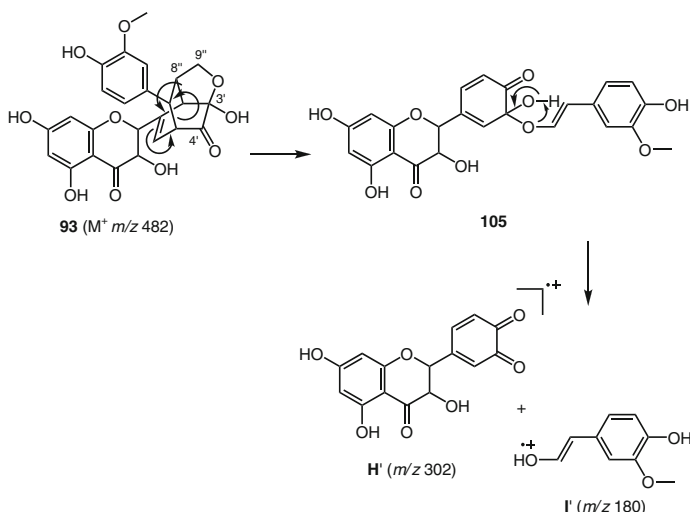
5,7-dihydroxydihydroflavonol derivatives (**95**). Hydnocarpin (**68**), which possesses a 5,7-dihydroxyflavone nucleus, exhibits the ion **H** at m/z 284, while palstatin (**83**), bearing an additional methoxy group at the 5' position, shows this peak at m/z 314 (**118**). The characteristic ion fragments **H** and **I** of some flavonolignans are shown in Table 16. Silydianin (**93**), an isomer of **1**, has a different ring system with a hemiacetal function, but this molecule also undergoes RDA cleavage (**92**), as shown in Fig. 19.

3.3.5. Circular Dichroism Spectra

CD spectroscopic analysis has been used frequently in natural product chemistry to determine the absolute configuration at the point of fusion of two rings.

Table 16. Mass spectrometric data of some flavonolignans having a 1,4-dioxane linkage

Flavonolignan	Fragment ion H (<i>m/z</i>)	Fragment ion I (<i>m/z</i>)
Dehydrosilybin (66)	300	180
Hydnocarpin (68)	284	180
5'-Methoxyhydnocarpin (77)	314	180
5''-Methoxyhydnocarpin (78)	286	210
Palstatin (83)	314	210
Silybin A (89)	302	180

**Fig. 19.** Mass fragmentation of silydianin (**93**)

In flavonolignans, the configurations of C-2, C-3, C-7'', and C-8'' have been studied through CD spectra, using model compounds having flavonoid and benzodioxane moieties for comparison (131–134). While the signs of Cotton effects at 326 and 292 nm have been used to determine the configuration at the 2 and 3 positions of the dihydroflavonol unit, that at 236 nm gives evidence for the configuration at the 7'' and 8'' positions of the benzodioxane moiety. Based on CD spectroscopic analysis, Kim *et al.* proposed the absolute stereostructures of silybins A and B as **89** and **90**

Table 17. CD spectroscopic data of some flavonolignans measured in MeOH

Compound	$\lambda_{\max}/\text{nm} (\Delta\epsilon/\text{mol}^{-1}\text{dm}^3\text{cm}^{-1})$
Isosilybin A (73)	330 (+2.9), 293 (−9.3), 236 (−1.9)
Isosilybin B (74)	328 (+2.8), 292 (−12.6), 229 (+9.0)
Isosilychristin (75)	326 (+1.4), 298 (−2.3), 226 (+44.3)
Silybin A (89)	326 (+4.9), 292 (−22.4), 236 (−9.3)
Silybin B (90)	328 (+2.1), 292 (−9.5), 230 (+10.1)
Silychristin A (91)	328 (+5.6), 298 (−5.2), 224 (+18.5)
Silydianin (93)	345 (+3.9), 295 (+2.1), 282 (−5.6)

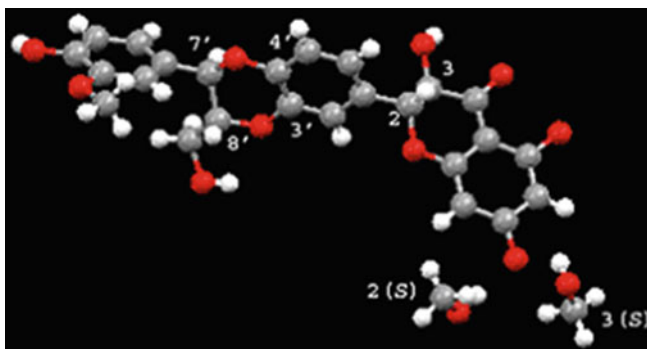


Fig. 20. Ball and stick model of X-ray structure of isosilybin A (**73**)

(**102**). The flavonolignan **65**, with a cyclohexane bridge, isolated from *Avena sativa*, showed a negative Cotton effect at 260 nm ($[\theta]_{260} - 918$), corroborating the absolute configurations at $7''$ and $8''$ as *S* (**118**). The CD data of some flavonolignans are presented in Table 17.

3.3.6. X-ray Crystallographic Analysis

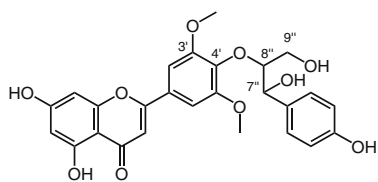
The molecular structure and the stereochemistry of some flavonolignans deduced through chemical and spectroscopic data have been confirmed by X-ray crystallographic analysis. The structure of silydianin (**93**) was confirmed through single-crystal X-ray analysis (**92**). The configurations of the diastereomers silybin A ($2R,3R,7''R,8''R$) (**89**), silybin B ($2R,3R,7''S,8''S$) (**90**), isosilybin A ($2R,3R,7''R,8''R$) (**73**), and isosilybin B ($2R,3R,7''S,8''S$) (**74**), were resolved unambiguously through X-ray crystallography (**124**). Although identical stereostructures for these compounds were also deduced from their CD spectroscopic analysis (**102**, **124**), it is not immediately obvious why **89** has been stated to have the ($2R,3R,7''S,8''S$) configuration (**102**). A ball and stick model picture for the X-ray structure of **73**, derived from the Cambridge Crystallographic Data Centre file is presented in Fig. 20.

3.4. Chemistry of Flavonolignans

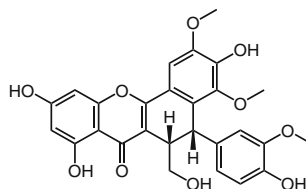
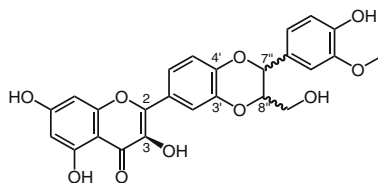
In this section of the chapter, the structure elucidation of some important flavonolignans and their syntheses will be discussed.

3.4.1. Silybins

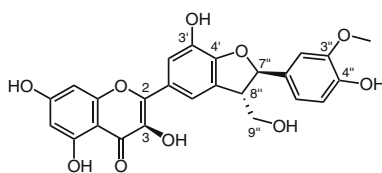
The structure of silybin (**1**), $C_{25}H_{22}O_{10}$, mp 158–160°C, $[\alpha]_D +12.6^\circ \text{cm}^2 \text{g}^{-1}$, the principal biologically active constituent of *S. marianum*, was first settled by *Pelter*



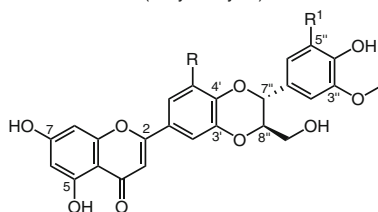
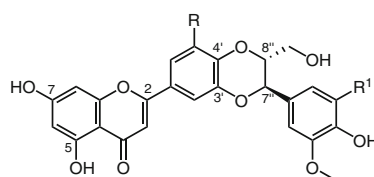
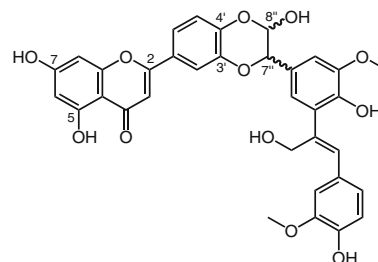
64 (aegicin)

65 (compound 2 from *Avena sativa*)

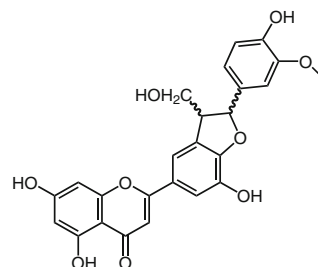
66 (dehydrosilybin)



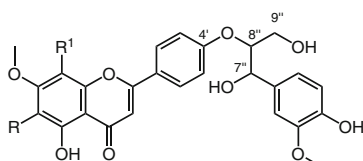
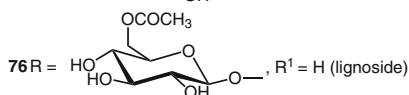
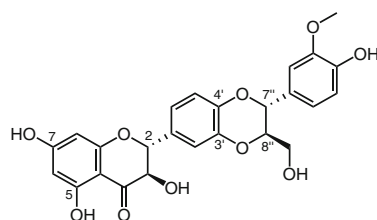
67 (dehydrosilychristin)

68 R = H, R¹ = H (hydnocarpin)77 R = OCH₃, R¹ = H (5'-methoxyhydnocarpin)78 R = H, R¹ = OCH₃ (5'-methoxyhydnocarpin)69 R = H, R¹ = H (isohydnocarpin D)79 R = OCH₃, R¹ = H (5'-methoxyisohydnocarpin D)83 R = OCH₃, R¹ = OCH₃ (palstatin)

70 (hydnowightin)

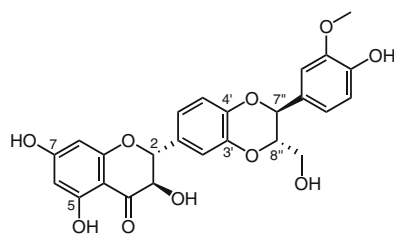


71 (isohydnocarpin)

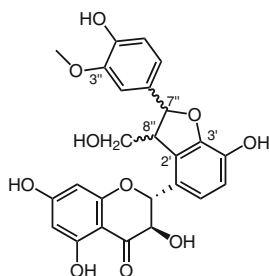
72 R = H, R¹ = H (isolignoside)76 R = H, R¹ = H (lignoside)

73 (isosilybin A)

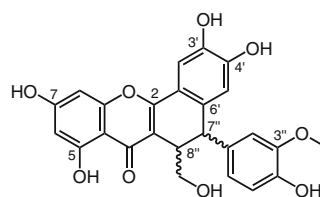
Chart 2. Structure formulas of flavonolignans



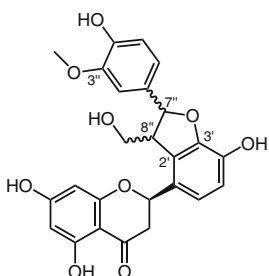
74 (isosilybin B)



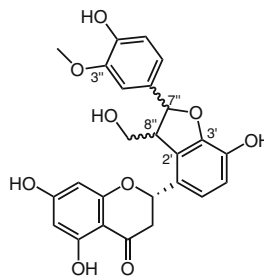
75 (isosilychristin)



80 (neohydrocarpin)



81 (neosilyhermin A)



82 (neosilyhermin B)

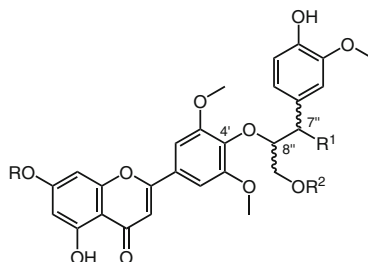
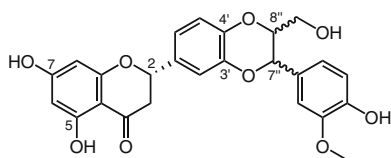
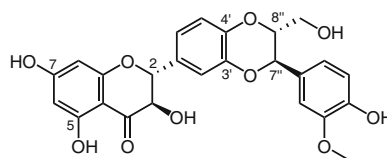
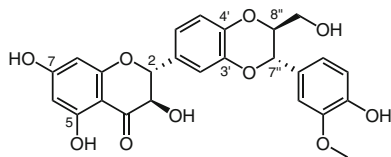
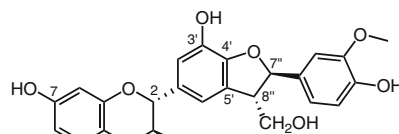
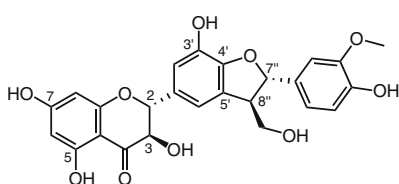
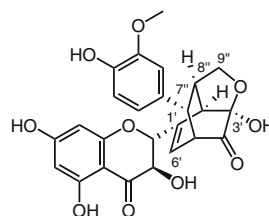
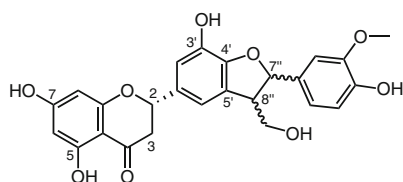
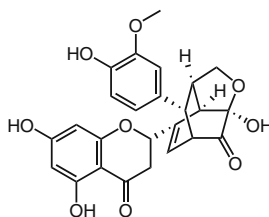
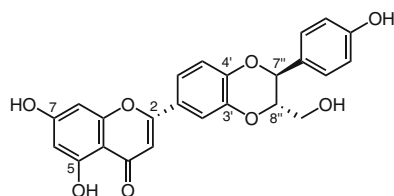
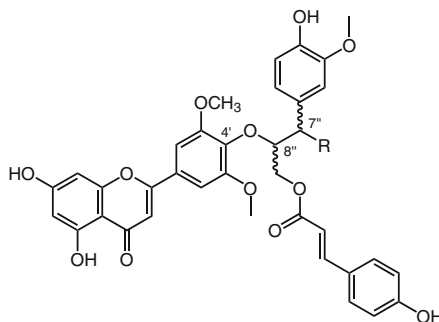
84 R = R² = H, R¹ = OH, *erythro* (salcolin A)85 R = β-Glc, R¹ = OH, R² = H, *erythro* (salcolin A glycoside)86 R = R² = H, R¹ = OH, *threo* (salcolin B)87 R = β-Glc, R¹ = OH, R² = H, *threo* (salcolin B glycoside)97 R = H, R¹ = OH, R² = Ac, *erythro* (Tricin 4'-O[*erythro*-β-guaiacyl-(9''-O-acetyl)-glyceryl] ether)98 R = H, R¹ = OH, R² = Ac, *threo* (Tricin 4'-O[*threo*-β-guaiacyl-(9''-O-acetyl)-glyceryl] ether)99 R = H, R¹ = OCH₃, R² = Ac, *erythro* (Tricin 4'-O[*erythro*-β-guaiacyl-(7''-O-methyl-9''-O-acetyl)-glyceryl] ether)100 R = H, R¹ = OCH₃, R² = Ac, *threo* (Tricin 4'-O[*threo*-β-guaiacyl-(7''-O-methyl-9''-O-acetyl)-glyceryl] ether)

Chart 2. (continued)

**88** (silandrin)**89** (silybin A)**90** (silybin B)**91** (silychristin A)**92** (silychristin B)**93** (silydianin)**94** (silyhermin)**95** (silymonin)**96** (sinaiticin)**101** R = OH (Tricin 4'-O-[*erythro*- β -guaiacyl-(9''-O-*p*-coumaroyl)-glyceryl] ether)**102** R = OH (Tricin 4'-O-[*threo*- β -guaiacyl-(9''-O-*p*-coumaroyl)-glyceryl] ether)**Chart 2.** (continued)

and Hänsel in 1975, from a comprehensive spectroscopic analysis of this compound, its derivatives, and its various degradation products (95). The compound bears an aromatic methoxy group and five acylable hydroxy groups, three of which are phenolic. Degradation experiments clearly indicated silybin to have a dihydroflavonol moiety, and, consistent with this assumption, treatment of this compound with iodine and potassium acetate yielded dehydrosilybin (66), $C_{25}H_{20}O_{10}$, which showed a quercetin-like UV spectrum. The presence of a quercetin unit in dehydrosilybin was confirmed by its degradation with boron tribromide. Silybin was thus proved to contain a 2,3-dihydroquercetin (*i.e.* taxifolin) (106) moiety. A comparison of the NMR spectrum of taxifolin methyl ether with that of the methyl ether of silybin was carried out to confirm this deduction. The presence of unit 106 in silybin accounted for 15 of 25 carbon atoms and the remaining ten-carbon unit was considered to be due to a coniferyl alcohol (45) moiety, on the basis of spectroscopic evidence. Accordingly, silybin was formed by union of 45 with 106 through a dioxane bridge, and two alternative gross structures, 1 and 1a, were advanced for this compound. A positive Cotton effect at 330 nm indicated a (2*R*,3*R*)-configuration. A choice between the alternative structures, 1 and 1b, was made through HMBC NMR spectroscopic analysis, and silybin was found to have a 3'-*O*-7''/4'-*O*-8'' linkage, which supported structure 1 (see Fig. 21).

The structure 1 proposed for silybin was supported from its mass spectrum, which showed fragment ions at m/z 302 (H) and 180 (I), formed by RDA cleavage of the dioxane bridge of the molecule (Table 16). The *trans* configurations of H-2, H-3 and H-7'', H-8'' were resolved from their coupling constant values ($J = 11.3$ and 7.9 Hz). Structure 1 assigned was finally confirmed by the synthesis of

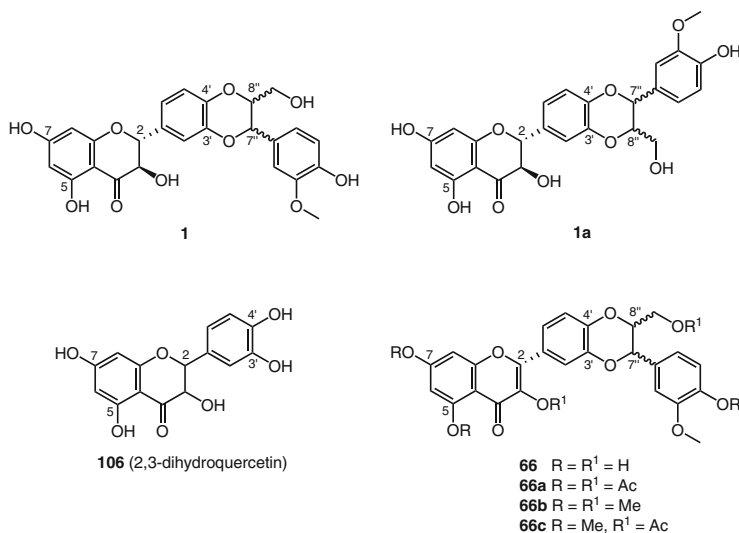
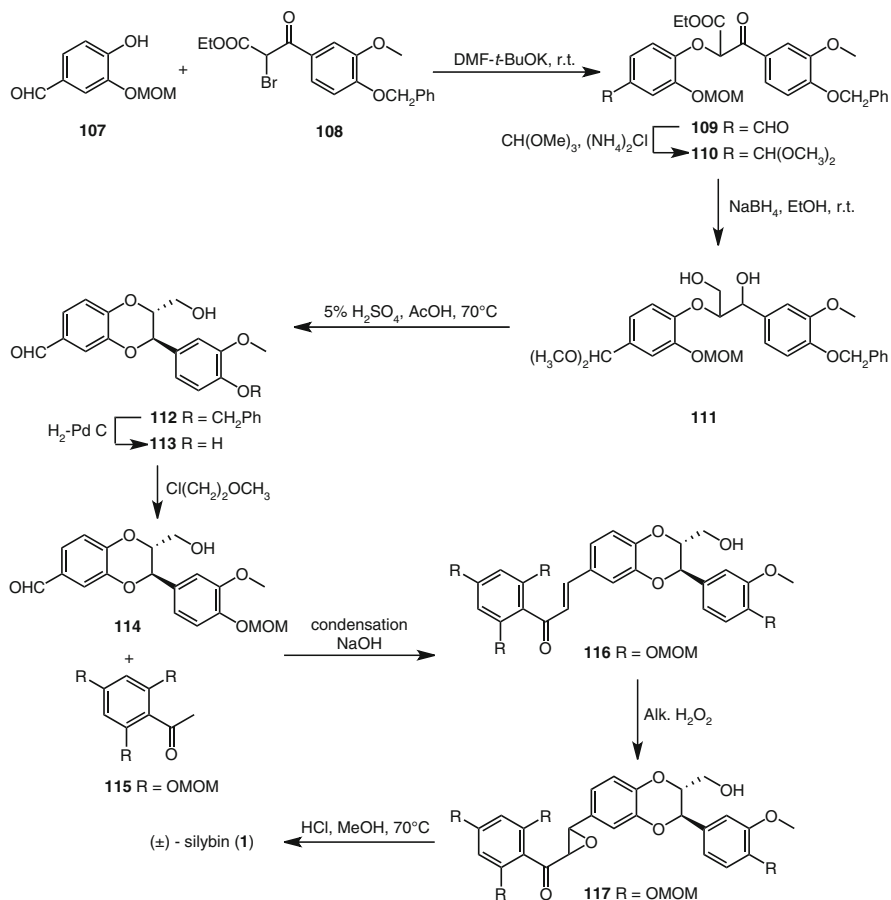


Fig. 21. Silybin (1) and its derivatives



Scheme 6. Outline of the synthesis of (±)-silybin

dehydrosilybin pentamethyl ether (**66b**) (*135*). A one-step biomimetic synthesis of natural **1**, by silver oxide-promoted oxidative coupling of **45** with (2*R*,3*R*)-**106**, also supported the structure proposed for silybin (**1**). However, this method yielded a mixture of regioisomeric pairs, silybin (78%) and isosilybin (*136*). A regioselective total synthesis of (±)-**1** from readily available starting materials, **107** and **108**, via the intermediate, 3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl-1,4-benzodioxan-6-carbaldehyde (**114**), was reported by *Tanaka et al.* (*137*), and is summarized in Scheme 6.

Isosilybin (**1a**) (mp $239\text{--}241^\circ\text{C}$) and silybin (**1**), isolated from the same source, showed closely comparable NMR spectroscopic and mass spectrometric data and similar TLC behavior. However, the TLC profiles of their dehydro derivatives were different and this observation suggested that the two compounds are not diastereoisomers but regioisomers. The structure of isosilybin was thus advanced as **1a**.

Both **1** and **1a** exist as diastereomeric pairs (**95**) and these were named as silybin A (**89**) and silybin B (**90**), and isosilybin A (**73**) and isosilybin B (**74**). All four compounds have the same configurations at C-2 and C-3 (*2R,3R*) but differ at the C-7'' and C-8'' positions. Compounds **89** and **90**, having a *2,3-cis* configuration, are also known, and have been isolated from *S. marianum* and named as *2,3-cis*-silybin A and *2,3-cis*-silybin B (**105**). Based on X-ray crystallographic analysis, the absolute configurations of **89** and **90** were identified as (*2R,3R,7''R,8''R*) and (*2R,3R,7''S,8''S*). Compounds **89** and **73** were shown to have the same configuration (*2R,3R,7''R,8''R*) (**124**). The purification of these diastereomers was achieved through semi-preparative reversed-phase HPLC methods (**102**, **124**) and various analytical methods (especially HPLC) have also been developed to quantify the ratio of isomers (**138**, **139**).

Besides the regioisomer of **1**, two other isomers, silychristin (**91a**) and silydianin (**93**), having different modes of fusion between the dihydroflavonol and coniferyl alcohol units, have been reported (**92**, **93**). Silydianin (**93**), C₂₅H₂₂O₁₀ (M⁺*m/z* 482), mp 190–192°C, [α]_D +175° cm² g⁻¹, showed in its IR spectrum an additional non-conjugated carbonyl group other than that present in **1**, and the structure was completely established as **93** through single-crystal X-ray analysis by Abraham *et al.* (**92**). The natural occurrence of 3-deoxysilydianin and 3-deoxyisosilybin, named silymonin (**95**) and silandrin (**88**), was also reported from a white-flowered variety of milk thistle (**99**).

Silychristin (**91a**), C₂₅H₂₂O₁₀ ([M-H]⁻ *m/z* 481.1167), mp 174–176°C, was recognized as being formed by fusion of taxifolin (**106**) with coniferyl alcohol (**45**) through a furan ring (**93**). This was evidenced from selected ¹H NMR signals, which showed a benzyl methine and a benzyloxy hydrogen at *vicinal* positions at δ 5.46 (d, *J* = 6.9 Hz) and 3.47 (dd, *J* = 12.5, 6.4 Hz) ppm and from ¹³C NMR (δ 129.0 (C-5'), 115.3 (C-6'), 53.3 (C-8'') ppm) spectroscopic data (**98**) (Tables **10** and **11**). Two possible structures, **91a** and **91b**, differing in the positions of linkage between the **106** nucleus and **45** nucleus were assumed (**97**) (see Fig. **22**). Comparison of the NMR signals of silychristin and its hexaacetate and anhydro derivatives as well as the comparison of the chemical shift values of C-7'', C-8'', and C-9'' with those of model compounds like dihydrodiconiferyl alcohol and its anhydro derivative, was used to confirm the structure of **91a**. The relative configuration of the dihydrofuran ring was resolved as *trans* (**97**).

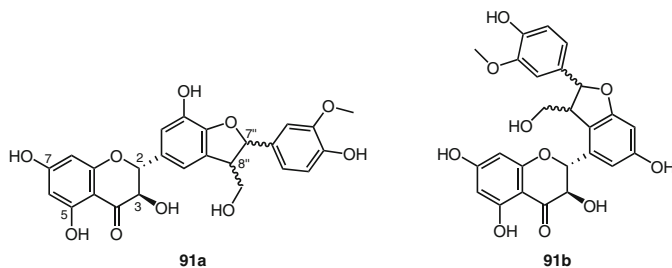
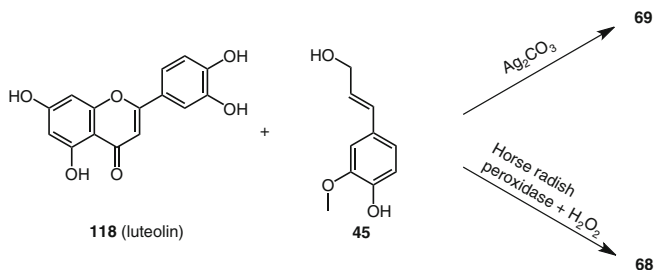


Fig. 22. Possible regioisomeric forms of silychristin (**91a**, **91b**)



Scheme 7. Biomimetic synthesis of hydnocarpin (**68**) and hydnocarpin D (**69**)

Compound **91a** was known previously as only one diastereomer, which was shown to have a (*7''R,8''S*) configuration. In 2005, *Smith et al.* isolated two silychristin-like compounds from the fruits of *S. marianum* using semi-preparative HPLC and found them to be a diastereomeric pair, silychristin A (**91**) and silychristin B (**92**) (*104*). The compounds differed in their optical rotations ($[\alpha]_{\text{D}} + 66^\circ$ and $+ 47^\circ \text{cm}^2 \text{g}^{-1}$).

Hydnocarpin (**68**) and hydnocarpin D (**69**) are regioisomers. While biomimetic radical coupling of luteolin (**118**) with **45** in the presence of Ag_2CO_3 yielded **69** as a major product, coupling in the presence of horseradish peroxidase/ H_2O_2 produced a good yield of hydnocarpin (**68**) (*123*) (Scheme 7).

She et al. reported the total synthesis of (\pm)-sinaiticin, *i.e.* 3''-demethoxyhydnocarpin (**96**). The major synthesis step involved the condensation of 2,4-dimethoxymethyl-6-hydroxyacetophenone with 3-hydroxymethyl-2-(4-hydroxyphenyl)-2,3-dihydrobenzo[1,4]dioxane-6-carbaldehyde to a chalcone, which, after cyclization with DDQ in dry dioxane and subsequent deprotection with 3 *N* HCl under reflux, yielded the desired product (*141*).

3.4.2. Aegicin and Related Flavonolignans

Aegicin (**64**), $\text{C}_{26}\text{H}_{24}\text{O}_{10}$ ($[\text{M}-\text{H}_2\text{O}]^+$ m/z 478), mp 235–236°C, isolated from *Aegilops ovata*, is the first member of the flavonolignans in which the flavonoid unit is linked with coniferyl alcohol (**45**) or its analog through a single ether bond (*114*). The flavone component of **64** was readily recognized as 5,7,4'-trihydroxy-3',5'-dimethoxyflavone, known by the trivial name tricrin (**119**), from ^1H - and ^{13}C NMR spectroscopic analysis, with supportive evidence from the observed base peak at m/z 330 and other fragment ions seen in the mass spectrum. On acetylation, **64** yielded a pentaacetate, having three phenolic and two alcoholic *O*-acetyl groups. All the proton signals present in the tricrin triacetate (**119a**) were discernible in the spectrum of aegicin pentaacetate, suggesting the union of a phenylpropanoid unit with **119** through an ether linkage. The unchanged chemical shifts of H-6 (δ 6.18 (d, $J = 2.0$ Hz) ppm) and H-8 (δ 6.51 (d, $J = 2.0$ Hz) ppm) in both **119** and **64** as well as in their acetates, eliminated the possibility of linkages either at C-5-O or C-7-O.

From the appearance of a singlet for two methoxy groups at δ 3.85 ppm, ring B was proved to be symmetrically substituted and hence the ether linkage was placed at C-4'. The remaining signals in the proton NMR spectrum (Table 10) corresponded to structure **120** for the phenylpropanoid component (see Fig. 23).

A 4'-O-8'' linkage in **64**, considered for biogenetic reasons, was supported by D₂O-exchange analysis and acetylation. The H-7'' signal, appearing as a broad doublet, sharpened upon addition of D₂O and was deshielded on acetylation. Similarly, the methylene protons at C-9'' were deshielded on acetylation but no such effect was observed for H-8'' and hence a 4'-O-8'' linkage was proposed. The configurations of the C-7'' and C-8'' substituents were confirmed as *erythro*, by comparing the coupling constant (6.5 Hz) of H-7'' and H-8'' in aegicin pentaacetate with model compounds like *threo*- and *erythro* 1-phenyl-1,2,3-propane triols. Aegicin was, therefore, characterized as tricin 4'-O-(β -*para*-hydroxyphenylglyceryl) ether (**64**).

Salcolins A (**84**) and B (**86**), isolated from the aerial parts of *Salsola collina*, are an epimeric pair of flavonolignans, structurally related to **64** and differ from the latter only by the presence of an additional methoxy group at the C-3'' position (*108*). These two compounds were also isolated from *Hyparrhenia hirta* (*119*), *Sasa veitchii* (*117*), and *Avena sativa* (*118*). Based on a method developed by Bouaziz and coworkers to distinguish the *threo* and *erythro* forms on the basis of the coupling constant values of H-7'' and H-8'', the structures of salcolins A and B were determined as tricin 4'-O-(*erythro*- β -guaiacylglyceryl) ether (**84**) and tricin 4'-O-(*threo*- β -guaiacylglyceryl) ether (**86**) (*119*). The configurational assignments made for **84** and **86** in the first report (*108*) was thus revised (see Fig. 24). The

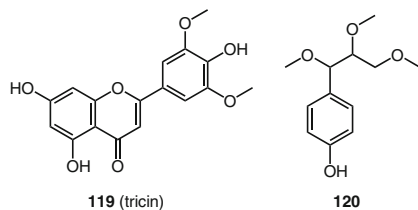
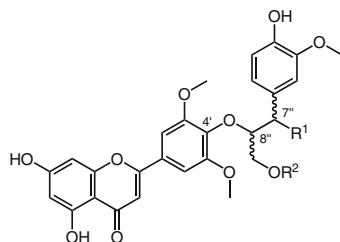


Fig. 23. The flavone and phenylpropanoid components of aegicin (**64**)



84 R¹ = OH, R² = H, *erythro*
86 R¹ = OH, R² = H, *threo*
84a R¹ = R² = H, *erythro*

Fig. 24. Salcolin epimers

epimeric relationship between these two flavonolignans was also confirmed by reduction of the benzylic hydroxy (OH-7'') group of each compound using $\text{Et}_3\text{SiH/TFA}$ as catalyst in CH_2Cl_2 at room temperature, with both these substances yielding the same reduction product **84a** (115).

Another three pairs of analogous flavonolignans have also been isolated from *Sasa veitchii* (115). These include two pairs of naturally acylated derivatives (**97–100**) and an epimeric flavonolignan pair (**101, 102**), constituting a tricin moiety (**119**), a guaiacylglyceryl group, and a *p*-coumaroyl group (115). The linkage of the *p*-coumaroyl group at the 9''-hydroxy oxygen was confirmed from HMBC spectroscopic correlations observed between $\text{H}_2\text{-}9''$ of the guaiacylglyceryl group and C-9''' of the *p*-coumaroyl group (115).

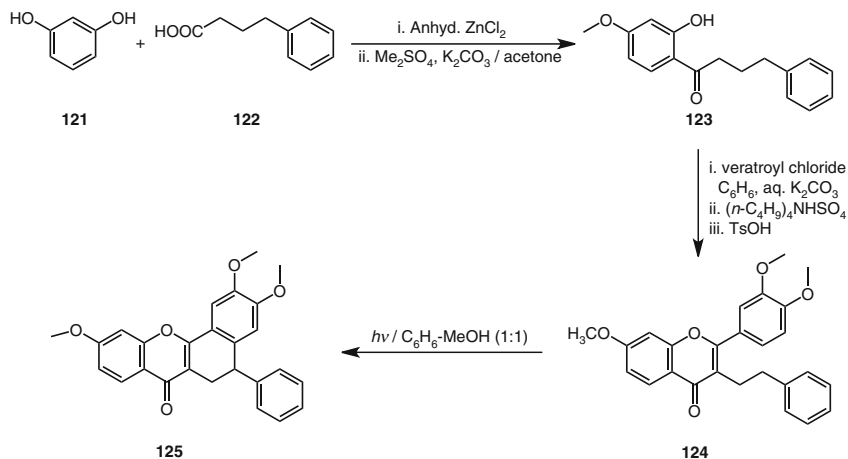
Natural acyl glycosides of this type of flavonolignans, viz. lignoside (**76**) and isolignoside (**72**), containing 6-acetylglucose and 8-acetylglucose residues, were reported from *Gratiola officinalis* (121, 122). Glycosides of **84** and **86** that were isolated from *Hyparrhenia hirta* (119) showed similar UV spectra and an identical molecular formula, $\text{C}_{33}\text{H}_{36}\text{O}_{16}$, with $[\text{M}+\text{H}]^+$ at m/z 689. This ion on MS-MS analysis yielded a major product ion at m/z 527 $[(\text{M}+\text{H})-162]^+$ corresponding to the loss of a hexose group, suggesting an O-glycosidic pair, characterized as tricin 4'-*O*-(erythro- β -guaiacylglyceryl) ether 7-*O*- β -glucopyranoside (**85**) and 4'-*O*-(threo- β -guaiacylglyceryl) ether 7-*O*- β -glucopyranoside (**87**).

3.4.3. Cyclohexane Bridged Flavonolignans

There are just two reports on the isolation of flavonolignans in which C-6' and C-3 of the flavone unit are linked, respectively, to C-7 and C-8 of a phenylpropanoid unit, to form a cyclohexane bridge. Neohydnocarpin (**80**), isolated from the seeds of *Hydnocarpus wightiana* (113), and compound **2** (**65**), isolated from *Avena sativa* (118), have such a C-C bond linkage between the flavone nucleus and the phenylpropanoid unit.

Neohydnocarpin (**80**), $\text{C}_{24}\text{H}_{20}\text{O}_9$, showed a UV absorption spectrum typical of a flavone. Detailed NMR spectroscopic analysis led to the proposal of the presence of a luteolin (**118**) and a coniferyl alcohol moiety (**45**). Careful examination of the ^{13}C NMR spectrum showed the absence of oxygen functions at C-7'' and C-8'', which resonated at δ_{C} 35.2 and 40.9 ppm, compared to those in silybin A (**89**) at δ_{C} 75.9 and 78.1 ppm (Table 15). The downfield shift of C-3 and C-6' (δ_{C} 104.8 and 133.9 ppm) of the flavone moiety in comparison to those of silybin-related compounds confirmed the involvement of these two carbons in the formation of a cyclohexane ring. A relatively downfield shift of the hydroxymethylene carbon (δ_{C} 69.2 ppm) was ascribed due to its proximity to the flavone carbonyl function. This finding favorably eliminated the consideration of an alternative formulation for neohydnocarpin (**80**).

Goyal and Parthasarathy have developed two different processes for the synthesis of the neohydnocarpin skeleton, **125**, through photocyclization (142). One of these involved irradiation of 7,3',4'-trimethoxy-3- β -phenylethylflavone (**124**), prepared by condensation of resorcinol (**121**) and γ -phenylbutyric acid (**122**) under



Scheme 8. Outline of the synthesis of the neohydrocarpin skeleton (**125**)

Nencki reaction conditions, followed by partial methylation, phase-transfer catalyzed *Baker–Venkataraman* reaction with veratroyl chloride, and photocyclization. An outline of this method is depicted in Scheme 8.

The UV spectrum of compound 2 (**65**), isolated from *A. sativa*, suggested a typical flavone chromophore and it showed a prominent peak at m/z 507.4, $[\text{M}-\text{H}]^-$, for $\text{C}_{27}\text{H}_{24}\text{O}_{10}$. The presence of a neohydrocarpin-type skeleton (**125**) in **65** was recognized from its ^1H and ^{13}C NMR spectra (Tables 14 and 15). All the proton and carbon resonances, including the axial or equatorial orientation of H-7'' and H-8'', were assigned using HSQC, HMBC, and NOESY correlations. CD measurement of this compound showed a negative *Cotton* effect at 261 nm $\{[\theta]_{261} - 918\}$, and was used to determine the absolute configuration at C-7'' and C-8'' as (*S*) (119).

3.5. Biological Activity of Flavonolignans

Several flavonolignans have been proved to be excellent hepatoprotectants and are also potential cancer chemopreventive agents. “Silymarin”, a standardized seed extract of *S. marianum*, is a mixture of silychristin (**91a**), silydianin (**93**), and silybin (**1**) in a ratio of 1:1:3 and is used for its liver protective action (139). Silymarin has shown no adverse side effects or harmful drug interactions, when tested pharmacologically and used clinically (140). It is considered as an alternative medicine, and, in 1969, silymarin was formally launched as hepatoprotective drug (FlavobionTM, LegalonTM). Since then, it has become commercially available in more than 100 countries, for the treatment of liver diseases (90, 143).

The liver-protective effect of silymarin is multifactorial and this may be due to its antidote, antifibrogenic, membrane stabilizing, protein synthesis inducing, and signal-evoking properties (90). Silymarin and silybin (**1**) have also shown potential

anticancer activity owing to their chemoprotective effect (88). Cardioprotective, chemopreventive, hypocholesterolemic, neuroactive, and neuroprotective activities of these compounds have been demonstrated (88). The fact that 2,3-dehydrosilybin (66) is 25 times more active than silybin as an antioxidant and as a free-radical scavenger indicates the importance of the presence of a double bond in the C-ring of the flavonolignan molecule for maximal biological efficacy of this type. Compound 66 is also a more potent (10 times) inhibitor of lipid peroxidation (144).

In order to increase the oral bioavailability of silybin (1) and its congeners, different formulations have been prepared using phosphatidyl choline (Siliphos™) and liposomes containing variable amounts of cholesterol and phospholipids, lipid microspheres, or surfactants (89). New series of analogs like silybin bis-hemisuccinate (Legalon-SIL™), silybin carboxylates (144), silybin glycosides (145, 146), and silybin 23-*O*-phosphate (145) have shown improved water solubility and consequent better therapeutic efficiency. *O*-Alkyl (methyl and benzyl) and *C*-alkyl (prenyl and geranyl) analogs of silybin (1) and of 2,3-dehydrosilybin (66) were found to be significant inhibitors of P-glycoprotein (91, 145, 147, 148).

In addition to silymarin, hydnocarpin (68) and sinaiticin (96) isolated from *Verbascum sinaiticum* exhibited cytotoxicity against cultured P-388 (murine lymphocytic leukemia) cells (120). 5'-Methoxyhydnocarpin-D (79) and silybin (1) have been identified as bacterial MDR pump inhibitors (107).

3.6. Biogenesis of Flavonolignans

The biogenesis of flavonoids in plants is well documented and known to take place by chain extension of 4-hydroxycinnamoyl-CoA (126) with malonyl CoA through the acetate pathway involving enzymes like chalcone synthase (86). Here, the initially formed polyketide 127 may undergo a Claisen-type condensation to form a chalcone and subsequently other flavonoids, as shown in Fig. 25. It is pertinent to

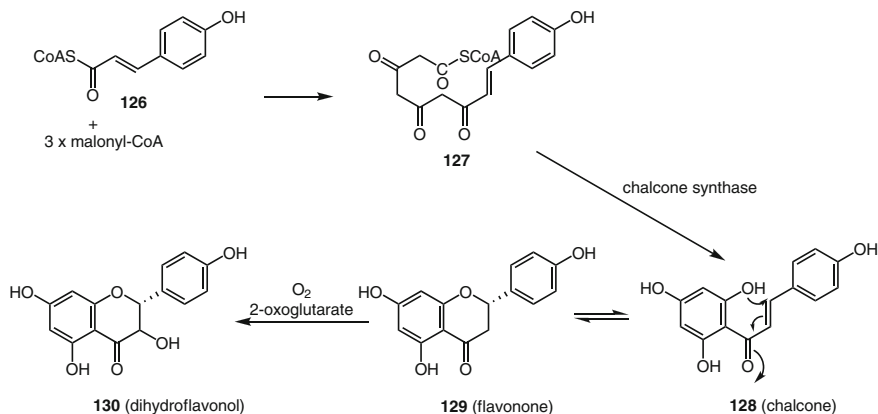


Fig. 25. Biogenesis of dihydroflavonol (130)

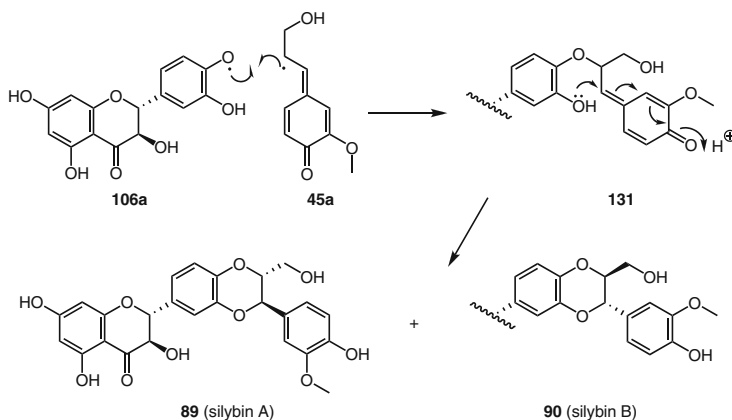


Fig. 26. Biogenesis of silybin A (**89**) and silybin B (**90**)

mention here that the A ring of flavonoids is malonyl-derived and therefore not a part of a C_6C_3 unit. For this reason, compounds formed by union of a C_6C_3 unit with the A ring of a flavonoid cannot be regarded as “non-conventional” lignans.

The biogenesis of silybins can be visualized by oxidative coupling of a 3',4'-dihydroxy group of a dihydroflavonol B-ring of taxifolin (**106**), with coniferyl alcohol (**45**) (Fig. 26). The free-radical coupling of **106a** and **45a** may form an adduct (**131**), which undergoes cyclization through nucleophilic attack of phenol on the quinone methide nucleus, creating two *trans* diastereomeric flavonolignan molecules, named silybin A (**89**) and B (**90**) (86). Similar radical coupling could also result in the formation of regioisomers, *e.g.* isosilybin A (**73**) and isosilybin B (**74**) isolated from the same plant, *S. marianum*.

The biogenesis of the furan-bridged flavonolignan, silychristin (**91a**), may occur through coupling of radicals **45a** with **106a**, holding an unpaired electron localized on C-5' (Fig. 27). In the case of the formation of silydianin (**93**), the first radical coupling takes place between the C-8 position of the incoming **45a** and C-2' of the flavonoid **106a**. The product (**133**) then cyclizes intramolecularly and undergoes hemiketal formation (Fig. 28). In the case of neohydnocarpin (**80**), radical coupling takes place between the C-8 position of **45** with the C-3 position of the flavonoid, **134**, which is followed up by bond formation between C-6' and C-7'' (Fig. 29).

4. Stilbenolignans

4.1. Occurrence and Features of Stilbenolignans

Stilbenolignans are a third interesting group of “non-conventional” lignans produced by plants. Stilbenes, like flavonoids, are believed to be metabolites of mixed biosynthetic origin and derived from a cinnamoyl CoA and three malonate units

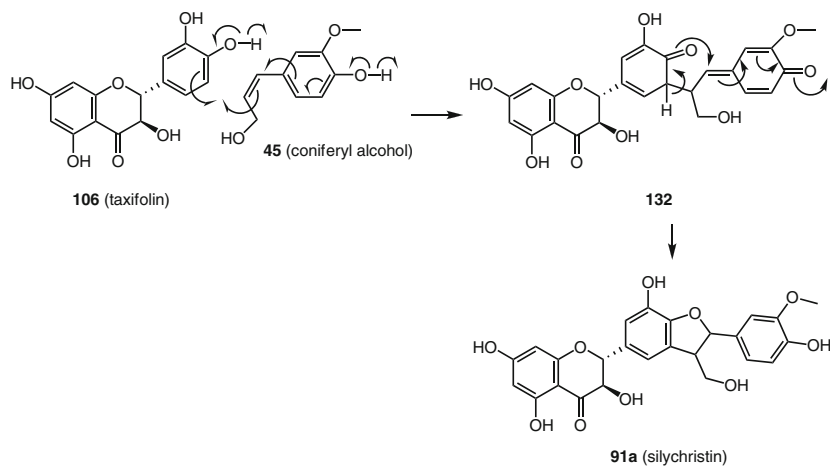


Fig. 27. Biogenesis of silychristin (91a)

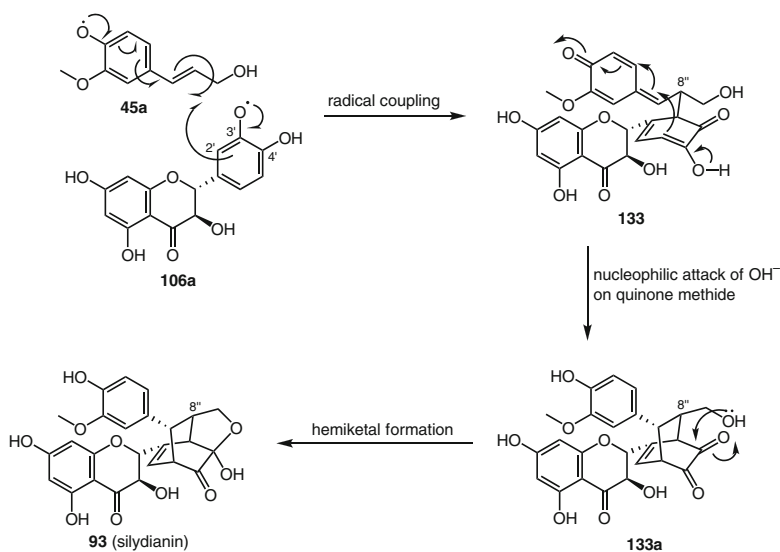


Fig. 28. Biogenesis of silydianin (93)

(86). Therefore, compounds formed by union of a C₆C₃ unit with the cinnamoyl part of stilbenes may be regarded as “stilbenolignans”. It may be argued that in stilbenes two aromatic rings are symmetrically disposed across the double bond and it is difficult to discern which one comes from the cinnamoyl unit and which one from malonate. The oxygen functionalities in malonate-derived aromatic rings are *meta*-oriented but those in cinnamoyl-derived aromatic rings are *ortho*-oriented. Based on this argument, only six true stilbenolignans are known so far. These have

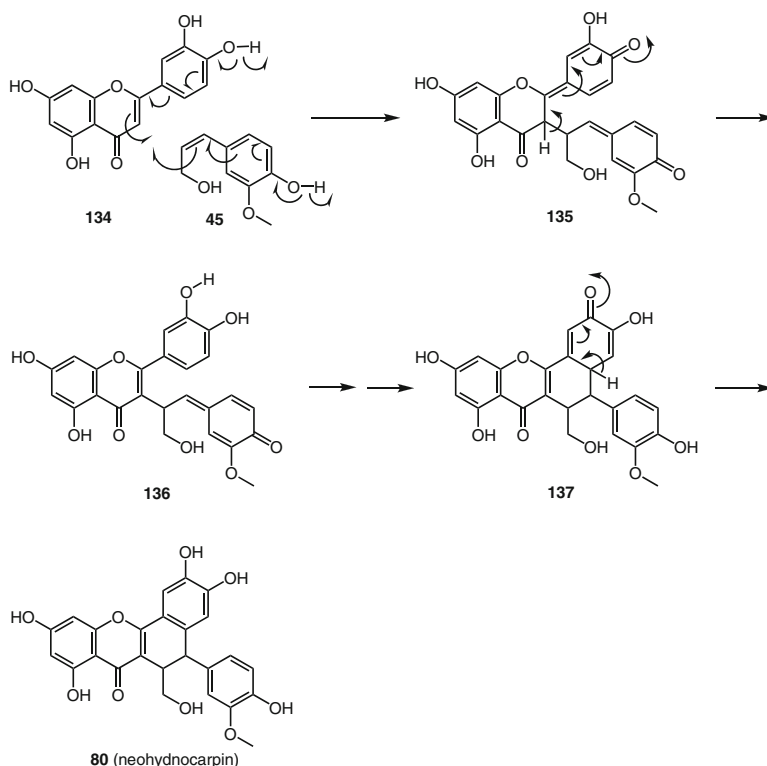


Fig. 29. Biogenesis proposal of neohydrocarpin (**80**)

been formed by union of a substituted stilbene with a C_6C_3 -unit through three different modes of fusion. Like coumarinolignans and flavonolignans, stilbenolignans may also exist as regioisomeric pairs but no such literature report has yet appeared, although such compounds have been obtained during the synthesis of natural stilbenolignans.

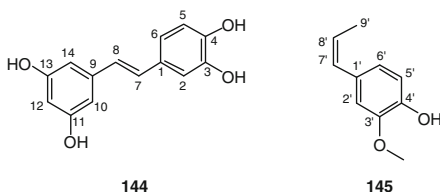
Plants from three different families, *viz.* Arecaceae, Fabaceae, and Gnetaceae, have so far been found to elaborate stilbenolignans. The individual plants known to yield stilbenolignans are listed in Table 18. Fig. 30 shows an image of *Maackia amurensis*.

4.2. Structure Elucidation and Synthesis of Stilbenolignans

The structures of stilbenolignans, elucidated almost exclusively by spectroscopic analysis, have often been confirmed through synthesis. The structure determination of a few important stilbenolignans together with their syntheses will be discussed in this section. It is pertinent to mention in this connection that different numbering

Table 18. Stilbenolignans and their sources

Compound (see Chart 3)	Source (Family)	References
Aiphanol (138)	<i>Aiphanes aculeata</i> (Arecaceae)	(151)
Gnetifolin F (139)	<i>Gnetum parvifolium</i> (Gnetaceae)	(150)
	<i>Gnetum klossii</i> (Gnetaceae)	(150)
	<i>Gnetum cleistostachyum</i> (Gnetaceae)	(152)
Gnetofuran A (140)	<i>Gnetum klossii</i> (Gnetaceae)	(151)
	<i>Gnetum cleistostachyum</i> (Gnetaceae)	(152)
Gnetucleistol F (141)	<i>Gnetum cleistostachyum</i> (Gnetaceae)	(152)
Lehmbachol D (142)	<i>Gnetum cleistostachyum</i> (Gnetaceae)	(152)
Maackolin (143)	<i>Maackia amurensis</i> (Fabaceae)	(153)

**Fig. 30.** *Maackia amurensis***Fig. 31.** Numbering system in stilbenolignans

systems have been used by different workers, but only a numbering based on biogenesis will be used here and it will start from the aromatic ring derived from cinnamoyl unit as shown in Fig. 31.

4.2.1. Aiphanol

Kinghorn and coworkers reported the isolation and characterization of aiphanol (**138**), the only stilbenolignan having a dioxane bridge (149). Aiphanol (**138**),

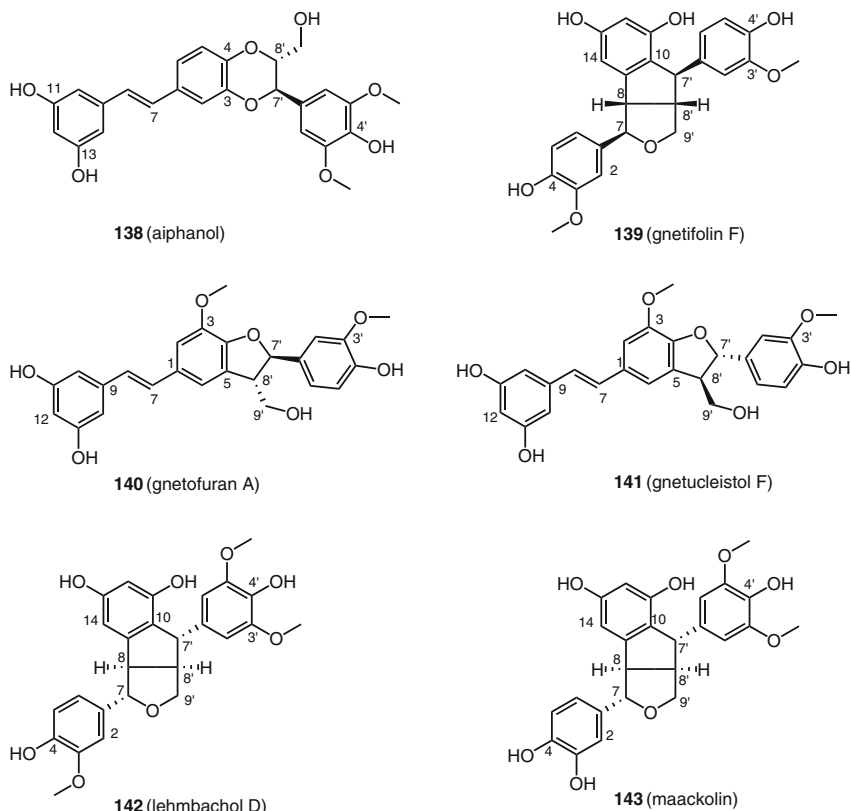


Chart 3. Structural formulas of stilbenolignans

$C_{25}H_{24}O_8$, $[\alpha]_D -21.8^\circ \text{cm}^2 \text{g}^{-1}$ showed in its ^1H NMR spectrum eight aromatic proton signals, of which six occurred in AMX and AX_2 patterns (Table 19), along with the signals of a *trans* double bond at δ 6.94 (d, $J = 16.4$ Hz) and δ 7.02 (d, $J = 16.3$ Hz) ppm, corroborating the presence of a stilbene moiety. The ^{13}C NMR spectrum (Table 20) supported by a HMQC experiment showed carbon resonances for two methoxy groups (δ_C 56.7 ppm) and a phenylpropanoid unit, in addition to the stilbene nucleus. This was further ascertained from the presence in the proton NMR spectrum of an aromatic singlet at δ 6.84 (2H) ppm, two *trans*-coupled oxymethine signals at δ 4.97 (d, $J = 8.1$ Hz) and 4.14 (m) ppm, oxymethylene signals at δ 3.53 (dd, $J = 4.1, 12.3$ Hz) and 3.74 (dd, $J = 2.3, 12.4$ ppm), and two methoxy singlets at δ 3.84 ppm. From the coupling between the two deshielded oxymethine signals (δ 4.97 and 4.14 ppm) in the HMBC spectrum, the linkage of the stilbene moiety with the phenylpropanoid unit was assigned through a 1,4-dioxane ring. The exact structure of aiphanol (3-O-7'/4-O-8' linkages) among the regioisomeric alternative formulations (3-O-7'/4-O-8' or 3-O-8'/4-O-7') was deduced through long-range NMR correlation studies. The large coupling constant

Table 19. ^1H NMR spectroscopic data (δ/ppm (J/Hz)) of compounds **138**, **139**, and **140** in $\text{Me}_2\text{CO}-d_6$

Proton(s)	Aiphanol (138)	Gnetifolin F (139)	Gnetofuran A (140)
H-2	7.13 d (1.9)	7.03 d (2.0)	6.98 br s
H-5	6.90 d (8.3)	6.82 d (8.0)	–
H-6	7.08 dd (8.4, 1.9)	6.89 dd (8.0, 2.0)	7.00 br s
H-7	6.94 d (16.4)	4.71 d (4.0)	6.89 d (16.4)
H-8	7.02 d (16.3)	3.81 dd (8.8, 4.0)	6.78 d (16.4)
H-10	6.56 d (2.0)	–	6.41 d (2.1)
H-12	6.28 brt (1.9)	6.27 d (2.0)	6.14 t (2.1)
H-14	6.56 d (2.0)	6.35 d (2.0)	6.41 d (2.1)
H-2'	6.84 s	6.73 d (2.0)	6.92 d (2.1)
H-5'	–	6.66 d (8.0)	6.67 d (8.1)
H-6'	6.84 s	6.50 dd (8.0, 2.0)	6.75 dd (8.1, 2.1)
H-7'	4.97 d (8.1)	4.18 s	5.45 d (5.4)
H-8'	4.14 m	3.03 q (8.8)	3.41 dt (12.2, 5.4)
H-9'	3.53 dd (12.3, 4.1)	4.46 t (8.8)	3.74 m
	3.74 dd (12.4, 2.3)	3.51 t (8.8)	
OCH_3-3	–	3.86 s	3.73 s
OCH_3-3'	3.86 s	–	–
OCH_3-5'	3.86 s	3.86 s	3.73 s

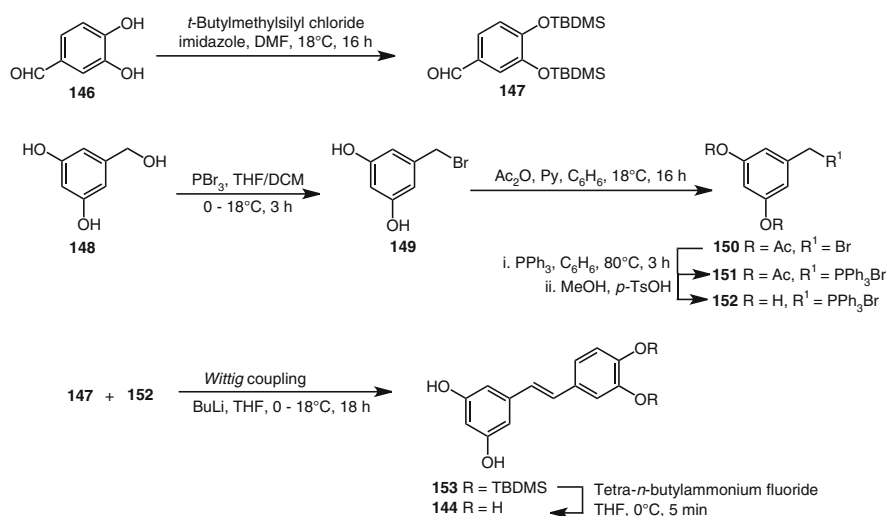
Table 20. ^{13}C NMR spectroscopic data (δ/ppm) of compounds **138**, **139**, and **140** in $\text{Me}_2\text{CO}-d_6$

Carbon	Aiphanol (138)	Gnetifolin F (139)	Gnetofuran A (140)
C-1	131.8	135.5	132.0
C-2	115.4	110.5	111.9
C-3	145.1	148.3	145.3
C-4	144.5	146.7	149.3
C-5	117.8	115.5	130.5
C-6	120.9	119.6	116.4
C-7	128.1	88.3	129.5
C-8	128.7	59.7	127.2
C-9	140.6	148.3	140.8
C-10	105.8	122.8	105.6
C-11	159.6	155.8	159.6
C-12	102.8	102.7	102.7
C-13	159.6	159.8	159.6
C-14	105.8	103.3	105.6
C-1'	128.1	138.0	134.3
C-2'	106.1	111.9	110.5
C-3'	148.8	148.0	148.4
C-4'	137.3	145.5	147.3
C-5'	148.8	115.4	115.7
C-6'	106.1	120.3	119.6
C-7'	77.5	51.0	88.6
C-8'	79.7	55.9	54.7
C-9'	61.9	74.5	64.6
OCH_3-3	–	56.2	56.4
OCH_3-3'	56.7	56.1	56.2
OCH_3-5'	56.7	–	56.2

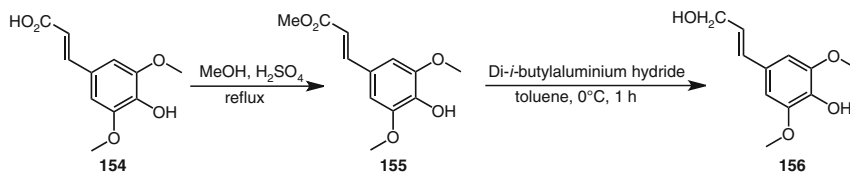
($J_{H-7',H-8'} = 8.1$ Hz) and the NOE correlation between H-8' and H-2', clearly showed the *trans* configuration of the chiral centers of the dioxane bridge. Thus, structure **138** was finalized for aiphanol (**149**), which was later confirmed through synthesis.

The structural novelty and various biological activities elicited by this compound prompted the synthesis of **138** by different workers following different strategies. Some of the successful reports are described here. *Banwell et al.* reported the synthesis of (\pm)-aiphanol (**154**) based on the standard procedures of *Stermitz's* modification (**124**). A mixture of (\pm)-**138**, its regioisomer (**157**), and their stereoisomers **158** and **159** was obtained by treatment of the stilbene, piceatannol (**144**), and 4-hydroxy-3,5-dimethoxycinnamyl alcohol (**156**) with silver carbonate in acetone-benzene (**155**). The reaction steps for the synthesis of starting materials **144** and **156**, and their oxidative coupling steps are shown in Schemes 9a, 9b, and 9c.

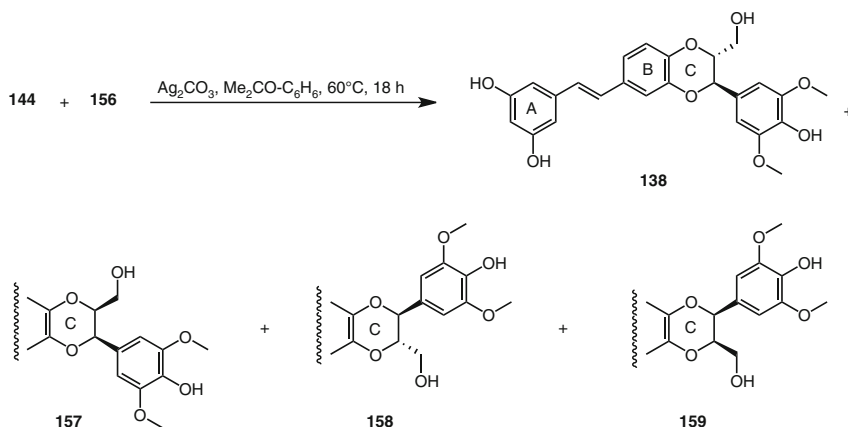
Kuboki and colleagues (**156**) reported a total synthesis of (\pm)-**138** following a strategy developed by *Pan et al.* (**141**) for the synthesis of (\pm)-sinaiticin (**96**).



Scheme 9a. Synthesis of piceatannol (**144**)



Scheme 9b. Synthesis of sinapic alcohol (**156**)

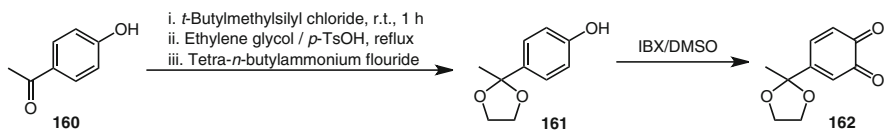


Scheme 9c. Coupling reaction involved in the synthesis of (±)-aiphanol (**138**)

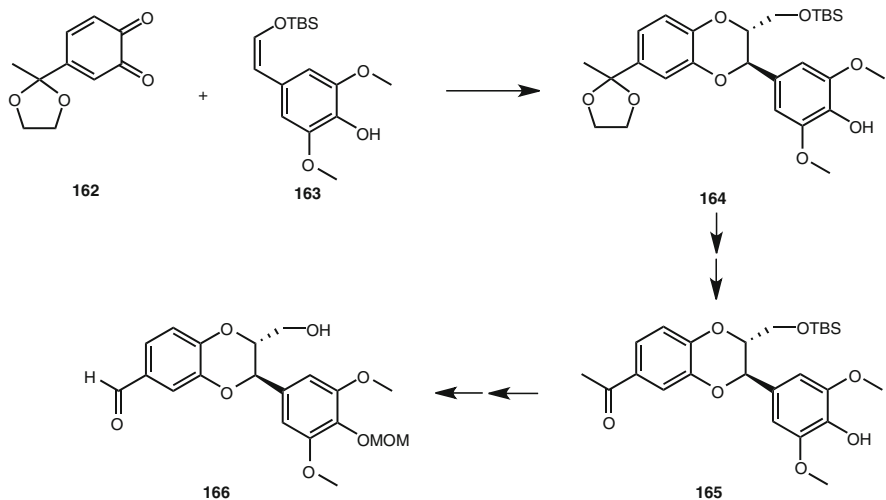
The key intermediate for this synthesis was an aromatic aldehyde, linked with sinapyl alcohol (**156**) through a dioxane bridge. The aldehyde **166**, on treatment with the appropriate phosphonium salt, yielded the desired stilbene. The essential steps of the preparation of the intermediate are summarized below.

1. Preparation of the *ortho*-quinone **162** from *p*-hydroxyacetophenone (**160**). This involved silylation of acetophenone, ketal protection, desilylation, and regioselective oxidation of phenol to *o*-quinone **162** using *o*-iodoxybenzoic acid (IBX) (Scheme 10a).
2. [4+2] Cycloaddition of *ortho*-quinone **162** with *tert*-butyldimethylsilyl ether of sinapyl alcohol (**163**) yielded the 1,4-dioxane-linked adduct **164**, as shown in Scheme 10b, but no regioisomer was detected. The ketal group in **164** was removed under acidic conditions and the derived ketone, a *trans-cis* mixture in the ratio of 2:1, on treatment with alkali, yielded the *trans*-isomer, **165**, in a good yield. The ketone **165** was converted to the key intermediate aldehyde **166** through the iodoform reaction, esterification, LAH reduction, and oxidation with *Dess-Martin* periodinane after protection of the hydroxy groups by MOM groups (Scheme 10b).
3. The stilbene skeleton of **138** was constructed by *Wittig* olefination between the key aldehyde (**166**) and phosphonium salt, **167**, as shown in Scheme 10c. The (*Z*)-isomer from the (*E*)/(*Z*) mixture (7:1), formed by the reaction, was separated by HPLC to give pure (±)-aiphanol (**138**).

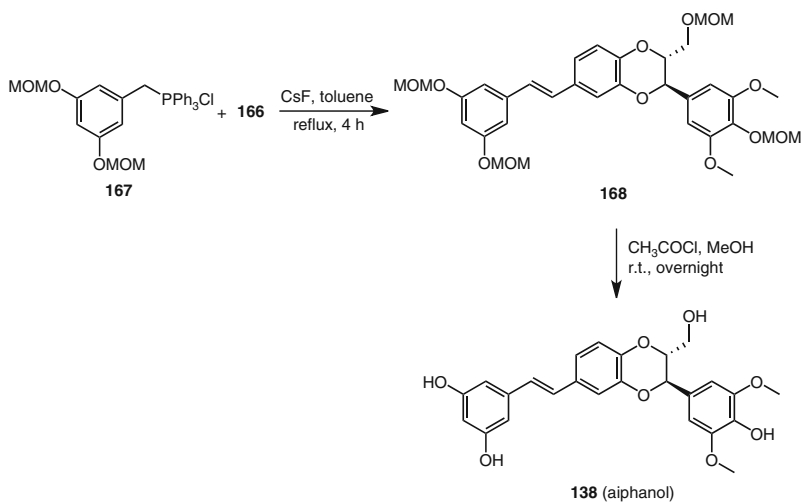
An enantioselective total synthesis of (–)-**138** was also achieved using (1*R*,2*R*)-1-(3',5'-dimethoxy-4'-methoxymethoxyphenyl)-2,3-dihydroxypropanol, the absolute stereochemistry of which was confirmed by single-crystal X-ray analysis of a readily available bromo- derivative. It was proved to have the (*S*)-configuration at both the 7' and 8' positions (**157**).



Scheme 10a. Preparation of *ortho*-quinone intermediate, **163**



Scheme 10b. Preparation of the key intermediate aldehyde, **166**



Scheme 10c. Wittig olefination of **166** and **167** to form aiphanol (**138**)

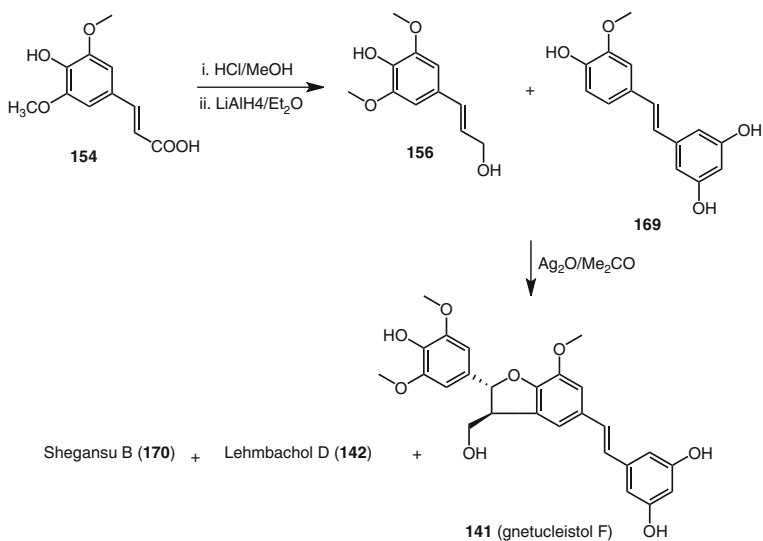
4.2.2. Gnetofuran A and Gnetucleistol F

Gnetofuran A (**140**) and gnetucleistol F (**141**) are stilbenolignans formed through a dihydrofuran bridge (151, 152). Gnetofuran A (**140**), C₂₅H₂₄O₇ [(M-H)⁻ *m/z* 435], [α]_D -7° cm² g⁻¹, showed the presence of a conjugated system (λ_{\max} 308, 288, 220 nm) in the UV spectrum. The ¹H NMR and ¹H-¹H COSY spectra revealed the presence of three substituted aromatic rings, with the protons appearing as AB, A₂B, and ABX systems (Table 19) and two methoxy groups at δ 3.73 and 3.69 (s) ppm. The presence of a pair of *trans*-coupled olefinic proton signals at δ 6.89 (d, *J* = 16.4 Hz) ppm and δ 6.78 (d, *J* = 16.4 Hz) ppm, taken together with the AB, A₂B aromatic proton systems and its UV spectrum, suggested the compound to be a stilbene derivative. The existence of a spin system of four aliphatic protons (benzylic methine at δ 5.45 (d, *J* = 5.4 Hz, 1H) ppm and 3.41 (dt, *J* = 12.2, 5.4 Hz, 1H) ppm and oxymethylene at δ 3.74 (m, 2H) ppm) and an aromatic ABX system indicated the presence of a phenylpropanoid unit in gnetofuran A. By considering the degrees of unsaturation, deshielding of the benzylic proton doublet (δ 5.45 ppm) and the HMBC correlation of H-7' (δ 5.45 ppm) with C-4 (δ 149.3 ppm), the linkage of the stilbene moiety with the phenylpropanoid unit through the dihydrofuran ring was confirmed. Based on findings from the COSY and HMBC NMR spectra, which revealed the linkages between C-7/C-1, C-8/C-9, C-8'/C-5, and C-7'/C-1', the structure of gnetofuran A was proposed as **140**. The interaction between H-7' and H-9' in the NOESY spectrum disclosed the relative configuration at the dihydrofuran ring as *trans*.

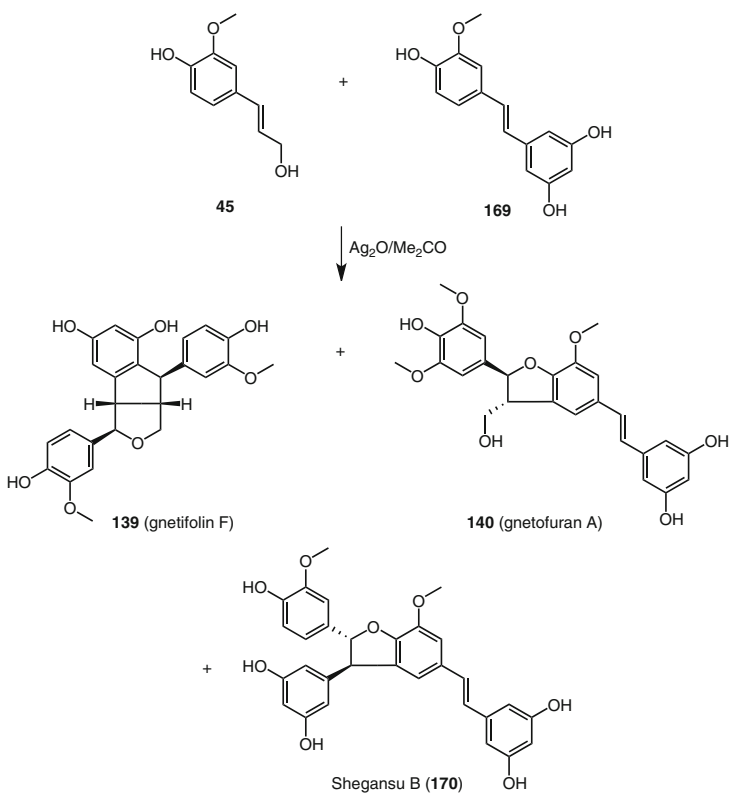
The congener, gnetucleistol F (**141**), C₂₆H₂₆O₈ [M⁺ *m/z* 466.160], showed absorption bands for hydroxy (3,350 cm⁻¹) and aromatic moieties (1,608, 1,518, 1,464 cm⁻¹) in the IR spectrum. Strong absorption bands at 310 (4.24) and 327 nm (4.29) in its UV spectrum indicated the presence of a stilbene-like conjugated system. The ¹H and ¹³C NMR spectroscopic data of **141** were comparable to those of **140**, but revealed the presence of an additional methoxy group, which was positioned at C-5' based on the HMBC spectrum. The structures determined for gnetucleistol F (**141**) and gnetofuran A (**140**) were eventually confirmed through their biomimetic synthesis (152). Oxidative coupling of 4-hydroxy-3,5-dimethoxyphenylpropanol (**156**), derived from sinapinic acid (**154**) through esterification and subsequent LAH reduction, with isorhapontigenin (**169**) in the presence of silver oxide as the catalyst, yielded two stilbenolignans, gnetucleistol F (**141**) and lehmbachol D (**142**), along with shegansu B (**170**), a dimer of **169** (Scheme 11). A similar reaction between ferulyl alcohol (**45**) and **169** yielded **140**, gnetifolin F (**139**) and the same dimer of isorhapontigenin, **170**, which is summarized in Scheme 12.

4.2.3. Gnetifolin F and Related Stilbenolignans

Gnetifolin F (**139**), C₂₅H₂₄O₇ [M⁺ *m/z* 436 (FDMS)]; [α]_D 0° cm² g⁻¹, isolated from the lianas of *Gnetum parvifolium*, is a novel stilbenolignan, the structure of which



Scheme 11. Biomimetic synthesis of gnetucleistol F (141)



Scheme 12. Biomimetic synthesis of gnetofuran A (140) and gnetifolin F (139)

was deduced mainly by the application of ^1H - ^1H COSY, ^{13}C - ^1H COSY, ^{13}C - ^1H COLOC, and NOE difference experiments (150). Compound **139** formed a tetraacetate, for which the mass spectrum showed the molecular ion peak at m/z 604 and fragment ions at m/z 562, 520, 368, 326, 284, and 137, with this fragmentation pattern comparable to that of compounds of the 2,6-diaryl-3,7-dioxabicyclo[3.3.0]-octane series. The presence of the 3-oxabicyclo[3.3.0]-octane system in gnetifolin F was supported by its ^1H NMR spectrum, which showed signals for eight aromatic hydrogens [δ 7.05 – 6.62 (2 x ABX systems, 2 *meta*-coupled Ar-H) ppm], six proton signals comprised of two methylene protons [δ 4.41 (dd, $J = 9.0, 7.0$ Hz, 1H) and 3.93 [dd, $J = 9.0, 5.5$ Hz, 1H) ppm], two benzylic protons [δ 4.81 (d, $J = 6.5$ Hz, 1H) and 4.30 (d, $J = 5.5$ Hz, 1H) ppm], and the bridgehead protons at δ 3.33 (m) ppm and δ 3.86 (m) ppm. The assignments made using the ^1H - ^1H COSY spectrum were supported by the ^{13}C - and other NMR spectra. The positions of the methoxy groups (δ 3.86 and 3.74 ppm, s) at C-3 and C-3', and the arrangement of the aromatic rings were ascertained through 2D NMR spectroscopic analysis. The NOE spectrum showed a *cis* relationship between the bridgehead hydrogens (H-8 and H-8'). The structure **139** deduced for gnetifolin F on the basis of the spectroscopic data of gnetifolin F tetraacetate was confirmed through single-crystal X-ray analysis (150). The biomimetic synthesis (152) of the compound is shown in Scheme 12. The ^1H and ^{13}C NMR data of gnetifolin F (151) are presented in Tables 19 and 20.

Maackolin, $\text{C}_{25}\text{H}_{24}\text{O}_8$, isolated from *Maackia amurensis*, was identified as 8-*epi*-8'-*epi*-3-*de-O*-methyl-5'-methoxygnatifolin F (**143**), based on the spectroscopic comparison between maackolin pentaacetate and gnetifolin F tetraacetate, and NOE difference NMR experiments on maackolin (153). Lehmbachol D (**142**), isolated from *G. cleistostachyum*, is structurally similar to **143** and it possesses a methoxy group in place of the hydroxy group at the C-3 position. The NOE correlation of H-8 and H-8' indicated their mutual *cis* orientation. The biomimetic synthesis of **142** has also been described (152) (Scheme 11).

4.3. Biological Activity of Stilbenolignans

Stilbenolignans have been proposed as anti-inflammatory lead compounds. Aiphanol (**138**) showed inhibitory activities against cyclooxygenases-1 and -2 (COX-1 and COX-2) with IC_{50} values of 1.9 and 9.9 μM , (149). Banwell and coworkers determined the anti-angiogenic properties of (\pm)-**138** and its isomers through an in vitro angiogenesis assay using the rat aorta. Racemic aiphanol completely inhibited blood vessel growth at 100 $\mu\text{g ml}^{-1}$ and, its isomers **157** and **159** also behaved similarly (154).

Yao *et al.* (153) reported the moderate anti-inflammatory potency of the stilbenolignans, gnetucleistol F (**141**), gnetofuran A (**140**), lehmbachol D (**142**), and gnetifolin F (**139**), as demonstrated in an inhibition bioassay on TNF- α production.

Compound **141** also exhibited inhibition of malondialdehyde and thereby qualified as a potent antioxidant.

4.4. Biogenesis of Stilbenolignans

The extension of a cinnamoyl-CoA chain (**126**) with malonate leads to the polyketide **127**, which may be folded differently, depending on the enzymes present in the plant (Fig. 32). Stilbene synthase, for example, converts the polyketide into a stilbene (**171**) through an aldol-type condensation (86).

Like the coumarinolignans, the dioxane-bridged stilbenolignan, aiphanol (**138**), may be derived biogenetically through oxidative radical coupling of the stilbene, piceatannol (**144**), with sinapic alcohol (**156**) (Fig. 33). During the biogenesis of gnetofuran A (**140**), the isorhapontigenin radical (**169a**) couples with the coniferyl alcohol radical (**45a**) to form intermediate **172**, which undergoes intramolecular cyclization to build the dihydrofuran bridge (**151**), as outlined in Fig. 34. In the case

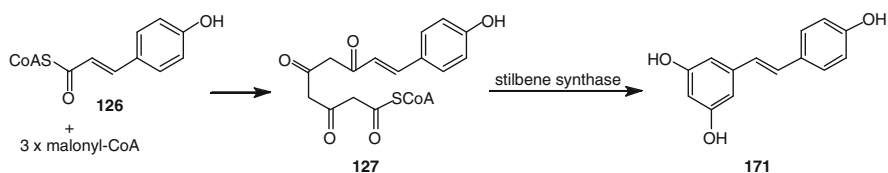


Fig. 32. Biogenesis of a stilbene **171**

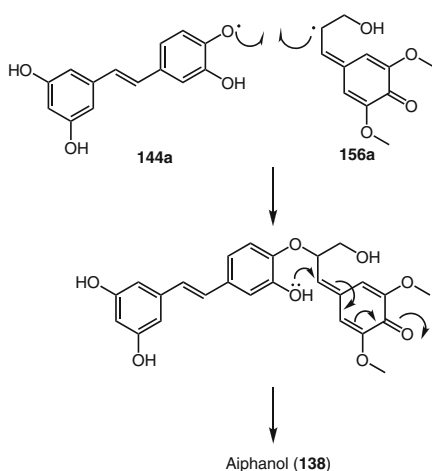
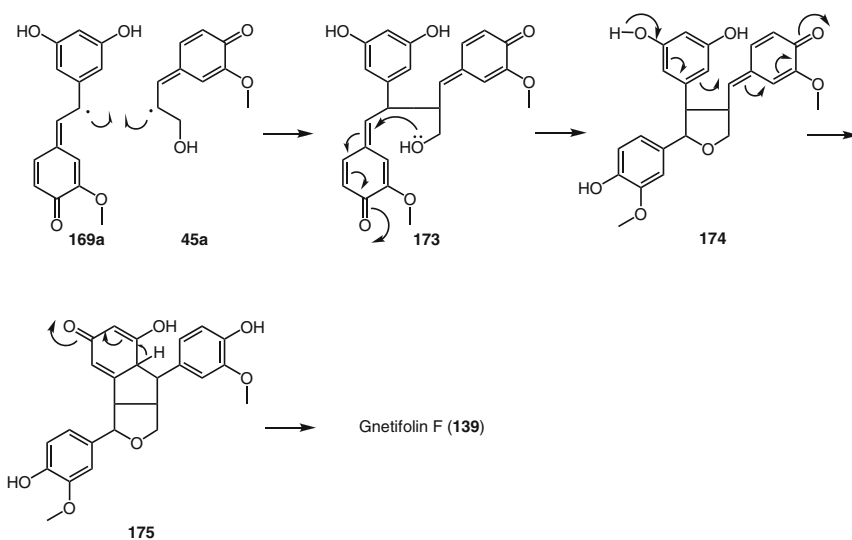
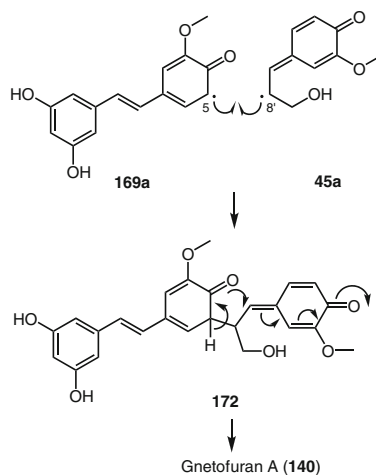


Fig. 33. Biogenesis of aiphanol (**138**)

Fig. 34. Biogenesis of gnetofuran A (**140**)**Fig. 35.** Biogenesis proposal for gnetifolin F (**139**)

of gnetifolin F (**139**) (*152*), radical coupling occurs between the 8 and 8' positions of the cinnamyl moieties of **169a** and **45a** (Fig. 35).

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USA for granting permission to reproduce the images of respectively, of *Cleome viscosa*, *Daphne oleoides*, *Sasa veitchii*, and *Maackia amurensis*. Thanks are also due to the Cambridge Crystallographic Data Centre for providing the X-ray crystallographic structure.

References

1. Haworth RD (1936) Natural Resins. *Annu Rep Prog Chem* **33**: 266
2. Haworth RD (1942) The Chemistry of the Lignan Group of Natural Products. *J Chem Soc* **448**
3. Gottlieb OR (1972) Chemosystematics of the Lauraceae. *Phytochemistry* **11**: 1537
4. Gottlieb OR (1978) Neolignans. In: Herz W, Grisebach H, Kirby GW (eds.) *Progress in the Chemistry of Organic Natural Products*, vol. 35, p. 1. Springer-Verlag, Vienna
5. Rao CBS (1978) *Chemistry of Lignans*. Andhra University Press, Visakhapatnam, India.
6. Hearon WM, McGregor WS (1955) The Naturally Occurring Lignans. *Chem. Rev.* **55**: 957
7. Stevenson R (1995) Some Aspects of Chemistry of Lignans. In: Atta-ur-Rahman (ed) *Studies in Natural Products Chemistry*, vol 17, p. 311. Elsevier, Amsterdam
8. Erdtman H (1955) *Moderne Methoden der Pflanzenanalyse*, p. 3. Springer-Verlag, New York
9. Ward RS (1982) The Synthesis of Lignans and Neolignans. *Chem Soc Rev* **11**: 75
10. Ayres DC, Loike JD (1990) *Lignans*. Cambridge University Press, Cambridge, UK
11. Carpenter J, Locksley HD, Scheinmann F (1969) Xanthones in Higher Plants: Biogenetic Proposals and a Chemotaxonomic Survey. *Phytochemistry* **8**: 2013
12. Bhat SV, Bhimsen A, Sivakumar NM (2005) *Chemistry of Natural Products*, p. 587. Springer Birkhauser and Narosa Publishing House, New Delhi, India
13. Kurkin VA, Zapesochnaya GG (1987) Flavolignans and Other Natural Lignoids: Problems of Structural Analysis. *Chem Nat Comp* **23**: 7
14. Chatterjee A, Das PC, Joshi PC, Mandal S (1994) Naturally Occurring Coumarino-lignoids. *J Indian Chem Soc* **71**: 475
15. Pelter A, Hansel R (1968) The Structure of Silybin (Silybum Substance E6). The First Flavonolignan. *Tetrahedron Lett* **25**: 2911
16. Bourgaud F, Hehn A, Larbat R, Doerper S, Gentier E, Kellner S, Mahern U (2006) Biosynthesis of Coumarins in Plants: A Major Pathway Still to Be Unravalled for Cytochrome P450 Enzymes. *Phytochem Rev* **5**: 293
17. Naoko F, Nagumo S, Inoue T, Nagai M (1988) Studies on the Constituents of Aceraceae Plants (VII). Coumarinolignans from the Wood of *Acer nikoense*. *Shoyakugaku Zasshi* **42**: 163; *Chem Abstr* 110: 101568
18. Iizuka T, Nagumo S, Yotsumoto H, Moriyama H, Nagai M (2007) Vasorelaxant Effects of *Acer nikoense* Extract and Isolated Coumarinolignans on Rat Aortic Rings. *Biol Pharm Bull* **30**: 1164
19. Jin WY, Thong PT, Su ND, Byung S, Son KH, Chang HW, Kim HP, Kang SS, Dai E, Bae K (2007) Antioxidant Activity of Cleomiscosin A and C Isolated from *Acer okamotoanum*. *Arch Pharm Res* **30**: 275
20. Tan XG, Zhang XR, Peng SI, Liao X, Ding LS (2003) Chemical Constituents from the Roots of *Biondia hemsleyana*. *Gaodeng Xuexiao Huaxue Xuebao* **24**: 436; *Chem Abstr* **138**: 365537
21. Mandal S, Das PC, Joshi PC, Chatterjee A (1991) Hemidesminine – A New Coumarinolignoid from *Hemidesmus indicus* R. Br. *Indian J Chem Sect B* **30**: 712
22. Das PC, Joshi PC, Mandal S, Das A, Chatterjee A (1992) New Coumarinolignoids from *Hemidesmus indicus* R. Br. *Indian J Chem Sect B* **31**: 342
23. Patnam R, Kadali SS, Koumaglo KH, Roy R (2005) A Chlorinated Coumarinolignan from the African Medicinal Plant *Mondia whitei*. *Phytochemistry* **66**: 683

24. Zhang Q, Zhao Y, Liu X, Zhang N, Cui Y, Cheng T (2000) Studies on Chemical Constituents of *Stelmocrypton khasianum* H. Baill. *Zhongguo Zhongyao Zazhi* **25**: 101; *Chem Abstr* **133**: 132439
25. Paula VF, Barbosa LCA, Demuner AJ, Howarth OW, Veloso DP (1996) Chemical Constituents of *Ochroma lagopus* Swartz. *Quim Nova* **19**: 225; *Chem Abstr* **124**: 337858
26. Almeida EX, Conserva LM, Lemos RPL (2002) Coumarins, Coumarinolignoids and Terpenes from *Protium heptaphyllum*. *Biochem System Ecol* **30**: 685
27. Zoghbi MDGB, Roque NF, Gottlieb OR (1981) Propacin, a Coumarinolignoid from *Protium opacum*. *Phytochemistry* **20**: 180
28. Magalhaes A, Zoghbi MDGB, Siani AC (2006) 5-Methoxypropacin, a Novel Coumarinolignoid from *Protium unifoliolatum*. *Nat Prod Res* **20**: 43
29. Ray AB, Chattopadhyay SK, Konno C, Hikino H (1980) Structure of Cleomiscosin A, a Coumarino-lignoid of *Cleome viscosa* Seeds. *Tetrahedron Lett* **21**: 4477
30. Ray AB, Chattopadhyay SK, Konno C, Hikino H (1982) Structure of Cleomiscosin B, a Coumarino-lignoid of *Cleome viscosa* Seeds. *Heterocycles* **19**: 19
31. Ray AB, Chattopadhyay SK, Konno C, Kiso Y, Hikino H (1985) Structures of Cleomiscosins, Coumarino-lignoids of *Cleome viscosa* Seeds. *Tetrahedron* **41**: 209
32. Kumar S, Ray AB, Konno C, Oshima Y, Hikino H (1988) Cleomiscosin D, a Coumarino-Lignan from the Seeds of *Cleome viscosa*. *Phytochemistry* **27**: 636
33. Chattopadhyay SK, Srivastava S, Negi AS, Gupta A, Khanuja SPS (2004) Hepatoprotective Pharmaceutical Composition Comprising a Mixture of Coumarinolignoids, Process for Preparation Thereof. *US Pat* 20040191343; *Chem Abstr* **141**: 301390
34. Proksa B, Uhrin D, Narantuyaa S, Batsuren D (1990) Cleomiscosins B and D, New Coumarino-lignoids from *Salsola laricifolia*. *Pharmazie* **45**: 804
35. Oueslati MH, Ben-Jannet H, Abreu P, Mighri Z (2004) Structure of a Coumarinolignan Isolated from the Aerial Part of the Plant *Salsola tetrandra*. *J Soc Alger Chim* **14**: 181; *Chem Abstr* **143**: 225960
36. Ahmad VU, Ullah F, Hussain J, Farooq U, Zubair M (2004) Tyrosinase Inhibitors from *Rhododendron colletianum* and Their Structure-Activity Relationship (SAR) Studies. *Chem Pharm Bull* **52**: 1458
37. Fozdar BI, Khan SA, Shamsuddin T, Shamsuddin KM, Kintzinger JP (1989) Aleuritn, a Coumarinolignoid and a Coumarin from *Aleurites fordii*. *Phytochemistry* **28**: 2459
38. Shamsuddin T, Rahman W, Khan SA, Shamsuddin KM, Kintzinger JP (1988) Moluccanin, a Coumarinolignoid from *Aleurites moluccana*. *Phytochemistry* **22**: 1908
39. Chen YC, Cheng MJ, Lee SJ, Dixit AJ, Ishikawa T, Tsai IL, Chen IS (2004) Coumarinolignans from the Root of Formosan *Antidesma pentandrum* var. *barbatum*. *Helv Chim Acta* **87**: 2805
40. Wang D, Liang X (1984) Structure and Stereochemistry of Maoyancaosu. *Acta Pharm Sin* **19**: 261; *Chem Abstr* **103**: 104162
41. Cheng XF, Chen ZL (2000) Coumarinolignoids of *Mallotus apelta*. *Fitoterapia* **71**: 341
42. Parthasarathy MR, Pardhasaradhi K (1984) A Coumarino-lignan from *Jatropha glandulifera*. *Phytochemistry* **23**: 867
43. Parthasarathy MR, Pardhasaradhi K (1984) Synthesis of Coumarinolignans. *Indian J Chem Sect B* **23**: 1005
44. Das R, Srinivas KVNS, Ramu R, Venkataiah B, Das B (2003) New Propacin Analogs from a Collection of the Whole Plant of *Jatropha gossypifolia*. *Int J Chem Sci* **1**: 159; *Chem Abstr* **140**: 90723
45. Das R, Srinivas KVNS, Mahender I, Venkataiah B, Das B (2003) Studies on Phytochemicals 42. New Coumarino-lignans from *Jatropha gossypifolia*. *Chemistry (Rajkot, India)* **1**: 9
46. Das B, Venkataiah B, Kashinatham A (1999) Venkatasin, a New Coumarino-lignoid from *Jatropha gossypifolia*. *Nat Prod Lett* **13**: 293
47. Das B, Kashinatham A, Venkataiah B, Srinivas KVNS, Mahender G, Reddy RM (2003) Cleomiscosin A, a Coumarino-lignoid from *Jatropha gossypifolia*. *Biochem System Ecol* **31**: 1189

48. Tanaka H, Kato I, Ichino K, Ito K (1986) Coumarinolignoids, Cleomiscosin A and Cleomiscosin B from *Aesculus turbinata*. J Nat Prod **49**: 366
49. Yun BS, Lee IK, Ryoo IJ, Yoo ID (2001) Coumarins with Monoamine Oxidase Inhibitory Activity and Antioxidative Coumarino-lignans from *Hibiscus syriacus*. J Nat Prod **64**: 1238
50. Qi S, Wu D, Ma Y, Luo X (2003) Novel Flavane from *Carapa guianensis*. Acta Bot Sin **45**: 1129
51. Mizuno M, Kojima H, Tanaka T, Iinuma M, Kimura R, Zhi-Da M, Murata H (1987) Phenolic Constituents from Seeds of *Coptis japonica* var. *dissecta*. Phytochemistry **26**: 2071
52. Chen JJ, Wang TY, Hwang TL (2008) Neolignans, a Coumarinolignan, Lignan Derivatives and a Chromene: Anti-inflammatory Constituents from *Zanthoxylum avicennae*. J Nat Prod **71**: 212
53. Chavez JP, David JM, Yang SW, Cordell GA (1997) 24-Norhopene Derivatives from *Diatenopteryx sorbifolia*. J Nat Prod **60**: 909
54. Ahmad VU, Fatima I, Fatima A (1987) The Sapogenins from *Dodonaea viscosa*. Fitoterapia **58**: 361
55. Hemlata, Kalidhar SB (1994) Chemical Constituents of *Dodonaea viscosa*. J Ind Chem Soc **71**: 213
56. Arisawa M, Handa SS, McPherson DD, Lankin DC, Cordell GA, Fong HHS, Farnsworth NR (1984) Plant Anticancer Agents. XXIX. Cleomiscosin A from *Simaba multiflora*, *Soulamea soulameoides* and *Matayba arborescens*. J Nat Prod **47**: 300
57. Lee KH, Hayashi N, Okano M, Nozaki H, Juichi M (1984) Antitumor agents, 65. Brusatol and Cleomiscosin A, Antileukemic Principles from *Brucea javanica*. J Nat Prod **47**: 550
58. Fukamiya N, Okano M, Miyamoto M (1992) Antitumor Agents, 127. Bruceoside C, a New Cytotoxic Quassinoid Glucoside and Related Compounds from *Brucea javanica*. J Nat Prod **55**: 468
59. Luyengi L, Suh N, Fong HHS, Pezzuto JM, Kinghorn AD (1996) A Lignan and Four Terpenoids from *Brucea javanica* that Induce Differentiation with Cultured HL-60 Promyelocytic Leukemia Cells. Phytochemistry **43**: 409
60. McChesney JD, Dou J, Sindelar RD, Goins DK, Walker LA, Rogers RD (1997) Tirucallanetype Triterpenoids: NMR and X-ray Diffraction Analyses of 24-Epi-piscidinol A and Piscidinol A. J Chem Crystallogr **27**: 283
61. Fastre RV, Luyengi L, Vanhaelen M, Declercq JP, Meerssche MV (1987) Two Quassinoids and Two Coumarinolignoids from *Hannoa klaineana* Roots. Phytochemistry **26**: 317
62. Arisawa M, Kinghorn AD, Cordell GA, Farnsworth NR (1983) Plant Anticancer Agents. XXIV. Alkaloid Constituents of *Simaba multiflora*. J Nat Prod **46**: 222
63. Begum SA, Sahai M, Suessmuth R, Teigo A, Fujimoto Y (2006) Hyosgerin, A New Optically Active Coumarinolignan from the Seeds of *Hyoscyamus niger*. Chem Pharm Bull **54**: 538
64. Bhandari P, Pant P, Rastogi RP (1982) Aquillochin, a Coumarinolignan from *Aquilaria agallocha*. Phytochemistry **21**: 2147
65. Kreher B, Neszmelyi A, Wagner H (1990) Triumbellin, a Tricoumarin Rhamnopyranoside from *Daphne mezereum*. Phytochemistry **29**: 3633
66. Cottiglia F, Bonsignore L, Loy G, Garau D, Floris C, Casu M (2002) Structure Elucidation and Antibacterial Activity of a New Coumarinolignoid from *Daphne gnidium* L. Magn Reson Chem **40**: 551
67. Ullah N, Ahmed S, Muhammad P, Ahmed Z, Nawaz HR, Malik A (1999) Coumarinolignoid Glycoside from *Daphne oleoides*. Phytochemistry **51**: 103
68. Lin-Gen Z, Seligmann O, Wagner H (1983) Daphneticin, a Coumarinolignoid from *Daphne tangutica*. Phytochemistry **22**: 617
69. Ma C, Zhang HJ, Tan GT, Hung NV, Cuong NM, Soejarto DD, Fong HHS (2006) Antimalarial Compounds from *Grewia bilamellata*. J. Nat. Prod. **69**: 346
70. Iqbal K, Anis I, Mukhtar N, Malik A, Fatima N, Choudhary MI (2003) Phosphodiesterase Inhibitory Coumarinolignoids from *Duranta repens*. Heterocycles **60**:151

71. Nair AGR (1979) Cleosandrin, a Novel 7-Phenoxycoumarin from the Seeds of *Cleome icosandra*. Indian J Chem **17B**: 438
72. Arnoldi A, Arnone A, Merlini L (1984) Synthesis of the Natural Coumarinolignoids Propacin and Cleomiscosin A and B. An Empirical Spectroscopic Method to Distinguish Regioisomers of Natural Benzodioxane Lignoids. Heterocycles **22**: 1537
73. Lin L-J, Cordell GA (1988) Synthesis and Structure Elucidation of Coumarinolignans. J Chem Res (M) 3046
74. Lin L-J, Cordell GA (1986) Application of the SINEPT Pulse Programme in the Structure Elucidation of Coumarinolignans. J Chem Soc Chem Commun **5**: 377
75. Tanaka H, Ichino K, Ito K (1985) Total Synthesis of Cleomiscosin A, a Coumarinolignoid. Chem Pharm Bull **33**: 3218
76. Das B, Thirupathi P (2007) A Highly Selective and Efficient Acetylation of Alcohols and Amines with Acetic Anhydride Using $\text{NaHSO}_4 \cdot \text{SiO}_2$ as a Heterogeneous Catalyst. J Mol Catal A: Chem **269**: 12
77. Ramesh C, Mahender G, Ravindranath N, Das B (2003) A Mild, Highly Selective and Remarkably Easy Procedure for Deprotection of Aromatic Acetates Using Ammonium Acetate as a Neutral Catalyst in Aqueous Medium. Tetrahedron **59**: 1049
78. Tanaka H, Ishihara M, Ichino K, Ito K (1985) Total Synthesis of Cleomiscosin B, a Coumarinolignoid. Heterocycles **23**: 1991
79. Tanaka H, Ishihara M, Ichino K, Ito K (1987) Definite Total Synthesis of Coumarinolignans, Daphneticin and its Regioisomer. Heterocycles **26**: 3115
80. Tanaka H, Ishihara M, Ichino K, Ito K (1988) Total Synthesis of Coumarinolignans, Propacin and its Regioisomer. Chem Pharm Bull **36**: 1738
81. Tanaka H, Ishihara M, Ichino K, Ito K (1988) Total Synthesis of Coumarinolignans, Aquillochin (Cleomiscosin C) and Cleomiscosin D. Chem Pharm Bull **36**: 3833
82. Tanaka H, Ishihara M, Ichino K, Ito K (1988) Synthesis of Natural Coumarinolignans: Oxidative Coupling of 7,8-Dihydroxycoumarins and Phenylpropenes in the Presence of Diphenyl Selenoxide. Heterocycles **27**: 2651
83. Ren XF, Peng K, Chen XC, Xie XG, Xia YM (2003) First Enantioselective Total Synthesis of Daphneticin. Chin Chem Lett **14**: 554
84. Bawankule DU, Chattopadhyay SK, Pal A, Saxena K, Yadav S, Yadav NP, Mani D, Tripathi AK, Beg SU, Srivastava A, Gupta AK, Khanuja SPS (2007) An in Vivo Study of the Immunomodulatory Activity of Coumarinolignoids from *Cleome viscosa*. Nat Prod Commun **2**: 923
85. Khanuja SPS, Pal A, Chattopadhyay SK, Daraokar MP, Patel RP, Gupta AK, Negi AS, Kaur T, Tandon S, Kahol AP, Garg A (2007) Immunomodulatory Pharmaceutical Composition Comprising Coumarinolignoid Isolated from Seeds of *Cleome* Species. PCT Int. Appl. PIXXD2 WO 2007066197 A2 20070614; Chem Abstr **147**: 58364
86. Dewick PM (2002) Medicinal Natural Products – a Biosynthetic Approach, 2nd Edn. John Wiley & Sons, New York
87. Schopen RD, Lange OK, Panne C, Kirnberger EJ (1969) Searching for a New Therapeutic Principle. Experience with Hepatic Therapeutic Agent. Legalon Med Welt **20**: 888
88. Kren V, Walterova D (2005) Silybin and Silymarin – New Effects and Applications. Biomed Papers **149**: 29
89. Kidd P, Head K (2005) A Review of the Bioavailability and Clinical Efficacy of Milk Thistle Phytosome: A Silybin – Phosphatidylcholine Complex (Siliphos). Altern Med Rev **10**: 193
90. Crocenzi FA, Roma MG (2006) Silymarin as a New Hepatoprotective Agent in Experimental Cholestasis: New Possibilities from an Ancient Medication. Curr Med Chem **13**: 1055
91. Pliskova M, Vondracek J, Kren V, Gazak R, Sedmera P, Walterova D, Psotova J, Simanek V, Machala M (2005) Effects of Silymarin Flavonolignans and Synthetic Silybin Derivatives on Estrogen and Aryl Hydrocarbon Receptor Activation. Toxicology **215**: 80

92. Abraham DJ, Takagi S, Rosenstein RD, Shino R, Wagner H, Horhammer L, Seligmann O (1970) The Structure of Silydianin, an Isomer of Silymarin (Silybin) by X-Ray Analysis. *Tetrahedron Lett* **31**: 2675
93. Wagner H, Seligmann O, Horhammer L, Seitz M (1971) Zur Struktur von Silychristin einem zweiten Silymarin-Isomeren aus *Silybum marianum*. *Tetrahedron Lett* **22**: 1895
94. Bandopadhyay M, Pardeshi NP, Seshadri TR (1972) Components of *Silybum marianum*. *Indian J Chem* **10**: 808
95. Pelter A, Hansel R (1975) Struktur Des Silybins: I. Abbauversuche. *Chem Ber* **108**: 790
96. Takemoto T, Ikegawa S, Nomoto K (1975) Studies on Constituents of *Silybum marianum* (L.) Gaertn. I. New Flavonolignans Named 2,3-Dehydrosilymonin and 2,3-Dehydrosilychristin. *Yakagaku Zasshi* **95**: 1017
97. Pelter A, Hansel R, Kaloga M (1977) The Structure of Silychristin. *Tetrahedron Lett* **51**: 4547
98. Wagner H, Chari VM, Seitz M, Riess-Mauerer J (1978) The Structure of Silychristin – a ¹³C-NMR Study. *Tetrahedron Lett* **19**: 381
99. Szilagyi I, Tetenyi P, Antus S, Seligmann O, Chari VM, Seitz M, Wagner H (1981) Struktur von Silandrin und Silymonin, zwei neuen Flavanolignanen aus einer Weißblühenden *Silybum marianum* Varietät. *Planta Med* **43**: 121
100. Arnone A, Merlini L, Zanarotti A (1979) Constituents of *Silybum marianum*. Structure of Isosilybin and Stereochemistry of Silybin. *J Chem Soc Chem Commun*: 696
101. Fiebig M, Wagner H (1984) Neue Antihepatotoxisch Wirksame Flavonolignane aus einer Weißblühenden *Silybum* – Varietät. *Planta Med* **51**: 310
102. Kim NC, Graf TN, Sparacino CM, Wani MC, Wall ME (2003) Complete Isolation and Characterisation of Silybins and Isosilybins from Milk Thistle (*Silybum marianum*). *Org Biomol Chem* **1**: 1684
103. Hasanloo T, Khavari-Nejab RA, Majidi E, Ardekani MRS (2005) Analysis of Flavonolignans in Dried Fruits of *Silybum marianum* (L.) Gaertn. from Iran. *Pak J Biol Sci* **8**: 1778
104. Smith WA, Lauren DR, Burgess EJ, Perry NB, Martin RJ (2005) A Silychristin Isomer and Variation of Flavonolignan Levels in Milk Thistle (*Silybum marianum*) Fruits. *Planta Med* **71**: 877
105. Lee DYW, Liu Y (2003) Three New Flavonolignans Isolated from the Seeds of *Silybum marianum*. In: 226th National Meeting of the American Chemical Society, New York, September 7–11, 2003. Abstract AGFD-057
106. Mericli AH (1988) Flavonolignans, Kaempferol 3-Sulphate and Other Flavonoids from *Silybum marianum* subsp. *anatolicum*. *Planta Med*: 44
107. Stermitz FR, Matsuda JT, Lorenz P, Mueller P, Zenewicz L, Lewis K (2000) 5'-Methoxyhydnocarpin-D and Pheophorbide A: *Berberis* Species Components that Potentiate Berberine Growth Inhibition of Resistant *Staphylococcus aureus*. *J Nat Prod* **63**: 1146
108. Syrchina AI, Gorshkov AG, Shcherbakov VV, Zinchenko SV, Vereshchagin AL, Zaikov KL, Semenov AA (1992) Flavonolignans of *Salsola collina*. *Khim Prir Soedin* **28**: 182
109. Cardono ML, Garcia B, Pedro JR, Sinisterra JF (1990) Flavonoids, Flavonolignans and a Phenylpropanoid from *Onopordon corymbosum*. *Phytochemistry* **29**: 629
110. Ranganathan KR, Seshadri TR (1973) A New Flavono-Lignan from *Hydnocarpus wightiana*. *Tetrahedron Lett*: 3481
111. Ranganathan KR, Seshadri TR (1974) Constitution of Isohydnocarpin Isolated from the Seed Hulls of *Hydnocarpus wightiana*. *Indian J Chem* **12**: 888
112. Ranganathan KR, Seshadri TR (1974) Minor Phenolic Components of Seed Hulls of *Hydnocarpus wightiana*: Constitution of Methoxyhydnocarpin. *Indian J Chem* **12**: 993
113. Parthasarathy MR, Ranganathan KR, Sharma DK (1979) ¹³C NMR of Flavonolignans from *Hydnocarpus wightiana*. *Phytochemistry* **18**: 506
114. Cooper R, Gottlieb HE, Lavie D (1977) A New Flavolignan of Biogenetic Interest from *Aegilops ovata* L.-Part I. *Israel J Chem* **16**: 12

115. Nakajima Y, Yun YS, Kunugi A (2003) Six New Flavonolignans from *Sasa veitchii* (Carr.) Rehder. *Tetrahedron* **59**: 8011
116. Kostova I, Rangaswami S (1977) Chemical Components of the Seeds of *Cassia absus*. *Indian J Chem.* **15B**: 764
117. Pettit GR, Meng Y, Stevenson CA, Doubek DL, Knight JC, Cichacz Z, Pettit RK, Chapuis JC, Schmidt JM (2003) Isolation and Structure of Palstatin from the Amazon Tree *Hymenaea palustris*. *J Nat Prod* **66**: 259
118. Wenzig E, Kunert O, Ferreira D, Schmid M, Schuhly W, Bauer R, Hiermann A (2005) Flavonolignans from *Avena sativa*. *J Nat Prod* **68**: 289
119. Bouaziz M, Veitch NC, Grayer RJ, Simmonds MSJ, Damak M (2002) Flavonolignans from *Hyparrhenia hirta*. *Phytochemistry* **60**: 515
120. Afifi MS, Ahmed MM, Pezzuto JM, Kinghorn AD (1993) Cytotoxic Flavonolignans and Flavones from *Verbascum sinaiticum* Leaves. *Phytochemistry* **34**: 839
121. Borodin LI, Litvinenko VI, Kurinnaya NV (1969). Flavonoid Acylglycosides of *Gratiola officinalis*. *Khim Prirod Soedin* **5**: 437
122. Borodin LI, Litvinenko VI, Kurinnaya NV (1970) Lignoside, a New Flavonoid Glycoside of *Gratiola officinalis*. *Farm Zh (Kiev)* **25**: 62
123. Guz NR, Stermitz FR (2000) Synthesis and Structures of Regioisomeric Hydnocarpin Type Flavonolignans. *J Nat Prod* **63**: 1140
124. Lee DYW, Liu Y (2003) Molecular Structure and Stereochemistry of Silybin A, Silybin B, Isosilybin A and Isosilybin B, Isolated from *Silybum marianum* (Milk Thistle). *J Nat Prod* **66**: 1171
125. Lee MW, Lee YA, Park HM, Toh SH, Lee EJ, Jang HD, Kim YH (2000) Antioxidative Phenolic Compounds from the Roots of *Rhodiola sachalinensis* A. Bor. *Arch Pharm Res* **23**: 455
126. Conserva LM, Yoshida M, Gottlieb OR, Martinez VJC, Gottlieb HE (1990) Iryantherins, Lignoflavonoids of Novel Structural Types from the Myristicaceae. *Phytochemistry* **29**: 3911
127. Silva DHS, Cavalheiro AJ, Yoshida M, Gottlieb OR (1995) Flavonolignoids from Fruits of *Iryanthera grandis*. *Phytochemistry* **38**: 1013
128. Liliana GN, Luis EC, Juan CM, Yoshida M, Gottlieb OR (1987) Flavonolignoid from the Fruit of *Iryanthera laevis*. *Phytochemistry* **26**: 2835
129. Silva DHS, Davino SC, Barros SBM, Yoshida M (1999) Dihydrochalcones and Flavonolignans from *Iryanthera lancifolia*. *J Nat Prod* **62**: 1475
130. Markham KR, Mabry TJ (1975) Ultraviolet-Visible and Proton Magnetic Resonance Spectroscopy of Flavonoids. In: Harborne JB, Mabry TJ, Mabry H (eds.) *The Flavonoids*, Part 1, p. 45. Academic Press, New York
131. Gaffield W (1970) Circular Dichroism, Optical Rotatory Dispersion and Absolute Configuration of Flavanones, 3-Hydroxyflavanones and their Glycosides: Determination of Aglycone Chirality in Flavanone Glycosides. *Tetrahedron* **26**: 4093
132. Arnoldi A, Merlini L (1985) Asymmetric Synthesis of 3-Methyl-2-phenyl-1,4-benzodioxanes. Absolute Configuration of the Neolignan Euserdin and Euseridin C and D. *J Chem Soc Perkin Trans I*: 2555
133. Da Silva MS, Barbosa-Filho JM, Yoshida M, Gottlieb OR (1989) Benzodioxane and β -Aryloxy-arylpropane Type Neolignans from *Licaria chrysophylla*. *Phytochemistry* **28**: 3477
134. Fang JM, Lee CK, Cheng YS (1992) Lignans from leaves of *Juniperus chinensis*. *Phytochemistry* **31**: 3659
135. Hansel R, Schulz J, Pelter A (1975) Struktur Des Silybins, II. Synthese Des Dehydrosilybin-pentamethylaethers und verwandter Verbindungen. *Chem Ber* **108**: 1482
136. Merlini L, Zanarotti A, Pelter A, Rocheport MP (1979) Biomimetic Synthesis of Natural Silybin. *J Chem Soc Chem Commun*: 695
137. Tanaka H, Shibata M, Ohira K, Ito K (1985) Total Synthesis of (\pm)-Silybin, an Antihepatotoxic Flavonolignan. *Chem. Pharm. Bull.* **33**: 1419

138. Quercia V, Pierini N, Incarnato G, Terracciano M, Papetti P (1980) Identification and Assay by HPLC of the Flavonoid Components of *Silybum marianum* in Medicinal Specialities. *Fitoterapia* **51**: 83
139. Quercia V, Pierini N, Incarnato GP, Papetti P, Gambero P (1980) HPLC Evaluation of the Ratio Between the Antihepatotoxic Constituents of *Silybum marianum*. *Fitoterapia* **51**: 297
140. Kuntz E (1998) Silymarin in the Treatment of Liver Diseases, p 8–50. Freiburg im Breisgau, Falk Foundation e. V, Freiburg, Germany.
141. She X, Jing X, Pan X, Chan ASC, Yang TK (1999) Total Synthesis of (\pm)-Sinaiticin. *Tetrahedron Lett* **40**: 4567
142. Goyal S, Parthasarathy MR (1992) Approaches Towards Synthesis of Neohydnocarpin Skeleton. *Indian J Chem* **31B**: 391
143. Hoofnagle JH (2005) Milk Thistle and Chronic Liver Disease. *Hepatology* **42**: 4
144. Gazak R, Svobodova A, Psotova J, Sedmera P, Prikrylova V, Walterova D, Kren V (2004) Oxidized Derivatives of Silybin and their Antiradical and Antioxidant Activity. *Bioorg Med Chem* **12**: 5677
145. Kren V, Kubisch J, Sedmera P, Halada P, Prikrylova V, Jegorov A, Cvak L, Gebhardt R, Ulrichova J, Simanek V (1997) Glycosylation of Silybin. *J Chem Soc Perkin Trans I*: 2467
146. Kubisch J, Sedmera P, Halada P, Gazak R, Skottova N, Simanek V, Kren V (2001) Chemoenzymatic Preparation of Oligoglycosides of Silybin, the Flavonolignan from *Silybum marianum*. *Heterocycles* **53**: 901
147. Maitrejean M, Comte G, Barron D, Kirat KL, Conseil G, Pietro AD (2000) The Flavanolignan Silybin and its Hemisynthetic Derivatives, a Novel Series of Potential Modulators of P-Glycoprotein. *Bioorg Med Chem Lett* **10**: 157
148. Dzubak P, Hajduch M, Gazak R, Svobodova A, Psotova J, Walterova D, Sedmera P, Kren V (2006) New Derivatives of Silybin and 2,3-Dehydrosilybin and Their Cytotoxic and P-glycoprotein Modulatory Activity. *Bioorg Med Chem* **14**: 3793
149. Lee D, Cuendet M, Schunke Vigo J, Graham JG, Cabieses F, Fong HHS, Pezzuto JM, Kinghorn AD (2001) A Novel Cyclooxygenase-Inhibitory Stilbenolignan from the Seeds of *Aiphanes aculeata*. *Org Lett* **3**: 2169
150. Lin M, Li JB, Wu B, Zheng QT (1991) A Stilbene Derivative from *Gnetum parvifolium*. *Phytochemistry* **30**: 4201
151. Ali Z, Tanaka T, Iliya I, Inuma M, Furusawa M, Ito T, Nakaya K, Murata J, Darnaedi D (2003) Phenolic Constituents of *Gnetum klossii*. *J Nat Prod* **66**: 558
152. Yao C, Lin M, Wang L (2006) Isolation and Biomimetic Synthesis of Anti-inflammatory Stilbenolignans from *Gnetum cleistostachyum*. *Chem Pharm Bull* **54**: 1053
153. Kulesh NI, Denisenko VA, Maksimov OB (1995) Stilbenolignan from *Maackia amurensis*. *Phytochemistry* **40**: 1001
154. Banwell MG, Bezos A, Chand S, Dannhardt G, Kiefer W, Nowe U, Parish CR, Savage GP, Ulbrich H (2003) Convergent Synthesis and Preliminary Biological Evaluations of the Stilbenolignan (\pm)-Aiphanol and Various Congeners. *Org Biomol Chem* **1**: 2427
155. Chand S, Banwell MG (2007) Biomimetic Preparation of the Racemic Modifications of the Stilbenolignan Aiphanol and Three Congeners. *Aust J Chem* **60**: 243
156. Kuboki A, Yamamoto T, Ohira S (2003) Total Synthesis of (\pm)-Aiphanol, a Novel Cyclooxygenase-Inhibitory Stilbenolignan. *Chem Lett* **32**: 420
157. Banwell MG, Chand S, Savage GP (2005) An Enantioselective Total Synthesis of the Stilbenolignan (–)-Aiphanol and the Determination of its Absolute Stereochemistry. *Tetrahedron Asymm* **16**: 1645

Picrotoxanes

Edda Gössinger

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1. Introduction

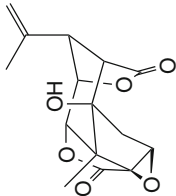
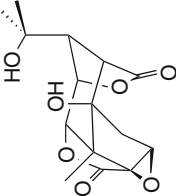
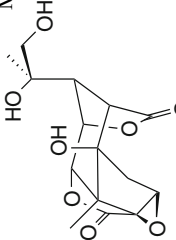
Shortly after *Sertürner*'s isolation of morphine in 1806, which signified the start of plant secondary products research, picrotoxin was isolated by the French scientist *Boullay* (1811) from the dried fruits of a liana growing in India and Southeast Asia (*1*). Although the plant had no value as therapeutic in Western medicine, picrotoxin was isolated even prior to such therapeutically most important plant constituents as emetine (1816) and quinine (1820). Its high toxicity and the ease of isolation by crystallization from water were responsible for the very early discovery of this first member of the picrotoxanes or tutinanolides. Picrotoxanes are a group of sesquiterpenes, sesquiterpene alkaloids, and "norditerpenes" with highly complex, mostly tetra- or pentacyclic structures and up to 12 stereogenic centers. Thus, it is not surprising that it took 70 years to learn that the crystalline substance picrotoxin is a molecular compound consisting of equal amounts of picrotoxinin (**1**), one of the most potent plant toxins, comparable in lethality with strychnine, and the less active hydrated derivative picrotin (**2**). An additional 80 years were to pass until the advent of modern spectroscopy allowed *Conroy* to complete his pioneering elucidation of the structure of picrotoxinin and picrotin. In the meantime more than ten new structural relatives had been isolated from very diverse plant families. In 1866, coriamyrtin (**9**) was isolated from the toxic berries of the tanner's brush, the only European plant known to contain picrotoxanes. Around the turn of the last century, loss of cattle in New Zealand prompted the investigation of the active principle of a shrub with succulent leaves called "tutu" or "toitoti" by the Maoris. The main toxin found was accordingly named tutin (**11**). Later, toxic honey from the same area was analyzed and found to contain a similar sesquiterpene the authors called "mellitoxin" (**15**). This represents a very early discovered example of a toxin passed through the food chain with a sap-sucking insect, with its honeydew and honeybees as intermediaries. In the first half of the twentieth century, Chinese and Japanese researchers looked at the active compounds of concoctions from orchids of the genus *Dendrobium* widely used in China and also in Japan as tonic and treatment for a variety of ailments. Thus, dendrobine (**82**), the first of the picrotoxane-type alkaloids, was detected. A highly toxic South African tree was the next source of picrotoxanes, when in 1920, hyenanchin (**15**) was isolated. When re-examined in the 1960s, this turned out to be identical with mellitoxin. Not only the existence of identical compounds in evolutionary distant plant families was remarkable but also the co-occurrence of picrotoxanes with analogs with an extended carbon skeleton, the so-called "norditerpene" picrotoxanes. Most picrotoxanes have shown a similar physiological activity. These neurotoxins are antagonists of neurotransmitters of various ligand gated chloride channels in the central nervous system and as such have played an important role as research tools in neurobiology. In two reviews (*2, 3*) knowledge gained on the picrotoxanes has been summarized

up to 1968. Despite the many interesting aspects of this group of sesquiterpenes, pseudoalkaloids, and “norditerpenes,” no comprehensive review has appeared since. Meanwhile the number of picROTOXANES has grown from 15 to 102, and 27 syntheses have been reported, and some of these have become classics of total synthesis. Publications of their biological activity have increased tremendously. In contrast, the number of species examined for picROTOXANES has only increased modestly, despite the fact that picROTOXANES are found in several angiosperm plant families growing world-wide in tropical and subtropical latitudes. The expectation of many more structures to be found among this compound class as well as the riddle of their occurrence in evolutionary distant plant families and the scant knowledge of the advantage gained by the plants that produce them are additional reasons for the present review. This chapter covers information discovered on the picROTOXANES since *Porter* and *Coscia* completed their reviews in 1967 and 1969, respectively.

2. Tabular Overview of the PicROTOXANES

The following five tables show the structures of all naturally occurring picROTOXANES (= tutinanolids), with their names, synonyms, and formulae in the first column, and the structures in the second column. Within the third column follow the plant (animal) source (family and name of species), and the geographical distribution. In the fourth column, the part of the plant and the solvent used for extraction are given and the yields of the purified substances complete the column. Analytical data collected and additional methods used for structure determination are included in the fifth column. The references in the sixth column are provided in detail only for structures determined after 1968. Earlier structure determinations were admirably covered by *Porter* and *Coscia* (2, 3). Table 1 lists the dilactone picROTOXANES, and, as in subsequent tables, the compounds are listed roughly according to their date of discovery. Table 2 records the monolactone picROTOXANES and is followed by a short table recording sesquiterpene picROTOXANES (Table 3) deviating from the structures of those presented in Tables 1 and 2. In Table 4, the “norditerpene” picROTOXANES (C18- and C19-picROTOXANES) are presented and the dendrobines are shown in Table 5. The structures are shown in their most stable conformation and they deviate from earlier attempts to represent stereostructures, which often presented the cyclohexane moiety of the picROTOXANES in the boat conformation with the methyl group and the isoprop(en)yl group in the flag pole position! In contrast to earlier drawings, the structures are presented with the convex face above. Thus, prefixes α and β are avoided and substituted by *exo* and *endo*. Numbering of the picROTOXANE skeleton has been unified; therefore this sometimes differs from that given in the original papers.

Table 1. Dilactone picrotoxanes

Trivial name formula	Structure	Natural source	Isolation	Structure determination	References
Picrotoxinin (1) C ₁₅ H ₁₆ O ₆		Menispermaceae: <i>Menispermum cocculus</i> (<i>Anamirta cocculus</i> = <i>A. paniculata</i>), India, Sri Lanka, Southeast Asia <i>Spirastrella inconstans</i> , marine sponge	Dried seeds of <i>M. cocculus</i> extracted with CH ₃ OH yielded after repeated chromatography 1.7 × 10 ⁻² %; shade-dried <i>S. inconstans</i> extracted with methanol yielded after chromatography 2.5 × 10 ⁻² %	Mp; IR; ¹ H-NMR; ¹³ C-NMR; X-ray analysis of picrotoxinin and of α ₁ -bromopicrotoxinin; [α] _D ; calculations of main conformation; synthesis	(2–10)
Picrotin (2) C ₁₅ H ₁₈ O ₇		Menispermaceae: <i>M. cocculus</i> , India, Sri Lanka, Southeast Asia <i>Spirastrella inconstans</i> , marine sponge Orchidaceae: <i>Dendrobium moniliforme</i> , Yunnan (China)	Dried seeds of <i>M. cocculus</i> extracted with CH ₃ OH yielded after repeated chromatography 7.3 × 10 ⁻² %; shade-dried <i>S. inconstans</i> extracted with methanol yielded after chromatography 1.7 × 10 ⁻² %; powdered dried stems of <i>D. moniliforme</i> extracted with ethanol yielded after partitioning and repeated chromatography 1.2 × 10 ⁻⁴ %	Mp; IR; ¹ H-NMR; ¹³ C-NMR; [α] _D ; conversion of picrotoxinin into picrotin; synthesis	(2–4, 7–12)
Dihydroxy-picrotoxinin (3) C ₁₅ H ₁₈ O ₈		Menispermaceae: <i>M. cocculus</i> , India, Sri Lanka, Southeast Asia	Dried seeds of <i>M. cocculus</i> extracted with CH ₃ OH yielded after repeated chromatography 2.2 × 10 ⁻³ %	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; [α] _D	(2, 3, 8)

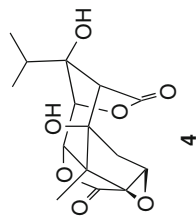
Aduncin (4)
C₁₅H₁₈O₇

Orchidaceae:
Dendrobium aduncum, nursery in West Bengal, India

Fresh plants extracted with CH₃OH yielded after further partitioning and crystallization 4 × 10⁻³%

Mp; MS; IR; ¹H-NMR; ¹³C-NMR; [α]_D; CD

(13-15)



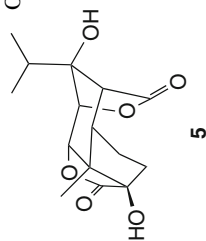
Amotin (5)
C₁₅H₂₀O₆

Orchidaceae:
Dendrobium amoenum, nursery in West Bengal, India

Fresh plants extracted with CH₃OH yielded after repeated chromatography 5 × 10⁻³%; dried plants extracted with CH₃OH yielded after chromatography 4 × 10⁻³%

Mp; MS; IR; ¹H-NMR; ¹³C-NMR; [α]_D; CD; aduncin was converted into amotin by hydrogenolysis

(15, 16)



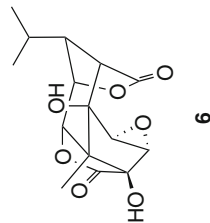
Dendrobiumane E (6)
C₁₅H₁₈O₇

Orchidaceae:
D. moniliforme, Yunnan (China)

Dried stems extracted with C₂H₅OH yielded after repeated chromatography 2.6 × 10⁻⁴%

Amorphous; MS; IR; ¹H-NMR; ¹³C-NMR; 2D-NMR; [α]_D

(11)



(continued)

Table I. (continued)

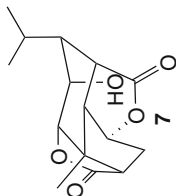
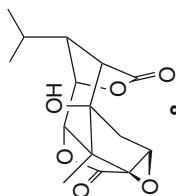
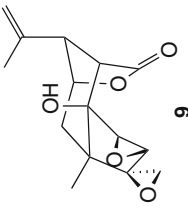
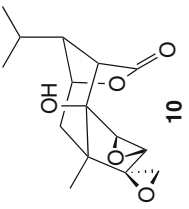
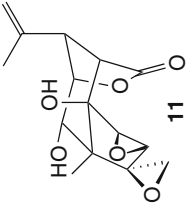
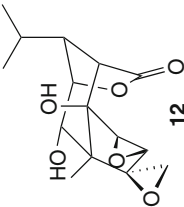
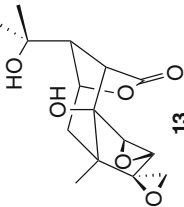
Trivial name formula	Structure	Natural source	Isolation	Structure determination	References
Flakinin A (7) C ₁₅ H ₂₀ O ₅		Orcchidaceae: <i>Dendrobium nobile</i> , cultivar <i>D. Snowflake</i> "Red Star"	Whole plants extracted with CH ₃ OH yielded after repeated chromatography 2 × 10 ⁻⁴ %	Amorphous; ESIMS; IR; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; [α] _D	(17)
α-Dihydro- picrotoxinin (8) C ₁₅ H ₂₀ O ₅		Orcchidaceae: <i>D. moniliforme</i> , Yunnan (China)	Dried stems extracted with C ₂ H ₅ OH yielded after partitioning and repeated chromatography 2 × 10 ⁻⁴ %	Amorphous; MS; IR; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; [α] _D ; comparison with picrotoxinin reduced with H ₂ /Pt	(2, 3, 11, 12, 15)

Table 2. Monolactone picrotoxanes

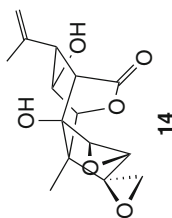
Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Coriamyrtin (9) C ₁₅ H ₁₈ O ₅		Coriariaceae: <i>Coriaria myrtifolia</i> , Europe; <i>C. ruscifolia</i> , Chile, South Africa; <i>C. sinica</i> (= <i>C. nepalensis</i>), China; <i>C. microphylla</i> (= <i>C. thymifolia</i>), <i>C. intermedia</i> , Taiwan; <i>C. japonica</i> , Japan Loranthaceae: <i>Loranthus parasiticus</i> , China	Dried aerial parts of <i>C. ruscifolia</i> extracted with C ₂ H ₅ OH yielded after LC 4 × 10 ⁻² %; dried aerial parts of <i>C. microphylla</i> (CH ₃ OH extract) 1.3 × 10 ⁻⁴ %; dried leaves of <i>C. intermedia</i> yielded 1.5 × 10 ⁻² %; dried powdered roots of <i>C. nepalensis</i> were extracted with water, chromatographed on polyamide and on silica gel yielding 1.3 × 10 ⁻³ %; dried leaves of <i>L. parasiticus</i> extracted with C ₂ H ₅ OH yielded 8.5 × 10 ⁻³ %	MS; IR; ¹ H-NMR; ¹³ C-NMR; [α] _D ; X-ray; calculations (AMI, MacroModel); synthesis	(2-4, 18-39)
Dihydrocoriamyrtin (10) C ₁₅ H ₂₀ O ₅		Coriariaceae: <i>C. nepalensis</i> , Nepal, China (Yunnan)	Dried powdered roots were extracted with water, chromatographed on polyamide and on silica gel yielding 7 × 10 ⁻⁵ %	MS; IR; ¹ H-NMR; ¹³ C-NMR; first identified by hydrogenation of coriamyrtin (9) over Adams catalyst	(24)
Tutin (11) C ₁₅ H ₁₈ O ₆		Coriariaceae: <i>C. arborea</i> , <i>C. sarmentosa</i> , <i>C. angustissima</i> , <i>C. pteroides</i> , <i>C. plumosa</i> , New Zealand (and toxic honey derived from these plants); <i>C. japonica</i> , Japan; <i>C. terminalis</i> , Tibet; <i>C. sinica</i> , China; <i>C. ruscifolia</i> , Chile Picrodendraceae: <i>Hyenanche globosa</i> , South Africa	Dried seeds of <i>C. japonica</i> yielded 8.5 × 10 ⁻³ %; toxic honey yielded between ~4 × 10 ⁻⁴ % and 1.7 × 10 ⁻³ %; dried aerial parts of <i>C. sarmentosa</i> yielded 8 × 10 ⁻³ %; dried aerial parts of <i>C. microphylla</i> (CH ₃ OH extract) yielded 1.4 × 10 ⁻¹ %; dried leaves of <i>L. parasiticus</i> extracted with C ₂ H ₅ OH yielded 3.5 × 10 ⁻² %; powdered dried fruits of <i>H. globosa</i> yielded after extraction with CHCl ₃ and	MS; IR; ¹ H-NMR; ¹³ C-NMR; ORD; [α] _D ; X-ray; calculations (AMI, MacroModel); synthesis; forms together with corianin (21) the molecular compound pseudotutin	(2-4, 9, 10, 19, 21-34, 37, 38, 40-42)

(continued)

Table 2. (continued)

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Dihydrotutin (12) C ₁₅ H ₂₀ O ₆		Loranthaceae: <i>L. parasiticus</i> , China Convulvulaceae: <i>Cuscuta japonica</i> , China, Korea, Japan Coriariaceae: <i>C. japonica</i> , Japan Toxic honey, New Zealand Picrodendraceae: <i>Picrodendron baccatum</i> , West Indies	chromatography $\sim 4 \times 10^{-1}\%$; dried powdered roots of <i>C. nepalensis</i> were extracted with water, chromatographed on polyamide and on silica gel yielding $1.2 \times 10^3\%$; dried seeds of <i>Cuscuta japonica</i> were extracted with C ₂ H ₅ OH, partitioned and chromatographed yielding $5 \times 10^{-4}\%$; Seeds of <i>C. japonica</i> isolated from ripe berries yielded $4 \times 10^{-5}\%$; toxic honey $2 \times 10^{-5}\%$; dried bark of <i>P. baccatum</i> (plants collected in Indonesia) extracted with CH ₃ OH yielded $1.6 \times 10^{-4}\%$	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; [α] _D ; first identified by hydrogenation of tutin (11) over Adams catalyst	(10, 28, 35, 36)
Coriatin (13) C ₁₅ H ₂₀ O ₆		Coriariaceae: <i>C. microphylla</i> (= <i>C. thymifolia</i>), Taiwan; <i>C. sinica</i> , China; <i>C. nepalensis</i> , Nepal, China Loranthaceae: <i>L. parasiticus</i> , China Convulvulaceae: <i>Cuscuta japonica</i> , China, Korea, Japan	Dried aerial parts of <i>C. microphylla</i> (CH ₃ OH extract) yielded $5 \times 10^{-2}\%$; dried powdered roots of <i>C. nepalensis</i> were extracted with water, chromatographed on polyamide and on silica gel yielding $2 \times 10^{-3}\%$; dried leaves of <i>L. parasiticus</i> extracted with C ₂ H ₅ OH yielded $2 \times 10^{-2}\%$; dried seeds of <i>Cuscuta japonica</i> were extracted with C ₂ H ₅ OH, partitioned and chromatographed yielding 1×10^{-4}	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; [α] _D ; X-ray	(21, 23, 24, 29, 41, 43)

Coriarin (14)
C₁₅H₁₈O₆



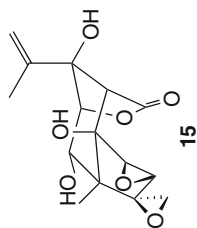
Coriariaceae:
C. japonica, Japan

Seeds isolated from ripe berries yielded $1.8 \times 10^{-4}\%$

Mp: MS; IR: ¹H-NMR; ¹³C-NMR; 2D-NMR; [α]_D; (28)

tutin (11) is converted into coriarin (14) with NaH in THF (19% yield)

Hyenanchin (15)
(Mellitoxin)
C₁₅H₁₈O₇



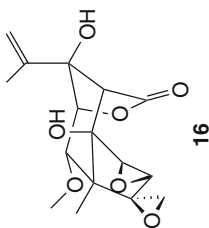
Picrodendraceae: *H. globosa* (*Toxidendrum capense*), South Africa

Toxic honey yielded from $4 \times 10^{-4}\%$ to $3.4 \times 10^{-3}\%$; powdered dried fruits of *H. globosa* yielded after extraction with CHCl₃ and chromatography $\sim 1 \times 10^{-1}\%$

(2, 3, 9, 10, 31, 32, 42, 44)

Ricaniidae: *Scolypopa australis* (Australian passion vine hopper) New Zealand. Toxic honey, New Zealand

2-Desoxy-2-methoxyhyenanchin (16)
(*O*-Methylhyenanchin)
C₁₆H₂₀O₇



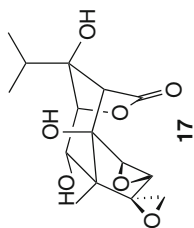
Picrodendraceae: *H. globosa* (*T. capense*), South Africa

Extraction of fruits with CHCl₃, was continued with C₂H₅OH. The alcoholic extract was dissolved in water, filtered, purified with Pb(OAc)₂ and extracted with ethyl acetate.

(3, 4, 45c, 134)

Chromatography on silica gel, crystallization

Dihydrohyenanchin (17) (Globosin, Picrodendrin C)
C₁₅H₂₀O₇



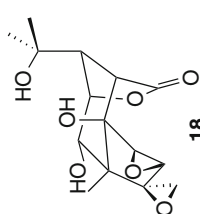
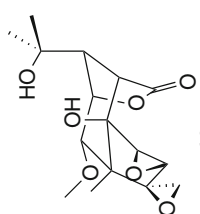
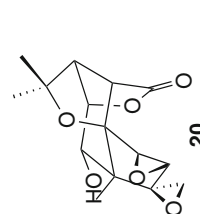
Toxic honey, New Zealand; Picrodendraceae: *H. globosa*, (*Toxidendrum capense*), South Africa Picrodendraceae: *P. baccatum*, West Indies

Toxic honey yielded $5.6 \times 10^{-5}\%$; powdered dried fruits of *H. globosa* yielded after extraction with CHCl₃ and chromatography $\sim 4 \times 10^{-1}\%$; dried bark (of *P. baccatum* collected in Indonesia) extracted with CHCl₃ yielded after repeated chromatography $1.4 \times 10^{-3}\%$

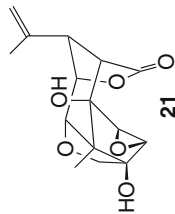
(3, 10, 42, 46)

Mp; IR; MS; ¹H-NMR; ¹³C-NMR; 2D-NMR; [α]_D

Table 2. (continued)

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Isohenanchin (18) Dihydro- isohenanchin, (8-Hydroxy- dihydro)tutin, Hydroxycoriatin, Isodihydro- hyenanchin) C ₁₅ H ₂₀ O ₇		Picrodendraceae: <i>H. globosa</i> , Lamb. (<i>T. capense</i> Thumb.) South Africa; <i>Celaenandron</i> <i>mexicanum</i> , Central America; <i>Picrodendron baccatum</i> , West Indies Coriariaceae: <i>C. nepalensis</i> , China, Nepal	Dried powdered bark of <i>C. mexicanum</i> yielded 1 × 10 ⁻² %; dried bark of <i>P. baccatum</i> (of plants collected in Indonesia) extracted with CHCl ₃ yielded 9.5 × 10 ⁻³ %; powdered dried fruits of <i>H. globosa</i> yielded after extraction with CHCl ₃ and chromatography ~1 × 10 ⁻¹ %; powdered roots of <i>C. nepalensis</i> were extracted with water, chromatographed on polyamide and on silica gel yielding 3 × 10 ⁻⁴ %	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; [α] _D ; X-ray	(2-4, 10, 24, 42, 45-48)
2-Desoxy-2- methoxy- isohenanchin (19) (Capensin, Substanz D) C ₁₅ H ₂₂ O ₇		Picrodendraceae: <i>Hyaenanche</i> <i>globosa</i> Lamb. (<i>T. capense</i> Thumb.) South Africa	Powdered dried fruits of <i>H. globosa</i> yielded after extraction with CHCl ₃ and chromatography ~5 × 10 ⁻¹ %	IR; ¹ H-NMR; ¹³ C-NMR; [α] _D	(2-4, 10, 42, 45b)
Apotutin (20) (β-Tutin) C ₁₅ H ₁₈ O ₆		Coriariaceae: <i>C. nepalensis</i> , China, Nepal; <i>C. intermedia</i> , Taiwan	CH ₃ OH extracts of dried leaves of <i>C. intermedia</i> were repeatedly chromatographed yielding 2.4 × 10 ⁻⁴ %; dried powdered roots of <i>C. nepalensis</i> were extracted with water, chromatographed on polyamide and on silica gel yielding 2 × 10 ⁻⁴ %	MS; IR; ¹ H-NMR; ¹³ C-NMR; partial synthesis	(2, 10, 24, 39)

Corianin (21)
C₁₅H₁₈O₆



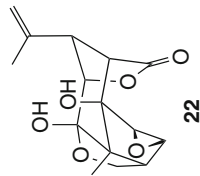
Coriariaceae: *C. japonica*, Japan;
C. sinica, *C. microphylla*
(*C. thymifolia*), South- and
Middle America, China;
C. ruscifolia, Chile
Loranthaceae: *L. parasitica*

Seeds isolated from ripe berries
yielded $6 \times 10^{-4}\%$; dried aerial
parts of *C. microphylla* extracted
with CH₃OH yielded 1.1
 $\times 10^{-1}\%$; dried fruits of *C.*
ruscifolia extracted with CH₃OH
yielded after repeated
chromatography $2 \times 10^{-3}\%$;
dried leaves of *L. parasitica*,
extracted with C₂H₅OH yielded
 $1.4 \times 10^{-3}\%$

Mp: MS; IR: ¹H-NMR;
¹³C-NMR; ORD; [α]_D;
chemical conversions;
partial and total synthesis
forms together with tutin
(11) the molecular
compound pseudotutin

(21, 23, 28, 29,
40, 49)

Asteromurin A (22)
C₁₄H₁₈O₆



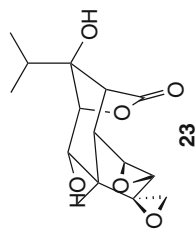
Cerocaroidae: *Asterocaroccus*
muratae scale insect

No isolation reported

Mp: [α]_D; X-ray of
its p-bromobenzoate;
synthesis

(50–52)

Picrodendrin D (23)
C₁₅H₂₀O₆



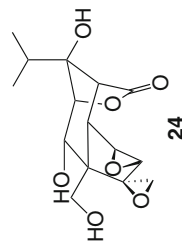
Picrodendraceae: *P. baccatum*,
West Indies

Dried bark (of plants collected in
Indonesia) extracted with
CHCl₃, yielded after repeated
chromatography $9 \times 10^{-3}\%$

Mp > 300° C; IR; MS;
¹H-NMR; ¹³C-NMR;
2D-NMR; [α]_D

(46)

Picrodendrin I (24)
C₁₅H₂₀O₇



Picrodendraceae: *P. baccatum*,
West Indies

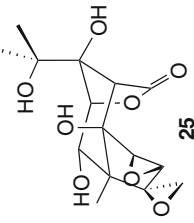
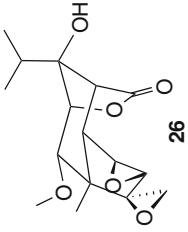
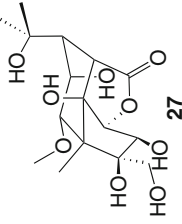
Dried bark (of plants collected in
Indonesia) extracted with
CH₃OH yielded after repeated
chromatography $4 \times 10^{-4}\%$

Mp; IR; MS; ¹H-NMR;
¹³C-NMR; 2D-NMR;
[α]_D; comparison with
picrodendrin D (23)

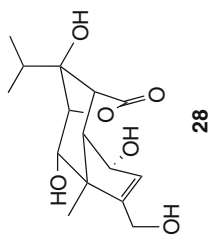
(53)

(continued)

Table 2. (continued)

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Picrodendrin J (25) C ₁₅ H ₂₀ O ₈		Picrodendraceae: <i>P. baccatum</i> , West Indies	Dried bark (of plants collected in Indonesia) extracted with CH ₃ OH yielded after repeated chromatography 4 × 10 ⁻⁴ %	Mp; IR; MS; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; [α] _D ; comparison with picrodendrin F (66)	(54)
Picrodendrin R (26) C ₁₆ H ₂₂ O ₆		Picrodendraceae: <i>P. baccatum</i> , West Indies	Dried stems (of plants collected in Indonesia) extracted with CH ₃ OH yielded after chromatography 2 × 10 ⁻⁵ %	Mp; IR; MS; ¹ H- NMR; ¹³ C-NMR; 2D-NMR; comparison with picrodendrin D (23)	(55)
Picrodendrin Z (27) C ₁₆ H ₂₆ O ₉		Picrodendraceae: <i>P. baccatum</i> , West Indies	Dried bark (of plants collected in Indonesia) extracted with CH ₃ OH yielded after repeated chromatography 5 × 10 ⁻⁴ %	Mp; IR; MS; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; [α] _D ; X-ray	(56)

Picrodendrin α (28)
C₁₅H₂₂O₆



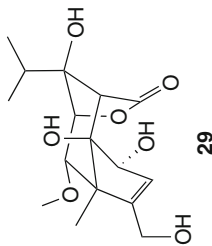
Picrodendraceae: *P. baccatum*,
West Indies

Dried leaves (of plants collected in
Indonesia) extracted with CH₂OH
yielded after repeated
chromatography $3 \times 10^{-4}\%$

Mp: IR; MS; ¹H-NMR;
¹³C-NMR; 2D-NMR;
[α]_D

(56)

Picrodendrin β (29)
C₁₆H₂₄O₇



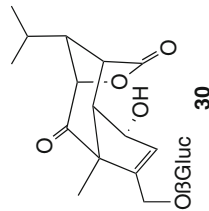
Picrodendraceae: *P. baccatum*,
West Indies

Dried leaves (of plants collected in
Indonesia) extracted with CH₂OH
yielded after repeated
chromatography $6.3 \times 10^{-4}\%$

Amorphous; IR; MS;
¹H-NMR; ¹³C-NMR;
2D-NMR; [α]_D

(56)

Picrodendrioxide A
(30)
C₂₁H₃₀O₁₀



Picrodendraceae: *Picrodendron*
baccatum, West Indies

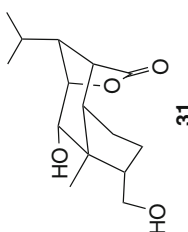
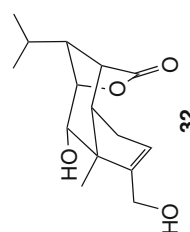
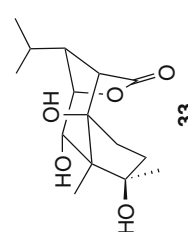
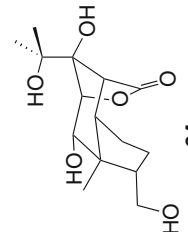
Dried leaves (of plants collected in
Indonesia) extracted with CH₂OH
yielded after repeated
chromatography $1.4 \times 10^{-4}\%$

Mp: IR; MS; ¹H-NMR;
¹³C-NMR; 2D-NMR; [α]_D;
X-ray; enzymatic hydrolysis
yielded the aglycon: mp: IR;
MS; ¹H-NMR; ¹³C-NMR

(56)

(continued)

Table 2. (continued)

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Dendrobiumane B (31) $C_{15}H_{24}O_4$		Orchidaceae: <i>Dendrobium moniliforme</i> , Yunnan (China)	Dried stems extracted with C_2H_5OH yielded after repeated chromatography $3.8 \times 10^{-4}\%$	Amorphous; MS; IR; 1H -NMR; ^{13}C -NMR; 2D-NMR; $[\alpha]_D$	(11)
Dendrobiumane C (32) $C_{15}H_{22}O_4$		Orchidaceae: <i>D. moniliforme</i> , Yunnan (China)	Dried stems extracted with C_2H_5OH yielded after repeated chromatography $1 \times 10^{-4}\%$	Amorphous; MS; IR; 1H -NMR; ^{13}C -NMR; 2D-NMR; $[\alpha]_D$	(11)
Dendrobiumane D (33) $C_{15}H_{24}O_5$		Orchidaceae: <i>D. moniliforme</i> , Yunnan (China)	Dried stems extracted with C_2H_5OH yielded after repeated chromatography $1.8 \times 10^{-4}\%$	Amorphous; MS; IR; 1H -NMR; ^{13}C -NMR; 2D-NMR; $[\alpha]_D$	(11)
Dendrodensiflorol (34) $C_{15}H_{24}O_5$		Orchidaceae: <i>Dendrobium densiflorum</i>	Fresh stems were extracted with CH_2Cl_2 and chromatographed	Amorphous; IR; MS; 1H -NMR; ^{13}C -NMR; 2D-NMR; aglycon of dendromonilide D (38)	(57)

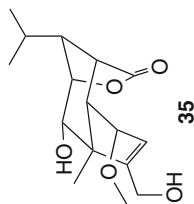
Flakinin B (35)
C₁₆H₂₄O₅

Orchidaceae: *Dendrobium nobile*,
cultivar Snowflake "Red
Star"

Crashed plants extracted with
CH₃OH; the basic fraction
yielded after repeated
chromatography 2 × 10⁻⁴%

Mp: IR; MS;
¹H-NMR; ¹³C-NMR;
2D-NMR; [α]_D

(77)



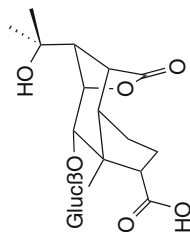
Dendromonilside
B (36)^a
C₂₁H₃₂O₁₁

Orchidaceae: *D. moniliforme*,
Yunnan (China)

Dried stems extracted with C₂H₅OH
yielded after repeated
chromatography 3 × 10⁻⁴%

Mp: IR; MS; ¹H-NMR;
¹³C-NMR; 2D-NMR;
[α]_D; X-ray^a

(58)



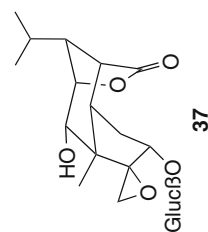
Dendromonilside C
(37)
C₂₁H₃₂O₁₀

Orchidaceae: *D. moniliforme*,
Yunnan (China)

Dried stems extracted with C₂H₅OH
yielded after repeated
chromatography 7 × 10⁻⁴%

Amorphous; IR; MS;
¹H-NMR; ¹³C-NMR;
2D-NMR; [α]_D

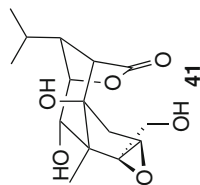
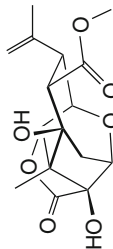
(58)



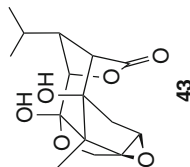
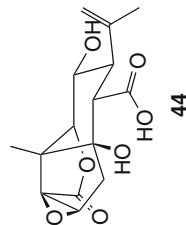
(continued)

Table 2. (continued)

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Dendromonilside D $C_{21}H_{34}O_{10}$ (38)		Orchidaceae: <i>D. moniliforme</i> , <i>D. nobile</i> , Yunnan (China)	Dried stems of <i>D. moniliforme</i> extracted with C_2H_5OH yielded after repeated chromatography $3 \times 10^{-4}\%$; dried stems of <i>D. nobile</i> extracted with C_2H_5OH yielded after repeated chromatography $1 \times 10^{-3}\%$	Amorphous; IR; MS; 1H -NMR; ^{13}C -NMR ^b ; 2D-NMR; [z]D; glucoside of dendrodisisiflorol (34)	(58, 59)
Dendroside F (39) $C_{21}H_{34}O_9$		Orchidaceae: <i>D. nobile</i> , <i>D. moniliforme</i> , China	Dried stems of <i>D. moniliforme</i> extracted with C_2H_5OH yielded after repeated chromatography $1.6 \times 10^{-4}\%$; dried stems of <i>D. nobile</i> extracted with C_2H_5OH yielded after repeated chromatography $1.6 \times 10^{-3}\%$	Amorphous; IR; MS; 1H -NMR; ^{13}C -NMR; 2D-NMR; [z]D; β -glucoside of dendrobiumane B (31)	(60)
Dendroside G (40) $C_{21}H_{32}O_{10}$		Orchidaceae: <i>D. nobile</i> , China	Dried stems of <i>D. nobile</i> extracted with C_2H_5OH yielded after repeated chromatography $6 \times 10^{-4}\%$	Amorphous; IR; MS; 1H -NMR; ^{13}C -NMR; 2D-NMR; [z]D; β -glucoside of dendronobilin B (46)	(60)

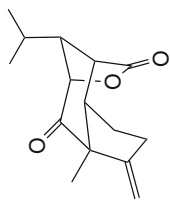
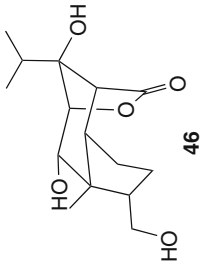
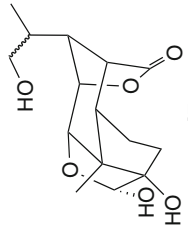
Amoenin (41)
C₁₅H₂₂O₆Orchidaceae: *Dendrobium amoenum*, IndiaDried plants extracted with CH₃OH yielded after repeated chromatography 1 × 10⁻³%; fresh plants extracted with CH₃OH yielded after repeated chromatography 2 × 10⁻³%Mp: MS; IR: ¹H-NMR; ¹³C-NMR; CD; [α]_D: conversion into α-dihydropicrotoxinin (8) by oxidation with O₂ on Pt (15, 16)Methyl picrotoxate (42)
C₁₆H₂₀O₇Menispermaceae: *Menispermum cocculus* (= *A. coccultus*), India, Sri Lanka, Southeast Asia *Spirastrella inconstans*, marine spongeDried seeds extracted with CH₃OH yielded after chromatography 3.4 × 10⁻³%; sun-dried *S. inconstans* extracted with CH₃OH yielded after chromatography 3 × 10⁻³%Mp: MS; IR: ¹H-NMR; ¹³C-NMR; 2D-NMR; [α]_D: first identified by conversion of picrotoxinin (1) with alkaline methanol synthesis; X-ray of the synthetic product (3, 7, 8, 61-63)

42

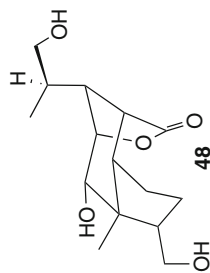
Picrotoximaesin (43)
C₁₅H₂₂O₆Phyllanthaceae: *Maesobotrya floribunda*, Cameroon, elsewhere in AfricaDried berries extracted with CH₃OH yielded after repeated chromatography 2 × 10⁻³%Mp: MS; IR: ¹H-NMR; ¹³C-NMR; 2D-NMR; [α]_D: X-ray abs. conf. (CuK_α) (64, 65)Picrotoxic acid (44)^b
C₁₅H₁₈O₇Menispermaceae: *M. coccultus* (= *A. coccultus*), India, Sri Lanka, Southeast AsiaDried seeds extracted with CH₃OH yielded after chromatography 1.4 × 10⁻³%Mp: MS; IR: ¹H-NMR; ¹³C-NMR; conversion of picrotoxinin (1) with NaOH/CH₃OH (8)

(continued)

Table 2. (continued)

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Nobilomethylene (45) $C_{15}H_{20}O_3$		Orchidaceae: <i>D. nobile</i> , China (probably artefact derived from dendrobine- <i>N</i> -oxide)	Fresh bulbs (stems) yielded after extraction, separation from non- basic substances! and repeated chromatography $3.6 \times 10^{-6}\%$	Mp; MS; IR; 1H -NMR; ORD; conversion of nobilone (90) to 45 via Cope-elimination	(66)
Dendronobilin B (46) $C_{15}H_{24}O_5$		Orchidaceae: <i>D. nobile</i> , Yunnan (China)	Dried stems extracted with 60% C_2H_5OH yielded after repeated chromatography $8 \times 10^{-5}\%$	Amorphous; MS; IR; 1H -NMR; ^{13}C -NMR; 2D-NMR; CD; $[\alpha]_D$; aglycon of dendroside G (40)	(67)
Dendronobilin C (47) $C_{15}H_{22}O_6$		Orchidaceae: <i>D. nobile</i> , Yunnan (China)	Dried stems extracted with 60% C_2H_5OH yielded after repeated chromatography $1 \times 10^{-4}\%$	Oil; MS; IR; 1H -NMR; ^{13}C -NMR; 2D-NMR; CD; $[\alpha]_D$; configuration of C-8 tentatively assigned	(67)

Dendronobilin D (48)
C₁₅H₂₄O₅

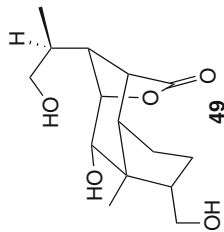


Orchidaceae: *D. nobile*, Yunnan (China)

Dried stems extracted with 60% C₂H₅OH, yielded after repeated chromatography 2 × 10⁻⁴%

Oil; MS; IR; ¹H-NMR; ¹³C-NMR; 2D-NMR; CD; [α]_D; configuration of C-8 tentatively assigned (67)

Dendronobilin E (49)
C₁₅H₂₄O₅

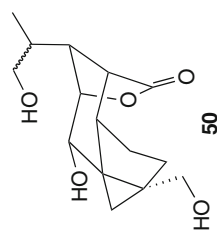


Orchidaceae: *D. nobile*, Yunnan (China)

Dried stems extracted with 60% C₂H₅OH, yielded after repeated chromatography 3 × 10⁻⁴%

Amorphous; MS; IR; ¹H-NMR; ¹³C-NMR; 2D-NMR; CD; [α]_D; configuration of C-8, tentatively assigned; epimer of dendronobilin D (48) (67)

Dendronobilin F (50)
C₁₅H₂₂O₅



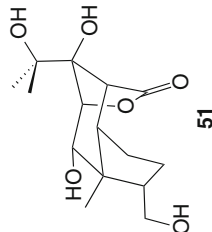
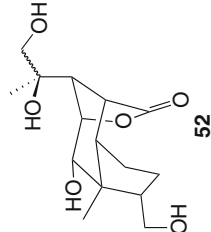
Orchidaceae: *D. nobile*, Yunnan (China)

Dried stems extracted with 60% C₂H₅OH yielded after repeated chromatography 3 × 10⁻⁵%

Oil; MS; IR; ¹H-NMR; ¹³C-NMR; 2D-NMR; CD; [α]_D; configuration of C-8 tentatively assigned (67)

(continued)

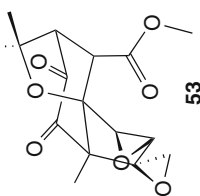
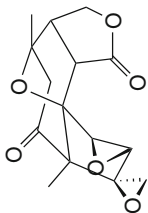
Table 2. (continued)

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Dendronobilin L (51) C ₁₅ H ₂₄ O ₆		Orchidaceae: <i>D. nobile</i> , Yunnan (China)	Dried stems extracted with 60% C ₂ H ₅ OH yielded after repeated chromatography 6 × 10 ⁻⁵ %	Oil; MS; IR; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; CD; [α] _D	(68)
Dendronobilin M (52) C ₁₅ H ₂₄ O ₆		Orchidaceae: <i>D. nobile</i> , Yunnan (China)	Dried stems extracted with 60% C ₂ H ₅ OH yielded after repeated chromatography 2.3 × 10 ⁻⁴ %	Oil; MS; IR; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; CD; [α] _D ; configuration of C-8 tentatively assigned	(68)

^aThe ORTEP drawing of the X-ray in ref (58) depicts **36** in its antipodal configuration recognizable by the L-sugar, because enzymatic hydrolysis yielded D-glucose. The authors depicted in consequence all picrotoxanes in the antipodal configuration inclusive dendrobine (**82**) of known absolute configuration (*11*, 58–60, 70, 71, 86). Therefore, in this review, the structures **8**, **34**, **36–40**, **55**, **56**, and **89** are depicted in the same absolute configuration as picrotoxinin and dendrobine.

^bThe name picrotoxic acid is identical with that of the acid obtained by hydrolysis of methylpicrotoxate (**42**) and thus should be changed.

Table 3. Structurally deviant picrotoxanes

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Coriatone (53) C ₁₆ H ₁₈ O ₇		Coriariaceae: <i>Coriaria nepalensis</i> , China	Aerial parts were extracted with C ₂ H ₅ OH yielding after repeated chromatography 2 × 10 ⁻⁴ %	Mp (yellow crystals); MS; IR; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; [α] _D	(69)
Corianlactone (54) C ₁₅ H ₁₆ O ₆		Coriariaceae: <i>C. nepalensis</i> , China	Aerial parts were extracted with C ₂ H ₅ OH yielding after repeated chromatography 2.4 × 10 ⁻⁴ %	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; [α] _D ; X-ray	(69)

(continued)

Table 3. (continued)

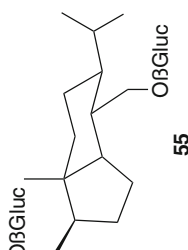
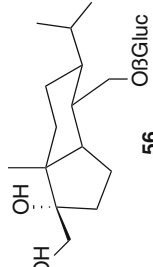
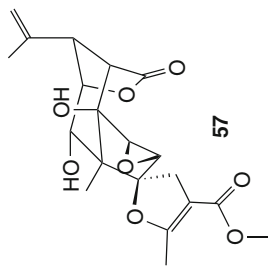
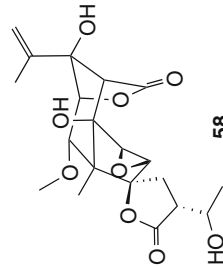
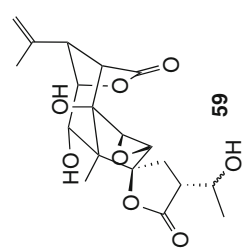
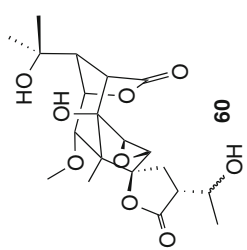
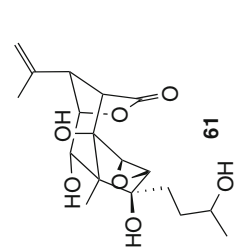
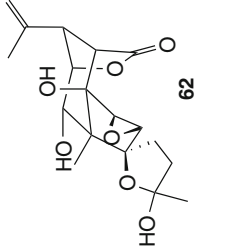
Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Dendronobitoside A (55) C ₂₇ H ₄₈ O ₁₂		Orchidaceae; <i>Dendrobium nobile</i> Lindl, China	Fresh stems extracted with C ₂ H ₅ OH yielded after repeated chromatography 6 × 10 ⁻⁴ %	Amorphous; MS; IR; ¹ H-NMR; ¹³ C-NMR; [α] _D ; enzymatic hydrolysis yielded the aglycon: amorphous; MS; IR; ¹ H-NMR; ¹³ C-NMR; 2D- NMR; [α] _D	(70, 71)
Dendronobitoside B (56) C ₂₁ H ₃₈ O ₈		Orchidaceae; <i>D. nobile</i> Lindl, China	Fresh stems extracted with C ₂ H ₅ OH yielded after repeated chromatography 2.8 × 10 ⁻³ %	Amorphous; MS; IR; ¹ H-NMR; ¹³ C-NMR; [α] _D ; enzymatic hydrolysis yielded the aglycon: amorphous; MS; IR; ¹ H-NMR; ¹³ C-NMR; 2D- NMR; [α] _D	(70, 71)

Table 4. “Norditerpene picrotoxanes” (C-18 and C-19 picrotoxanes)

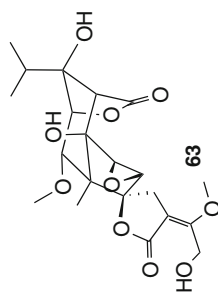
Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Capenicin (57) (Toxicodendrin) C ₂₀ H ₂₄ O ₈		Picrodendraceae: <i>Hybanche globosa</i> Lamb. (= <i>Toxicodendrum capense</i>) South Africa	Powdered dried fruits of <i>H. globosa</i> yielded after extraction with CHCl ₃ and chromatography ~4 × 10 ⁻¹ %	MS; UV; IR; ¹ H-NMR; [α] _D ; chemical degradation; comparison with tutin determined the absolute configuration too.	(2, 3, 42, 72-75)
Celaenodendrolide I (58) C ₂₀ H ₂₆ O ₉		Picrodendraceae: <i>Celaenodendron mexicana</i> Standl Jalisco (Mexico)	Stem bark extracted with CHCl ₃ , yielded after chromatography 8.6 × 10 ⁻³ %	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; [α] _D ; comparison with picrodendrin E; chemical transformation	(48)
Pretoxin (59) C ₁₉ H ₂₄ O ₈		Picrodendraceae: <i>H. globosa</i> Lamb. (= <i>T. capense</i>) South Africa	Fruits were extracted with alcohol, repeatedly chromatographed on Al ₂ O ₃ and SiO ₂	Mp; MS; IR; ¹ H-NMR; [α] _D ; chemical transformation; comparison with tutin, capenicin, and dendrotoxin	(2, 3, 76)

(continued)

Table 4. (continued)

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Dendrotoxim (60) C ₂₀ H ₂₈ O ₉		Picrodendraceae: <i>H. globosa</i> Lamb. (= <i>T. capense</i> Thumb.) South Africa	Powdered dried fruits of <i>H. globosa</i> yielded after extraction with CHCl ₃ and chromatography ~5 × 10 ⁻³ %	Mp; MS; IR; ¹ H-NMR; [α] _D	(2, 3, 42, 75)
Codendrin (61) C ₁₈ H ₂₆ O ₇		Picrodendraceae: <i>H. globosa</i> Lamb. (= <i>T. capense</i>) South Africa	Powdered dried fruits of <i>H. globosa</i> were extracted with CHCl ₃ and chromatographed (product with lowest R _F)	Mp; MS; IR; ¹ H-NMR; [α] _D	(3, 75)
Lambicin (62) C ₁₈ H ₂₄ O ₇		Picrodendraceae: <i>H. globosa</i> Lamb. (= <i>T. capense</i>) South Africa	Fruits were extracted with alcohol, repeatedly chromatographed on Al ₂ O ₃ and SiO ₂	Mp; MS; IR; ¹ H-NMR; [α] _D ; chemical transformation; correlation with pretoxin	(2, 3, 76)

Picrodendrin A (**63**)
C₂₁H₂₈O₁₀

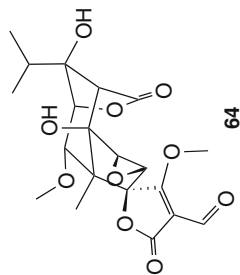


Picrodendraceae:
Picrodendron
baccatum,
West Indies

Dried bark (of plants
collected in Indonesia)
extracted with CHCl₃
yielded after
chromatography
 $9 \times 10^{-3}\%$

Mp; MS; UV; IR; ¹H-NMR; (**77**)
¹³C-NMR; 2D-NMR;
[α]_D; X-ray

Picrodendrin B (**64**)
C₂₀H₂₄O₁₀

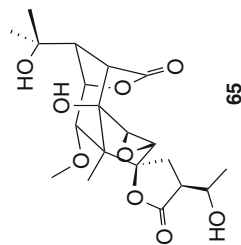


Picrodendraceae:
P. baccatum,
West Indies

Dried bark (of plants
collected in Indonesia)
extracted with CH₃OH
yielded after repeated
chromatography
 $2.5 \times 10^{-4}\%$

Mp; IR; UV; MS; ¹H-NMR; (**54**)
¹³C-NMR; NOE; 2D-
NMR; [α]_D; comparison
with picrodendrin A (**63**)

Picrodendrin E (**65**)
C₂₀H₂₈O₉



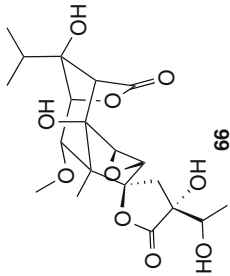
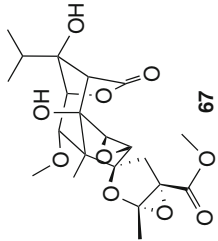
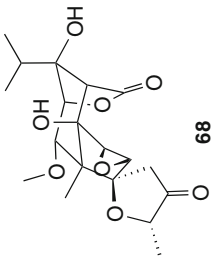
Picrodendraceae:
P. baccatum,
West Indies

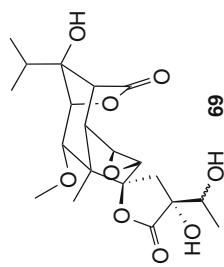
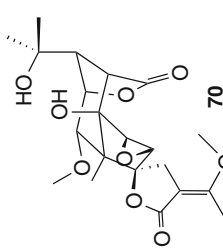
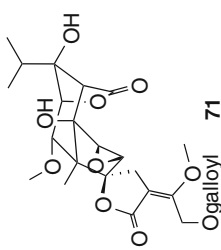
Dried bark (of plants
collected in Indonesia)
extracted with CH₃OH
yielded after repeated
chromatography
 $2 \times 10^{-3}\%$

Mp; IR; MS; ¹H-NMR; (**53**)
¹³C-NMR; 2D-NMR;
NOE; [α]_D; comparison
with picrodendrin F (**66**)

(continued)

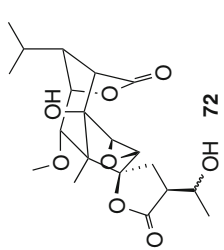
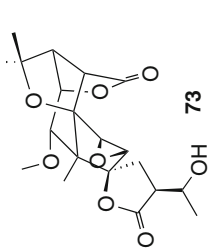
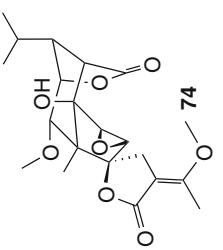
Table 4. (continued)

Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Picrodendrin F (66) C ₂₀ H ₂₈ O ₁₀		Picrodendraceae: <i>P. baccatum</i> , West Indies	Dried bark (of plants collected in Indonesia) extracted with CH ₃ OH, yielded after repeated chromatography 5 × 10 ⁻⁴ %	Mp; ¹ H-NMR; ¹³ C-NMR; COSY; IR; [α] _D ; X-ray of its 18- <i>p</i> - bromobenzoate; CD-spectrum	(53)
Picrodendrin G (67) C ₂₁ H ₂₈ O ₁₀		Picrodendraceae: <i>P. baccatum</i> , West Indies	Dried bark (of plants collected in Indonesia) extracted with CH ₃ OH yielded after repeated chromatography 2.5 × 10 ⁻³ %	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; H-H COSY; NOE; LSPD; [α] _D ; and comparison with picrodendrin A (63)	(54)
Picrodendrin K (68) C ₁₉ H ₂₆ O ₈		Picrodendraceae: <i>P. baccatum</i> , West Indies	Dried stems (of plants collected in Indonesia) extracted with CH ₃ OH yielded after repeated chromatography 7 × 10 ⁻⁵ %	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; NOE; [α] _D	(55)

<p>Picrodendrin L (69) C₂₀H₂₈O₉</p>	 <p style="text-align: right;">69</p>	<p>Picrodendraceae: <i>P. baccatum</i>, West Indies</p>	<p>Dried stems (of plants collected in Indonesia) extracted with CH₃OH yielded after repeated chromatography 3.3 × 10⁻³%</p>	<p>Mp (>300°C); MS; IR; ¹H-NMR; ¹³C-NMR; NOE; [α]_D</p> <p style="text-align: right;">(55)</p>
<p>Picrodendrin M (70) C₂₁H₂₈O₉</p>	 <p style="text-align: right;">70</p>	<p>Picrodendraceae: <i>P. baccatum</i>, West Indies</p>	<p>Dried stems (of plants collected in Indonesia) extracted with CH₃OH yielded after repeated chromatography 3.3 × 10⁻⁵%</p>	<p>Mp; MS; IR; UV; ¹H-NMR; ¹³C-NMR; NOE; 2D-NMR; [α]_D</p> <p style="text-align: right;">(55)</p>
<p>Picrodendrin N (71) C₂₈H₃₂O₁₄</p>	 <p style="text-align: right;">Ogalloyl 71</p>	<p>Picrodendraceae: <i>P. baccatum</i>, West Indies</p>	<p>Dried stems (of plants collected in Indonesia) extracted with CH₃OH yielded after repeated chromatography 7 × 10⁻³%</p>	<p>Mp; MS; MS/FAB; IR; ¹H-NMR; ¹³C-NMR; NOE; 2D-NMR; COLOC; [α]_D; methanolysis with NaOCH₃/CH₃OH led to picrodendrin A (63)</p> <p style="text-align: right;">(55)</p>

(continued)

Table 4. (continued)

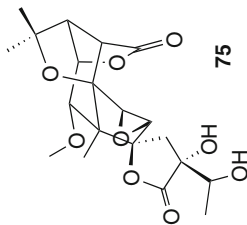
Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Picrodendrin O (72) C ₂₀ H ₂₈ O ₈		Picrodendraceae; <i>P. baccatum</i> , West Indies	Dried stems (of plants collected in Indonesia) extracted with CH ₃ OH yielded after repeated chromatography 4 × 10 ⁻⁵ %	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; NOE; 2D-NMR; [α] _D	(55)
Picrodendrin P (73) C ₂₀ H ₂₆ O ₈		Picrodendraceae; <i>P. baccatum</i> , West Indies	Dried stems (of plants collected in Indonesia) extracted with CH ₃ OH yielded after repeated chromatography 1.5 × 10 ⁻⁴ %	Mp; MS/FAB; IR; ¹ H- NMR; ¹³ C-NMR; COSY; NOESY; HMQC; COLOC; [α] _D	(36, 55)
Picrodendrin Q (74) C ₂₁ H ₂₈ O ₈		Picrodendraceae; <i>P. baccatum</i> , West Indies	Dried stems (of plants collected in Indonesia) extracted with CH ₃ OH yielded after repeated chromatography 4 × 10 ⁻⁵ %	Mp; MS; IR; ¹ H-NMR; ¹³ C-NMR; NOE; 2D-NMR; [α] _D	(55)

Picrodendrin S (75)
C₂₀H₂₈O₉

Picrodendraceae:
P. baccatum,
West Indies

Dried bark (of plants collected in Indonesia) extraction with CH₃OH yielded after repeated chromatography 4 × 10⁻³%

Mp (>300°C); IR; MS/FAB; ¹H-NMR; ¹³C-NMR; 2D-NMR; [α]_D; sequential COSMIC-force field calculations (Nemesis program) (36)

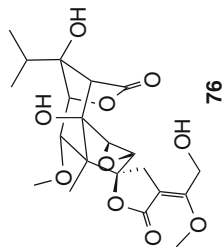


Picrodendrin T (76)
C₂₁H₂₈O₁₀

Picrodendraceae:
P. baccatum,
West Indies

Dried bark (of plants collected in Indonesia) extraction with CH₃OH yielded after repeated chromatography 4.4 × 10⁻⁴%

Mp; IR; MS/FAB; UV; ¹H-NMR; ¹³C-NMR; COSY; NOESY; [α]_D (36)

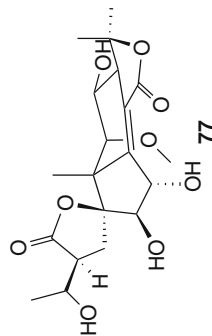


Picrodendrin U (77)
C₂₀H₂₈O₉

Picrodendraceae:
P. baccatum,
West Indies

Dried bark (of plants collected in Indonesia) extraction with CH₃OH yielded after repeated chromatography 1.5 × 10⁻⁴%

Mp (dec); MS/FAB; IR; UV; ¹H-NMR; ¹³C-NMR; 2D-NMR; X-ray; absolute configuration: by nonempirical exciton CD of the *p*-bromobenzoate; [α]_D (78)



(continued)

Table 4. (continued)

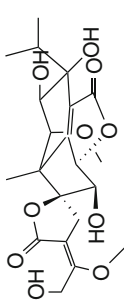
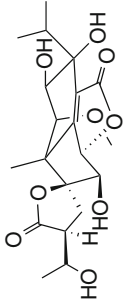
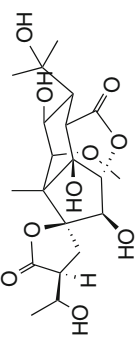
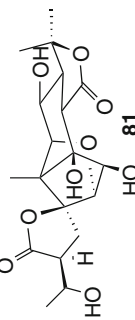
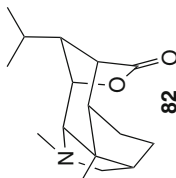
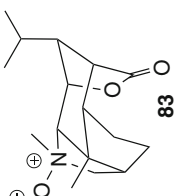
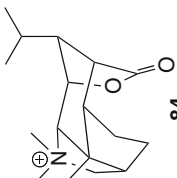
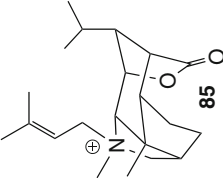
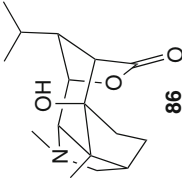
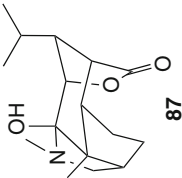
Trivial Name Formula	Structure	Natural Source	Isolation	Structure Determination	References
Picrodendrin V (78) C ₂₁ H ₂₈ O ₁₀		Picrodendraceae; <i>P. baccatum</i> , West Indies	Dried bark (of plants collected in Indonesia) extraction with CH ₃ OH yielded after repeated chromatography 2.5 × 10 ⁻⁵ %	Amorphous; MS/FAB; IR; UV; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; [α] _D	(78)
Picrodendrin W (79) C ₂₀ H ₂₈ O ₉		Picrodendraceae; <i>P. baccatum</i> , West Indies	Dried bark (of plants collected in Indonesia) extraction with CH ₃ OH yielded after repeated chromatography 6.3 × 10 ⁻³ %	Mp; MS; IR; UV; ¹ H-NMR; (78) ¹³ C-NMR; 2D-NMR; absolute configuration: by nonempirical exciton CD of the <i>p</i> -bromobenzoate; [α] _D	(78)
Picrodendrin X (80) C ₂₀ H ₃₀ O ₁₀		Picrodendraceae; <i>P. baccatum</i> , West Indies	Dried bark (of plants collected in Indonesia) extracted with CH ₃ OH yielded after repeated chromatography 6.3 × 10 ⁻³ %	Amorphous; MS/FAB; IR; ¹ H-NMR; ¹³ C-NMR; 2D-NMR; [α] _D ; comparison with picrodendrin W (79)	(56)
Picrodendrin Y (81) C ₁₉ H ₂₆ O ₉		Picrodendraceae; <i>P. baccatum</i> , West Indies	Dried bark (of plants collected in Indonesia) extracted with CH ₃ OH yielded after repeated chromatography 2 × 10 ⁻³ %	Mp; MS/FAB; IR; ¹ H-NMR; (56) ¹³ C-NMR; 2D-NMR; [α] _D ; X-ray	(56)

Table 5. Dendrobines

Trivial Name	Formula	Structure	Natural Source	Isolation	Structure Determination	References
Dendrobine (82)	$C_{16}H_{25}O_2N$		Orchidaceae: <i>Dendrobium noble</i> Lindl and cultivars; <i>D. linawianum</i> ; <i>D. findlayanum</i> ; <i>D. hildebrandii</i> ; <i>D. friedricksianum</i> ; <i>D. wardianum</i> , China	Fresh plants of a <i>D. noble</i> cultivar were extracted with CH_3OH ; separation from lipophilic, non, basic substances and repeated chromatography yielded $2 \times 10^{-2}\%$; <i>D. noble</i> was extracted with C_2H_5OH , after evaporation partitioned; the <i>n</i> -BuOH-phase was repeatedly chromatographed and yielded after crystallization $4 \times 10^{-3}\%$	Mp; MS; IR; 1H -NMR; ^{13}C -NMR; [α] _D ; chemical degradation; synthesis	(2, 3, 17, 70, 79)
Dendrobine- <i>N</i> -oxide (83)	$C_{16}H_{25}O_3N$		Orchidaceae: <i>D. noble</i> Lindl, China	Fresh plants were extracted with CH_3OH ; separation from lipophilic compounds by organic solvents, ion exchange, and chromatography on Al_2O_3 yielded $1.7 \times 10^{-3}\%$	Mp; MS; IR; 1H -NMR; ^{13}C -NMR; [α] _D ; identified by dendrobine (82) with peracid	(79, 81)
<i>N</i> -Methyl-dendrobinium salt (84)	$C_{17}H_{28}O_2N^+X^-$		Orchidaceae: <i>D. noble</i> Lindl, China	Fresh plants were extracted with CH_3OH ; separation from lipophilic compounds by organic solvents, ion exchange chromatography, and countercurrent distribution yielded $3 \times 10^{-3}\%$	Mp (X = I); MS; IR; 1H -NMR; ^{13}C -NMR; [α] _D ; identified by methylation of dendrobine (82)	(2, 3, 79-81)

(continued)

Table 5. (continued)

Trivial Name	Formula	Structure	Natural Source	Isolation	Structure Determination	References
<i>N</i> -Isopentenyl-dendrobium salt (85)	$C_{21}H_{34}O_2N^+X^-$		Orchidaceae: <i>D. nobile</i> Lindl, China	Fresh plants were extracted with CH_3OH ; separation from lipophilic compounds by organic solvents, ion exchange chromatography, and countercurrent distribution yielded $5 \times 10^{-3}\%$	Mp ($X = Br$); MS; IR; 1H -NMR; ^{13}C -NMR; $[\alpha]_D$; partial synthesis starting with dendrobine (82)	(79–81)
Dendramine (86) (6-Hydroxy-dendrobine)	$C_{16}H_{25}O_3N$		Orchidaceae: <i>D. nobile</i> Lindl; <i>D. hildebrandii</i> ; <i>D. friedricksianum</i> , China	Fresh plants of a cultivar of <i>D. nobile</i> were extracted with CH_3OH ; separation from lipophilic non basic substances and repeated chromatography yielded $1 \times 10^{-3}\%$	Mp; MS; IR; 1H -NMR; ^{13}C -NMR; $[\alpha]_D$	(2, 3, 79, 82)
2-Hydroxy-dendrobine (87)	$C_{16}H_{25}O_3N$		Orchidaceae: <i>D. fındlayanum</i> , China	Plants were extracted with CH_3OH ; crystallization removed the main alkaloid (dendrobine), chromatography of the mother liquor yielded $7 \times 10^{-3}\%$	Mp; MS; IR; 1H -NMR; ^{13}C -NMR; $[\alpha]_D$; synthesis	(79, 83, 84)

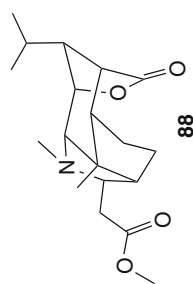
Dendrine (**88**)
C₁₉H₂₉O₄N

Orchidaceae: *D. nobile*
Lindl, China

Finely cut plant material was
extracted with CH₃OH and
chromatographed on Al₂O₃

Mp; MS; IR;
¹H-NMR; [α]_D;
partial synthesis
starting with
dendrobine (**82**)

(2, 3, 79, 85)



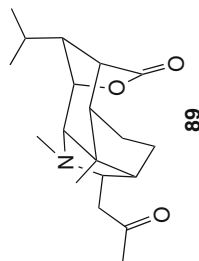
Dendronobiline A
(**89**) C₁₉H₂₉O₃N

Orchidaceae *D. nobile*
Lindl, China

Stems of *D. nobile*;
isolation not reported

Mp; HRMS;
¹H-NMR;
¹³C-NMR;
2D-NMR; [α]_D

(43, 86)



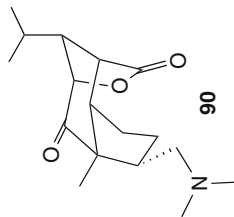
Nobilonine (**90**)
(Nobiline)
C₁₇H₂₇O₃N

Orchidaceae: *D. nobile*
Lindl; *D. hillebrandii*;
D. friedricksianum

Fresh plants of a *D. nobile*
cultivar were extracted with
CH₃OH; separation from
lipophilic non basic
substances and repeated
chromatography yielded 6
× 10⁻³%; fresh plants (*D.*
hillebrandii) extracted with
CH₃OH yielded after
repeated chromatography
1.4 × 10⁻³%

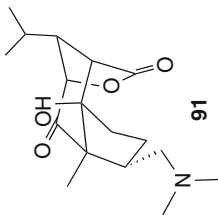
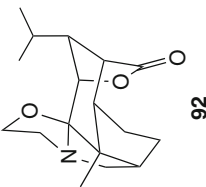
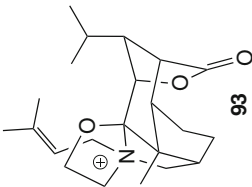
Mp; MS; IR; ¹H-
NMR; [α]_D;
partial synthesis
starting with
dendrobine (**82**);
synthesis

(2, 3, 17, 79,
84)

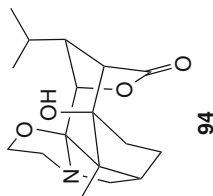


(continued)

Table 5. (continued)

Trivial Name	Formula	Structure	Natural Source	Isolation	Structure Determination	References
6-Hydroxynobiline (91) (6-Hydroxynobilomine)	$C_{17}H_{27}O_4N$		Orchidaceae: <i>D. nobile</i> Lindl; <i>D. hildebrandii</i> ; <i>D. friedricksianum</i> ; <i>D. moniliforme</i>	Fresh plants (<i>D. hildebrandii</i>) were extracted with CH_3OH yielded after chromatography on Al_2O_3 $1.4 \times 10^{-5}\%$; whole plants (<i>D. moniliforme</i>) were percolated with C_2H_5OH , separation from lipophilic non-basic substances and purification by repeated chromatography yielded $4 \times 10^{-5}\%$	MS; IR; 1H -NMR; [α]D	(79, 87, 88)
Dendroxime (92)	$C_{17}H_{25}O_3N$		Orchidaceae: <i>D. nobile</i> Lindl	Fresh bulbs were extracted with CH_3OH and yielded after separation $8.8 \times 10^{-5}\%$	Mp; MS; IR; 1H -NMR; [α]D	(2, 3, 66, 79)
N-Isopentenyl-dendroxinium salt (93)	$C_{22}H_{34}O_3N^+X^-$		Orchidaceae: <i>D. nobile</i> Lindl; <i>D. hildebrandii</i> ; <i>D. friedricksianum</i>	Fresh plants were extracted with CH_3OH ; separation from lipophilic compounds by organic solvents; ion-exchange chromatography, and countercurrent distribution yielded $1.7 \times 10^{-3}\%$	Mp (X = Cl); MS; IR; 1H -NMR; ^{13}C -NMR; [α]D	(79–81)

6-Hydroxy-dendroxine (94)
C₁₇H₂₅O₄N



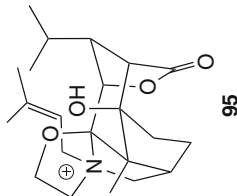
Orchidaceae: *D. nobile*
Lindl

Fresh plants of *D. nobile* cultivar were extracted with CH₃OH; separation from lipophilic non-basic substances and repeated chromatography yielded 2 × 10⁻⁴%

Mp; MS; IR;
¹H-NMR; [α]_D

(2, 3, 17, 66, 79)

N-Isopentenyl-6-hydroxydendroxinium salt (95)
C₂₂H₃₄O₄N⁺ X⁻



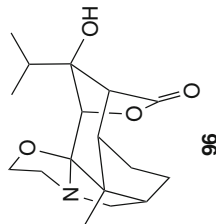
Orchidaceae:
D. nobile Lindl;
D. friedricksianum;
D. hildebrandii;

Fresh plants (*D. nobile*) were extracted with CH₃OH; separation from lipophilic compounds by organic solvents; ion-exchange chromatography and countercurrent distribution yielded 1.7 × 10⁻³%

Mp (X = Cl); MS;
IR; ¹H-NMR;
¹³C-NMR; [α]_D

(79-81)

4-Hydroxy-dendroxine (96)
C₁₇H₂₅O₄N



Orchidaceae: *D. nobile*
Lindl

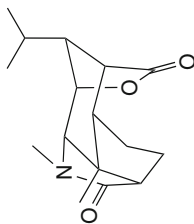
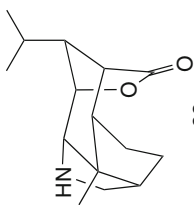
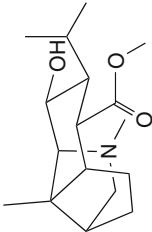
Fresh bulbs (stems) yielded after extraction, separation from non-basic substances and repeated chromatography 1.8 × 10⁻⁵%

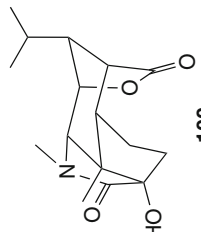
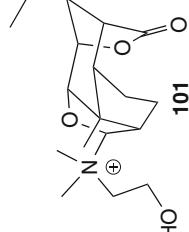
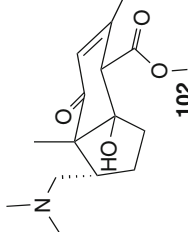
Mp; MS; IR;
¹H-NMR

(66)

(continued)

Table 5. (continued)

Trivial Name	Formula	Structure	Natural Source	Isolation	Structure Determination	References
Mubironine A (97) (14-Oxo-dendrobine) $C_{16}H_{23}O_3N$		Orchidaceae: <i>D. Snowflake</i> "Red Star" (a cultivar of <i>D. nobile</i>)	Fresh plants of a cultivar of <i>D. nobile</i> were extracted with CH_3OH ; separation from lipophilic non-basic substances and repeated chromatography yielded 1 $\times 10^{-4}\%$	Amorphous; HRESIMS; IR; 1H -NMR; ^{13}C -NMR; $[\alpha]_D$; oxidation of Dendrobine (82) with $KMnO_4$ led to 97; synthesis	(17, 84)	
Mubironine B (98) (Nordendrobine, <i>N</i> -demethyl-dendrobine) $C_{15}H_{23}O_2N$		Orchidaceae: <i>D. Snowflake</i> "Red Star" (a cultivar of <i>D. nobile</i>)	Fresh plants of a cultivar of <i>D. nobile</i> were extracted with CH_3OH ; separation from lipophilic non-basic substances and repeated chromatography yielded 7 $\times 10^{-4}\%$	Amorphous; HRFABMS; IR; 1H -NMR; ^{13}C -NMR; $[\alpha]_D$; also degradation product of dendrobine (82)	(17)	
Mubironine C (99) $C_{17}H_{29}O_3N$		Orchidaceae: <i>D. Snowflake</i> "Red Star" (a cultivar of <i>D. nobile</i>)	Fresh plants of a cultivar of <i>D. nobile</i> were extracted with CH_3OH ; separation from lipophilic non-basic substances and repeated chromatography yielded 2 $\times 10^{-2}\%$	Amorphous; HRESIMS; IR; 1H -NMR; ^{13}C -NMR; $[\alpha]_D$; hydrolysis and methylation of dendrobine led to an identical product	(17)	

<p>13-Hydroxy-14-oxodendrobine (100) (3-Hydroxy-2-oxodendrobine) C₁₆H₂₃O₄N</p>	 <p style="text-align: center;">100</p>	<p>Orchidaceae: <i>D. nobile</i> Lindl</p>	<p>Fresh stems prior to effluorescence were extracted with EtOH yielded 9 × 10⁻⁵% (and 1.1 × 10⁻³% dendrobine)</p> <p>Mp; MS; IR; UV; ¹H-NMR; ¹³C-NMR; 2D-NMR; [α]_D (89)</p>
<p>Dendrowardine (101) C₁₆H₂₅O₂N⁺Cl⁻</p>	 <p style="text-align: center;">101</p>	<p>Orchidaceae: <i>D. wardianum</i></p>	<p>Fresh plants extracted with CH₃OH were purified by ion exchange chromatography, and crystallisation as Reineckate and again ion exchange chromatography yielded 6 × 10⁻³% as chloride</p> <p>Mp; MS; IR; ¹H-NMR; CD; [α]_D; chemical degradation (90)</p>
<p>Moniline (102) C₁₇H₃₁O₃N</p>	 <p style="text-align: center;">102</p>	<p>Orchidaceae: <i>D. montiforme</i></p>	<p>Whole plants were percolated with C₂H₅OH, separation from lipophilic non basic substances and purification by repeated chromatography yielded 2.3 × 10⁻³%</p> <p>Oil; MS; IR; UV; ¹H-NMR; ¹³C-NMR; 2D-NMR; [α]_D (88)</p>

3. Occurrence

3.1. Systematic and Geographic Occurrence of Picrotoxane-Containing Plants

When *Porter* published his comprehensive review in 1967, fifteen picrotoxanes were known (2). Even then, the strangely scattered occurrence of these sesquiterpene lactones within the families of the angiosperms was noticed. Since this time, due to modern cladistics, especially through DNA sequencing, families and orders of the angiosperms have been regrouped (91–93) emphasizing the evolutionary distance of these plant families containing picrotoxanes. In this review, I shall follow the classification as demonstrated in AGP II (92). Thus, in several cases, the family and even the order can differ from that given in the original papers, or in *Porter's* and *Coscia's* accounts (2, 3) as well as in *Hegnauer's* “Chemotaxonomy of Plants” (94). Since *Porter's* account (2) nearly 90 new picrotoxanes have been reported, with most of these isolated from plants within families, in which picrotoxanes were detected already.

3.1.1. Menispermaceae

The first picrotoxanes discovered were isolated from the dried seeds of *Menispermum cocculus* (*Anamirta cocculus*) (1). Their characteristic features are two γ -lactones and an oxirane. *M. cocculus* belongs to the Menispermaceae (“moon seed” family), which is in the order Ranunculales, and thus part of the primitive eudicots. So far, *M. cocculus* is the only species of the primitive eudicots recorded to contain picrotoxanes, and this species can be found in India, Sri Lanka, and southeast Asia.

3.1.2. Coriariaceae

Within the core eudicots two orders belonging to the rosids are known to accumulate picrotoxanes. The Coriariaceae are a family with only one genus, allocated to the order Cucurbitales (subclass eurosids II). The characteristic features of their picrotoxanes are the monolactone picrotoxanes containing one or two oxiranes. According to *Yokoyama et al.*, *Coriaria* sp. have one of the most disjunct distributions within the families of angiosperm (95). Despite this fact, the picrotoxanes isolated from species found in the Mediterranean countries, South and Middle America, South Africa, New Zealand, Nepal, China, Tibet, Taiwan, and Japan are tutin (11) and/or coriamyrtin (9), as the main toxic sesquiterpenes, accompanied by a few very similar structures. Of all the species investigated so far: *Coriaria myrtifolia* (southern Europe, North Africa), *C. ruscifolia* (South Africa, Chile), *C. sinica*, *C. nepalensis* (China), *C. microphylla* (= *C. thymifolia*) (high altitudes of Central America and northern South America), *C. intermedia* (Taiwan), *C. japonica* (Japan), *C. arborea*, *C. sarmentosa*,

C. angustissima, *C. pteroides*, *C. plumosa* (New Zealand), and *C. terminalis* (Tibet), only *C. nepalensis* contains structurally deviant picrotoxanes, coriatone (**53**) and corianlactone (**54**) (69). Evidence of diurnal or seasonal fluctuation of picrotoxanes in plants has never been sought intentionally, but Jommi *et al.* (96) mentioned variable concentration levels of coriamyrtin and tutin in *C. japonica*. Thus the main picrotoxane, coriamyrtin, disappears in the autumn, when tutin (2-hydroxycoriamyrtin) becomes the main picrotoxane.

3.1.3. Picrodendraceae and Phyllanthaceae (Formerly Euphorbiaceae)

Within the subclass eurousids I, two closely related families of the order Malpighiales have been found to contain picrotoxanes. In turn, within the Picrodendraceae, (Euphorbiaceae s.l.), three genera (*Hyenanche*, *Picrodendron* and *Celaenodendron*) are known to contain monolactone picrotoxanes and the structurally related “norditerpene” picrotoxanes. Their most characteristic picrotoxanes are the “norditerpene” picrotoxanes. So far this structural variant of the picrotoxanes is restricted to this family. In the sister clade, the Phyllanthaceae (formerly Euphorbiaceae) one species of the genus *Maesobotrya* is known to produce a sesquiterpene picrotoxane. Only one species each in South Africa (Cape region), West Africa (south and east Cameroon to the Congo basin, and southern Nigeria), Mexico (Pacific Ocean coast), and the West Indies has been found to contain picrotoxanes. Different parts of these plants have been examined. Whereas dried fruits were extracted from *Hyenanche* and *Maesobotrya*, in *Picrodendron* and *Celaenodendron* mainly the stem bark was examined.

3.1.4. Orchidaceae

The structurally most diverse picrotoxanes occur within one large genus, *Dendrobium* (1,100 species), of the Orchidaceae (order: Asparagales) belonging to the monocotyledons. The most characteristic structures are the sesquiterpene alkaloids, the dendrobines. Next to these pseudoalkaloids, *Dendrobium* species biosynthesize monolactone as well as dilactone picrotoxanes, and additionally picrotoxanes, in which the lactone functionalities are reduced formally to alcohols and in some cases glucosylated. Remarkably no picrotoxanes with an extended carbon skeleton (norditerpenes) have been isolated so far from this genus, although two examples of sesquiterpene alkaloids with an extended carbon skeleton were found. *Dendrobium* species grow in Asia, Australia, and Oceania (with over 500 species in New Guinea). They are very popular ornamental plants and thus many cultivars are grown industrially mainly in India, Japan, and especially in Hawaii. According to Wang *et al.*, *Dendrobium nobile* cultivated on trees may contain one to three unknown alkaloids not found in wild *D. nobile*. The overall content of alkaloids is lower than in wild *D. nobile* (3.2% in stems, 0.8% in leaves and 0.08% in roots) (97). So far, the following *Dendrobium* species have been examined and

found to contain picrotoxanes: *D. aduncum*, *D. amoenum*, *D. densiflorum*, *D. nobile* and cultivars, *D. linawianum*, *D. findlayanum*, *D. hildebrandii*, *D. friedricksianum*, *D. moniliforme*, and *D. wardianum*.

3.2. Parasitic Plants

3.2.1. Convolvulaceae

Guo et al. reported the isolation of picrotoxanes from *Cuscuta japonica* (Japanese dodder), a parasitic vine of many shrubs, growing in China, Korea, and Japan (41). This was the first report of a picrotoxane detected within the Asterid subclass of the core eudicots. The picrotoxanes of this member of the Convolvulaceae are also found as constituents of *Coriaria* species. As this plant is a parasite, it is possible that the picrotoxanes present were received from the sap of its host.

3.2.2. Loranthaceae

That such a transfer as hypothesized in section 3.2.1 above does occur within *Coriaria* species and their parasites, plants, or animals, has been proven. Loranthaceae, relatives of the mistletoe (order: Santalales; core eudicots) grow on the twigs of *Coriaria* species. With the help of their haustorias they participate in the sap of their host. The picrotoxanes were extracted from the leaves of this parasitic plant.

3.3. Picrotoxanes Found in Animals and Animal Products

The Australian passion vine hopper, *Scolypopa australis*, a sap-sucking insect (homoptera) indigenous to eastern Australia, was accidentally introduced to New Zealand around 1870. Being a generalist, it was able to adapt to New Zealand's native plants, and it has developed into a veritable pest, especially in kiwi fruit orchards. In years of limited nectar sources (mostly in conditions of drought), it can be found on New Zealand's most toxic plant *Coriaria arborea*. Honeydew excreted by this plant hopper when feeding on *C. arborea* was examined and found to contain picrotoxanes (32, 98). In addition to the main toxic constituent of *C. arborea*, tutin (11), its oxidation product hyenanchin (15) was detected. Honeybees collect the honeydew these insects excrete only under unfavorably dry conditions storing it as toxic honey (10, 99, 100). Nowadays, New Zealand honey from hazardous areas is strictly controlled. This very early-discovered example of the transfer of toxins via the food chain demonstrates the rapid adaptability of insects and the possible danger of insect immigrants.

Unfortunately, the next isolation of a picrotoxane from a sap-sucking insect, *Asterococcus muratae* (Homoptera), has only been presented at two scientific

conferences. Thus, knowledge about the plant-host of this scale insect is as yet preliminary in nature (50, 51).

The strangest report so far is the isolation of the three main picrotoxanes of *M. cocculus* in the haplosclerid marine sponge *Spirastrella inconstans* (= *Cliona inconstans*) (7). Aware of the fact that *M. cocculus* was formerly used to stun fish, a practice nowadays forbidden, the authors tried to ascertain that the sponge could not have harvested those toxins from the surrounding sea water. Therefore, the seawater was tested too, but no trace of picrotoxinin was detected in the water samples (7). There still remains some doubt as to how the sponge could biosynthesize identical picrotoxanes to those of *M. cocculus*. Also, nothing is known about how long the sponge is able to store such toxins unharmed.¹

3.4. *The Riddle of the Scattered Taxonomic Occurrence of Picrotoxanes*

How can this scattered taxonomic occurrence of the picrotoxanes be explained? Many of the apparent chemical convergences in animal toxins have been explained as toxins received via the food chain (e.g. brevetoxin, pederin, saxitoxin, tetrodotoxin, and the toxins of the arrow-poison frogs). This may explain the occurrence of picrotoxanes in both parasitic animals and plants, but further research will be necessary for a better understanding of this phenomenon.

Since the biosyntheses of secondary metabolites in plants needs a range of enzymes (usually 10–20), thus the corresponding genes including those for regulation, makes the occurrence of identical or very similar highly complex structures in evolutionary distant clades by independent biosynthesis pathways (chemical convergence) rather unlikely. However, scattered secondary metabolite distribution in distant clades is not unknown (e.g. camptothecin (101) and the cardiac glycosides (102)). Explanations other than chemical convergence are slowly emerging, such as transfer of secondary metabolism by ecto- or endophytic microorganisms (103, 104), general (dormant) biosynthetic pathways (102, 105), and horizontal gene transfer by intracellular bacteria and viruses. So far no evidence for any of these possibilities has emerged for the picrotoxanes.

4. Isolation of Picrotoxanes

The isolation procedures for the picrotoxanes have been mostly quite conventional. Plant material has been extracted with alcohols, and in few cases with chloroform. On concentration, water was added in many cases with subsequent

¹For other unexplained occurrences of secondary metabolites in marine sponges and phylogenetically very distant species see references (282, 283).

partitioning with hexane, a halogenated solvent, and butanol. Successful chromatographic separation on silica gel or alumina has not been easily achieved in most cases, with rechromatography necessary for final compound purification.

A few of the more recent isolation protocols are given as examples in the paragraphs below.

4.1. Examples of Recent Isolation Procedures

4.1.1. Isolation from *M. cocculus* (*A. cocculus*)

Picrotoxin, a crystalline compound mixture consisting of picrotoxinin (**1**) and picrotin (**2**) in equal amounts, is commercially available. Separation of these two substances is possible by chromatography, crystallization in organic solvents, and obviously most easily by bromination, chromatography, and debromination. The last-recorded isolation from *A. cocculus* (**8**) started with air-dried seeds that were extracted with methanol at room temperature. The filtered solution was evaporated below 60°C. Separation was carried out by chromatography on silica gel with hexane/ethyl acetate mixtures of increasing polarity. The fractions cut were rechromatographed yielding 0.017% picrotoxinin (**1**), 0.0034% methyl picrotoxate (**42**), 0.073% picrotin (**2**), and two new picrotoxanes, 0.0022% dihydroxypicrotoxinin (**3**), and 0.0014% picrotoxic acid (**44**).

4.1.2. Isolation from the Sponge *S. inconstans* (*C. inconstans*)

The shade-dried sponges were extracted first with *n*-hexane and then with ethyl acetate. The latter extract was evaporated to dryness and chromatographed on silica gel with benzene/ethyl acetate mixtures yielding 0.025% picrotoxinin (**1**), 0.003% methyl picrotoxate (**42**), and 0.017% picrotin (**2**) (**7**).

4.1.3. Isolation from *Coriaria* Species

As mentioned in the last section, *Coriaria* species accumulate few but similar picrotoxanes. When the separation of ethanol extracts of the fruits of *Coriaria* species has been afforded by crystallization, a third compound, “pseudotutin,” in addition to tutin (**11**) and coriamyrtin (**9**) was isolated by crystallization from water. *Okuda et al.* (**21, 40**) revealed that pseudotutin is a molecular compound comparable to picrotoxin. These authors extracted *Coriaria japonica* and recrystallized pseudotutin from chloroform. Interestingly, in this organic solvent with marginal hydrogen-binding propensities this highly oxygenated molecular compound dissociates and tutin (**11**) crystallizes. Repeated recrystallization from the mother liquor yielded corianin (**21**). The most recent investigation of *C. japonica* was

reported by *Kinoshita et al.* (28). These researchers proved that the flesh (sarcocarp) of the berries (pseudocarps) of *C. japonica* is free of the toxic picrotoxanes and only the achenes (seeds) contain the toxins. These authors suggested that this may be true for other *Coriaria* species also, because human usage of their sarcocarp is recorded. The dried and crushed achenes (seeds) were extracted with acetone at room temperature. After evaporation of the solvent, the residue was suspended in methanol and percolated with benzene. The aqueous methanol solution was evaporated and chromatographed on silica gel with *n*-hexane, *n*-hexane/acetone mixtures, and methanol as eluents. The *n*-hexane/acetone (2:1) fraction was recrystallized from *n*-hexane/dimethyl ether and then chromatographed on octadecyl silica gel (ODS) with water/acetonitrile mixtures. This afforded after crystallization and rechromatography of the mother liquors on ODS 0.0084% tutin (11), 0.001% corianin (21), and 0.000045% dihydrotutin (12). Another fraction from this separation yielded the then unknown coriarin (14) in 0.00015% yield.

4.1.4. Isolation from the Parasitic Plant *Loranthus parasiticus*

The dried leaves (32 kg) of *L. parasiticus* parasiting on *C. japonica* were extracted with ethanol, concentrated, filtered, treated with chloroform, and chromatographed on polyamide. The crystalline eluate was recrystallized yielding 0.02% coriatin (13), 0.035% tutin (11), 0.0085% coriamyrtin (9), and 0.0014% corianin (21) (21). Note the high amount of toxins of the host plant in the parasite! A comparison of the main picrotoxanes of *L. parasiticus* and its host plant *Coriaria sinica* by TLC-densitometry revealed that the parasite contained 0.012% coriamyrtin (9), ~0.075% tutin (11), and ~0.05% coriatin (13), and the seeds of the host plant no coriamyrtin, 0.063% tutin (11), and 0.023% coriatin (13) (106). It is known that leaves, stems, and roots of *Coriaria* spp. contain picrotoxanes in even higher concentration than found for achenes. Thus, it would be desirable to compare the picrotoxane content of the stems and leaves with that of the picrotoxanes of the parasitic plant harvested at the same time.

4.1.5. Isolation from Toxic Honey and Quantitative Analysis of its Main Picrotoxanes

Via a plant hopper and honeybees, partly metabolized picrotoxanes from *Coriaria* spp. growing in New Zealand are found in toxic honey. The rather difficult isolation of picrotoxanes from honey has been described several times (2, 3, 10, 99, 100) and even now the procedure seems not entirely satisfactory (100). Originally, the isolation of picrotoxanes was achieved by extraction of honey with acetone. Later, ethyl acetate was the preferred solvent. In the late 1960s, the two main picrotoxanes were identified as tutin (11) and hyenanchin (15). Originally, the detection was governed by the physical properties of crystals obtained, color reactions, and bromoether formation. *Palmer-Jones* then developed a method

using guinea pigs as assay animals, because most other laboratory animals were not sensitive enough. Later intercerebral injections into mice were used to detect picrotoxanes in honey. Eventually, a TLC method was introduced that allowed qualitative detection of the two main picrotoxanes. By-products with very similar R_f values prevented the utilization of this method to quantify the amount of tutin (**11**) and hyenanchin (**15**). The by-products were identified, although not totally purified, by *Blunt et al.* (10) as dihydrotutin (**12**) and dihydrohyenanchin (**17**). The attempt to use GLC for detection and quantification to avoid animal experiments was only partly successful as tutin (**11**) could reliably be quantified but hyenanchin (**15**) gave erratic results (10). Finally, HPLC was used, but these picrotoxanes have no chromophores for reliable quantification. Usually, UV radiation at 210 nm is employed for detection when a C_{18} column with water/methanol mixtures as eluent is used for elution. However, this method is unsatisfactory for samples with a small picrotoxane content. In this instance, each toxin is separately examined by HPLC. This allows reliable control of honey in hazardous areas, but, as *Sutherland* pointed out (100), the honey after extraction with organic solvents still contains some toxicity. This is understandable because detoxification in animals usually occurs by conjugation to highly water-soluble sulfates, glucosides, or glucuronides.

4.1.6. Isolation from *Hyenanche globosa*

In 1858, *Henkel* first examined an alcoholic extract of the fruits of the gift bom (*H. globosa*) (Picrodendraceae; Euphorbiaceae s.l.), the most toxic shrub of the Cape area of South Africa and found that it contained toxic substances (107). *Henry* then isolated the picrotoxanes hyenanchin (**15**) and isohyenanchin (**18**) in crystalline form in very small quantities from the leaves and stems, but in nearly 0.2% w/w, yield from the fruits of this species (108). *Jommi et al.* (76 and the references cited therein) and *Arigoni et al.* (42, 75, 109) succeeded in isolating and elucidating the structures of these two compounds **15** and **18** and the sesquiterpenes *O*-methylhyenanchin (**16**), dihydrohyenanchin (globosin) (**17**), and, for the first time substances with the “norditerpene” picrotoxane skeleton, capenicin (**57**), pretoxin (**59**), dendrotoxin (**60**), codendrin (**61**), and lambicin (**62**) (3).

4.1.7. Isolation from *Picrodendron baccatum*

The most thorough examination of a species in the Picrodendraceae was conducted by *Koike, Ohmoto*, and coworkers on *P. baccatum* (36, 46, 53–56, 77, 78). This tree is endemic to the West Indies. The plants investigated by the authors were harvested in the Botanical Garden of Bogor, Indonesia in 1986. In the next 15–20 years this Japanese group isolated 9 monolactone sesquiterpenes and 19 “norditerpene” picrotoxanes from this plant material. At first, these researchers examined a relatively small sample of the bark of the tree to determine the structural type of the toxins. Thus, 1.2 kg of dried bark were extracted successively with *n*-hexane,

chloroform, and methanol. The evaporated chloroform extract (18 g) was chromatographed on silica gel with chloroform containing increasing amounts of methanol. The fraction eluted with chloroform–methanol (9:1) yielded after further purification on silica gel 0.009% picrodendrin A (**63**). The fraction eluted with chloroform–methanol (9:1 to 5:1) yielded after a second chromatography on silica gel 0.0095% of the known isohyenanchin (**18**), 0.0014% picrodendrin C (dihydrohyenanchin; **17**) (a compound identical with globosin (**17**) from *H. globosa*), and 0.009% picrodendrin D (**23**). After this successful preliminary experiment, 8 kg of dried bark were extracted with methanol at room temperature. To the dried extract was added water and the aqueous solution was partitioned with chloroform, ethyl acetate, and *n*-butanol. The dried chloroform extract (80 g) was chromatographed on silica gel, using chloroform containing increasing amounts of methanol for elution. The fraction eluted with chloroform–methanol (9:1) was rechromatographed on silica gel CQ-3, with benzene–ethyl acetate (9:1) as eluent, yielding 0.00025% picrodendrin B (**64**) and 0.0025% picrodendrin G (**67**). The dried ethyl acetate extract (260 g) was chromatographed on the polymeric resin Diaion HP-20, with water containing increasing amounts of methanol. Fractions eluted with water–methanol (9:1), (5:1), (7:3), (1:1), and (3:7) were purified separately. The 5:1 fraction yielded after medium-pressure chromatography (MPLC) on silica gel CQ-3, using benzene–ethyl acetate (9:1) for elution, 0.002% picrodendrin E (**65**), 0.0005% picrodendrin F (**66**), 0.0004% picrodendrin I (**24**), and 0.0004% picrodendrin J (**25**). In turn the 7:1 fraction yielded after MPLC with benzene/ethyl acetate 0.00004% picrodendrin S (**75**). Finally, the 1:1 fraction yielded after MPLC with benzene–ethyl acetate 0.000045% picrodendrin T (**76**) and 0.00015% dihydrotutin (**12**).

The *n*-butanol extract (302 g) of *P. baccatum* was treated in a similar manner. After reversed-phase chromatography with Diaion HP-20, fractions eluted with water, water–methanol (8:2), (6:4), (4:6), (2:8), and methanol were collected. The 8:2 fraction after MPLC on silica gel with chloroform–methanol (20:1) furnished 0.00015% picrodendrin U (**77**) and 0.00006% picrodendrin W (**79**). The fraction eluted with chloroform–methanol (10:1) was further purified by high pressure LC (Capcell pack C₁₈ SG120) with water–methanol (2:1) yielding 0.00006% picrodendrin X (**80**). The water eluent of this HPLC chromatography was chromatographed on silica gel with methylene chloride–methanol (9:1), leading to a pure fraction of picrodendrin Z (**27**) (0.0005%). The residue of the 9:1 eluate was further purified by MPLC with benzene–ethyl acetate (4:1) yielding 0.002% picrodendrin Y (**81**). The 4:6 fraction of the reversed-phase LC was purified by TLC affording 0.000025% picrotoxin V (**78**).

The work-up of the stems of *P. baccatum* corresponds with that of the bark and yielded 0.0007% picrodendrin K (**68**), 0.00003% picrodendrin L (**69**), 0.00003% picrodendrin M (**70**), 0.00007% picrodendrin N (**71**), 0.00004% picrodendrin O (**72**), 0.00015% picrodendrin P (**73**), and 0.00004% picrodendrin Q (**74**). The dried leaves, when treated in a corresponding manner, yielded additional picrotoxanes (0.0003% picrodendrin α (**28**), 0.0006% picrodendrin β (**29**), and 0.00014% of the picrotoxane glucoside, picrodendrioside A (**30**)).

4.1.8. Isolation from *Dendrobium* Species

The most intensely studied species of the genus *Dendrobium* is *D. nobile* due to its use as a major component of the traditional Chinese medicine “Chin-Shih-Hu”. The first reports of alkaloids in this ornamental orchid in the 1930s by *Suzuki et al.* and *Chen et al.* (110–112) were followed by numerous attempts to isolate further picrotoxane-type compounds. Even now the search seems not to have been completed, although no less than 30 picrotoxanes have been isolated. Of these 17 are pseudoalkaloids and 13 sesquiterpenes. According to *Wang et al.* (113), the amounts of picrotoxanes are equally distributed in the roots, stems, and leaves of *D. nobile*. Several detection methods, mainly TLC and colorimetric evaluation, have been developed by Chinese researchers due to the popular use of this species as a tonic. A detailed flow chart for the isolation and purification of the main alkaloids, dendrobines and dendrobium salts, was published by *Inubushi et al.* in 1966 (114). As examples of recent isolation and purification procedures, *Kobayashi et al.* examination of a *D. nobile* cultivar, *Dendrobium* Snowflake “Red Star” (17), and the recent study on *D. nobile* by *Yao et al.* (67), have been chosen. The fresh plants of the cultivar “Red Star” were crushed and extracted with methanol. This extract was acidified with aqueous tartaric acid (3%) and partitioned with ethyl acetate. The aqueous layer was adjusted to pH 10 and extracted with chloroform. Successive column chromatography on silica gel with chloroform–ethyl acetate–methanol mixtures as eluents, on a C₁₈-column, using methanol–0.1% trifluoroacetic acid, and on C₁₈ HPLC, with nitromethane–0.1% trifluoroacetic acid as eluent yielded small amounts of two picrotoxane sesquiterpenes: 0.0002% flakinin A (7) and 0.0002% flakinin B (35), and of seven sesquiterpene alkaloids: 0.0007% mubironine B (98), 0.00007% mubironine C (99), 0.0001% mubironine A (97), 0.02% dendrobine (82), 0.001% dendramine (86), 0.0002% 6-hydroxydendroxine (94), and 0.006% nobilonine (90). In 2007, *Yao et al.* reported the isolation of several new picrotoxanes from *D. nobile*. They extracted the powdered air-dried stems with 60% aqueous ethanol at reflux. After evaporation of ethanol, the aqueous solution was partitioned with ethyl acetate and then with *n*-butanol. The ethyl acetate fraction was chromatographed on silica gel with chloroform and increasing amounts of methanol. Several fractions were separately chromatographed on Sephadex LH-20 with chloroform–methanol (1:1). These purification steps were followed by MPLC (silica gel: cyclohexane with increasing amounts of acetone as solvents), chromatography on ODS (methanol with increasing amounts of water) and HPLC (water/methanol mixtures as solvents). This resulted in five new picrotoxanes: 0.00008% dendronobilin B (46), 0.0001% dendronobilin C (47), 0.0002% dendronobilin D (48), 0.0003% dendronobilin E (49), and 0.003% of the structural variant, dendronobilin F (50), having a cyclopropane moiety. Other fractions revealed that the picrotoxanes are accompanied by several other structural types of sesquiterpenes (*e.g.* copacamphanes, cyclocopacamphanes, cadinanes, cyclocadinanes (60), alloaromadendranes) (67).

So far, *Dendrobium* species have yielded 21 pseudoalkaloids of the picrotoxane type and 27 sesquiterpene picrotoxanes (5 dilactones, 20 monolactones,

and 2 picrotoxanes having lower oxidation levels). As mentioned above, the first dendrobines were isolated in 1932, while the first sesquiterpene of the genus *Dendrobium*, the dilactone aduncin (**4**), was detected by *Leander et al.* more than 40 years later (*13*).

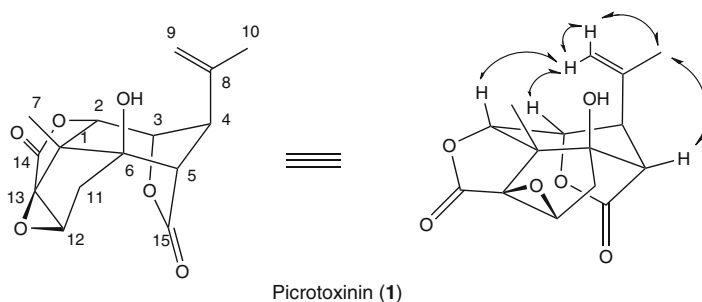
5. Structure Determination of Picrotoxanes

The most important and difficult part of elucidating the basic structure of picrotoxanes was achieved prior to introduction of modern spectroscopic methods. Moreover, work on the picrotoxanes was pioneering in introducing spectroscopic methods to complete the structure determination of natural products. It is a pleasure to follow the description of *Coscia* of these early efforts (*3*), as he was able to encapsulate in an elegant manner the enormous amount of knowledge on the chemistry of these highly compact and constrained compounds gained by transformation and degradation. It will be indicated later, how important this knowledge, especially the anchimeric effects and thus neighboring-group directed reactions, has been for planning their syntheses. In view of this very clearly written account (*3*) the work done up to 1968 shall only be mentioned briefly herein.

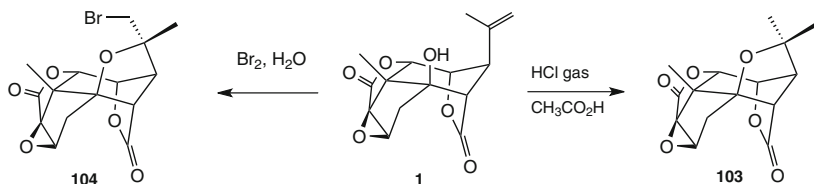
5.1. Main Picrotoxanes of the Menispermaceae

5.1.1. Picrotoxinin

One of the anchimeric effects that hampered the structure elucidation of picrotoxinin (**1**) considerably was the high stability of its oxirane (Scheme 1). When combined with the fact that this functionality seemed highly unusual for a naturally occurring compound at the time, tests to confirm the presence of this functionality failed. For example, even gaseous hydrochloric acid in acetic acid did not cleave this epoxide, but led to anhydropicrotoxinin (**103**) by intramolecular ether formation (Scheme 2). Under strongly basic condition the many functionalities of the



Scheme 1



Scheme 2

compound led to retroaldol-type fragmentation. *Conroy*, who eventually determined the structure of picrotoxinin (**1**) (115), used IR and UV spectroscopy in his efforts to characterize the functionalities of this compound. *Coscia* (3) stated that these efforts marked “the end of diagnostic derivatives. As attendant structural difficulties multiplied, the necessity of the more powerful spectroscopic methods of analysis were emphasized. In a brilliant treatise, *Conroy* conveyed organic chemistry into its modern form.” In the early 1960s, *Craven* confirmed *Conroy*’s structure determination of compound **1** by X-ray analysis of α_1 -bromopicrotoxinin (**104**) (Scheme 2).

The use of Cu K_α radiation permitted the absolute configuration of this picrotoxinin derivative to be established (116, 117). Since then, picrotoxinin (**1**) has been synthesized four times (118–122). Picrotoxinin itself was examined by X-ray analysis, especially to gain information about its conformation and to compare these data with those gained by NMR spectroscopy and theoretical calculations. The ultimate aim was to determine structure–activity correlations (5, 6). The conformation found by X-ray analysis, NMR spectroscopy, and calculations shows the cyclohexane in a distorted chair conformation with C(2), C(3), C(5), C(6) coplanar, with C(1) only slightly out of the plane spanned by these atoms (0.17 Å), whereas C(4) deviates from this plane by 0.89 Å. This requires the five-membered rings to be in an envelope conformation. According to these calculations, two low energy conformations with respect to the isopropenyl group exist. More refined calculations and NOESY experiments by *Perry et al.* (Scheme 1) revealed that the main conformation in solution and the conformation in the crystal are the same, with the methyl moiety C(10) of the isopropenyl group *syn* to C(4)–C(5) and the methylene *syn* to C(4)–C(3) (9). An electrostatic potential map of picrotoxinin (**1**) was contributed by *Schmidt et al.* (123).

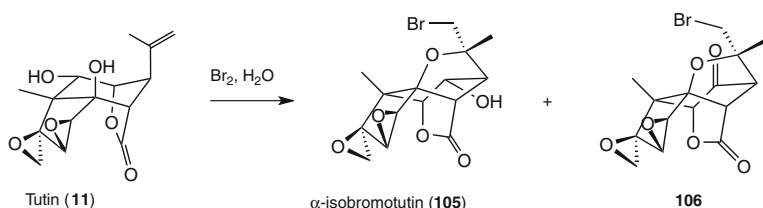
5.1.2. Picrotin

The structure of picrotin (**2**) was determined by the chemical transformation of this compound as well as of picrotoxinin (**1**) into identical structures. Later, a method was developed to convert picrotoxinin (**1**) into picrotin (**2**) in three steps (Scheme 38) (124). Thus, the absolute configuration of picrotin (**2**) was also confirmed. Specialized NMR techniques and calculations revealed hydrogen bonding between the hydroxy groups at C(6) and C(8), which *Schmidt et al.* assumed to be the reason for the low physiological activity of picrotin (**2**) (9, 123).

5.2. Main PicROTOXANES of the Coriariaceae

5.2.1. Coriamyrtin and Tutin

Comparison with the structure and chemistry of picROTOXIN (**1**) facilitated the structure elucidations of all the other picROTOXANES. Thus, *Riban* who isolated coriamyrtin from *C. myrtifolia* (tanner's brush) in 1864 (*18a*), concluded that its structure had to be similar to that of picROTOXIN due to its analogous reversible halogenation reaction (*18b*). *Okuda* and *Yoshida* proposed the correct formula of coriamyrtin (**9**) (*125*, *126*). This was confirmed by correlation with the structure of tutin (**11**) (*37*), which had been determined by X-ray analysis of its derivatives, α_1 -bromotutin (= α -bromoisotutin) (**105**) (*25*, *127*) and α -bromoisotutinone (**106**) (*26*) (Scheme 3).

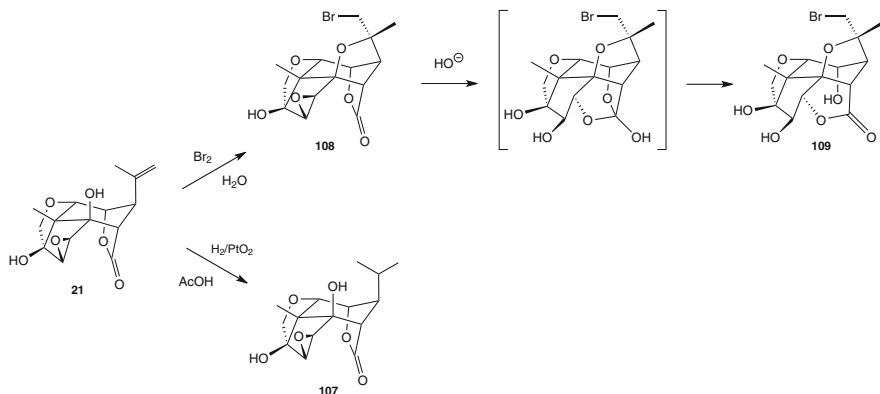


Scheme 3

The structures and absolute configurations of both natural products **9** and **11** were confirmed by synthesis (*52*, *119*, *128–130*). To gain better knowledge of the conformation of these two monolactones for structure–activity correlation, X-ray spectra of the compounds themselves and calculations (AM1 and MacroModel) were evaluated (*22*). The conformations of the two compounds are very similar and close to that of picROTOXIN (**1**). When compared with picROTOXIN (**1**) they are slightly less strained and lack the second γ -lactone, which leads to a more regular chair conformation of the cyclohexane unit. Thus, C(1) is 0.47 Å out of the plane spanned by C(2), C(3), C(5), and C(6) and C(4) is 0.89 Å out of plane. The cyclopentane unit exhibits an envelope form. Calculations provided an electrostatic potential energy surface. The conformation of tutin (**11**) was reexamined using NOESY and more refined calculation techniques (*9*). The earlier finding that the isopropenyl group has the same main conformation in all three compounds was confirmed. Newer calculations of their electrostatic potential energy surface appeared in 1999 (*123*).

5.2.2. Corianin

The propensity of tutin (**11**) to form a molecular compound with corianin (**21**), pseudotutin, which crystallizes from water, was mentioned earlier in this chapter.



Scheme 4

The isolation and structure determination of corianin (**21**) represent a fine example on the melding of the fields of crystallization and chemical transformations to modern methods of separation and structure elucidation of natural products (*21*, *40*). By repeated crystallization starting with pseudotutin in halogenated solvents as well as by chromatography of alcoholic extracts of *L. parasiticus*, pure corianin (**21**) was obtained. Its structure determination was based on comparison with the spectra of tutin (**11**). As with tutin (**11**), the IR spectrum showed absorption bands of hydroxy groups ($3,450\text{ cm}^{-1}$), γ -lactones ($1,760\text{ cm}^{-1}$, $1,740\text{ cm}^{-1}$), and double bonds ($1,640\text{ cm}^{-1}$). Diagnostic ^{13}C -NMR data in the sp^2 -region were only one lactone carbonyl ($\delta = 175.7\text{ ppm}$) and the terminal methylene group signal (112.4 ppm) of the isopropenyl group. Two D_2O -exchangeable protons in the ^1H -NMR spectrum indicated two hydroxy groups. Differences from the ^1H -NMR spectrum of tutin (**11**) were found in the region of the methylene protons at C(14). The spectroscopic data were supplemented by the chemical transformations shown in Scheme 4. Hydrogenation over Adams' catalyst afforded dihydrocorianin (**107**), which showed a typical isopropyl pattern in its ^1H -NMR spectrum. Treatment with bromine/water, one of the most useful reactions to detect and classify picrotoxanes prior to the advent of modern spectroscopy, led to the cyclic β -bromoether, **108**, revealing the proximity of one of the two hydroxy groups to the isopropenyl group, as found in tutin (**11**). Base treatment of this bromoether demonstrated a further neighboring group-directed reaction, which ascertained the configuration of the endocyclic epoxy group. Attack of HO^- at the γ -lactone led to hemioorthoester formation via substitution at the epoxide. This unstable intermediate is stabilized by formation of the isomeric γ -lactone (**109**). The position of the second hydroxy group of corianin (**21**) was revealed by a quaternary carbon atom and the fact that under normal conditions no acetylation occurred and treatment with Jones reagent formed no ketone. By acetylation at elevated temperatures, a monoacetate was formed revealing the less hindered position of this hydroxy group. The combination of these data with the signal pattern in the ^1H -NMR spectrum permitted the assignment of the position and relative configuration of the second hydroxy

group. Thus, corianin (**21**) is formally generated from tutin (**11**) by intramolecular substitution at the *spiro*-oxirane. Enantiomerically pure compound (EPC) synthesis of corianin (**21**) confirmed its structure and absolute configuration (*131*, *132*).

5.3. *Picrotoxanes Isolated from Toxic Honey*

Tutin (**11**) is metabolized to “mellitoxin” (**15**) in the plant hopper *S. australis*, thus making it plausible that mellitoxin is an oxidation product of tutin (**11**). The IR spectrum and especially the fragmentation pattern in the mass spectrum suggested hydroxylation at C(4) (*133*). Later it was recognized that mellitoxin is identical with hyenanchin (**15**), isolated from seeds of the highly toxic South African tree, *H. globosa* (Sect. 4.1.6) (*2*, *3*, *44*). The spectroscopic data obtained were later supplemented by the ¹³C-NMR spectrum (*10*). NOESY spectra and supporting calculations were used to determine the relative configuration and conformation of this compound (*9*). Neither X-ray structure analysis nor total synthesis as ultimate structure confirmation for **15** have been performed, however.

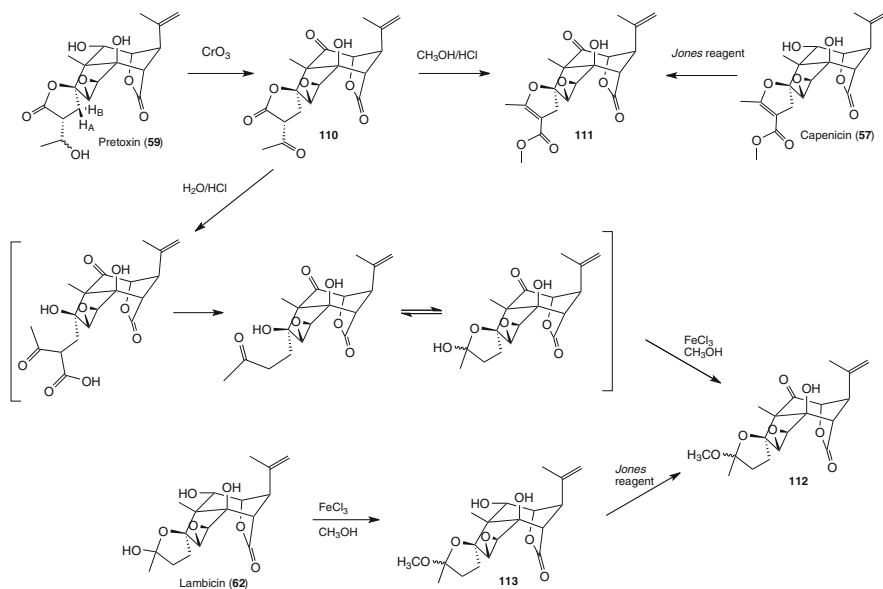
As mentioned above, tutin (**11**) and hyenanchin (**15**) are accompanied by small amounts of very difficult to separate satellites. Their structure determination consisted mainly of the interpretation of NMR difference spectra, and comparison with ¹³C-NMR spectroscopy series of similar picrotoxanes. This allowed the assignment of structure **12** to dihydrotutin and structure **17** to dihydrohyenanchin (*10*).

5.4. *Picrotoxanes of the Picrodendraceae*

5.4.1. *Picrotoxanes of H. globosa*

Along with hyenanchin (**15**), isohyenanchin (= isodihydrohyenanchin) (**18**), and tutin (**11**) several other picrotoxanes were isolated from the South African “gift bom” *H. globosa* (= *Toxidendron capense*). In the 1960s, degradation and spectroscopic analysis by *Jommi et al.* revealed the structures of hyenanchin (**15**) (*44*) and isodihydrohyenanchin (**18**) (*45*). In the years following the groups of *Jommi* and *Arigoni* isolated and determined the structures of the 2-*O*-methyl ether of isohyenanchin capensin (**19**), as well as the 2-*O*-methyl ether of hyenanchin (**16**), dihydrohyenanchin (=globosin) (**17**) and most interestingly capenicin (**57**) (*2*, *3*). *Scolastico* presented a thorough correlation of the ¹H-NMR spectra of these picrotoxanes (*134*). With capenicin (**57**), a new type of picrotoxane derivative was discovered, with an additional short side chain attached at C(14) of the sesquiterpene picrotoxane, which is cyclized to a spirodihydrofuran.² Continued work on the constituents of *H. globosa* by *Arigoni*'s group led to the structure elucidation of

²Note that the structures of capenicin and its derivatives are incorrectly depicted in (*2*).



Scheme 5

dendrotoxin (**60**)³ and codendrin (**61**), two further “norditerpene” picrotoxanes (3). Müller determined (75) the sesquiterpene part of those two structures mainly by comparison of their spectra with those of hyenanchin (**15**), tutin (**11**), and capenicin (**57**). The absolute configuration was determined by degradation to known sesquiterpene picrotoxane derivatives. The relative configuration of the short side chain at the *spiro*- γ -lactone of dendrotoxin (**60**) was determined by the shift difference of the geminal protons at C(14) and their coupling constants with the proton at C(17). (The comparable geminal protons H_A and H_B of pretoxin (**59**) are shown in Scheme 5) (3, 75).

In 1969, Jommi *et al.* reported isolation and structure determination of two further picrotoxanes (76). Elemental analysis showed them to be “norditerpene” picrotoxanes. Neither pretoxin (**59**) nor lambicin (**62**) were found to possess a chromophore. Their IR spectra revealed signals for hydroxy groups, γ -lactones, and olefins. The $^1\text{H-NMR}$ spectrum of pretoxin (**59**) established the presence of an isopropenyl group and an angular methyl group. Comparison with the spectra of tutin (**11**), capenicin (**57**), and dendrotoxin (**60**) allowed structure **59** to be proposed. Acetylation led to a diacetate still containing the signal of one O–H stretch band in the IR spectrum, indicating a tertiary alcohol. Oxidation of pretoxin (**59**) led to diketone **110** (Scheme 5). One of the ketones could be identified as a methyl ketone by $^1\text{H-NMR}$ spectroscopy. This methyl ketone is part of a β -dicarbonyl unit, confirmed by its UV absorption, which shifted bathochromically when treated with base, and by its color

³Unfortunately this name has also been given to a peptide toxin.

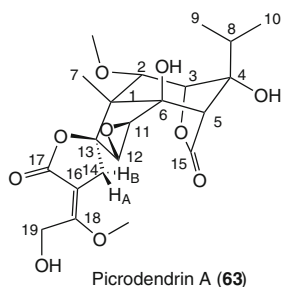
reaction with FeCl_3 . The second keto group is positioned at C(2). When this diketone was treated with methanolic hydrochloric acid methanolysis led to the instable β -ketoester, which by cyclization to the hemiketal and dehydration was transformed to the spirodihydrofuran **111**, identical to the oxidation product of capenicin (**57**). Thus, the absolute configuration was ascertained for compound **59** (**76**).

The determination of the relative configuration at C(16) of pretoxin (**59**) followed *Scolastico*'s reasoning (**134**) which corresponds with the argumentation *Müller* used in the structure determination of dendrotoxin (**60**) (**75**). Due to deshielding by the C(2) hydroxy group, proton H_B of the geminal protons at C(14) was shifted downfield. The coupling constants of the geminal protons with the proton at C(16) were used to establish the relative configuration of C(16). Lambicin (**62**) exhibited a similar ^1H -NMR spectrum to those of tutin (**11**) and pretoxin (**59**). Moreover compound **62**, showed mutarotation, and treatment with methanol catalyzed by FeCl_3 transformed it into two epimeric cyclic methyl ketals **113**. Hence the hemiketal moiety of lambicin (**62**) was confirmed (Scheme 5). Correlation with pretoxin (**59**) was achieved by oxidation of the epimeric methyl ketals **113** and treatment of oxidized pretoxin **110** with aqueous acid, and consequently with methanol and catalytic amounts of FeCl_3 . This reaction sequence converted the oxidized pretoxin via the β -keto acid and immediate decarboxylation into the methyl ketone, which using acidic methanol yielded the identical derivative **112** as was derived from lambicin (**62**). These transformations not only allowed the structure determinations of pretoxin (**59**), lambicin (**62**), and picrodendrin B (**64**) but also revealed their biogenetic interrelationship.

5.4.2. PicROTOXANES OF *P. baccatum* AND THREE RELATED STRUCTURES

Picrodendron A and its One-Dimensional NMR Spectra

When looking for the toxic principle of *P. baccatum*, another species of the Picrodendraceae, *Ohmoto*, *Koike et al.* discovered a wealth of picROTOXANES, which they painstakingly isolated and characterized within the next 15–20 years (**36**, **46**, **53–56**, **77**, **78**). The most abundant of these toxic compounds was picrodendrin A (**63**) a “norditerpene” picROTOXANE according to its high-resolution mass spectrum (**77**). Its IR spectrum revealed the occurrence of hydroxy groups and γ -lactones. A UV absorption maximum ($\lambda = 258$ nm) indicated that one of the lactones is unsaturated. The ^1H -NMR spectrum revealed the presence of an isopropyl group. Its methine proton signal was split into a septet, indicating that one of the hydroxy groups is attached to C(4). One of the two isolated methylene groups showed chemical shift values equivalent to a primary allylic alcohol in the ^1H -NMR spectrum. Indeed, D_2O exchange simplified its signal pattern to an AB-system. The second methylene signal corresponded with the C(14)-H signals of capenicin (**57**). Three singlets accounted for two methoxy groups and one angular methyl group. The typical signals of the oxirane fused to the cyclopentyl group were found at 4.18 ppm and 3.66 ppm with small coupling constants ($J = 2.9$ Hz). The low-field signal at 5.15 ppm showed the typical *W*-long-range coupling of the C(3)-H.



NMR data in d_5 -pyridine (δ /ppm):		
position	$^1\text{H-NMR}$	$^{13}\text{C-NMR}$
1		53.0
2	3.70 (1H, s)	87.9
3	5.15 (1H, d, $J = 1.1\text{Hz}$)	83.2
4		82.2
5	3.47 (1H, d, $J = 1.1\text{Hz}$)	57.8
6		78.2
7	1.89 (3H, s)	25.1
8	2.74 (1H, sept, $J = 6.2\text{Hz}$)	31.0
9	1.21 (3H, d, $J = 6.2\text{Hz}$)	15.6
10	1.48 (3H, d, $J = 6.2\text{Hz}$)	18.2
11	4.18 (1H, d, $J = 2.9\text{Hz}$)	62.9
12	3.66 (1H, d, $J = 2.9\text{Hz}$)	63.2
13		89.3
14	H _A : 3.44 (1H, d, $J = 17.2\text{Hz}$) H _B : 4.74 (1H, d, $J = 17.2\text{Hz}$)	34.3
15		175.8
16		104.3
17		170.1
18		166.3
19	H _A : 5.29 (1H, dd, $J_{\text{gem}} = 13.2\text{Hz}$, $J_{\text{OH}} = 4\text{Hz}$) H _B : 5.37 (1H, dd, $J_{\text{gem}} = 13.2\text{Hz}$, $J_{\text{OH}} = 4\text{Hz}$)	53.6
2-OCH ₃	3.96 (3H, s)	59.1
18-OCH ₃	3.31 (3H, s)	55.8
4-OH	7.41 (1H, s)	
6-OH	8.09 (1H, s)	
19-OH	7.35 (1H, d, $J = 4\text{Hz}$)	

Scheme 6

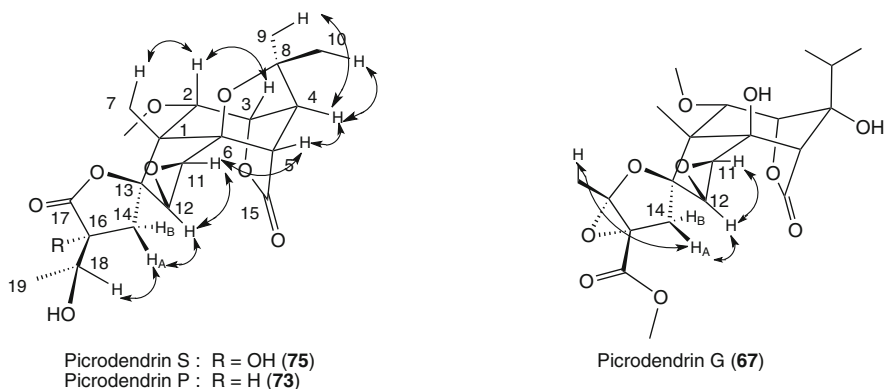
The $^{13}\text{C-NMR}$ spectrum showed six quaternary carbon signals, four of them in the range of sp^2 -hybridized carbon atoms, which accounted for the two carbonyl functions and the double bond. The upfield shift to 104.3 ppm of one of the olefinic carbons revealed an enol ether. The two low-field sp^3 signals belonged to the carbons of the tertiary alcohols. As an example of the NMR spectra of a picrotoxane the chemical shift values and, in the case of the $^1\text{H-NMR}$ spectrum the coupling constants of picrodendrin A (**63**) are shown in Scheme 6.

These data were supplemented by 2D-NMR ($^1\text{H-}^{13}\text{C-COSY}$ and two- and three-bond correlations). Picrodendrin A (**63**) crystallized as colorless prisms. Its X-ray diffraction analysis confirmed the proposed structure and allowed the configuration of the double bond to be determined.

Picrodendrins B, C, D, I, J, and G and the Use of NOE in the Structure Determination of Picrotoxanes

The structures of picrodendrins C (**17**) and D (**23**) were determined by analysis of their spectra, which strongly resembled those of tutin (**11**) and coriamyrtin (**9**) (46). Picrodendrin C (**17**) has been isolated previously as globosin from *H. globosa* (3, 42) and was identified as a constituent of toxic honey in New Zealand (10). Additional 2D-NMR (e.g. the NOESY spectrum) data allowed the assignment of their structures as dihydrohenanchin and 4-hydroxydihydrocoriamyrtin.

NOE interactions:



Scheme 7

Picrodendrin B (**64**) deviates from other picrotoxanes in containing an aldehyde group. The usual spectra were supplemented by complete, partial, and non-decoupled ^{13}C -NMR spectra, NOE-difference spectra, and long-range ^1H - ^{13}C -correlation (**54**). The same techniques were used to determine the structures of picrodendrin J (**25**), which turned out to be 4,8-dihydroxydihydrotutin (**54**), picrodendrin I (**24**), the first picrotoxane found with an oxidized angular methyl group (**53**), and picrodendrin G (**67**) (**54**). The stereocenters of C(13), C(16), C(17) of picrodendrin G (**67**) were established by NOE of the high-field signal of one of the geminal protons at C(14) with the proton signals at C(12) and the methoxy group. As an example of the use of the nuclear *Overhauser* effect in the structure determination of picrotoxanes, the more significant correlations of this type for picrodendrines G (**67**), P (**73**), and S (**75**) are shown in Scheme 7.

Picrodendrins E, F, L-T, and Celaenodendrolide I

Picrodendrins E (**65**) and F (**66**) more closely resemble pretoxin (**59**) and dendrotoxin (**60**) (**53**). X-ray analysis of the monobromobenzoate of picrodendrin F (**66**) was impeded by the fact that the two molecules of the asymmetric unit have different conformations. However, it was possible to determine the relative configuration of the stereogenic centers.⁴ The CD exciton chirality method conducted on the 16,18-bisbromobenzoate of picrodendrin F (**66**) revealed its absolute configuration. Comparison of the spectra of picrodendrin F (**66**) and of pretoxin (**59**) with those of picrodendrin E (**65**) allowed the determination of its structure. Shifts and

⁴However, the authors depicted **65** and **66** with C(18) in the (*R*)-configuration in this publication (**53**) whereas in a later publication (**55**) **65** and **66** are depicted with C(18) in the (*S*)-configuration without comment.

coupling constants of the C(14) and C(16) proton signals in the $^1\text{H-NMR}$ spectrum showed that the side chain at C(16) is epimeric to that of pretoxin (**59**). It is worth mentioning that another picROTOXANE, celaenodendrolide I (**58**), strongly resembles the above-mentioned picROTOXANES. The structure of **58** was determined by comparison of its spectra with those of picRODENDRIN E (**65**) (**48**). Compound **58** differed from compound **65** by the configuration at C(16), thus corresponding with the configuration of C(16) found in the *H. globosa* “norditerpene” picROTOXANES. Further deviations from picRODENDRIN E (**65**) were a hydroxy group at C(4), and an isopropenyl group instead of a 2-hydroxyisopropyl group.

Spectroscopic analysis and comparison with the closely related spectra of picRODENDRINS A (**63**), E (**65**), and F (**66**) sufficed to propose the structure of picRODENDRIN L (**69**) as 6-desoxypicRODENDRIN F, picRODENDRIN Q (**74**) as 4,19-didesoxypicRODENDRIN A, picRODENDRIN M (**70**) as 4,19-didesoxy-8-hydroxypicRODENDRIN A, and picRODENDRIN N (**71**) as the gallic acid ester of picRODENDRIN A (**63**) (**55**). To complete the structure determination of picRODENDRIN N (**71**) the molecular peak was obtained by FAB mass spectrometry and methanolysis of the ester afforded picRODENDRIN A (**63**), while COLOC experiments allowed the galloate ester unit to be located at C(19). PicRODENDRIN O (**72**) is 8-desoxypicRODENDRIN E. In picRODENDRIN K (**68**), the *spiro-γ*-lactone of picRODENDRIN F (**66**) is replaced by a *spiro*-tetrahydrofuranone (compare with the rearrangement of pretoxin (**59**) to lambicin (**62**)). The structure of the sesquiterpene picRODENDRIN R (**26**) was resolved by comparison of its spectroscopic data with those of the 2-*O*-methyl derivative of picRODENDRIN D (**23**) (**55**). The structures of the next batch of isolates from the bark of *P. baccatum* were presented two years later (**36**). PicRODENDRIN P (**73**) can be viewed as the anhydride of picRODENDRIN E (**65**), and picRODENDRIN S (**75**) as 16-hydroxypicRODENDRIN P. In fact, it was the structure of picRODENDRIN S, elucidated by spectroscopic data analysis including 2D-NMR spectra that led to revision of the structure of picRODENDRIN P (**73**) by comparison (**36**, **55**). Force-field calculations (COSMIC, Nemesis program) were applied to clarify the conformation.

PicRODENDRIN T (**76**) differs only in the configuration of the double bond from picRODENDRIN A (**63**).

PicRODENDRINS with Fused γ -Lactones

PicRODENDRINS with rearranged fused γ -lactones were elucidated next (**56**, **78**). Formally, hydrolysis of the bridged γ -lactone of picRODENDRIN A (**63**) is followed by inversion of the conformation. The hydroxy group is now axially positioned, so that dehydration is facilitated and attack of the carboxyl group at the newly formed allylic epoxide completes the rearrangement to picRODENDRIN V (**78**). The same formal retrograde reaction sequence would lead from picRODENDRIN W (**79**) to a so far unknown picRODENDRIN derivative. In similar reaction sequences, starting with the hydrolysis of the bridged γ -lactone of picRODENDRIN E (**65**), picRODENDRIN U (**77**) and picRODENDRIN X (**80**) may be formed. The pentacyclic picRODENDRIN Y (**81**) might correlate with 2-desmethyl picRODENDRIN E, whereas the sesquiterpene picRODENDRIN

Z (**27**) can be traced back formally to isodihydrohyenanchin (**18**). These structures were determined by spectroscopic and X-ray diffraction data analyses. For picrodendrin U, V, and W, the absolute configurations of the stereocenters were determined from non-empirical exciton circular dichroism spectroscopy.

Picrodendrins from the Leaves of *P. baccatum*, Picrotoximaesin and Asteromurin A

The leaves of *P. baccatum* have been found to contain only sesquiterpene picrotoxanes (**56**). None of these picrodendrins possesses the characteristic oxirane fused to the cyclopentane. They are less highly oxidized than the picrotoxanes of the stem and bark of *P. baccatum* as is easily recognized from their IR and NMR spectroscopic data. The structure determination of the amorphous picrodendrins α (**28**) and β (**29**) relied on their NMR spectra, including 2D-NMR methods. These are *cis*-hydrindenenes with a bridging γ -lactone and a primary allylic alcohol and a saturated isopropyl group at C(4). The structure of crystalline picrodendrioside A (**30**), the only picrotoxane glycoside found in the family Picrodendraceae so far, was ascertained by X-ray diffraction analysis, and its aglycon and glucose were released by enzymatic hydrolysis. A special feature of this aglycon is the keto group at C(2). This same feature was found in the structure of picrotoximaesin (**43**), a constituent of a further member of the former family of Euphorbiaceae (**64**, **65**). In this case, the keto group is transformed by the primary alcohol at C(14) to a cyclic hemiketal. Its structure was determined by X-ray diffraction analysis. Interestingly, the only other sesquiterpene picrotoxane with this cyclic hemiketal is asteromurin A (**22**), isolated from a scale insect. Its structure was determined by spectroscopic analysis (**50**) and by X-ray diffraction analysis of its bromobenzoate derivative (**51**). Total synthesis confirmed its structure (**135**).

5.5. Picrotoxanes from *Dendrobium* Species

5.5.1. Dilactones

Many of the structural features found in the sesquiterpene picrotoxanes of the plant families mentioned so far can be seen again within only a few species of the genus *Dendrobium*. The first picrotoxane sesquiterpene, aduncin (**4**), was isolated from *D. aduncum* (**13**). Its spectra showed strong similarities with those of α -dihydropicrotoxinin (**8**) obtained from picrotoxinin (**1**) by hydrogenation over Adams' catalyst. The molecular mass is the same as that of picrotoxinin (**2**) thus only the position of one of the hydroxy groups of this dilactone sesquiterpene had to be determined. This was easily concluded from the patterns of proton-NMR signals at C(2) and C(8) of aduncin (**4**) and dihydropicrotoxinin, respectively. The configuration of C(4) was assigned by comparison of the CD curves of several picrotoxinin derivatives, especially α - and β -dihydropicrotoxinin, epimers at C(4) with the CD curve of

aduncin (**4**) (13). Later, the Swedish researchers involved described amotin (**5**), a closely related dilactone from *D. amoenum* (15). They determined by spectroscopic analysis that amotin (**5**) is dihydroaduncin, and, instead of the epoxy group [C(12), C(13)] only the tertiary alcohol at C(13) remained. The only dilactone with an isomerized γ -lactone found among the *Dendrobium* species is flakinin A (**7**) (17). The structure of this amorphous compound had to be determined by spectroscopic data analysis: The molecular formula was obtained by HRESIMS. IR spectra revealed the hydroxy group and the γ -lactones. The ^{13}C -NMR spectrum showed signals of three methyl groups, eight sp^3 -methines (three in the range of alcohols), one sp^3 methylene, and two carboxy groups and one quaternary carbon atom. The ^1H - ^1H COSY, HOHAHA, HMBC, and NOESY NMR-spectra allowed the complete structural assignment of flakinin A (**7**). *D. moniliforme* yielded two dilactone picrotoxanes, of which one of them turned out to be identical with α -dihydropicrotoxinin (**8**) (11). Thus, its absolute configuration was confirmed also. The other, dendrobiumane E (**6**), was elucidated by detailed spectroscopic analysis including 2D-NMR methods.

5.5.2. Monolactones and Structurally Deviant Picrotoxanes

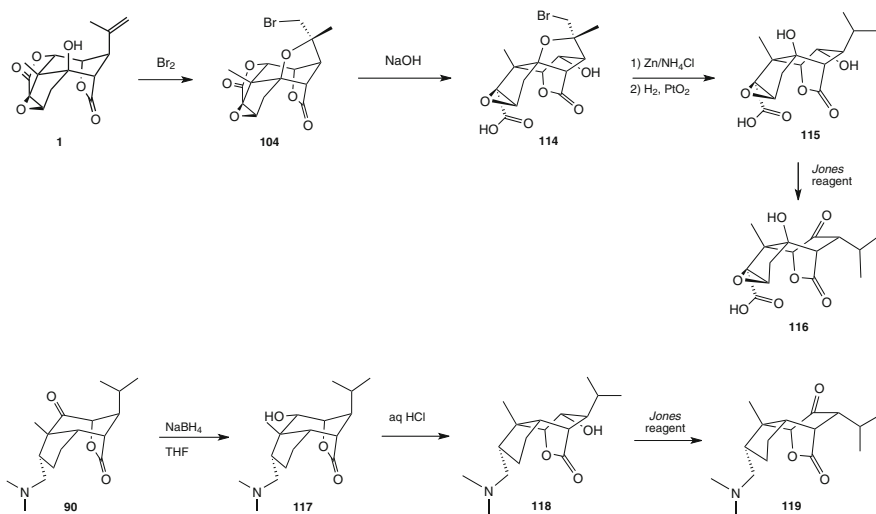
The dilactones described thus far were accompanied by several monolactone picrotoxanes with a low oxidation level, but their small amounts isolated prevented crystallization. Thus, the structures of dendrobiumanes B-D (**31**–**33**) were determined by spectroscopic analysis. Their structures resemble those of the picrodendrins from the leaves of *P. baccatum*, with even lower oxidation levels. In turn, the structure of crystalline dendromonilide B (**36**), isolated from *D. moniliforme* (58), was resolved by X-ray diffraction analysis. The structures of dendromonilides C (**37**) and D (**38**) were assigned by comparison of their spectra and the spectra of their aglycons with those of dendromonilide B (**36**) and its aglycon. The structure of amoenin (**41**) was determined by spectroscopic data analysis and confirmed by oxidation with molecular oxygen over platinum yielding α -dihydropicrotoxinin (**8**) (15). As before, the most intensely investigated species is *D. nobile* and its cultivars. Spectroscopic analysis including COSY, HOHAHA and HMBC, and NOESY NMR spectra allowed a determination of the structure of flakinin B (**35**). Its absolute configuration was determined according to the Mosher ester method. Esterifying the C(2)-OH with Mosher's reagent allowed by means of a chemical shift difference the assignment of the (*S*)-configuration to C(2) (17). Two of the compounds found in *D. moniliforme* were also identified in *D. nobile*. The first of these was dendroside F (**39**) (58, 60), which is the C(14)*O*- β -glucoside of dendrobiumane B (**31**). The second one is most likely dendromonilide D (**39**) (58, 59), although the spectra were run in different solvents and the IR spectrum in KBr showed very small differences, so that identity of the two compounds was tentative but not conclusive. Dendroside F was accompanied by a second glucoside, dendroside G (**40**) (60), which differs from dendroside F by having a hydroxy group at C(4). Recently, the aglycon of dendroside G was isolated from *D. nobile* and named

dendronobilin B (**46**) (*67*). The relative configuration of C(13) between the methylene protons of the primary alcohol and the protons of the angular methyl group was proven by NOE observations. The same relative configuration at C(13) was found in dendronobilins D (**48**) and E (**49**). Since the dendronobilins were isolated in small amounts as amorphous powders or oils, their structure determination is incomplete because the spectroscopic data obtained did not warrant the definitive assignment of relative configuration at C(8), which was only presented tentatively. Dendronobilins D and E are epimers at C(8). Dendronobilin C (**47**) is a cyclic hemiacetal and the angular methyl group at C(1) is converted into the methylene unit of a cyclopropane in dendronobilin F (**50**). Again, the configuration of C(8) is tentatively given. Most recently, *Yao et al.* added two further dendronobilins, L (**51**) and M (**52**), to an expanding list of sesquiterpene picROTOXANES from *D. nobile* (*68*). HR-TOF-MS, ¹³C-NMR, DEPT, ¹H-¹H COSY, HSQC, HMBC, and NOESY spectra were used to determine their structures. Dendronobilin M (**52**) is either 9-hydroxydendronobilin D or E. An even lower oxidation level is exhibited by two glucosides, dendronobilosides A (**55**) and B (**56**), which are dihydroxy and trihydroxy *cis*-perhydroindane derivatives (*71*). In the case of dendronobiloside A (**55**), both hydroxy groups are etherified to *O*- β -D-glucopyranosides. Dendronobiloside B (**56**) is a monoglucoside. The glucosides as well as their aglycones, obtained by enzymatic hydrolysis, were investigated by spectroscopic analysis including COSY, TOCSY, HMQC, and HMBC. The position of the attachment of the glucose units was revealed according to the correlation of their HMBC spectra. Although nobilemethylene (**45**) was isolated and characterized as long ago as 1972, it is still not clear if this compound is a constituent of *D. nobile* or an artefact generated from dendrobine-*N*-oxide (**83**) by the method of solvent extraction used (*66*).

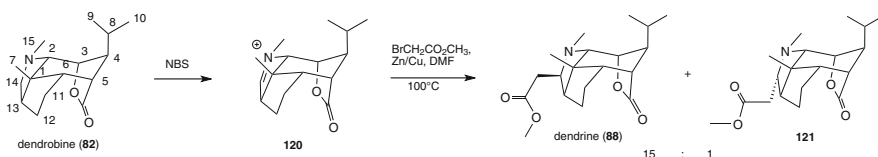
5.5.3. The Dendrobines, Sesquiterpene Alkaloids

Porter (*2*) and *Coscia* (*3*) described the structures of six dendrobine alkaloids and one dendrobium salt. Although the structures of the dendrobines are less complex than those of most other picROTOXANES, their structure determination became feasible only after spectroscopic methods became generally available in the 1960s. Thus, in 1964, no less than three Japanese research groups completed independently the structure determination of dendrobine (**82**), the main alkaloid of *D. nobile*, using a combination of degradation reactions and spectroscopic data analysis (*136–138*).

Inubushi et al. and *Huang* (*139, 140*), using the lactone rule and the octant rule, found that the absolute configuration of dendrobine (**82**) correlated with that of picROTOXININ (**1**), which had been established by X-ray diffraction analysis using Cu K α radiation. An X-ray analysis of nobilenine methiodide seemed to contradict these results (*141*), which motivated *Leander et al.* to attempt a renewed correlation between a degradation product of nobilenine (**90**), δ -nobilenine (**119**), and of picROTOXININ (**1**), ketopicROTOXININIC ACID (**116**) (*79*). The structure and configuration of nobilenine itself had been correlated to dendrobine by converting dendrobine into nobilenine (*142*).



Scheme 8



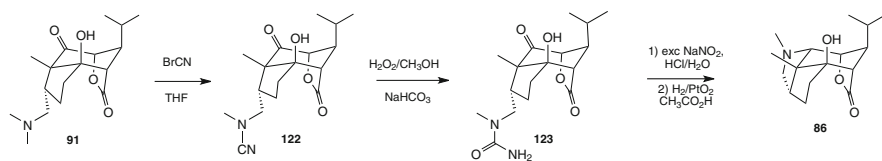
Scheme 9

Picrotoxinin (**1**) was converted into α_1 -bromopicrotoxinin (**104**), which by base treatment rearranged to **114** (Scheme 8). Reductive cleavage of the α -bromo ether with zinc was followed by hydrogenation of the isopropenyl group. The secondary alcohol of the resulting δ -lactone **115** was oxidized with *Jones* reagent that triggered immediate epimerization at C(4) leading to **116**. Nobiletin (**90**) was reduced with sodium borohydride to **117**. With aqueous hydrogen chloride the γ -lactone rearranged to the more stable δ -lactone **118**. *Jones* oxidation yielded δ -nobilinone (**119**). The similarity of the circular dichroism curve of both derivatives confirmed *Inubushi's* and *Huang's* designation. Since then, two EPC-syntheses of dendrobine (**82**) have completely removed any remaining doubts about the absolute configuration of dendrobine (**82**) (*143*, *144*). Of the dendrobines described in the 1960s, dendrine was assigned structure **88**, but the configuration at C(13) was still unknown. *Leander et al.* solved this problem by correlating dendrine (**88**) with dendrobine (**82**), so not only the relative but also the absolute configuration of dendrine (**88**) was revealed (*85*).

Dendrobine was oxidized with NBS to the imminium salt **120** (Scheme 9). A *Reformatsky* reaction then converted the imminium salt into dendrine (**88**) and

in small amounts to its epimer **121**. *Leander* argued that the attack from the convex face must be much favored. Since dendrine (**88**) was identical with the main product, its side chain had to be *exo*-positioned. This reaction sequence constitutes a partial synthesis of dendrine (**88**). With dendrine the first sesquiterpene alkaloid with extended carbon skeleton was discovered. *Zhao et al.* have isolated a further dendrobine alkaloid with an extended carbon skeleton, dendronobiline A (**89**) (43, 86). Its structure was determined from the high-resolution mass spectrum and its NMR spectra, including a set of several 2D-NMR data. An identical molecular formula and very similar IR and $^1\text{H-NMR}$ spectra to those of dendramine (**86**) (= 6-hydroxydendrobine) guided *Leander et al.* in the structure determination of 2-hydroxydendrobine (**87**). The most diagnostic differences in the $^1\text{H-NMR}$ spectrum were the simplified pattern of the C(3)-H signal and the lack of a geminal proton to nitrogen at C-2 in the newly found compound. These data convinced the authors that they had isolated 2-hydroxydendrobine (**87**) (83). Indeed, hydrogenolysis in acetic acid transformed 2-hydroxydendrobine (**87**) into dendrobine (**82**), thus confirming the absolute configuration of 2-hydroxydendrobine (**87**) as well. In the same year, *Leander et al.* (87) determined the structure of 6-hydroxynobiline (**91**) by comparison of its spectroscopic data with those of nobilonine and conversion of 6-hydroxynobiline (**91**) into dendramine (**86**).

After several trials, successful conversion was achieved by treating 6-hydroxynobiline (**91**) with cyanobromide (Scheme 10). The resulting aminocyanide, **122**, hydrolyzed with hydrogen peroxide to the urea derivative **123**, which was treated with excess sodium nitrite in dilute aqueous hydrogen chloride and subsequently hydrogenated via 2,6-dihydroxydendrobine to dendramine (**86**). The absolute configuration of dendramine (**86**) was correlated with that of dendrobine (**82**) by comparison of their very similar CD curves. Thus, the absolute configuration of 6-hydroxynobiline (**91**) was also ascertained. 6-Hydroxynobiline (**91**) was isolated also from *D. moniliforme* together with moniline (**102**) (88). Its structure was determined by spectroscopic means. The elemental formula was obtained from the molecular ion peak in the mass spectrum. The IR spectrum revealed a hydroxy group and carbonyl groups that were not compatible with a γ -lactone and a double bond. The UV spectrum corresponded with the presence of an α,β -unsaturated ketone, and the $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectra aided the completion of the structure determination of the bicyclic methyl ester **102**. NOESY spectra were used to ascertain the relative configuration of the ester group at C(5) and the dimethylaminomethyl group at C(14). Its close structural relationship with 6-hydroxynobiline (**91**) allowed its absolute

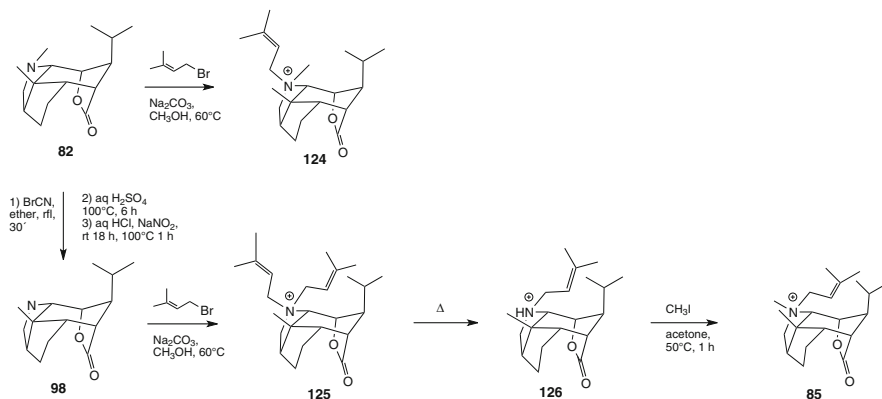


Scheme 10

configuration to be ascertained. *Okamoto, Shimizu* and their groups, who determined the structures of dendrobine (**82**), nobilonine (**90**), dendroxine (**92**), dendramine (**86**), and 6-hydroxydendroxine (**94**) (2, 3), reported the structure elucidation of 4-hydroxydendroxine (**96**) and the controversial nobilomethylene (**45**) in 1972 (66). Both structures were postulated by comparison of spectroscopic data. In the case of 4-hydroxydendroxine (**96**), the very similar spectra of dendroxine (**92**) and 6-hydroxydendroxine (**94**) facilitated its structure determination. 4-Hydroxydendroxine (**96**) has the same molecular formula as 6-hydroxydendroxine (**94**) and it differs by exhibiting a singlet for the proton at C(3) in its $^1\text{H-NMR}$ spectrum from the doublets evident in the respective $^1\text{H-NMR}$ spectra of dendroxine (**92**) and 6-hydroxydendroxine (**94**). This was indicative that a hydroxy group is attached at C(4). The relative configuration was assigned tentatively. Nobilomethylene (**45**) was found exclusively in the alkaloid fraction and not in the neutral fraction and so is thought to have been generated from nobilonine-*N*-oxide. With 13-hydroxy-14-oxodendrobine (**100**), the first γ -lactam was characterized (89). The molecular peak and the resemblance with the spectra of dendrobine (**82**) established that the compound belongs to the picrotoxanes. The double peak at $1,676\text{ cm}^{-1}$ and $1,693\text{ cm}^{-1}$ in the IR spectrum and a singlet at 2.88 ppm in the $^1\text{H-NMR}$ spectrum, which constitutes a 0.33 ppm downfield shift compared with dendrobine (**82**), indicated the presence of the lactam moiety. Furthermore, the stretching bands of the hydroxy group were visible in the IR spectrum. The signal of a quaternary carbon at 86.0 ppm in the $^{13}\text{C-NMR}$ spectrum indicated a tertiary alcohol. The position of this tertiary hydroxy group at C(13) was established by the signal pattern of C(5)H, which was consistent with two *vicinal* protons, thus neither C(4) nor C(6) were attached to a hydroxy group. Since the methyl groups of the isopropyl moiety were doublets, the hydroxy group had to be at C(13). An examination of the constituents of a cultivar of *D. nobile* increased the number of dendrobine alkaloids by three (17). Their structures showed significant similarities with dendrobine (**82**) and spectroscopic analysis including several 2D-NMR spectra revealed that mubironine A (**97**) is 14-oxodendrobine, which was synthesized from dendrobine (**82**) by oxidation with KMnO_4 . Mubironine B (**98**) is nordendrobine, a compound *Leander et al.* had synthesized previously (Scheme 11) (81). Mubironine C (**99**) showed an additional methoxy group and no γ -lactone signals. Its structure was proven by its identity with methanolized dendrobine. Since all three alkaloids were connected with dendrobine (**82**) by synthesis, their absolute configuration is in accord with that of dendrobine (**82**).

5.5.4. Dendrobinium Salts, Quaternary Sesquiterpene Alkaloids

N-Methyldendrobinium chloride (**84**) was the first quaternary picrotoxane alkaloid isolated and characterized by *Inubushi* (114). The next dendrobinium salt isolated was *N*-isopentenyl-dendrobinium chloride (**85**) (81). Its structure was determined by pyrolysis to dendrobine (**82**). The configuration of its quaternary nitrogen was determined by synthesis (81).



Scheme 11

When dendrobine (**82**) was alkylated with 1-bromo-3-methyl-2-butene, the resulting compound **124** was not identical with the isolated *N*-isopentenyl-dendrobium salt (Scheme 11). To prove that **124** indeed is the epimer of *N*-isopentenyl-dendrobium chloride (**85**) dendrobine (**82**) was demethylated using *Inubushi's* method (*136*). The resulting nordendrobine (mubironine B) (**98**) was diallylated with 1-bromo-3-methyl-2-butene. Pyrolysis yielded *N*-isopentenyl-nordendrobine (**126**), which was methylated yielding the ammonium salt, **86**, identical with that isolated from *D. nobile* (**81**). *N*-Isopentenyl-dendrobium chloride (**85**), first isolated by *Inubushi et al.* (**82**) in the 1960s, belongs to the small group of *N*-prenylated alkaloids and it may have been quite possibly the first one isolated from a plant (*145*). The structure of dendrobine-*N*-oxide (**83**) was proven by treatment of dendrobine (**82**) with *m*-chloroperbenzoic acid. Because the synthetic product was identical with the natural product, the oxygen at the nitrogen is on the convex face of the compound. The structures of *N*-isopentenyl-dendrobium salt (**93**) and *N*-isopentenyl-6-hydroxydendrobium salt (**95**) were determined by pyrolysis of their chlorides at 160°C for 15–20 min, yielding compounds indistinguishable from dendroxine (**92**) and 6-hydroxydendroxine (**94**) (*80*). Alkylation of dendroxine (**92**) and 6-hydroxydendroxine (**94**) with 1-bromo-3-methyl-2-butene led to the naturally occurring products, **93** and **95**. Since attack from the convex face should be favored and because *cis*-diquinanes are more stable than *trans*-diquinanes and hence also their aza analogs, the configuration of the quaternary nitrogen is established. When dendrowardine (**101**) in its hydroxy form was pyrolyzed, *Leander et al.* isolated *N,N*-dimethylethanolamine. Treatment of dendrowardine (**101**) with lithium hydride in DMF resulted in an unsaturated tertiary amine. Hydrogenation over *Adams' catalyst* then led to a product identical with dihydronobilonine gained by reduction of nobilonine (**90**) with sodium borohydride. The relative configuration of C(14) was assigned tentatively due to the coupling constants of its proton with C(13)H in the ¹H-NMR spectrum (*90*).

6. Total Syntheses of Picrotoxanes

6.1. Overview

When *Porter* and *Coscia* published their reviews (2, 3) no total synthesis of a picrotoxane had come forth. Even the partial syntheses were not well developed, as mubironine A (97), an oxidation product of dendrobine (82), was not known as natural product at that time. The same is true for nordendrobine, the demethylation product of dendrobine (82), now known as mubironine B (98). Partial syntheses in the 1970s were made to determine the structure or absolute configuration of new picrotoxanes and are described earlier in this chapter.

When the first picrotoxane syntheses were published *Corey's* rules for synthesis planning and the *Woodward–Hoffmann* rules had just become common knowledge as well as the necessity to prepare physiologically active products in an enantiomerically pure form.

Due to their more simple structures, the syntheses of the sesquiterpene alkaloids, the dendrobines, were mastered first.

6.1.1. Syntheses of Dendrobines

Eight years after the structure elucidation of dendrobine (82) was completed, two total syntheses of this highly crowded tetracyclic compound with its seven stereogenic centers were published. To date, seven total syntheses and five formal syntheses have been reported. Those synthesis efforts nicely reflect trends in total synthesis over the last decades, which shall be demonstrated by the stereoselective construction of the quaternary center, a formidable task even today.

The first syntheses relied on *Michael* addition and aldol-like reactions. *Yamada et al.*, who presented their synthesis as full paper in 1972, used the intramolecular *Robinson* annulation (*Johnson's* tandem reaction) as a key step (146). This allowed the stereoselective construction of the crucial quaternary center in a reaction generating three stereogenic centers in the correct relative configuration. Their starting material was a cheap arene, and although it is a first synthesis the stereoselectivity of its steps was with one exception remarkably high. *Yamada* demonstrated the versatility of his synthetic strategy by also synthesizing nabilonine (90) and 2-hydroxydendrobine (87) (84). *Inubushi*, who had spearheaded the structure determination of dendrobine (82), published a short communication in the same year, which was followed by a full paper 2 years later (147, 148). This synthesis started with a hydrindenone, which was prepared by *Robinson* annulation, including the construction of the quaternary center. Subsequent hydrogenation led stereoselectively to the *cis*-hydridanone. The further pathway suffers from several steps with no or incorrect stereoselectivity. Both syntheses need more than 20 steps to reach the target. A further attempt to use a *Michael* reaction to construct the quaternary center of

dendrobine (**82**), starting with the monoterpene carvotanacetone (dihydrocarvone) was reported by *Heathcock et al.*, but was abandoned later on (149).

The predictability of the stereochemistry of concerted reactions made the *Diels–Alder* reaction a very attractive key step, especially in the 1970s and 1980s. Several authors used this reaction to advantage in their dendrobine syntheses. In 1970, *Kaneko* had used the monoterpene carvone as dienophile and butadiene in a *Lewis* acid-catalyzed intermolecular *Diels–Alder* reaction to construct the quaternary center (150). By oxidative fragmentation of the newly formed cyclohexene and renewed cyclization, ring contraction was achieved. *Kaneko et al.* concluded their synthesis efforts after constructing the tricyclic skeleton of dendrobine (**82**). A similar concept was used by *Kende* and *Bentley* (151). They started with the cheap thymol. Using a quinone for the intermolecular *Diels–Alder* reaction with butadiene allowed high yields, despite the trisubstituted dienophile. Ring contraction was analogous to that of *Kaneko*. The choice of the starting material shortened the synthesis pathway considerably, compared with the above-mentioned syntheses (146, 147), so that *Kende's* synthesis with its cheap starting material is still one of the most economic dendrobine syntheses. This was emphasized when *Corey et al.* demonstrated how easily and without loss of material, *Kende's* racemic synthesis can be transformed into an asymmetric synthesis (152).

In the early 1970s, intramolecular *Diels–Alder* reactions (IMDA) had emerged as powerful method in natural product synthesis, but little was known about the steric requirements. At this early stage, *Borch* and *Roush* chose IMDA as a key step of their synthesis efforts toward dendrobine (**82**). Both constructed an open-chained triene. *Borch* used a trisubstituted (*Z*)-double bond as part of the diene moiety to ascertain the stereochemistry of the quaternary center (153, 154). Despite the expected advantages of IMDA, lower temperature and higher selectivity, the author needed high temperatures that led to isomerization and in consequence to 4-*epi*-dendrobine. *Roush* avoided *Borch's* problems by using only disubstituted double bonds for the IMDA reaction (155–157). The missing methyl group of the quaternary center was introduced by alkylation; thereby relying on the thermodynamic preference of *cis*-hydrindanes for the stereoselectivity. This enabled *Roush* to synthesize dendrobine (**82**) but this was disadvantageous in including a higher number of steps. Ten years later, *Martin et al.* presented their formal dendrobine synthesis (158, 159). Their IMDA precursor is formed in a convergent synthesis with a preformed methylcyclopentene derivative as dienophile tethered to the diene by an amide moiety. Cyclization afforded the substituted tricyclic skeleton of dendrobine (**82**), which then was transformed into an intermediate of *Inubushi's* synthesis. Cascade reactions, reactions with multiple bond changes, have become an important component of modern total synthesis. This is reflected in the latest attempt toward dendrobine (**82**), *Padwa's* formal synthesis (160, 161). Again, the key step is an IMDA reaction. With an isopropyl-substituted furan as diene, a preformed methylcyclopentene moiety as dienophile, and an amine as tether, the quaternary center is constructed stereospecifically. The cyclization initiates successive isomerization, so that no less than ten bonds are changed in one step. *Padwa*

ends his synthetic efforts with an intermediate of *Kende's* synthesis. Despite the many variations of the *Diels Alder* reaction as key step, all of those syntheses needed more than 15 steps to reach the target, dendrobine (**82**), starting from commercially available compounds. Also, when compared with the first synthesis of dendrobine the yields were not improved.

Heathcock used as concerted cycloaddition intramolecular photochemical (2 + 2) addition to create the quaternary center in his projected synthesis. A cationic rearrangement (*Wagner–Meerwein* rearrangement) was intended as further key step but failed (162).

Cationic olefin cyclization as a key step led to the shortest racemic dendrobine synthesis reported so far (163, 164). *Livinghouse et al.* used an intramolecular acylnitrilium attack on a preformed methylcyclopentene moiety to construct the quaternary center and a reductive radical cyclization as second key step in a convergent synthesis to construct the fully substituted skeleton of dendrobine. A few additional steps led then to dendrobine (**82**). In most of the syntheses following *Livinghouse's* efforts the intention was less focused on strategy (economy) of the synthesis of dendrobine (**82**) than on demonstrating the versatility of the (newly developed) key step.

After the intense use of concerted reactions for natural product synthesis, the related metal-catalyzed cyclizations gained ground in the 1980s and 1990s. Several authors demonstrated the effectiveness of these reaction types for the synthesis of dendrobine (**82**). *Takano et al.* and *Zard et al.* used the *Pauson–Khand* reaction as a key step for their EPC-synthesis efforts (144, 165, 166). *Mori et al.* relied on the more stable zirconacycle in a related key step (167–169), while *Trost et al.* employed a palladium-catalyzed alkylation as well as a palladium-catalyzed ene reaction as key steps (170). *Takano's* efforts ended with the tricyclic skeleton of dendrobine, whereas *Mori* and *Trost* finished their formal EPC-syntheses with intermediates of *Kende's* and *Roush's* racemic syntheses, respectively. Both completed dendrobine synthesis would have necessitated more than 20 steps.

Knowledge of polar and stereoelectronic effects of radical reactions became more transparent and the toxic stannanes could be used catalytically or be replaced by more convenient propagators, which led to a renaissance of radical reactions in total synthesis. *Livinghouse* made use of a reductive radical cyclization. Two enantiomerically pure syntheses of dendrobine (**82**) also reflect this trend. The above-mentioned EPC-synthesis by *Zard* contains one radical reaction that changed four bonds. However, the construction of the quaternary center originally planned as a radical reaction was changed to the *Pauson–Khand* reaction. Although the demand for enantiomerically pure syntheses became ever more pressing starting with the late 1960s, the first complete EPC-synthesis of dendrobine (**82**) was published by *Sha et al.* in 1997 (143). Their key step generating the quaternary center was an intramolecular radical addition reaction. Both *Zard's* and *Sha's* linear EPC-syntheses need more than 15 steps. *Sha's* synthesis includes EPC-syntheses of nobilomethylene (**45**), mubironine B (**98**), and dendrobine (**82**), and it has the second highest overall yield. The four earliest dendrobine syntheses have been reviewed previously (171).

6.1.2. Syntheses of Sesquiterpene PicROTOXANES

Due to the more complex structures of most sesquiterpene picROTOXANES compared with the dendrobines, fewer syntheses have been reported. Their structures with up to nine stereogenic centers were too complex to be used as test molecules for newly developed reactions. Three of the syntheses reported beginning with 1979 followed new strategies (two picROTOXININ syntheses and one corIAMYRTIN synthesis). The other syntheses of picROTOXININ (**1**), picROTIIN (**2**), corIAMYRTIN (**9**), TUTIN (**11**), corIANIN (**21**), methyl picROTOXATE (**42**), and asterOMURIN A (**22**) were extensions either of successful dendrobine syntheses or partial syntheses. Remarkably, with one exception, all the syntheses are EPC-syntheses.

The first picROTOXININ synthesis developed by *Corey and Pearce* is still the shortest EPC-synthesis of picROTOXININ (**1**) (*118*). The starting material was (–)-carvone. The construction of the quaternary center was achieved regio- but not stereoselectively by alkylation in an α -position to a *N,N*-dimethylhydrazone. The following cyclizations were based on aldol reactions. The most surprising step of this 20-step synthesis was the quantitative double lactone formation with lead tetraacetate. The authors extended their synthesis by converting picROTOXININ in four steps into picROTIIN (**2**) (*124*). *Inubushi et al.* were the next to publish a sesquiterpene picROTOXANE synthesis (*128*). These authors refrained from using the strategy they developed for dendrobine (**82**). Their protocol for racemic corIAMYRTIN (**9**) started with 5-methylenebutanolide. Then, 1,6-addition of the 2-methyl-1,3-cyclopentadione anion generated the quaternary center unselectively. Introducing the isopropenyl group by 1,4-addition initiated intramolecular *Claisen* condensation. Several nonselective steps counterbalance the advantage of this relatively short racemic synthesis. *Yamada* extended the strategy used for his dendrobine synthesis to synthesize picROTOXININ (**1**), corIAMYRTIN (**9**), TUTIN (**11**), and asterOMURIN A (**22**) (*52, 119*). The common starting material was a substituted tetralone, the first key step the *Johnson*'s tandem reaction, which generated the quaternary center. Optical resolution at a rather late step led to enantiomerically pure products. These syntheses of **1**, **9**, **11**, and **22** necessitated approximately 40 steps. The next EPC-synthesis of picROTOXININ (**1**) started with a derivative of (–)-carvone (*120*). The quaternary center was stereospecifically constructed via a *Claisen* rearrangement by *Yoshikoshi et al.* Altogether, 31 steps were necessary to afford picROTOXININ (**1**). These authors improved the partial synthesis of picROTIIN (**2**) from picROTOXININ (**1**) considerably. Once again, (–)-carvone was used as starting material in *Trost*'s EPC-synthesis of picROTOXININ (**1**) (*122*), employing the strategy developed for the synthesis of dendrobine (**82**). With newly developed palladium catalysts an ene reaction afforded the quaternary center, and the synthesis was completed after 29 steps. An intermediate of this synthesis was the starting point for the first EPC-synthesis of corIANIN (**21**), and a variant of this synthesis was published 3 years later. The number of steps and consequently the low yields of these two syntheses inspired *Trost* to look for a partial synthesis of this potential therapeutic starting with commercially available picROTOXININ. Here corIANIN (**21**) was obtained within nine steps in good yield. A further intermediate of this picROTOXININ synthesis was the starting point for the first

EPC-synthesis of methyl picrotoxate (**42**). *Trost's* syntheses were the last attempts toward sesquiterpene picrotoxanes. No efforts to synthesize C-18- or C-19-picrotoxanes ("norditerpene" picrotoxanes) have been reported so far.

The sequence in which the ensuing syntheses are described is according to their publication date, because this seems to be the best way to evaluate the novelty of the strategies and reactions and how much each author benefited from the experiences of earlier work in the field.

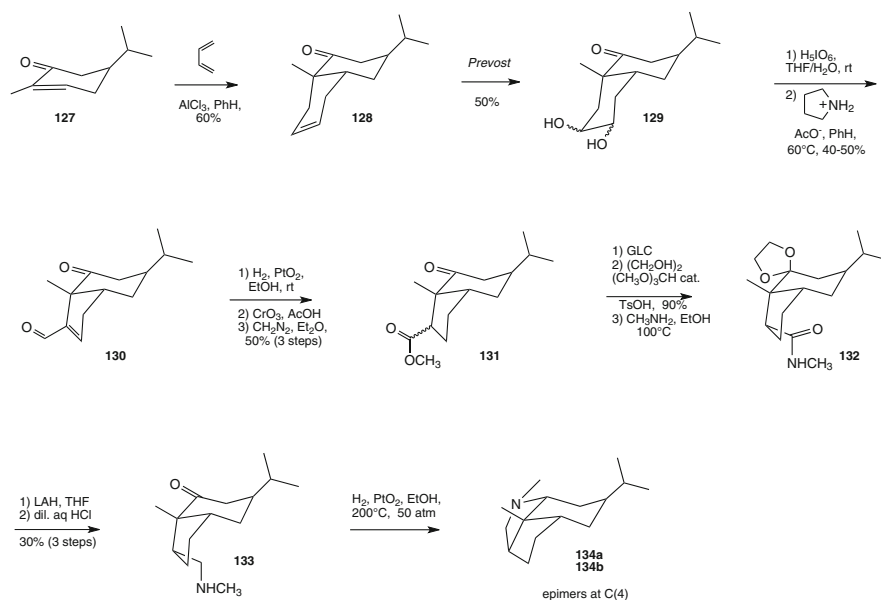
6.2. Description of the Syntheses

6.2.1. Syntheses of Dendrobine

Synthesis of the Skeleton of Dendrobine by *Kaneko et al.*

In 1970, the first attempt toward dendrobine was reported by *Kaneko et al.* (**150**). These authors synthesized the tricyclic skeleton of dendrobine (**82**) as an isomeric mixture.

The starting material was dihydrocarvone (carvotanacetone (**127**)), which is easily prepared from carvone (Scheme 12) (**172**). Thus, this strategy bears the prerequisite for an EPC-synthesis. The key step was the Lewis acid-catalyzed



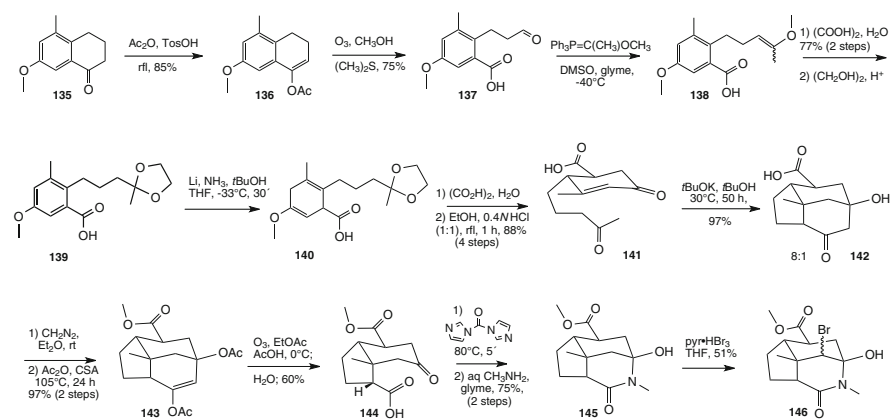
Scheme 12

intermolecular *Diels–Alder* reaction of butadiene and dihydrocarvone (**127**). The relative configuration of two of the stereogenic centers of dendrobine (**82**) was secured with this step. No data of the face selectivity of the reaction were given. Ring contraction of the newly formed cyclohexene of **128** was achieved by dihydroxylation to the isomeric mixture, **129**, periodate cleavage to the dial, and aldol reaction catalyzed by pyrrolidinium acetate. The desired regioisomer, **130**, was isolated in 20–25% overall yield. Hydrogenation over *Adams'* catalyst was followed by oxidation to the epimeric acids and esterification with diazomethane. The isomeric mixture of ketoesters **131** was separated by GLC. The *endo*-ketoester was protected as a cyclic ketal prior to conversion into the amide **132** with methylamine in ethanol in a sealed tube. Reduction to the amine was followed by deprotection of the ketone. The resulting aminoketone **133** was cyclized to the tricyclic amine **134** using hydrogen over platinum oxide at 200°C and 50 atm. Compound **134** turned out to be a mixture of two diastereomers (**134a** and **134b**).

Total Synthesis of (\pm)-Dendrobine by Yamada *et al.*

In 1971, Yamada *et al.* published their preliminary studies toward dendrobine (**82**) (**173**). Their starting material was the cheap, commercially available arene thymol, and the key step was an intramolecular *Robinson* annulation also known as *Johnson's* tandem reaction. After unexpected rearrangements within an ozonization, their protocol was modified and in 1972 Yamada *et al.* were able to present the first synthesis of (\pm)-dendrobine (**82**) (**146**).

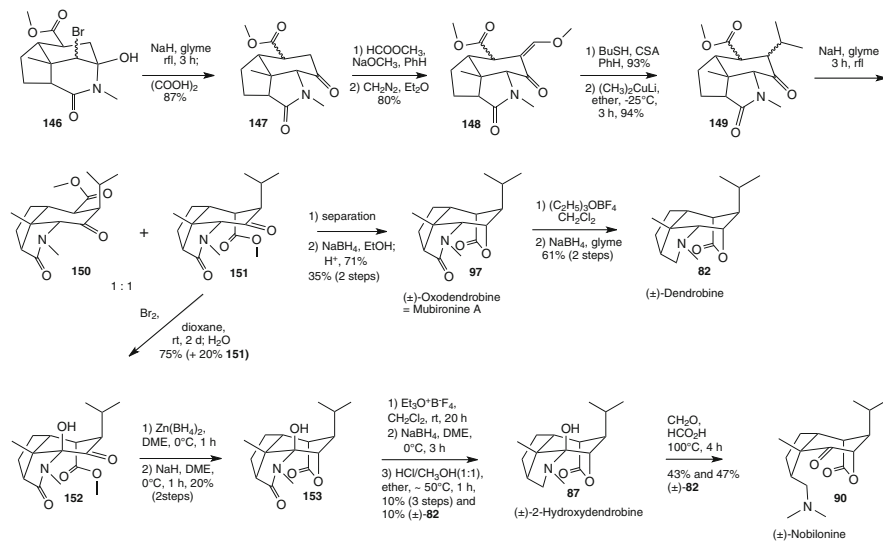
The authors had changed the starting material to the substituted tetralone **135** (**174**), which was converted into the enol acetate **136** when treated with acetic anhydride and acid (Scheme 13). Subsequent ozonization afforded aldehyde **137**. Chain elongation was achieved by *Wittig* condensation using *Corey–Chaykovsky*



Scheme 13

conditions. The resulting enol ether **138** was hydrolyzed with aqueous oxalic acid yielding the methyl ketone in 49% overall yield. After protection of the ketone as cyclic ketal **139**, the arene was reduced by *Birch* reaction and the resulting unsaturated enol ether **140** was hydrolyzed to the cyclohexenone. In a second step, the cyclic ketal was hydrolyzed with concomitant double bond shift to the conjugated enone attaining intermediate **141**, necessary for the key step of *Yamada's* synthesis (175). *Johnson's* tandem reaction afforded the tricyclic keto acid **142** in very high yield. After esterification with diazomethane, peracetylation under harsh conditions yielded the enol acetate **143** quantitatively. The following critical ozonization, which had forced the modification of the original synthesis plan, proceeded with this less crowded molecule (the isopropyl group is missing) in acceptable yield (60%) to the expected keto ester **144**. Then, the acid was converted into the secondary amide via the imidazolide and treatment with aqueous methylamine. This amide immediately cyclized to the tricyclic hydroxylactam **145**. The two missing substituents at the cyclohexane moiety were introduced starting with α -bromination using pyridinium hydrobromide perbromide. A possible explanation for the regioselectivity of this reaction is initial attack of bromine on the nitrogen of the lactam/amide and intramolecular transfer of bromine to the enol.

The monobromide(s) **146** were treated under strong basic conditions at elevated temperature, slowly generating the γ -lactone **147** by ring opening, and consecutive S_N2 reaction in 87% yield (Scheme 14). The isopropyl group was introduced in a four-step sequence. *Claisen* condensation with methyl formate led to a mixture of the enol and the enol ether, which by treatment with diazomethane, yielded in 80% the enol ether, **148**. To introduce the methyl groups, the enol ether was converted into the thioenol ether. 1,4-Addition of dimethyl copper lithium afforded the



Scheme 14

epimeric mixture of the isopropyl-substituted ketone **149** in 87% yield. Base treatment at elevated temperature isomerized this mixture to a 1:1 mixture of *cis* and *trans* disubstituted ketoesters **150** and **151**. After separation, ketone **151** was stereoselectively reduced by sodium borohydride and immediately cyclized to 14-oxodendrobine (**97**) in 35% yield from **149**. 14-Oxodendrobine (**97**) was first characterized as an oxidation product of dendrobine (**82**) and much later isolated as constituent of a cultivar of *D. nobile* and named mubironine A. Thus, this dendrobine synthesis is also a racemic synthesis of mubironine A (**97**). Transformation of 14-oxodendrobine (**97**) followed *Borch's* protocol (*176*). The intermediate lactim ether, which was produced with *Meerwein's* salt, was reduced with sodium borohydride affording racemic dendrobine (**82**) in 61% yield. Starting with substituted tetralone **135** *Yamada et al.* needed 23 steps to afford racemic dendrobine (**82**) in 1.2% total yield without recycling.

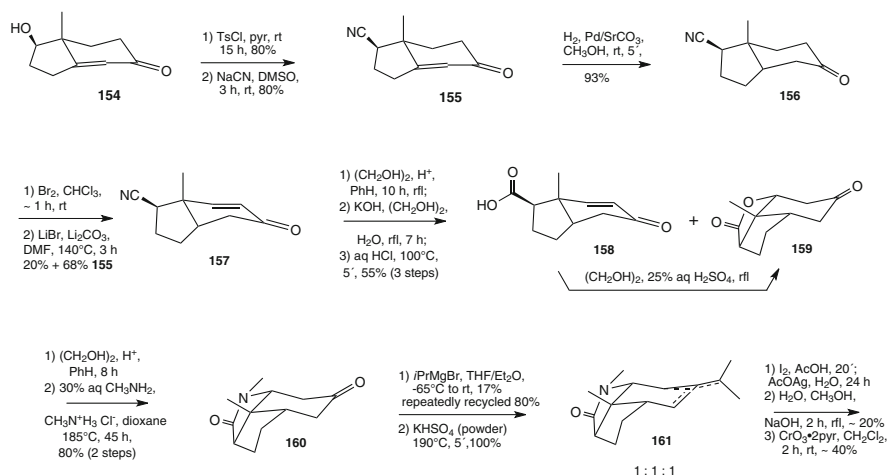
Synthesis of 2-Hydroxydendrobine and Nobilonine by *Yamada et al.*

To demonstrate the versatility of his synthesis strategy *Yamada* used ketoester **151** as relay substance to synthesize two further picrotoxane alkaloids isolated from *Dendrobium* species, nobilonine (**90**) and 2-hydroxydendrobine (**87**) (Scheme 14) (*84*). Monobromination of **151** with bromine in dioxane and subsequent treatment with water resulted in hydroxy- γ -lactam **152**, whereas attempts to hydroxylate **151** by Barton oxidation led to rearrangements. Chemo- and stereoselective reduction with zinc borohydride converted **152** into the *endo*-alcohol. To counterbalance the unfavorable conformational equilibrium this alcohol had to be converted into the alcoholate to achieve lactonization. Chemoselective reduction of the hydroxylactam moiety of lactone **153** again followed *Borch's* protocol, which led in this case to boron complexed amino compounds necessitating successive acid treatment to obtain racemic 2-hydroxydendrobine (**87**) in low yield accompanied by dendrobine (**82**). 2-Hydroxydendrobine (**87**) was converted into nobilonine (**90**) by *Eschweiler-Clark* methylation.

Total Synthesis of (\pm)-Dendrobine by *Inubushi et al.*

Inubushi announced the successful synthesis of dendrobine (**82**) in a short communication in the same year as *Yamada et al.* (*147*). In 1974, a full description of these efforts followed (*148*).

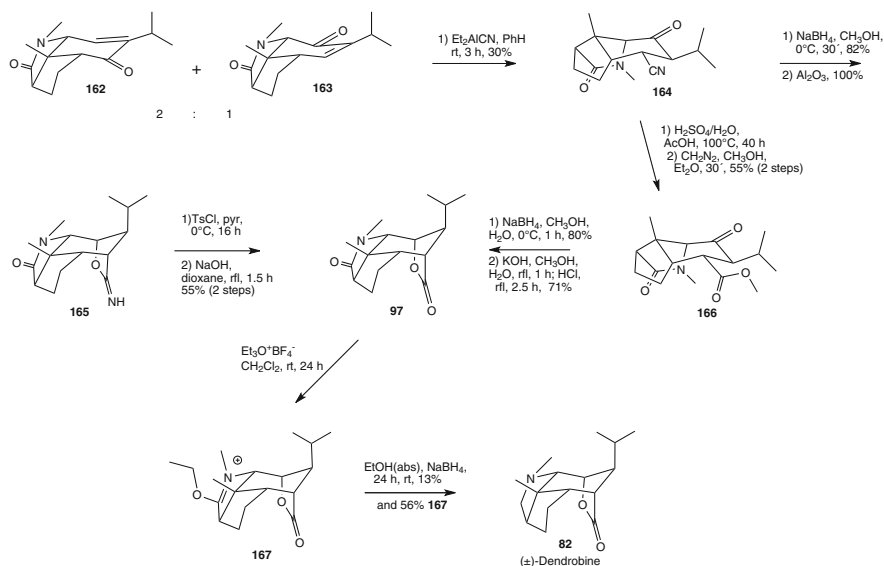
The synthesis started with the known hydrindenone derivative, **154** (Scheme 15) (*177*). Surprisingly, the group transformation of the secondary alcohol to the cyanide **155** via the tosylate occurred under retention of configuration in 64% yield. The authors were able to exclude a retroaldol-aldol reaction sequence as the reason for the unusual stereochemistry but did not further elaborate. To form the annelated pyrrolidinone, the enone was chemo- and stereoselectively reduced by



Scheme 15

hydrogenation with palladium on strontium carbonate leading in high yields to the *cis*-hydrindanone, **156**. Several methods were tested to introduce the unsaturation at $\Delta^{2,3}$ (picrotoxane numbering). The authors had to settle for bromination with one equivalent of bromine and elimination with lithium bromide and lithium carbonate in DMF at elevated temperatures. Even then, bromination at C(5) was preferred. Only 20% of the desired enone **157** were obtained next to 68% of the isomeric enone **155**. After protection of the keto group of **157**, the cyanide was hydrolyzed by strong aqueous base. Treatment with strong acid at elevated temperature led via carboxylic acid **158** to ketolactone **159**. After renewed protection of the keto group, harsh conditions were necessary to convert the lactone into the desired lactam **160**. Under these conditions, aqueous methylamine, methylammonium chloride at 185°C for 2 days, not only the amide was formed but the ketone was regenerated, which initiated dehydration to the conjugated enone and subsequent 1,4-addition of the amide afforded **160** in 80% yield. The introduction of the isopropyl group proved laborious. Addition of isopropyl magnesium bromide at room temperature to **160** led mainly to enolization. Since this work was done prior to *Luche's* discovery of the benefit of cerium trichloride, the authors had to be content to add the *Grignard* reagent at low temperatures and separate the starting material from 17% of adduct. Recycling of the recovered starting material multiple times led to good yields. After several trials, dehydration of the tertiary alcohol succeeded with powdered potassium hydrogen sulfate at elevated temperatures yielding quantitatively a 1:1:1 mixture of olefins **161**.

These olefins were transformed into unsaturated acetates by *Woodward's* protocol with iodine, silver acetate, and acetic acid. Saponification and subsequent oxidation of the liberated alcohols with *Collin's* reagent resulted in a 2:1 mixture of conjugated enones, **162** and **163**, in mediocre yields (Scheme 16). The minor enone **163** was transformed into the cyanoketone **164** by 1,4-addition using



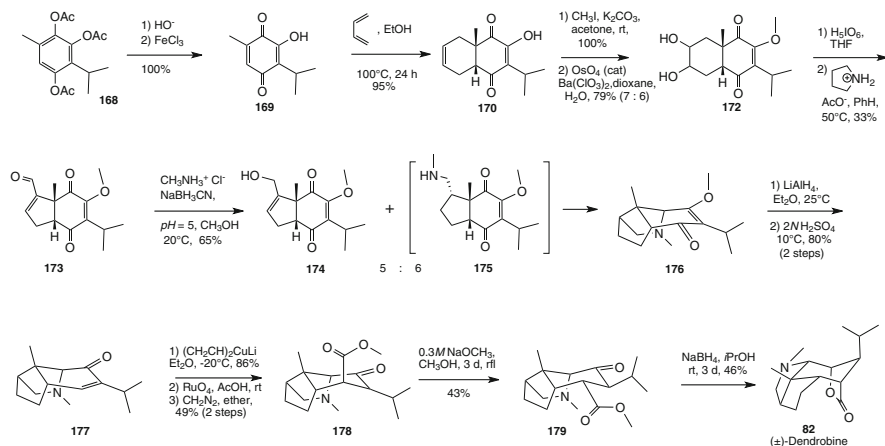
Scheme 16

Nagata's method. Reduction of the ketone with sodium borohydride stereoselectively led to the *endo*-alcohol, which on repeated chromatography on basic alumina cyclized to the iminoether, **165**. Its conversion into 14-oxodendrobine (**97**) was achieved by tosylation to the tosylamide and subsequent basic hydrolysis. The authors developed an alternative route from cyanoketone **164** to 14-oxodendrobine (**97**) by hydrolyzing the nitrile under acidic conditions. The acid formed was esterified with diazomethane and the ketone **166** was reduced stereoselectively with sodium borohydride. Subsequent saponification and acidic lactonization led to 14-oxodendrobine (**97**). *Inubushi et al.* also used *Borch's* method to convert 14-oxodendrobine (**97**) into dendrobine (**82**) via the lactimether **167** and reduction.

Yamada's and *Inubushi's* pioneering total syntheses proved that this complex, crowded molecule could be synthesized by means available in the late 1960s and early 1970s. Although the number of steps is high and selectivity and yields are low in *Inubushi's* synthesis, the knowledge gained by these authors was indispensable for the development of more economic synthesis planning.

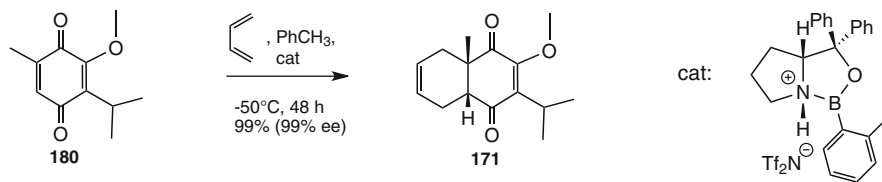
A Simple Total Synthesis of (\pm)-Dendrobine by *Kende et al.*

Kende's elegant racemic synthesis is still one of the shortest total synthesis of dendrobine (**82**), although no less than eight syntheses and four additional synthesis attempts followed (*151*). As *Yamada* in his first attempt, *Kende et al.* started with cheap, commercially available thymol, which was converted via thymoquinone and *Thiele-Winter*-acetoxylation into the known 1,2,4-triacetoxy-3-isopropyl-6-methylbenzene (**168**) (Scheme 17) (*178*).



Scheme 17

After saponification, the triol was oxidized to hydroxyquinone **169** with ferric chloride. Intermolecular *Diels–Alder* reaction of a quinone with butadiene secured not only the relative configuration of the two newly generated stereogenic centers but also quantitative yields even with the trisubstituted double bond (compare with *Kaneko's* key step). After methylation of enol **170** with methyl iodide to enoether **171**, ring contraction was achieved by dihydroxylation to **172**, periodate cleavage, and consecutive aldol reaction. As in *Kaneko's* case, the aldol reaction showed no regioselectivity even when catalyst, solvent, and temperature were varied. Thus, the authors had to be content with a 33% yield of the desired aldehyde, **173**. Nitrogen was introduced by reductive amination of **173** with methylamine and sodium cyanoborohydride under mild acidic conditions. Although sodium cyanoborohydride is known to reduce imines faster than aldehydes, the neighboring quaternary center decreased the rate of imine formation of the unsaturated aldehyde in this instance, so that reduction of the aldehyde to allyl alcohol **174** competed with iminium formation. The iminium generated was immediately reduced to the unstable saturated amine **175** by 1,4-addition of hydride, acidic enamine–iminium isomerization with protonation of the double bond from the convex face, and subsequent 1,2-addition of hydride. The saturated amine **175** spontaneously cyclized with the keto group of the enedione unit to the iminium salt, which was reduced to the tricyclic skeleton **176** of dendrobine (**82**). The mediocre yield of **176** was increased to 45% by one time recycling of allylic alcohol **174**. To introduce the missing carboxylic acid, the methoxy enone **176** was reduced to the enone **177** with lithium aluminum hydride. 1,4-Addition of divinylcopper lithium at -20°C was followed by oxidative fragmentation with ruthenium tetroxide. Esterification completed this reaction sequence in 42% overall yield. Ketoester **178** was epimerized with sodium methanolate yielding a mixture of starting material and epimer **179**, which was stereoselectively reduced to the *endo*-alcohol, which is identical with mubironine C (**99**). By prolonged reaction time the alcohol cyclized to racemic



Scheme 18

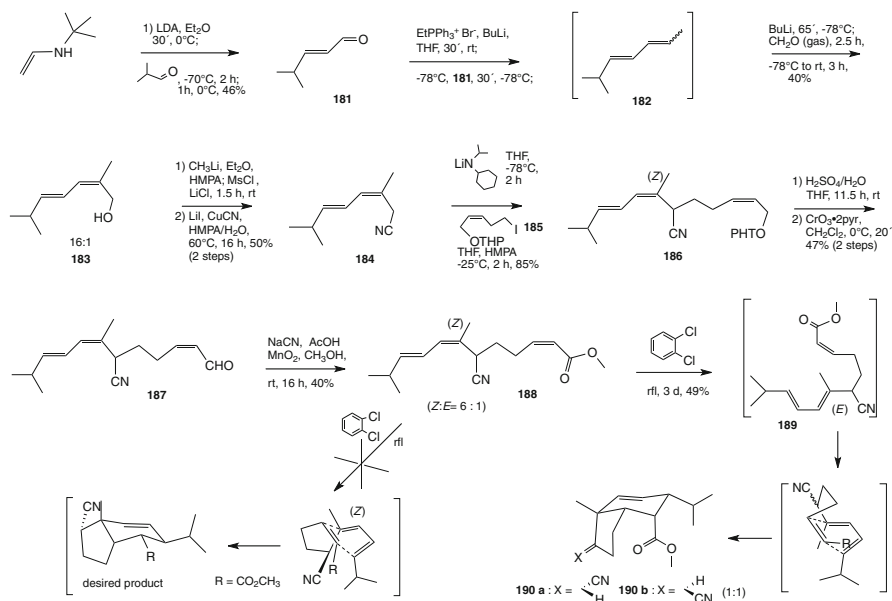
dendrobine (**82**) in 46% yield. This 15-step synthesis (with 0.6% overall yield including recycling, 16–18 steps from commercially available starting materials (**178**)) inspired two research groups (*Trost, Mori*) to plan their EPC-syntheses of dendrobine (**82**) with enone **177** as relais substance and *Corey et al.* proved the easy conversion of *Kende*'s racemic synthesis into an asymmetric synthesis (**152**). To demonstrate the versatility and power of chiral oxazaborolidinium cations as catalysts, *Corey et al.* chose key steps of racemic “classical syntheses of the twentieth century” and converted them into asymmetric reactions with very high enantiomeric excess. One of the chosen examples was *Kende*'s intermolecular *Diels–Alder* reaction.

Starting with quinone **180**, which was obtained by methylation of hydroxyquinone **169**, the oxazaborolidine-catalyzed intermolecular *Diels–Alder* reaction with butadiene was conducted at -50°C in toluene. Within 48 hours, 99% of the starting material was converted into adduct **171** in 99% optical purity (Scheme 18).

Synthesis of 4-Epi-Dendrobine by *Borch et al.*

The first synthesis using an intramolecular *Diels–Alder* reaction (IMDA) as key step was *Borch*'s intended (\pm)-dendrobine synthesis, which culminated in the synthesis of (\pm)-4-*epi*-dendrobine (**193**) (**153, 154**).

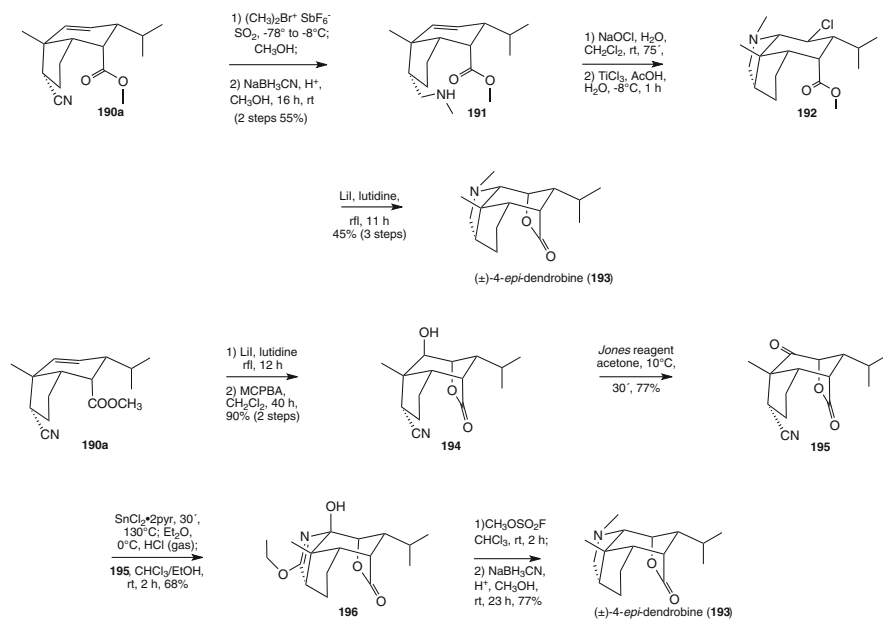
The convergent synthesis of the open chained precursor **188** started with a directed aldol condensation of 2-methylpropanal with *tert*-butyl(vinyl)amine (Scheme 19). In a one-pot reaction according to *Corey*, the generated enal **181** was transformed to dienol **183** (**179**). *Wittig* condensation of **181** with ethyl(triphenyl)phosphonium bromide and butyllithium as base formed diene **182**, which was deprotonated by a second equivalent of butyllithium. At low temperature the least-hindered vinyl proton was abstracted. Bubbling gaseous formaldehyde through this solution afforded the primary allylic alcohol **182** in high stereoselectivity (*(Z):(E)* = 16:1). This high stereoselectivity is obviously restricted to the more reactive gaseous formaldehyde as paraformaldehyde yielded a 1:1 mixture under identical conditions. The primary alcohol was substituted by cyanide via the mesylate and subsequent addition of lithium iodide and copper cyanide under highly polar conditions. Combining the resulting cyanide **184** with iodide **185**, prepared from *O*-protected propargyl alcohol in four steps and 40% overall yield, proved difficult. After many trials the following protocol met with success. Intermediate **184** was deprotonated by



Scheme 19

lithium isopropylcyclohexylamide. To this anion at -25°C was added precooled iodide **185** in HMTA/THF as solvent. The resulting dodecatriene **186** was deprotected with aqueous acid and the primary allylic alcohol was oxidized with a variant of *Collin's* reagent. The aldehyde **187** was converted into methyl ester **188** via the α -hydroxynitrile, oxidation to the aclylnitrile and methanolysis to the ester (**180**). The resulting mixture of *cis* and *trans* conjugated unsaturated esters ((Z):(E) = 6:1) was separated. Unexpected for an IMDA reaction, methyl (Z,Z,E)-6-cyano-7,11-dimethyl-2,7,9-dodecatrienoate (**188**) had to be heated to 170°C for 3 days to obtain a cyclized product. As it turned out, even those harsh conditions were not sufficient for cyclization, due to extreme steric hindrance. However, at 170°C the conjugated (Z,E)-dienyl cyanide unit became unstable. Monitoring the reaction by HPLC and NMR spectroscopy revealed isomerization to the (Z,E,E)-dodecatrienoate **189** in approximately 10%. The higher population of the *cisoid* diene conformation in **189** and the possibility of support by second order interactions (*endo* addition) permitted cyclization to the bicyclic nitriles, **190**. At the temperature necessary for the cyclization no face selectivity could be observed. Thus, the 1:1 mixture of *endo* nitrile **190a** and *exo* nitrile **190b** was separated and two synthesis pathways for **190a** to 4-*epi*-dendrobine (**193**) were designed.

Within the first pathway, reduction of the nitrile was facilitated by preliminary methylation with dimethylbromonium hexafluoroantimonate in liquid sulfur dioxide and subsequent methanolysis to the iminoether, which was treated with sodium cyanoborohydride (Scheme 20). The secondary amine **191** was chlorinated with sodium hypochlorite to the corresponding *N*-chloroamine to enable titanium

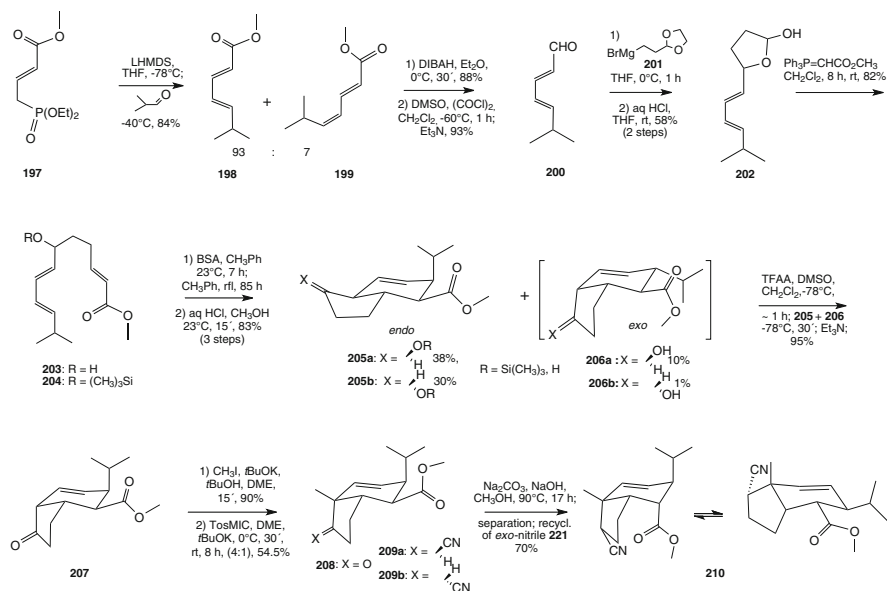


Scheme 20

trichloride-catalyzed radical addition to the double bond. Lithium iodide in lutidine at elevated temperature liberated the carboxylate of ester **192**, which formed the desired lactone, 4-*epi*-dendrobine (**193**), by substituting the chloride in 25% overall yield from **190a**. Although this first route is one step shorter than the second, the latter pathway achieved higher yields. Here, the alkyl saponification started the reaction sequence, which was continued by epoxidation with *m*-chloroperbenzoic acid. The resulting epoxide was attacked intramolecularly by the carboxylic acid affording lactone **194**. Jones oxidation yielded ketone **195**. Attempted reduction of the nitrile according to *Stephen* unexpectedly formed lactim ether **196** by *Lewis* acid catalysis. *N*-Methylation of **196** by methyl fluorosulfonate was followed by reduction with sodium cyanoborohydride affording 4-*epi*-dendrobine (**193**) in 36% overall yield from **190a**. The overall yield of route 1 (14 steps) is 0.18% and that of route 2 (15 steps) 0.26%.

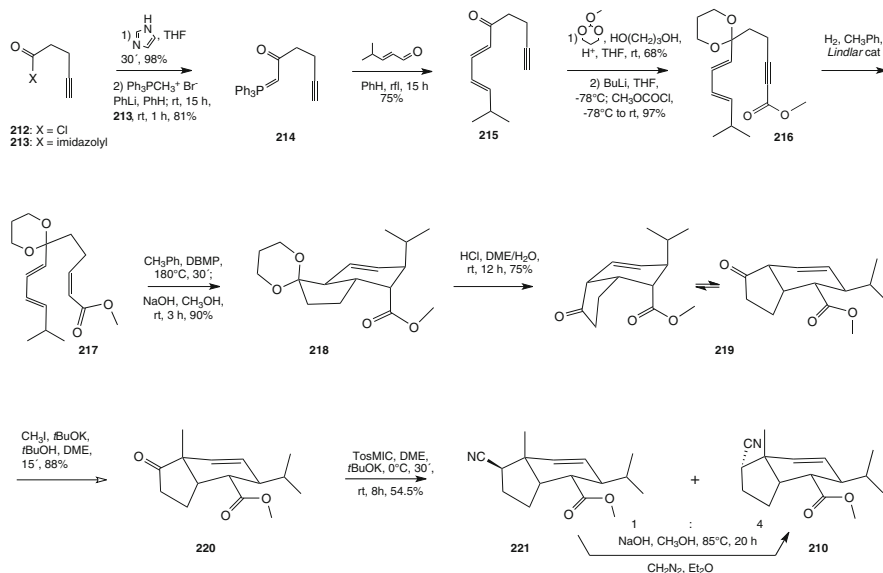
Total Synthesis of (±)-Dendrobine by Roush

Roush single-handedly succeeded in synthesizing racemic dendrobine (**82**) with IMDA as key step ([155](#), [156](#)). He diminished the steric hindrance of the key step by avoiding trisubstituted double bonds, thereby accepting a higher number of steps. An advantage of the belated introduction of the angular methyl group was that an (*E,E*)-diene could be used, thus raising the population of the *cisoid* conformation and enabling positive second order effects by *endo* addition.



Scheme 21

The necessary triene for the IMDA reaction was prepared starting with methyl 4-(diethylphosphono)crotonate (**197**) in 25% overall yield (181). Using a lithium base in the *Horner–Emmons* reaction of **197** with 2-methylpropanal increased the (*E*)-selectivity of the diene formation considerably (Scheme 21). After separation, methyl (*E,E*)-6-methylhepta-2,4-dienoate (**198**) was reduced to the primary alcohol and reoxidized to aldehyde **200**. Chain extension with *Grignard* reagent **201** was succeeded by hydrolysis leading to spontaneous cyclization to hemiacetal **202**. A second chain extension was achieved by *Wittig* reaction affording the (*E,E,E*)-2,7,9-triene **203** next to 5% of the corresponding (*Z,E,E*)-diene **211**. Protection of the secondary allyl alcohol of **203** as a silyl ether improved the yield of the IMDA reaction. Thus, bistrimethylsilylacetaamide (BSA) and **203** were heated in toluene to reflux for 3.5 days affording a mixture of *endo* adducts, **205**, and *exo* adducts, **206** (~6:1) in 79% yield. Although partial separation of the mixture of adducts was possible, the author proceeded with the whole mixture. After deprotection, the alcohols **205** and **206** were oxidized to the mixture of *cis*- and *trans*-hydrindenones. The unstable *trans*-hydrindenones immediately epimerized, and the mixture of *cis*-hydrindenones with ketone **207** as the main component was treated with exactly one equivalent of base, followed by an excess of methyl iodide. Now, separation was possible and pure ketone **208** was transformed into nitriles **209**. After several methods had failed, the nitrile was introduced by means of tolylsulfonylmethylisocyanide. The 1:1 mixture of nitriles attained was treated with base, thereby epimerizing the carboxylic group and partially the nitrile group, leading to the tricyclic nitriloester **210**. The remaining *exo*-nitriloester was

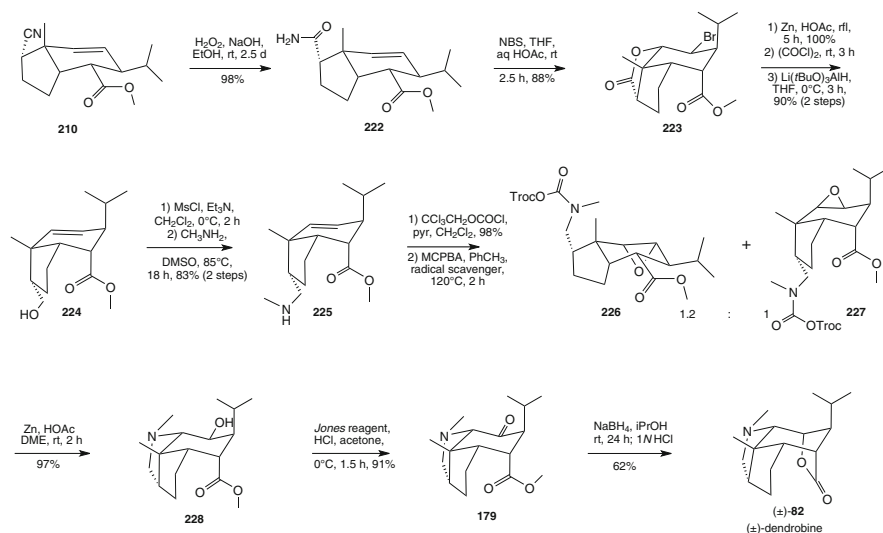


Scheme 22

recycled, accounting for 70% overall yield of **210**. To improve the yield of this reaction sequence, *Roush* examined the above-mentioned methyl (*Z,E,E*)-6-hydroxy-11-methyl-2,7,9-dodecatrienoate (**211**) for its use in an IMDA reaction. When he discovered that cyclization occurred mainly in the *exo* mode, he designed a shorter, higher yielding route to **210** (*157*) (Scheme 22).

Pent-4-ynoic chloride (**212**) was transformed to the imidazolyl derivative **213** to facilitate the introduction of triphenylphosphane methylene ylide. Refluxing the α -ketophosphane ylide **214** with (*E*)-4-methylpent-2-enal provided (*E,E*)-dienone **215** in 75% yield. After protection of the keto group as 1,3-dioxane, abstraction of the alkyne proton permitted addition to methyl chlorocarbonate. The resulting ester **216** was reduced with hydrogen and *Lindlar's* catalyst to the conjugated ester (*Z*)-ester **217**, the precursor of the IMDA reaction. Cyclization was achieved at 180°C in toluene with catalytic amounts of a radical scavenger within 30 min. Treatment of the resulting reaction mixture with sodium hydroxide in methanol selectively saponified the unchanged starting material **217**. Workup yielded an inseparable mixture of *exo*-adduct **218** and the *endo*-adduct (83:17). The adducts were deprotected with aqueous acid. Under these conditions the originally formed *trans*-hydrindenone isomerized to the *cis*-hydrindenone **219** in 75% yield. α -Methylation to **220** and transformation of the ketone to the nitriles **221** and **210** in a 1:4 ratio followed the former approach as did epimerization of **221** to **210**. This improved route yielded 12% of **210** within 12 steps starting with 4-pentynoic acid.

The next aim was the preparation of the secondary amine **225** (Scheme 23). Reduction of the nitrile as well as of the nitrilium salt led only to mediocre yields. Therefore, the author saponified the nitrile **210** chemoselectively to the amide **222**

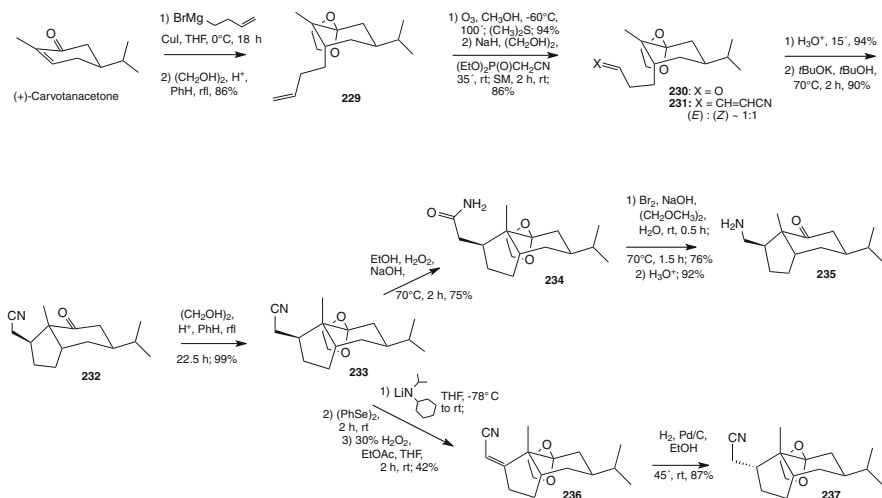


Scheme 23

with basic hydrogen peroxide. Bromolactonization to **223** was achieved with *N*-bromosuccinimide. Subsequent reductive elimination with zinc restored the double bond setting the carboxylic acid free. This acid was reduced to the primary alcohol **224** via the acid chloride and chemoselective reduction with lithium tri(*tert*-butoxy)aluminum hydride in excellent yield. Finally, the primary alcohol was esterified and the mesylate was substituted by methylamine. Several routes to the desired tricyclic amine **228** were examined with amine **225**. The best of these efforts was to protect the amine as trichloroethylcarbamate and subsequently oxidize the double bond to a mixture of *exo*- and *endo*-epoxides, **227** and **226**, respectively, with very low stereoselectivity. The *endo*-epoxide, **226**, was recycled to an amine, **225**, by epoxide cleavage with hydrogen bromide and successive reduction with zinc. The carbamate moiety of *exo*-epoxide, **227**, was removed with zinc and the liberated amine immediately cyclized to pyrrolidine **228**. Oxidation with *Jones* reagent formed ketone **179**, an intermediate of *Kende*'s dendrobine synthesis, which yielded racemic dendrobine (**82**) by selective reduction with sodium borohydride and chromatography on silica gel. The overall yield of the first version (26 steps) was 1.1% with recycling of **226**, whereas the improved version (24 steps) led with recycling of **226** to 1.7% overall yield.

Two of the Attempted Syntheses of (\pm)-Dendrobine by *Heathcock et al.*

In 1975, *Heathcock* and *Brattesani* presented failed attempts toward the basic skeleton of dendrobine (**82**) (*149*). From their efforts to achieve this task via two 1,4-additions, the more advanced is reported.

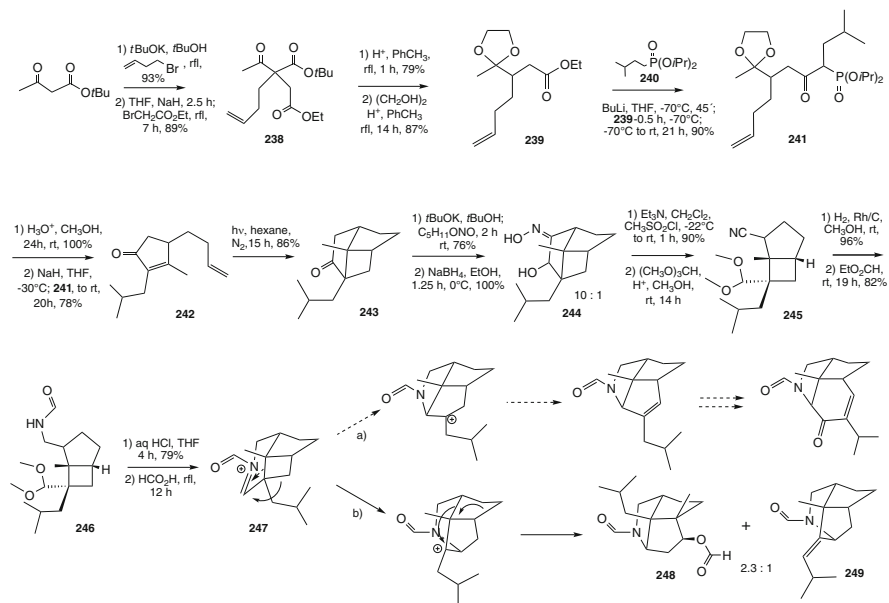


Scheme 24

(+)-Carvotanacetone (dihydrocarvone) was the chiral starting material. When added to but-4-enylmagnesium bromide and copper iodide, axial attack led stereoselectively to the desired ketone (Scheme 24). To extend the newly introduced unsaturated side chain, the cyclic ketone was protected as ketal **229**. Now, ozonolysis afforded aldehyde **230**. *Horner–Emmons* reaction with diethyl (cyanomethyl)phosphonate led unselectively to the (*E*):(*Z*) mixture of the unsaturated nitriles **231**. The protecting group was removed by aqueous acid and subsequent base treatment led to the second 1,4-addition in very good yield. As expected, the single stereoisomer **232** was the *cis*-hydrindanone, whereas the configuration of the second new stereocenter could not be determined at this stage. Thus, the ketone was protected again and the nitrile **233** was hydrolyzed under basic conditions to amide **234**. *Hofmann* degradation then led to the amine **235**. However, no cyclization occurred when the ketone was deprotected with aqueous acid. This fact revealed that the side chain at the cyclopentane moiety of **232** as well as of **235** was *exo*-positioned. The authors proved that epimerization of the side chain was possible by dehydrogenation and hydrogenation. Dehydrogenation was accomplished via selenation, oxidation, *cis* elimination, and hydrogenation with hydrogen over palladium on charcoal. The yields achieved for this reaction sequence to the desired nitrile **237** convinced the authors to abandon this synthesis plan.

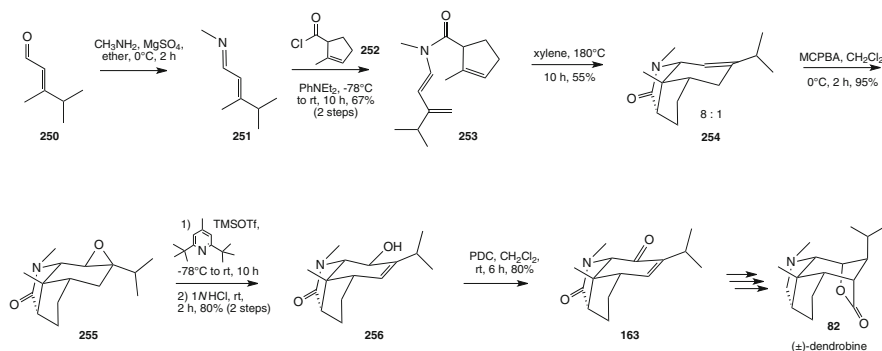
A quite different, ambitious strategy for the synthesis of dendrobine (**82**) presented by *Heathcock et al.* in 1985 (*162*). The key step was a cationic rearrangement of the tricyclic acyliminium ion **247** containing a four-, a five-, and a six-membered ring to the less strained system containing three five-membered rings, analogous to the cyclobutanecarbinyl rearrangement (*Cargill* rearrangement) (*182*). However, the synthesis pathway to **246** is quite lengthy.

tert-Butyl acetoacetate was alkylated twice, first with but-3-enyl bromide and then with ethyl α -bromoacetate, yielding the α,α -disubstituted acetoacetate **238** in



Scheme 25

83% yield (Scheme 25). Acid treatment removed the *tert*-butyl group and initiated the decarboxylation. The resulting ketoester was converted into the ketal **239** to permit addition of the anion of phosphonate **240** to the ester group. After hydrolysis of the ketal **241**, cyclization by intramolecular *Horner–Emmons* reaction afforded the tetrasubstituted double bond of the cyclopentenone **242** in good yield. Irradiation of **242** led to intramolecular (2 + 2) addition in very good yield. Of the two regiochemical possibilities, the less-strained tricycle **243** was formed exclusively. Ring expansion of the cyclopentanone **243** to the piperideinium **247** required ring cleavage by *Beckmann* fragmentation. To achieve this aim, the deprotonated ketone was treated with isoamyl nitrite, which led to the oxime via the nitroso compound. After reduction to alcohol **244**, mild conditions permitted selective mesylation of the oxime that immediately fragmented to the bicyclic nitrilaldehyde. The aldehyde was protected as dimethyl acetal **245**. Now, reduction of the nitrile preceded acylation to the formamide **246**. When the ketal was hydrolyzed, cyclization to the unstable acyl iminium **247** occurred. Strong acidic conditions initiated the rearrangement. It should be mentioned that the acylation is essential because the iminium ion itself is too stable to rearrange. The outcome of the rearrangement was by no means clear. Models predicted maximum continuous orbital overlap for pathway b whereas force–field calculations predicted less strain for the intermediate carbocation of pathway a. The experiment showed that the better alignment of orbitals influenced the activation energy more than the difference in sterical hindrance. Thus, the products **248** and **249** were formed exclusively by pathway b thwarting the synthesis plan.



Scheme 26

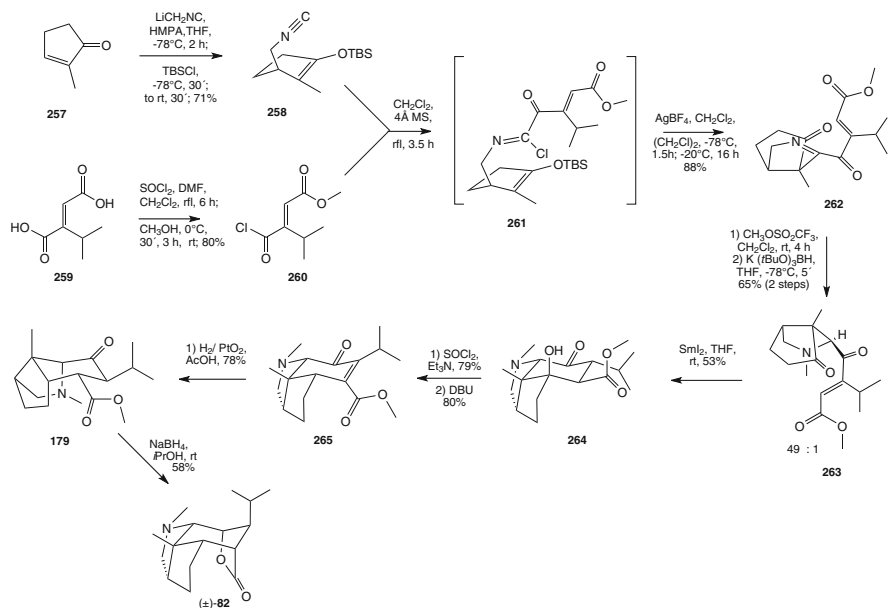
A Formal Synthesis of (±)-Dendrobine by *Martin et al.*

The next attempt was *Martin's* formal synthesis of racemic dendrobine (**82**) (*158, 159, 183*). Again, IMDA was used as the key step. *Martin et al.* replaced the usually lengthy linear synthesis of the IMDA precursor by a more convenient, convergent synthesis. After testing the feasibility of their key step, the intramolecular addition of a dieneamide to an unactivated trisubstituted olefin, with model compounds, the authors started with the synthesis of the diene unit (Scheme 26).

In a short known reaction sequence, enal **250** was obtained from commercially available material (*184*). With methylamine and magnesium sulfate imine **251** was formed and combined with acyl chloride **252** (*185*) (≥ 4 steps). The use of low temperatures for this acylation led exclusively to the less substituted dienamide **253**. The desired basic skeleton of dendrobine **254** was obtained by cyclizing **253** at 180°C in an acceptable 50% yield. *endo*-Adduct **254** was accompanied by small amounts of the *exo*-adduct. Epoxidation led exclusively to *exo*-epoxide **255**, which by means of trimethylsilyltriflate was converted into the allylic silyl ether. Acid treatment liberated the hydroxy group and subsequent oxidation of alcohol **256** led to enone **163**, an intermediate of *Inubushi's* dendrobine synthesis and thus concluded this formal synthesis. The intermediate **163** was obtained from commercially not available materials in seven steps in 22.5% overall yield. To reach (±)-dendrobine according to *Inubushi et al.* would afford six additional steps, reducing the overall yield to 0.4% without including the preparation of the starting materials from commercially available compounds.

An Efficient Total Synthesis of (±)-Dendrobine by *Livinghouse et al.*

In 1987, *Livinghouse* and *Westling* published a general method to prepare 2-acylpyrrolines via acylnitrilium alkyne cyclization (*163*). In 1992, they presented



Scheme 27

the advantageous use of their method in a short convergent synthesis of racemic dendrobine (**82**) (164).

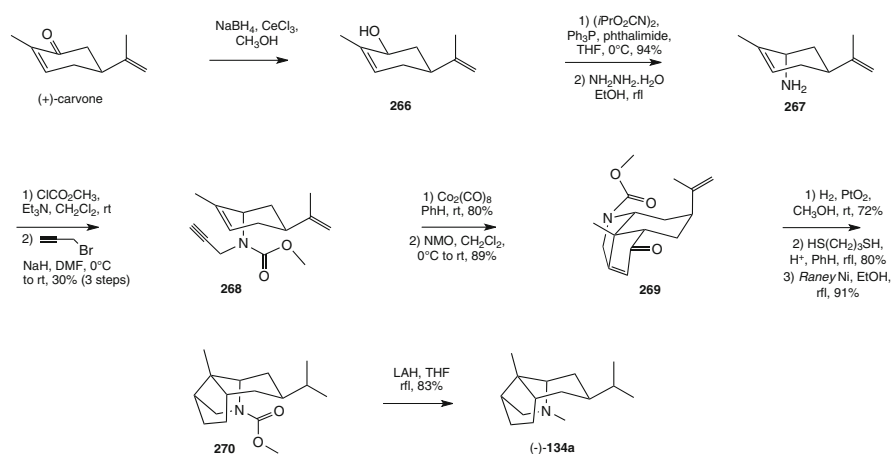
Methylisocyanide lithium was added to commercially available 2-methylcyclopent-2-enone **257** (Scheme 27). The enolate formed by this 1,4-addition was silylated *in situ*. Note that, despite the low temperatures and the use of lithium ions, addition of HMPA to the reaction mixture sufficed to largely suppress the product of 1,2-addition. Fumaric acid was converted into 2-isopropylfumaric acid (**259**) in a known four-step sequence (186). This diacid was converted into the desired monoacyl chloride monomethyl ester **260** via its bisacyl chloride and selective methanolysis. At slightly elevated temperature, acid chloride **260** formed the chloroimine acyl adduct **261** with isocyanide **258**. Silver ion abstracted the chloride of **261** and the highly reactive acyl nitrilium salt attacked the electron rich enol ether double bond forming bicyclic pyrroline **262** in very good yield. Methylation with methyl triflate led to the *N*-methyl iminium salt. Its reduction to the tertiary amine **263** proved difficult. Finally, a highly stereoselective reduction to **263** was achieved with voluminous potassium tri(*tert*-butoxy)borohydride. The following cyclization to the tricyclic ketoester was originally planned by 1,4-addition of alkyl phosphite and successive intramolecular *Horner–Emmons* reaction but failed. Thus, one electron reduction with samarium diiodide was examined. Whereas reaction at low temperature led to 5-*exo-trig*-cyclization and subsequent lactonization, reaction at room temperature gave tricycle **264** via the desired 6-*endo-trig*-cyclization accompanied by up to 10% of the saturated bicyclic compound. This result does not permit one to decide between ketyl

radical 1,4-addition or reduction of the double bond and subsequent aldol-like reaction. The stereochemistry of the reaction was verified by X-ray diffraction analysis of **264**. Dehydration with thionyl chloride was followed by double bond isomerization with strong base yielding tricyclic compound **265**. Hydrogenation over *Adams'* catalyst in acetic acid occurred from the convex side of the molecule. Subsequent acid assisted epimerization led to keto ester **179**, an intermediate of *Kende's* synthesis. Following *Kende's* reduction led to racemic dendrobine (**82**). This short convergent synthesis is the most economic synthesis (10 steps from **259** (or 14 steps from fumaric acid), 7% overall yield) of racemic dendrobine (**82**) published so far.

A Facile EPC-Approach to the Dendrobine Skeleton by *Takano et al.*

With the EPC-synthesis of the tricyclic skeleton of dendrobine, **134a**, *Takano et al.* intended to demonstrate the effectiveness of the *Pauson–Khand* reaction (**165**).

(*S*)-Carvone was stereoselectively reduced to allylic alcohol **266** according to *Luche* (Scheme 28). *Mitsunobu* reaction with phthalimide and successive hydrazinolysis transformed **266** into the allylic amine **267**. The authors examined the optical purity of **267** and found a slight decrease (3% racemization) compared to the starting material that they explained with modest participation of S_N2' reaction in the *Mitsunobu* reaction. After the amine **267** was converted into its carbamate, subsequent substitution of propargyl bromide under strongly basic conditions set the stage for the *Pauson–Khand* reaction. The tricyclic carbamate **269** was produced in 71% yield via the enyne cobalt complex, cyclization with insertion of carbon monoxide, and removal of the heavy metal by oxidation with *N*-methylmorpholine *N*-oxide. The double bonds were reduced by hydrogenation with *Adam's* catalyst to avoid epimerization of the isopropyl group. Subsequently, the ketone was removed



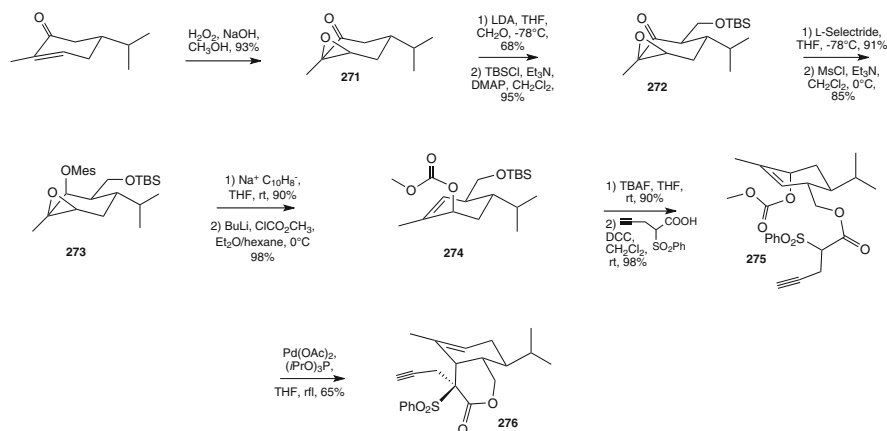
Scheme 28

via dithioacetal formation and successive treatment with *Raney* nickel. Reduction of carbamate **270** with lithium aluminum hydride yielded the tricyclic skeleton of (–)-dendrobine **134a**.

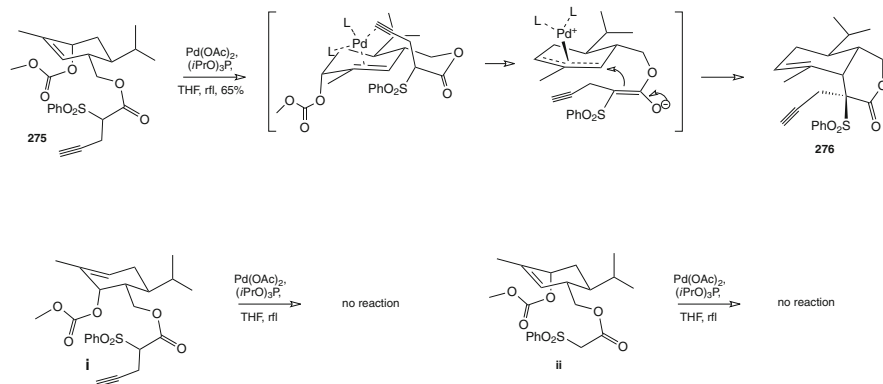
Formal Total Synthesis of (±)-Dendrobine by *Trost et al.*

Trost and *Krische* reported the first formal synthesis of (–)-dendrobine (**82**) (*170*, *187*). Their intention was to demonstrate the versatility and broad range of palladium catalyzed reactions (*188*). Two different types of palladium-catalyzed cyclizations mark the central part of this EPC-synthesis.

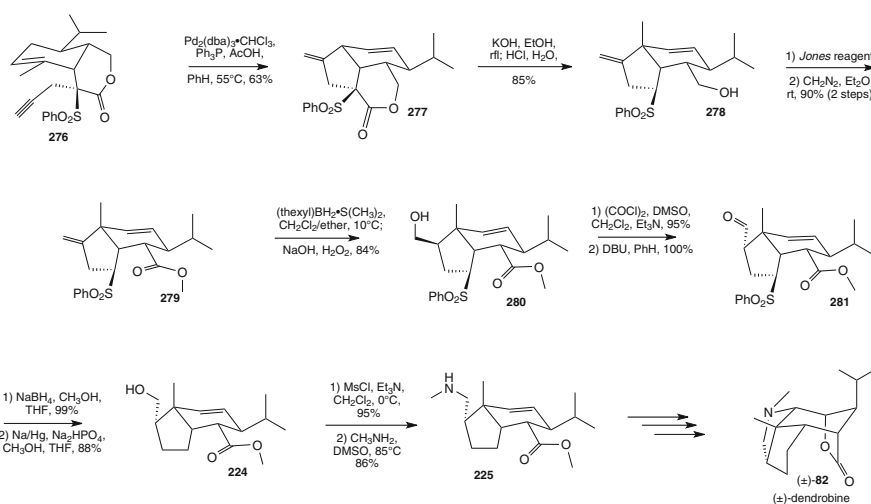
The starting material was the easily available, (–)-dihydrocarvone, which was epoxidized with basic hydrogen peroxide prior to introduction of a C-1 unit (Scheme 29). Formaldehyde was added to the lithium enolate of ketoepoxide **271** at low temperatures and the carbinol generated was protected as silyl ether **272**. Note the high stereoselectivity of this aldol reaction, which suggests that the enolate of **271** is attacked as conformer with the isopropyl group in the pseudoaxial position. Chemo- and stereoselective reduction of the ketone was achieved with *L*-selectride at low temperatures and the *axial* alcohol formed was esterified to mesylate **273**. Reductive elimination by sodium naphthalide proceeded via one electron attack of the mesylate and cleavage of the epoxide. The allylic alcohol was protected as methyl carbonate **274**. To obtain high yields and stereoselectivity, the projected palladium-catalyzed allyl alkylation was conducted intramolecularly. Therefore, silyl ether **274** was deprotected and the resulting primary alcohol was esterified with 2-(phenylsulfonyl)pent-4-ynoic acid and *N,N'*-dicyclohexyl carbo-diimide. This nine step sequence led in 36% overall yield to the enyne, **275**, the precursor of the first palladium-catalyzed cyclization. Palladium diacetate and triisopropyl phosphite as ligand led in high stereoselectivity to lactone **276**. *Trost et al.* discovered an interesting effect when experimenting with this allyl alkylation.



Scheme 29



Scheme 30



Scheme 31

Their original option was to use isomer **i**, because less sterical hindrance for the complexation of the double bond with the palladium was expected (Scheme 30). When no reaction occurred with several ligands, **275** was chosen, which produces the same (allyl)palladium complex. *Trost* assumed that preliminary complexation of the palladium to the alkyne and intramolecular transposition to the double bond as the reason for this surprising result. Indeed, when **ii**, where the alkyne is absent, was treated under identical conditions as **275**, no reaction occurred (Scheme 30).

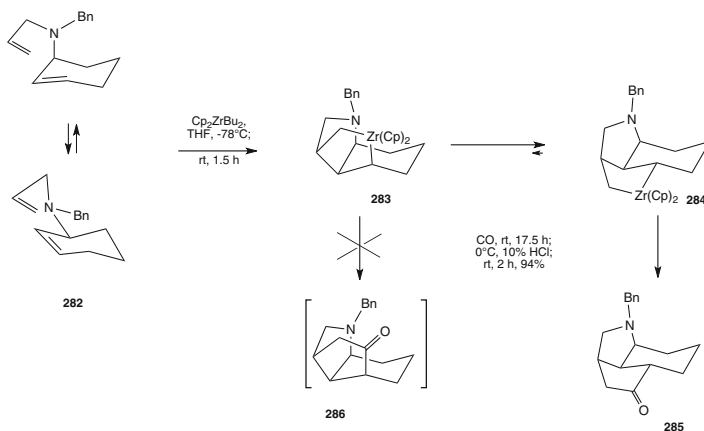
Palladium-catalyzed cycloisomerization of lactone **276** produced the tricyclic lactone **277** (Scheme 31). The lactone was saponified and the generated acid was decarboxylated to the bicyclic sulfone **278**. Oxidation of the primary alcohol to the acid with *Jones* reagent was followed by esterification yielding **279**. Now, the

methylene group was selectively hydrated to the undesired *exo*-carbinol **280** by hydroboration with voluminous dimethylsulfide complexed hexyl borohydride and oxidative removal of the borane. Note that the angular methyl and the phenylsulfonyl group prevented attack from the convex face of the molecule. To epimerize the carbinol, it was oxidized to the aldehyde, which epimerized under basic conditions to *endo*-aldehyde **281**. After reduction of the aldehyde with sodium borohydride, the obsolete sulfone was removed with sodium amalgam yielding **224**, an intermediate in *Roush's* racemic dendrobine synthesis. Thus, *Trost et al.* achieved a formal total EPC-synthesis of dendrobine (**82**) in 20 steps and 8% overall yield. To reach dendrobine (**82**) following *Roush's* synthesis from **224** on would increase the number of steps to 27 and decrease the overall yield to 1.5% (without recycling).

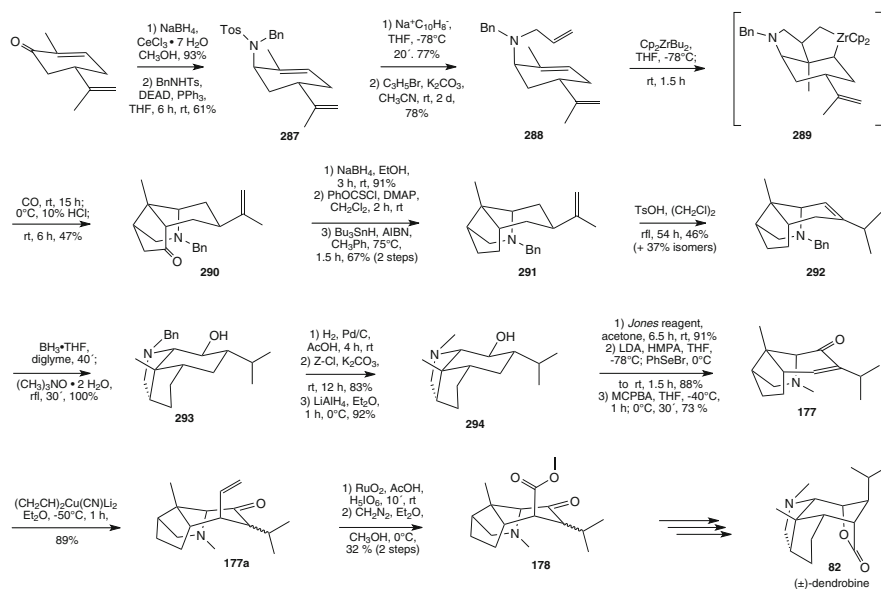
Formal Total Synthesis of (–)-Dendrobine by *Mori et al.*

Whereas *Trost* and *Takano* established the usefulness of late transition metals and their complexes for the construction of the dendrobine skeleton, *Mori et al.* demonstrated the advantage of the more stable early transition-metal complexes in the synthesis of dendrobine (**82**) (167–169). They presented their formal EPC-synthesis centered on *Negishi's* (*Taber's*) zirconium-assisted cyclization in several publications (189, 190). *Mori et al.* tried to test their key step with a model synthesis (191).

Dibutylbis(cyclopentadienyl)zirconium was added to *N*-allyl-*N*-(benzyl)cyclohex-2-enylamine (**282**) and the generated zirconacycle was treated with carbon monoxide (Scheme 32). The metal was removed with acid and the tricyclic ketone **285** was isolated in excellent yield. The relative configuration of the newly formed stereogenic centers could not be determined at this point, which turned out to be advantageous. The authors assumed according to kinetic preference that the tricyclic **286** with all *cis* stereochemistry had been formed and started the



Scheme 32



Scheme 33

actual EPC-synthesis. However, it was known that 1,6-heptadiene and 1,7-octadiene formed *cis*-fused zirconacycles, which equilibrated to the *trans*-fused ones. *Taber's* semiempirical ZINDO calculation predicted equilibration and thus structure **285** (192). Eventually, an X-ray diffraction analysis confirmed this structure. Meanwhile, *Mori et al.* had succeeded with their formal dendrobine synthesis, because the additional substituents in the actual zirconacycle **289** changed the equilibrium or prevented equilibration (Scheme 33). This is a nice reminder of the ambiguity of model reactions.

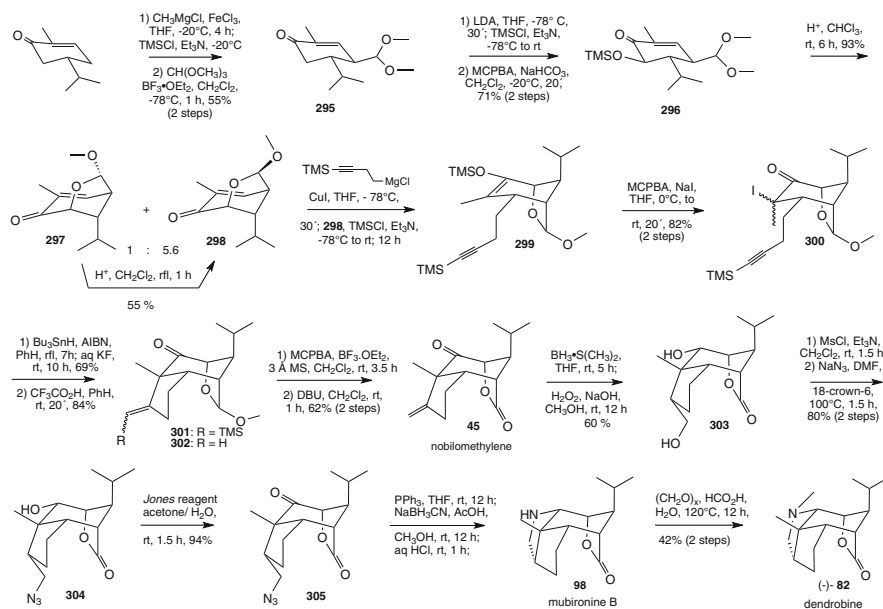
The EPC-synthesis started with (+)-carvone, which was reduced according to *Luche* (Scheme 33). The equatorial hydroxyl group was substituted by benzylsulfonamide via *Mitsunobu* reaction. The now obsolete sulfonate of **287** was removed by one-electron reduction with sodium naphthalide and the liberated amine was allylated with allyl bromide under basic conditions yielding the precursor **288** of the zirconium-assisted cyclization. After addition of dibutylbis(cyclopentadienyl)zirconium, the atmosphere was changed to carbon monoxide. Addition of acid completed the reaction sequence toward the tricyclic dendrobine skeleton **290** in 47% yield. This structure was verified by X-ray analysis. Deoxygenation of the keto group proceeded by reduction with sodium borohydride, esterification of the alcohol, and reduction of the thionocarbonic ester with tributylstannane. Strong acid isomerized the double bond of unsaturated amine **291** to the mixture of unsaturated amines with **292**, the isomer with the double bond in the desired $\Delta^{3,4}$ position, as the main isomer in 46% yield. The preference of the trisubstituted double bond is presumably due to $\sigma^*-\pi$ interaction with the highly polar C–N⁺ bond. Recycling also transformed the residual isomers to **292**. Hydroboration and subsequent

oxidation afforded alcohol **293**. Now, the benzyl substituent at nitrogen was exchanged against methyl by hydrogenolysis, transformation to the carbamate, and reduction with lithium aluminum hydride. To introduce the missing carboxylic group, alcohol **294** was converted into the enone **177** by oxidation to the ketone, α -selenation, oxidation to the selenoxide, and spontaneous *cis* elimination. With **177**, an intermediate of *Kende's* racemic synthesis was obtained. *Mori et al.* then followed *Kende's* synthesis to ketoester **178**. This formal synthesis required 17 steps in 4% overall yield. If supplemented with five steps according to *Kende*, this dendrobine synthesis needs 22 steps in 0.75% overall yield.

First Total EPC-Synthesis of (–)-Dendrobine by *Sha et al.*

The first total synthesis of (–)-dendrobine (**82**) was presented by *Sha et al.* (**143**). The key step of their synthesis was a newly developed radical cyclization (**193**, **194**).

The authors used (*S*)-carvotanacetone (dihydrocarvone) as starting material (Scheme 34). To prepare the linearly conjugated silylenol ether, they used the *Kharash* protocol and attained γ -alkylation by *Mukaiyama* aldol reaction with trimethylorthoformate (**195**). The ketoacetal **295** was α -hydroxylated according to *Rubottom* by silylenol ether formation followed by epoxidation and silyl migration. Acid treatment transformed **296** to the epimeric cyclic acetals **297** and **298**. *endo*-Acetal **297** was equilibrated thereby increasing the amount of *exo*-acetal **298**. The necessary unsaturated side chain for the prospected radical cyclization was introduced by 1,4-addition of a (trimethylsilyl)butynylcopper compound.



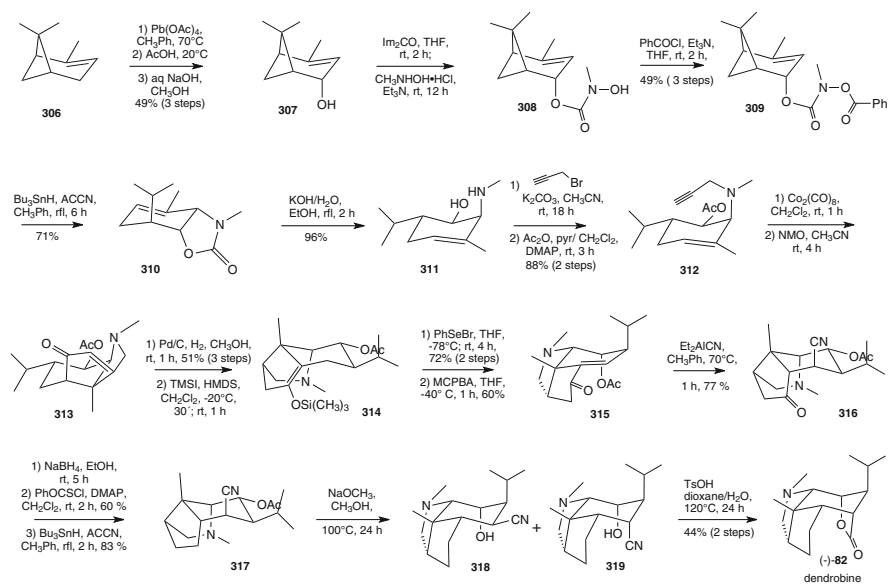
Scheme 34

Subsequent silylation of the enolate led to silylenol ether **299**. α -Iodoketone **300**, the precursor of the radical cyclization, was formed by a variation of the *Rubottom* reaction with peracid and sodium iodide in 30% overall yield from carvotanacetone (dihydrocarvone). Tributylstannane with AIBN as starter abstracted the iodide and the α -ketoradical added to the alkyne. Consecutively, the vinyl radical abstracted hydrogen from the stannane. The tricyclic vinyltrimethylsilane **301** was desilylated with trifluoroacetic acid. Acetal **302** was converted into nobilomethylene (**45**) by *Lewis* acid-catalyzed S_N1 reaction to the peroxyacylal and subsequent base treatment. Hydration with borane and successive oxidation afforded *endo*-carbinol and simultaneously reduced the ketone, yielding **303**, the C(13)-epimer of naturally occurring dendrobiumane B (**31**). Selective esterification of the primary alcohol of **303** with mesyl chloride permitted exchange against azide anion. To overcome the steric hindrance, the reaction was run at 100°C in a polar solvent with added crown ether. The secondary alcohol of azide **304** was reoxidized with *Jones* reagent to ketone **305**. *Staudinger* reaction led via azaphosphorane to the cyclic imine, which was reduced to the tetracyclic amine, **98**, a compound isolated as mubironine B from a cultivar of *D. nobile*. *Eschweiler-Clark* reaction led to (–)-dendrobine (**82**). Starting with (*S*)-dihydrocarvone, the first EPC-synthesis of dendrobine (**82**) was achieved within 17 steps in 3.3% overall yield. This EPC synthesis is the second highest yielding synthesis of dendrobine but is also the first EPC synthesis of nobilomethylene (**45**) and mubironine B (**98**).

Formal Total Synthesis of (–)-Dendrobine by *Zard et al.*

This EPC-synthesis of dendrobine (**82**) is based on (+)-*trans*-verbenol (**307**) as starting material and *Pauson-Khand* reaction as the key step ([144](#), [166](#)).

Commercially available (+)- α -pinene (**306**) was converted by a known reaction sequence into **307** (Scheme 35) ([284](#), [166](#)). The hydroxy group of this monoterpene was transformed to the *N*-hydroxycarbamate **308** via the imidazylcarbamate and treatment with *N*-methylhydroxylamine. After *O*-benzoylation to **309**, the cyclic carbamate was formed by homolysis of the N–O bond with tributylstannane and addition of the nitrogen-centered radical to the double bond ([196](#)). The tertiary carbon radical attacked the strained single bond of the cyclobutane moiety and the reaction cascade was completed by saturation of the isopropyl radical. The generated oxazolidinone **310** was saponified to the *vicinal cis*-hydroxyamine **311**. *Zard* originally planned to use a nickel mediated radical cyclization of α -chloroacylamides formed from **311**. After this plan failed, the authors turned to the *Pauson-Khand* reaction, which *Takano* had employed successfully in his synthesis of the tricyclic skeleton of dendrobine (**82**). Thus, the secondary amine **311** was converted into the tertiary propargylamine with propargyl bromide. Subsequently, the secondary alcohol was protected as acetate **312**. Whereas *Takano* had used the neutral amide, *Zard et al.* wanted to examine the *Pauson-Khand* reaction in the presence of an amine. When they used cobaltoctacarbonyl and *N*-methylmorpholine-*N*-oxide in methylene chloride, the desired tricyclic product, **313**, was obtained in low yield accompanied by an

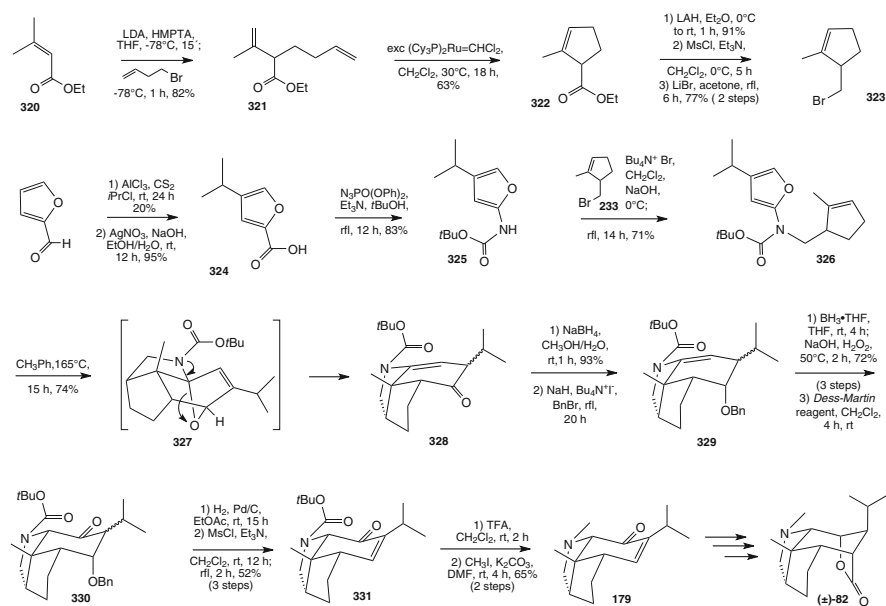


Scheme 35

unexpected bicyclic compound. Employing solvents with increasing complexation power increased the yield of the regular *Pauson–Khand* reaction product. Acetonitrile as solvent suppressed the by-product and yielded the unstable tricyclic enone **313** in acceptable yield. After hydrogenation over palladium on charcoal as catalyst, the saturated ketone was regioselectively transformed into the higher substituted silylenol ether **314** with trimethylsilyl iodide and hexamethyldisilazane as base. Crude **314** was treated with phenylselenyl bromide resulting in the unstable α -selenyl ketone. Oxidation to the selenoxide led to spontaneous *cis*-elimination. The enone **315** was transformed to ketonitrile **316** by 1,4-addition with *Nagata's* reagent. Despite the neighboring alkyl groups, the reagent attacked from the convex face of the molecule. The now obsolete ketone was removed by reduction to the *endo*-alcohol, which was esterified to the thionocarbonate and reductively removed with tributylstannane and ACCN as starter. The acetate of **317** was transesterified with methanolate yielding the alcohol and partially epimerizing the nitrile. The mixture of hydroxynitriles **318** and **319** was heated with acid in dioxane/water. Hydroxynitrile **319** led via the lactimether to (–)-dendrobine (**82**), whereas **318** was inert under these conditions but could be recycled. Starting with α -pinene, (–)-dendrobine (**82**) was reached in 21 steps and 0.5% overall yield. Starting with verbenol, the target was accomplished in 18 steps and 1% overall yield.

Formal Total Synthesis of (±)-Dendrobine by *Padwa et al.*

The most recent attempt toward a synthesis of dendrobine (**82**) was reported by *Padwa et al.* (160, 161). Their formal racemic synthesis of dendrobine is a typical



Scheme 36

example of probing the versatility of a newly developed key step by constructing a highly complex molecule. Their key step was an IMDA reaction that triggered a cascade of consecutive reactions (197, 198). The precursor of the key step was prepared in a convergent manner.

Ethyl 3-methylbut-2-enoate (**320**) was α -alkylated by but-4-enyl bromide (Scheme 36). Ring-closing metathesis of diene **321** with an excess of first generation *Grubbs*' catalyst afforded the trisubstituted double bond of racemic cyclopentenyl ester **322**. The ester was converted into methyl bromide **323** by reduction to the primary alcohol, mesylation, and substitution by lithium bromide. Then, the bromide was substituted by carbamate **325**, under basic conditions assisted by a phase transfer catalyst, yielding the desired precursor **326** of the key step. The heteroaromatic carbamate **325** was prepared starting with furfural. *Friedel-Crafts* alkylation introduced the isopropyl group in β -position of the furan in mediocre yield. The aldehyde was now oxidized by silver nitrate yielding acid **324**. *Curtius* rearrangement was achieved by *Shiori*'s protocol leading to carbamate **325** (199). With **326** in hand, the stage was set for the cascade reaction by heating the compound in toluene to 165°C over 15 h. Under these conditions, IMDA reaction produced tetracycle **327**, which rearranged to the conjugated unsaturated acyliminium alcohol. Double bond isomerization to the conjugated hydroxydieneamide and subsequent ketone formation completed the cascade yielding the tricyclic skeleton **328** of dendrobine (**82**) as 2:1 mixture of epimers at C(4). Eight further steps were necessary to reach *Kende*'s intermediate, **179**. At first, the keto group of **328** was selectively reduced with sodium borohydride and

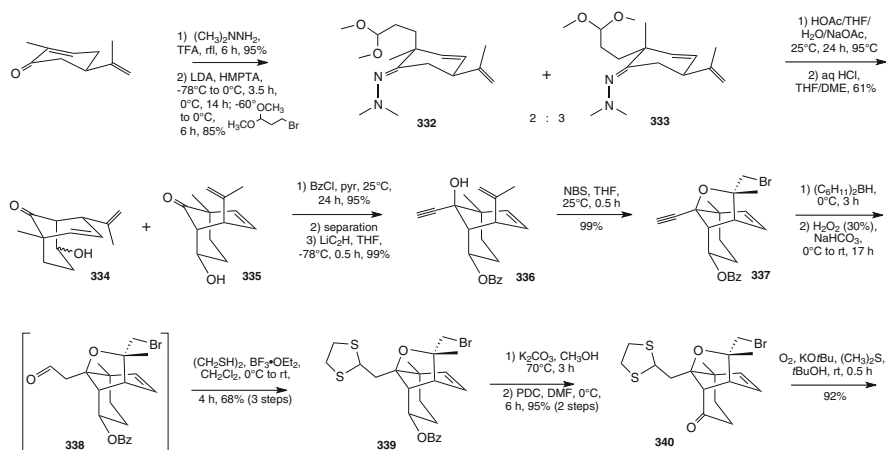
protected as benzyl ether **329**. Now, *anti*-Markovnikov hydration with borane and successive oxidation yielded the secondary alcohol, which was oxidized to ketone **330**. After removal of the protective benzyl group by hydrogenolysis, dehydration to the enone **331** was achieved via mesylation and $E1_{cB}$ reaction. The carbamate was removed with strong acid and reaction with methyl iodide led to the tertiary amine, **179**. Thus, the authors needed 18 steps in an overall yield 2.6% to reach *Kende's* intermediate **179** starting from commercially available materials. Supplemented by five additional steps according to *Kende*, this synthesis of (\pm)-dendrobine (**82**) would need 23 steps in 0.5% overall yield. These figures belittle *Padwa's* synthetic efforts, as starting as most other authors did with literature known compounds (**323** and **324**) would have reduced the number of steps to *Kende's* intermediate to 11 steps and thus increased the overall yield considerably to 10%.

6.2.2. Syntheses of Sesquiterpene PicROTOXANES

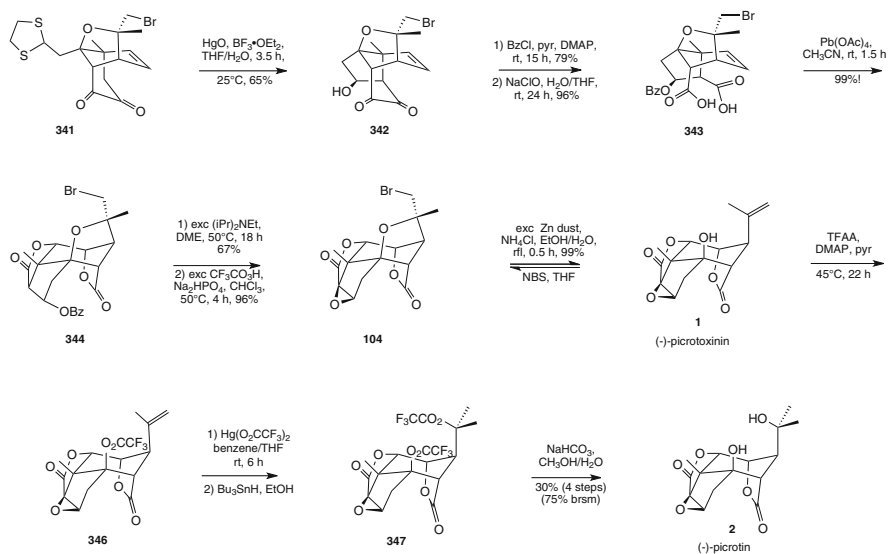
Total EPC-Synthesis of PicROTOXININ and PicROTIN by *Corey et al.*

The first synthesis of this group of sesquiterpenes was *Corey's* and *Pearce's* EPC-synthesis of picROTOXININ (**1**), which, due to the originality of its steps and relative shortness, has become a classic (*118, 200, 201*). To form the quaternary center they used a method partly developed for this synthesis.

To achieve this goal, (–)-carvone was transformed into the dimethylhydrazone, and the unsaturated hydrazone was deprotonated by LDA (Scheme 37). Due to complexation of the lithium ion with nitrogen, 3,3-dimethoxypropyl bromide was used to alkylate the α -position (**202**). A 3:2 mixture of the two possible stereoisomers **332** and **333** was obtained. The authors claimed that the ketone was



Scheme 37



Scheme 38

recovered from the hydrazone by buffered acidic hydrolysis and that stronger acid was necessary to hydrolyze the acetal. The mixture of the ketoaldehydes generated cyclized spontaneously under these conditions. Although the reaction was carried out at room temperature, the *endo*-alcohol **335** was formed stereoselectively. After benzylation, the mixture of aldols was separated and lithium acetylde was added to the O-protected **335** at -78°C over a short period, affording exclusively the tertiary alcohol, **336**. To differentiate between the unsaturated bonds, bromoetherification protected the exocyclic double bond as well as the tertiary alcohol and shielded the endocyclic double bond. Now, the triple bond of **337** was converted into the unstable aldehyde **338** via hydroboration and subsequent oxidation with basic hydrogen peroxide. The aldehyde was protected as cyclic dithioacetal **339** and transesterification with methanolate yielded the secondary alcohol, which was oxidized with pyridinium dichromate to ketone **340**.

Barton oxidation was the key to form the 1,2-diketone **341** in surprisingly high yield, in order to close the five-membered ring (Scheme 38). The conditions chosen for the deprotection of the aldehyde, mercuric oxide and boron trifluoride etherate, at room temperature, immediately led to aldol **342**. After protection of the newly formed secondary alcohol as a benzoate, the diketone was fragmented quantitatively with excess sodium hypochlorite. Cyclization of the generated diacid **343** to the desired dilactone **344** proved very difficult. After a variety of methods failed, the use of lead tetraacetate (**203**), preceded by work performed within the structure determination of picrotoxinin (**1**), was spectacularly successful (**204**). In 99% yield, the simultaneous formation of both lactones was achieved. E1_{cB} reaction with an excess of tertiary amine removed the benzoate of **344** and the double bond formed was epoxidized with peracid affording β -oxirane **104** stereoselectively. Treatment of

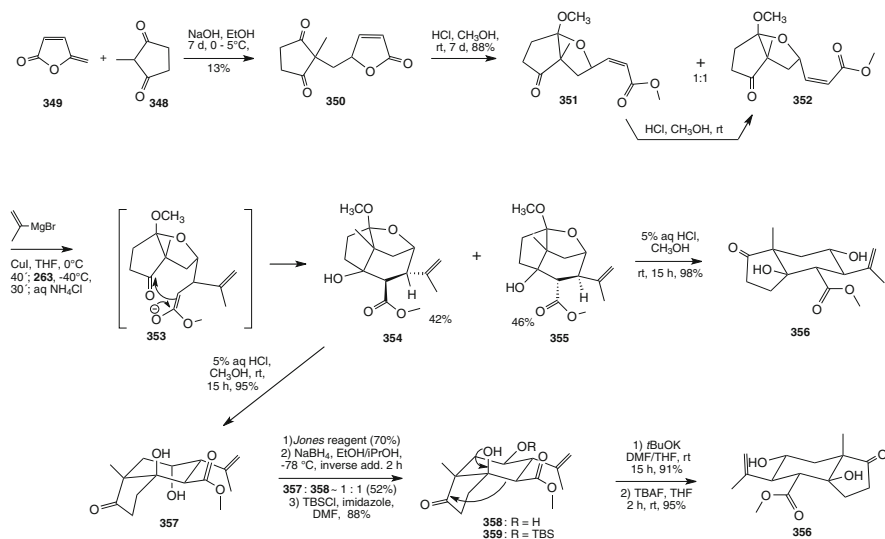
the bromoether with zinc under mild conditions afforded picrotoxinin (**1**) quantitatively. Thus, the first EPC-synthesis of picrotoxinin (**1**) was achieved within 20 steps in an unbelievable 5.2% overall yield.

Conversion of synthetic picrotoxinin (**1**) to picrotin (**2**) was achieved by *Corey and Pearce* in four steps and 30% overall yield (*124*). To prevent intramolecular oxymercuration, the tertiary alcohol of picrotoxinin (**1**) was protected as trifluoroacetate **346** prior to addition of mercury trifluoroacetate in a benzene/THF mixture as solvent. Demercuration with sodium borohydride failed, thus the covalent C–Hg bond was cleaved by tributylstannane in ethanol. Mild hydrolysis of the bistrifluoroacetate **347** afforded picrotin (**2**) in 30% overall yield (75% corrected for recovered **1**) from picrotoxinin (**1**).

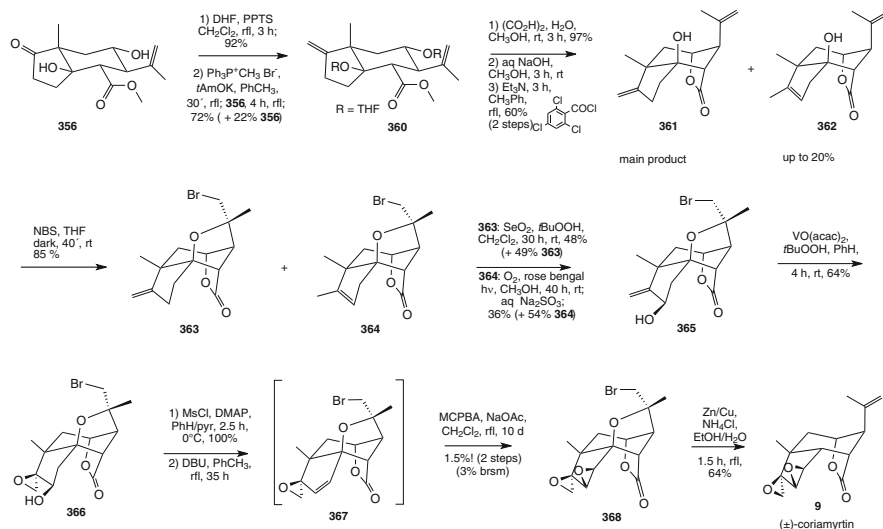
Total Synthesis of (±)-Coriamyrtin by *Inubushi et al.*

The first synthesis of racemic coriamyrtin (**9**) was presented by *Inubushi et al.* (*128, 129, 205, 206*). To achieve this aim, the authors developed a new protocol for the preparation of highly substituted *cis*-hydrindanones.

1,6-Addition of the anion of 2-methyl-1,3-cyclopentadione (**348**) to protoanemonin (**349**), easily prepared from levulinic acid in four steps (*207*), led to adduct **350** in low yield due to ready self-condensation (Scheme 39). Transesterification with acidic methanol set the alcohol free, which cyclized spontaneously to the 1:1 mixture of ketals **351** and **352**. To enhance the yield of the ketal **352**, ketal **351** was recycled by equilibration in acidic methanol. Under *Kharash–Grignard* conditions, the isopropenyl group added unselectively in 1,4-mode and the ester enolate **353**



Scheme 39



Scheme 40

generated attacked the ketone. The mixture of the tricyclic esters **354** and **355** was converted into the desired *cis*-hydrindanone, **356**. Whereas **355** hydrolyzed quantitatively to **356**, the conversion of **354** necessitated several steps. After hydrolysis to the *cis*-hydrindanone **357**, the secondary alcohol was inverted by oxidation and subsequent unselective reduction with sodium borohydride. After separation and recycling, the secondary alcohol of **358** was protected as silyl ether **359**. Treatment with a strong voluminous base led to an interesting rearrangement. Retro-*Claisen* condensation was followed by attack of the ester enolate at the original keto group forming the isomer of **359**. The small differences in steric hindrance between the two isomeric silyl ethers point to the unfavorable orbital overlap for retro-*Claisen* reaction in the main conformation of the silyl ether of **356** as the reason for the surprisingly high yields of this isomerization. The high population of this conformer explains the lack of lactonization under the desilylation conditions. The removal of the silyl group completed the laborious detour to *cis*-hydrindanone **356**.

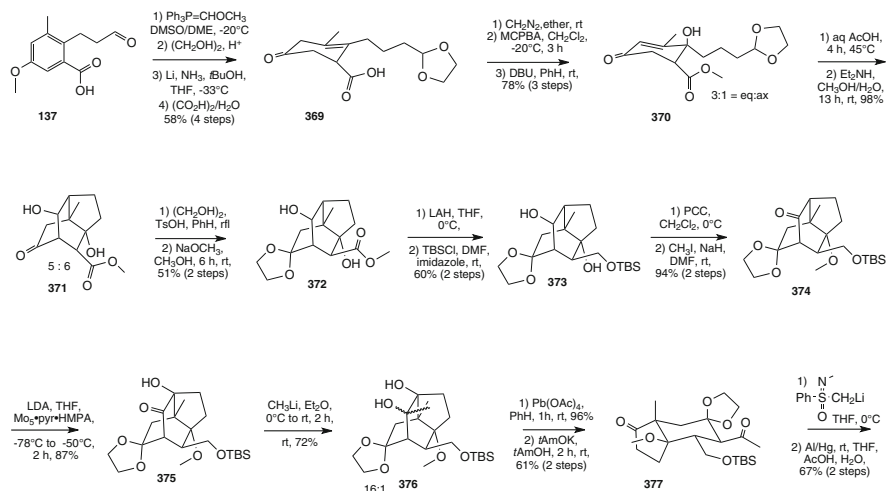
Both hydroxy groups of **356** were protected as tetrahydrofuranyl ethers to gain high yield in the subsequent methylenation by Wittig condensation (Scheme 40). After the protecting groups of **360** were removed with aqueous oxalic acid, the methyl ester was saponified to enable lactonization with Yamaguchi's reagent. Unfortunately, the reaction conditions chosen for this reaction sequence destabilized the newly introduced methylene group, leading up to 20% to the endocyclic double bond. To differentiate between the double bonds of **361** and **362**, the double bond of the respective isopropenyl groups were protected as cyclic bromoethers **363** and **364**. The methylene group of the main product **363** was α -hydroxylated with selenium dioxide and *tert*-butyl hydroperoxide, whereas the endocyclic double bond of the minor product **364** underwent allylic oxidation with singlet oxygen

and subsequent reduction of the hydrogen peroxide with sulfite. The allylic alcohol **365** attained with both isomers was epoxidized. To ascertain attack from the convex face of **365**, despite the vicinity of the angular methyl group, neighboring group assisted *Sharpless* oxidation was chosen. Dehydration of epoxide **366** via mesylation and base treatment led to the unstable allylic epoxide, **367**. Epoxidation with peracid in deplorably low yield afforded bisepoxide, **368** and reductive elimination with a zinc/copper couple completed the synthesis of racemic coriamyrtin (**9**). *Inubushi*'s synthesis of (\pm)-coriamyrtin (**9**) is the shortest sesquiterpene picrotoxane synthesis (16 steps) published so far. Unfortunately, non-selective and low yielding steps lessen this accomplishment. With recovery of the side products, thus increasing the number of steps to at least 21, the overall yield remains at $\sim 0.01\%$.

Total Synthesis of (–)-Picrotoxinin and (+)-Coriamyrtin by *Yamada et al.*

As described above, *Yamada et al.* have published the first racemic synthesis of dendrobine (**82**). They demonstrated the versatility of this synthesis strategy by synthesizing two further *Dendrobium* alkaloids. Experience gained in their execution and variation of the synthetic strategy enabled *Yamada et al.* to design syntheses of the more complex sesquiterpene picrotoxanes. Again, the authors proved the flexibility of this synthetic protocol by synthesizing not only picrotoxinin (**1**) and transforming the synthesis to an EPC-synthesis by separation of a racemic intermediate but also by synthesizing coriamyrtin (**9**) (*119*), tutin (**11**) (*130*), and a constituent of a scale insect, asteromurin A (**22**) (*52, 135, 208*).

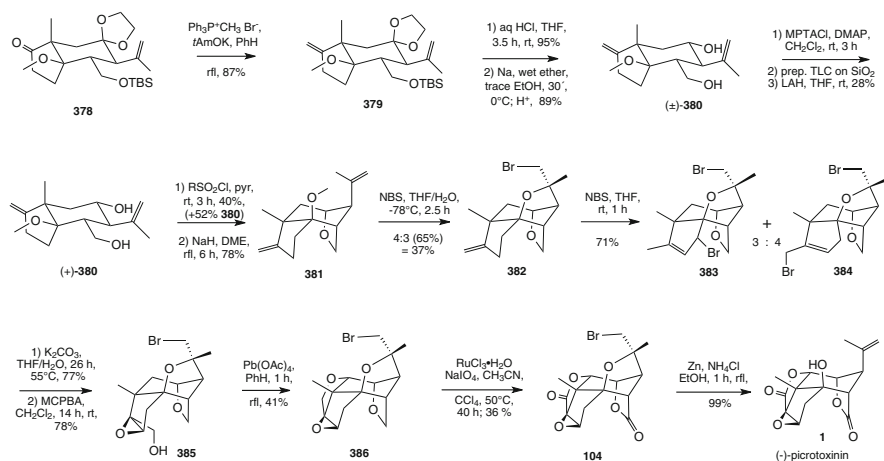
Their picrotoxinin synthesis started with cheap aromatic compounds and followed the pathway elaborated for dendrobine (**82**) to the tetrasubstituted aromatic aldehyde **137** (Scheme 41). Chain elongation with phosphanemethoxymethylide to the methyl enolether, ketalization, *Birch* reduction, and selective hydrolysis of the endocyclic enolether are analogous to the dendrobine synthesis. The β,γ -unsaturated keto acid **369** was converted into the methyl ester before the tetrasubstituted double bond was oxidized with peracid yielding a 3:1 mixture of *exo*- to *endo*-epoxide, then $E1_{cB}$ reaction led to γ -hydroxyenones **370**. Deprotection of the aldehyde with aqueous acid afforded the precursor of the key step, *Johnson*'s tandem reaction, which led, assisted by diethylamine, in near quantitative yield to a 5:6 mixture of the epimeric tricyclic esters, **371**. After protection of the keto group as cyclic ketal to prevent retroaldol reactions, base treatment epimerized the *endo*-ester, affording exclusively *exo*-ester **372** in 51% yield. Now, the ester was reduced to the primary alcohol, which was selectively protected by *tert*-butyl(dimethyl)silyl chloride. The secondary alcohol of **373** was oxidized and the tertiary alcohol protected as a methyl ether. Ketone **374** was α -hydroxylated according to *Vedejs* (*209*). Note that despite the *anti-Bredt* conditions the reaction proceeded in 87% yield to hydroxyketone **375**. This oxidation preceded the fragmentation to the *cis*-hydrindanone, **377**. To achieve this goal, methyl lithium was added to the ketone affording the tertiary alcohol **376** with high stereoselectivity. Lead tetraacetate fragmented the *vicinal*



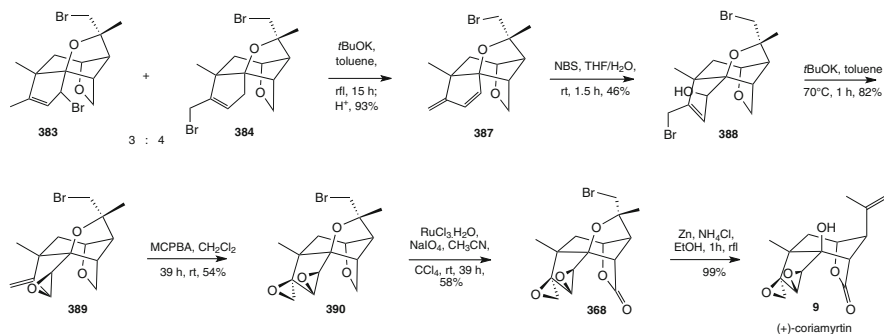
Scheme 41

diol and base treatment epimerized the newly formed methyl ketone. The equatorial acetyl group of **377** was converted into the isopropenyl group by means of [(*N*-methyl)phenylsulfimidoyl]methyl lithium and successive reduction with aluminum amalgam according to Johnson (210).

The sterically more hindered ketone of **378** was transformed also to the methylene group. Here, Wittig condensation met with success. Attempts to shorten the synthesis by methylenation of both ketones of **376** simultaneously led to a very low yield, whereas the three-step variant achieved 58% overall yield of *cis*-hydrindane **379**. Its ketal was hydrolyzed and reduced subsequently to the secondary alcohol. All attempts to effect this reduction with hydrides led to the undesired axial alcohol. Thus, the authors turned to one-electron reduction with sodium in wet ether, which indeed gave stereoselectively the equatorial alcohol, and acidic workup cleaved the silyl ether. Optical resolution of the racemic diol **380** followed Mosher's protocol (211). Thus, excess MPTA Cl with DMAP as catalyst led to the diastereomeric diesters, which were separated by preparative TLC. The less polar product was then reduced with lithium aluminum hydride yielding pure (+)-**380**. The enantiomerically pure diol was cyclized to the tricyclic ether **381** via esterification with camphorsulfonyl chloride and successive base treatment. Differentiation of the two double bonds and simultaneous cleavage of the tertiary methyl ether was achieved by cyclic bromoether formation with *N*-bromosuccinimide at low temperatures in a polar solvent. Surprisingly, in contrast to other reports of bromoether formation of picrotoxanes, very low stereoselectivity was observed in the bromoether formation of **381**. The 4:3 mixture of epimers **382** was separated and both epimers could be converted into picrotoxinin (**1**). However, the authors reported only the experimental data of the epimer depicted in Scheme 42. Allylbromination with *N*-bromosuccinimide of **382** led to a 3:4 mixture of the primary and secondary bromide, **383** and **384**. Aqueous base substituted both



Scheme 42



Scheme 43

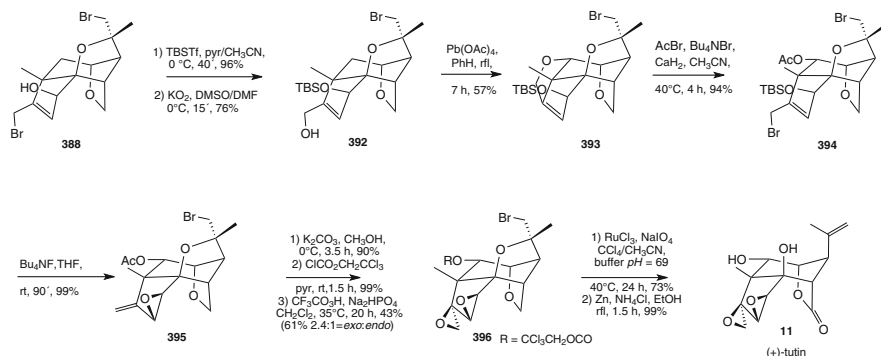
bromides, yielding by $\text{S}_{\text{N}}2$, and $\text{S}_{\text{N}}2'$ respectively, the primary allylic alcohol. Epoxidation with peracid occurred at the convex face leading to the *exo*-epoxide **385**. Functionalization of the unactivated methylene group with lead tetraacetate afforded the tetrahydrofuran subunit of **386**. The cyclic ethers were converted into the bislactone **104** with *in situ*-produced ruthenium tetroxide in 36% yield (212). Reductive elimination with zinc under mild conditions afforded (–)-picrotoxinin (**1**). This synthesis needed 37 steps with 0.004% overall yield.

Allylic bromides **383** and **384** were the relay substances for the synthesis of coriamyrtin (**9**) (Scheme 43). The allylic bromides were eliminated with potassium *tert*-butoxide in toluene affording diene **387**. 1,4-Addition with *N*-bromosuccinimide in polar solvent at room temperature yielded the unsaturated α -bromo- δ -hydroxy unit of **388**, which formed the unsaturated epoxide **389** by base treatment. Epoxidation with peracid occurred as expected from the convex face. The cyclic ether moiety of bisepoxide **390** was oxidized to the lactone **368** with ruthenium

tetroxide. Reductive elimination with zinc completed the total synthesis of (+)-coriamyrtin (**9**) in 38 steps and 0.007% overall yield.

First Total Synthesis of (+)-Tutin and (+)-Asteromurin A by *Yamada et al.*

An intermediate of the coriamyrtin synthesis (*119*) was chosen to synthesize a further main picROTOXANE sesquiterpene of *Coriaria* species, (+)-tutin (**11**).



Scheme 44

Allyl alcohol **388** was protected as silyl ether and the allylic primary bromide was substituted according to *Corey et al.* (*213*) affording primary allyl alcohol **392** (Scheme 44). Functionalization through space with lead tetraacetate led to cyclic ether **393** and considerable amounts of the unsaturated aldehyde (~29%), which could be recycled via reduction to alcohol **392**. The cyclic ether **393** was cleaved with acetyl bromide affording the acetoxy bromide, **394**. Note that addition of CaH₂ was crucial for the success of this reaction. Deprotection of the silyl ether led via intramolecular S_N2' reaction to the allylic epoxide, **395**. The protective group of the secondary alcohol at C(2)-was exchanged to the more easily removable trichloroethyl carbonate prior to epoxidation of the exocyclic double bond with peracid. A mixture of the *exo*-oxirane **396** and the *endo*-oxirane was isolated in a 2.4:1 ratio. Ruthenium tetroxide oxidized the tetrahydrofuran to the γ -lactone. Finally, reductive cleavage of the bromoether and the trichloroethylcarbonate with zinc gave (+)-tutin (**11**) in 45 steps and 0.001% overall yield starting with tetralone **135**.

(+)-Asteromurin A (**22**), a compound isolated from a scale insect was the next target of *Yamada's* picROTOXANE synthesis project. An intermediate of the synthesis of tutin (**11**), the unsaturated epoxide **395**, was the relais substance.

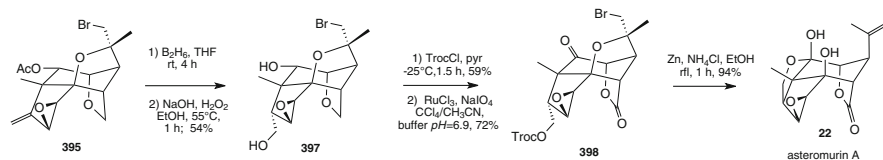
Hydration with boranes proved difficult due to extensive reductive cleavage of the epoxide (Scheme 45). The best results (54%) were achieved with borane in THF. The subsequent oxidation with basic hydrogen peroxide led to the primary alcohol as well as to saponification of the acetate. Selective protection of the primary alcohol **397** as trichloroethyl carbonate was followed by oxidation with RuCl₃/NaIO₄, thereby producing not only the γ -lactone but also the cyclic ketone.

Zinc removed the protective group of the primary alcohol and cleaved the bromoether of **398** reductively under mild conditions. The ketoalcohol thus formed spontaneously cyclized to asteromurin A (**22**) in 45 steps and 0.001% overall yield.

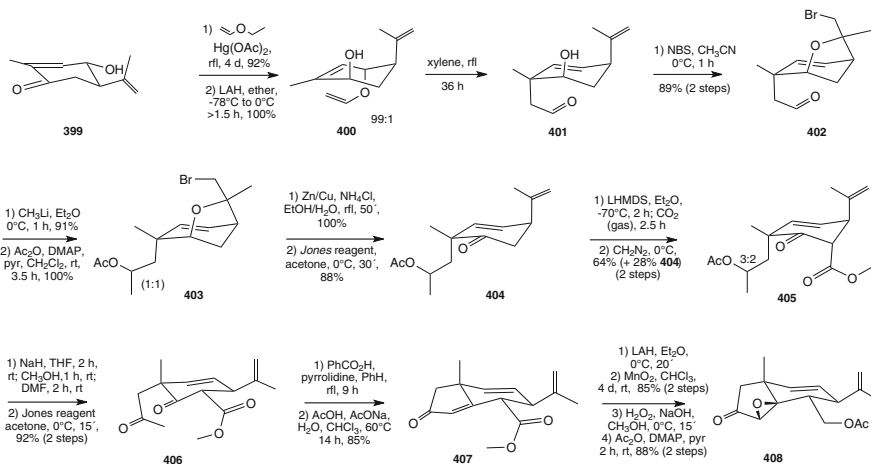
Total EPC-Synthesis of Picrotoxinin and Picrotin by *Yoshikoshi et al.*

The third new strategy for the construction of picrotoxinin (**1**) was presented by *Yoshikoshi et al.* (*120*). The chiral starting material, **399**, of the EPC-synthesis of picrotoxinin (**1**) and picrotin (**2**), was derived from (–)-carvone in four steps (*214*).

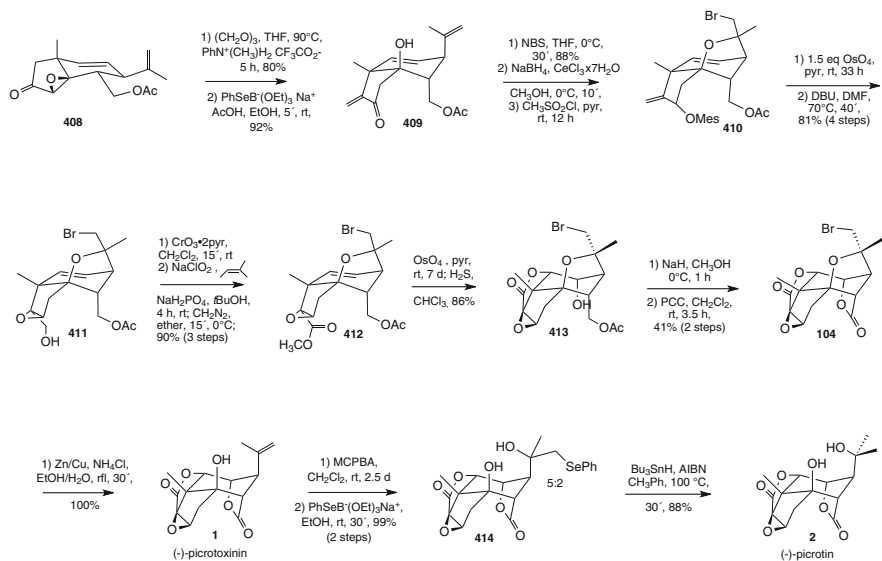
The quaternary center was constructed stereospecifically by *Claisen* rearrangement (Scheme 46). The necessary enol ether was obtained by reaction of the secondary alcohol of **399** with ethyl vinyl ether and mercuric acetate. To change the polarity of the endocyclic double bond, the unsaturated ketone was reduced with lithium aluminum hydride to the allylic alcohol, **400**, at low temperature. Then, prolonged heating with xylene led to the aldehyde, **401**. Protection of the secondary alcohol was achieved by bromoether formation with *N*-bromosuccinimide in acetonitrile before the aldehyde of **402** was reacted with methyllithium. The epimeric mixture of secondary alcohols was protected as acetates **403**. Then, the cyclic ketone



Scheme 45



Scheme 46



Scheme 47

was prepared by reductive elimination with a zinc/copper couple and successive oxidation. This allowed the stereoselective introduction of the carboxylic group by enolate formation and addition of gaseous carbon dioxide. The corresponding ester **405** was formed with diazomethane. The next task was the construction of the cyclopentenone **407** by intramolecular aldol reaction. Saponification led to spontaneous cyclic hemiketal formation preventing the oxidation to diketone **406**. Therefore, the following protocol was designed. The enolate of the β -ketoester was formed with sodium hydride before addition of methanol set the secondary alcohol free by transesterification. Inverse addition to *Jones* reagent afforded methyl ketone **406**, which cyclized to the conjugated dienamine with pyrrolidinium benzoate. Hydrolysis with aqueous acid led to a 4.5:1 mixture of the unsaturated α,β -ketone **407** and the β,γ -ketone. The latter could be converted into the α,β -ketone **407** by treatment with Al_2O_3 in benzene. To allow selective epoxidation with basic hydrogen peroxide, the ester was reduced by lithium aluminum hydride. The concomitant reduction of the ketone necessitated selective reoxidation with active manganese dioxide. Then, basic hydrogen peroxide converted the enone in surprisingly high stereoselectivity into the *exo*-ketoepoxide, which the authors explained by the favored *cis*-hydrindanone formation. After acetylation, the ketone **408** was methylenated according to *Gras* with formaldehyde under phase-transfer catalysis (Scheme 47) (215).

This reaction was followed by a reductive epoxide cleavage the authors had developed (216). Sodium phenylselenyl triethoxyboronate in acidic methanol cleaved the epoxide within 5 min in high yield. The resulting tertiary alcohol **409** was once more simultaneously protected with the isopropenyl double bond as

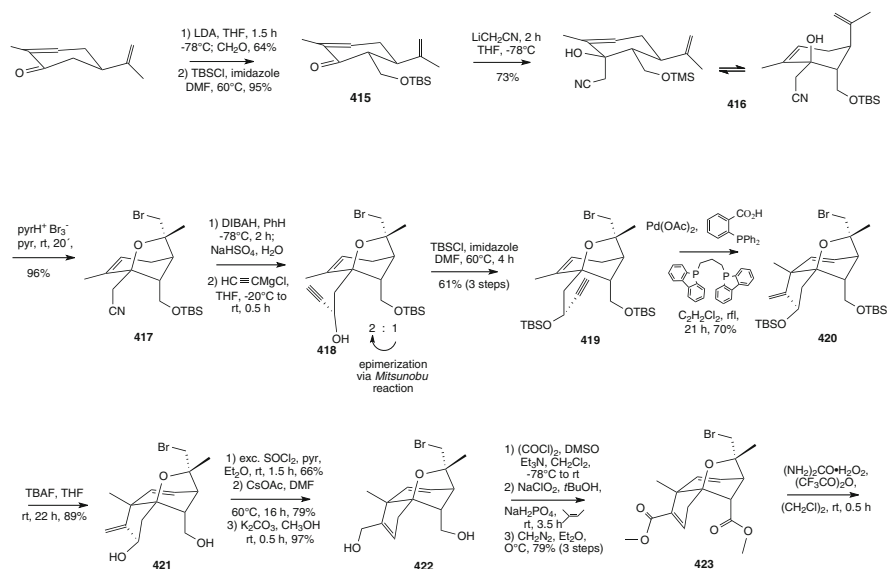
bromoether prior to reduction of the unsaturated ketone according to *Luche*. The *endo*-alcohol formed was esterified with mesyl chloride. Selective dihydroxylation of the methylene group of **410** was achieved with the voluminous osmium tetroxide. Base treatment of the diol led to epoxide **411**. The primary alcohol was now oxidized to the acid via the aldehyde with *Collin's* reagent and subsequent *Pinnick* oxidation. After methyl ester formation, the hindered double bond of **412** was stereoselectively oxidized to lactone **413** within 1 week when using equivalent amounts of osmium tetroxide in pyridine. Methanolysis of the acetate allowed oxidation of the carbinol at C(5) with pyridinium chlorochromate to the aldehyde and after spontaneous hemiacetal formation to the known α_1 -bromopicrotoxinin (**104**). Reductive elimination of **104** afforded (–)-picrotoxinin (**1**) in 31 steps and 5% overall yield (+4 steps from (–)-carvone).

Starting with picrotoxinin (**1**) *Yoshikoshi* developed an improved synthesis of (–)-picrotoxin (**2**). Epoxidation of picrotoxinin (**1**) with peracid at room temperature led to a 5:2 mixture of the epimeric epoxides. Regioselective cleavage of the epoxide was achieved with sodium phenylselenenyl triethoxy boronate. Radical reduction of the phenyl selenides **414** with stannane completed this three-step sequence to picrotoxin (**2**) in 87% overall yield.

Total EPC-Synthesis of Picrotoxinin and Corianin by *Trost et al.*

The most recent EPC-syntheses of picrotoxane sesquiterpenes were reported by *Trost* and his coworkers (*63*, *121*, *122*, *131*, *132*). In the 1980s of the last century, *Trost* developed an efficient palladium(II)-catalyzed ene reaction (*217*). To demonstrate the broad range and mild reaction conditions of their new method, they chose the picrotoxanes due to their high structural complexity. As in *Trost's* dendrobine synthesis, the ene reaction has to generate the quaternary center in *anti-Markovnikov* mode at a densely substituted molecule without the assistance of strong electron-donating and electron-accepting substituents at the reaction centers. In 1987, *Trost et al.* reported the successful EPC-synthesis of the highly substituted *cis*-hydrindene system and, in consequence, the EPC-synthesis of dendrobine (**82**). After considerable research to improve the capacity of the catalyst by varying the ligands (*218*), *Trost et al.* were able to use this key step for the syntheses of picrotoxinin (**1**), corianin (**21**), and methyl picrotoxate (**42**). These synthetic efforts included several structurally analogs for physiological tests and a partial synthesis of corianin (**21**) starting with picrotoxin.

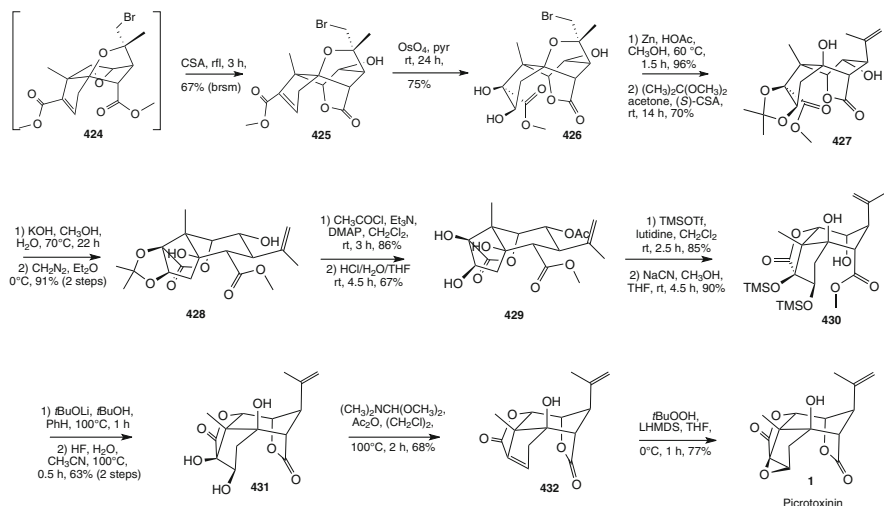
Once again, (–)-carvone was chosen as starting material (Scheme 48) (*121*). Aldol reaction with formaldehyde led to 6-hydroxymethylcarvone. The primary alcohol was protected as silylether **415** to permit addition of (cyanomethyl)lithium. The 5:1 mixture of epimeric tertiary alcohols was separated and the main product, **416**, the alcohol generated by *axial* attack, was protected stereoselectively as bromoether **417** utilizing pyridinium hydrobromide perbromide. To introduce the necessary alkyne, the nitrile was reduced to the aldehyde with diisobutylaluminum hydride and subsequent hydrolysis. Addition of the alkyne led to a 2:1 mixture of



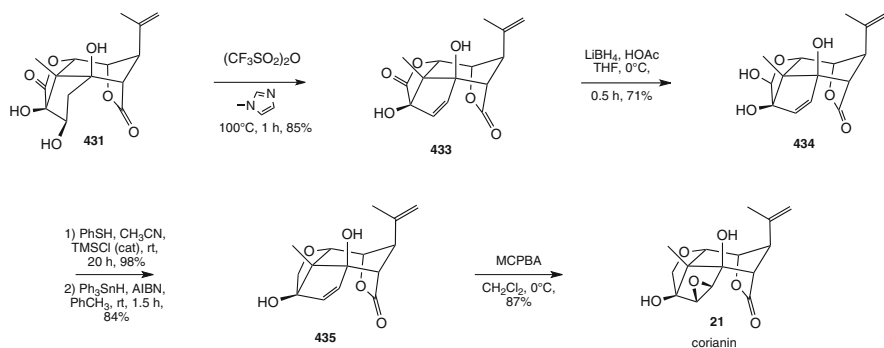
Scheme 48

propargylic alcohols. The minor alcohol was converted into the main alcohol **418** via *Mitsunobu* reaction and hydrolysis. After the propargylic alcohol was protected as silylether **419**, the palladium-assisted ene reaction afforded tricyclic compound **420**. The ligand 1,3-bis(dibenzophosphoryl)propane was chosen due to its small volume compared to other ligands and its stronger π -acceptor and lesser σ -donor properties and 2-(diphenylphosphino)benzoic acid as internal proton source. Desilylation with tetrabutylammonium fluoride afforded allyl alcohol **421**. 1,3-Hydroxyl shift was achieved within three steps. Excess thionyl chloride transformed the secondary allyl alcohol into the primary allyl chloride, which was substituted by acetate with cesium acetate. Saponification completed the reaction sequence. Diol **422** was oxidized to the diacid by *Swern* oxidation followed by *Pinnick* oxidation. The attempt to use *Corey's* protocol to convert the unsaturated diacid with lead tetraacetate into the bislactone failed. Thus, the following much longer route was designed. After ester formation with diazomethane, the electron rich double bond of diester **423** was stereoselectively epoxidized.

The epoxide **424** was treated with strong acid that led most probably via neighboring group assistance of the bromide to cleavage of the oxirane and immediate attack of the ester group to δ -lactone **425** (Scheme 49). Dihydroxylation with osmium tetroxide led to diol **426**. Reductive cleavage of the bromoether did not initiate the desired lactonization to the bislactone. Thus, the *vicinal* diol was protected as acetonide **427**. Now, potassium hydroxide in methanol formed the γ -lactone. The newly generated acid was converted into the methyl ester **428**. The fact that the second lactone was not formed indicated that the acetonide preferred the conformation with the acid and the alcohol in equatorial position. Therefore, the



Scheme 49



Scheme 50

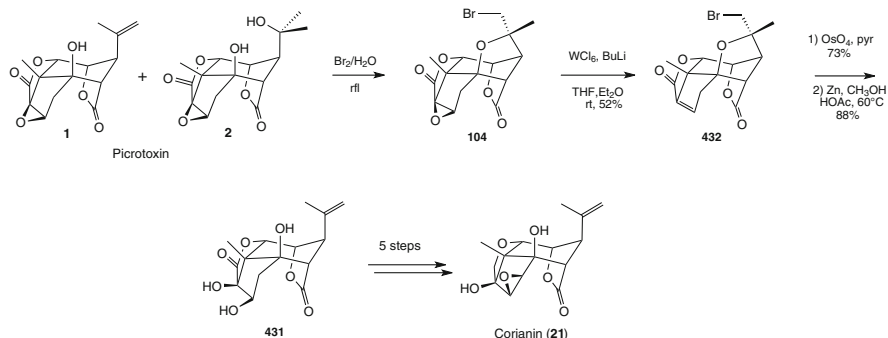
secondary alcohol was protected as an acetate prior to removal of the acetonide. Renewed protection of the *vicinal* diol as silyl ethers and cyanide-assisted selective removal of the acetate led to ester lactone **430**. Drastic basic conditions afforded the bislactone, and hydrogen fluoride removed the silyl groups. Relais substance **431** led to picrotoxinin (**1**) in two steps. The *vicinal* diol was converted into the double bond according to Eastwood (219) and epoxidation with basic *tert*-butyl peroxide completed the EPC-synthesis in 29 steps and 0.3% overall yield.

When relais substance **431** was selectively dehydrated via the triflate of the secondary alcohol the path toward corianin (**21**) was opened (Scheme 50). The authors used the fact that neighboring hydroxy groups enhance the reducing power of borohydrides to selectively reduce one of the lactones to the hemiacetal **434**. Reduction to tetrahydrofuran **435** was achieved by thioacetal formation with

phenylthiol catalyzed by trimethylsilyl chloride and subsequent radical reduction with triphenyl stannane. Epoxidation with peracid afforded the first total synthesis of corianin (**21**) in 32 steps and 0.27% overall yield.

Partial Synthesis of Corianin by *Trost et al.*

The possible therapeutic use of corianin (**21**) in the treatment of schizophrenia and the general influence of picROTOXANES on the central nervous system make more economic pathways desirable. *Trost* and *Krische* have shown that relais substance **431** can be easily prepared from commercially available picROTOXIN (**131**). This constitutes a partial synthesis of corianin (**21**) in nine steps (Scheme 51).



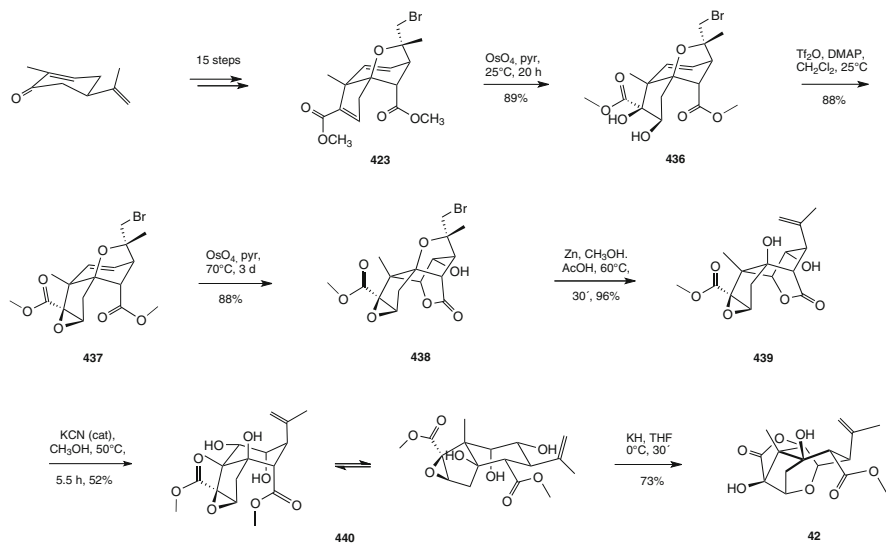
Scheme 51

The molecular compound, picROTOXIN, was treated with bromine in water, thereby not only permitting easy separation from unchanged picROTOXIN (**2**) but also protecting the double bond of the isopropenyl group as a bromoether. Then, low-valent tungsten (**220**) was used to transform the oxirane of **104** into the double bond without affecting the bromoether. The double bond of **432** was dihydroxylated by osmium tetroxide in pyridine resulting in relais substance **431**. Five steps, already described above, completed the partial synthesis of corianin in 14.5% overall yield starting with picROTOXIN (**1**).

Total Synthesis of Methyl PicROTOXATE by *Trost et al.*

A further intermediate of the above-described synthesis of picROTOXIN (**1**), diester **423**, inspired the authors to attempt the first EPC-synthesis of methyl picROTOXATE (**42**) (**63**). Originally, this compound was found as a degradation product of picROTOXIN (**1**) (**2**, **3**). It was reported later as a minor constituent in both the plant *M. cocculus* and in the sponge *S. inconstans*.

Starting with (–)-carvone, the diester **423** was reached in 15 steps (see Scheme 48). Interestingly, the conjugated double bond of **423** was inert against nucleophilic

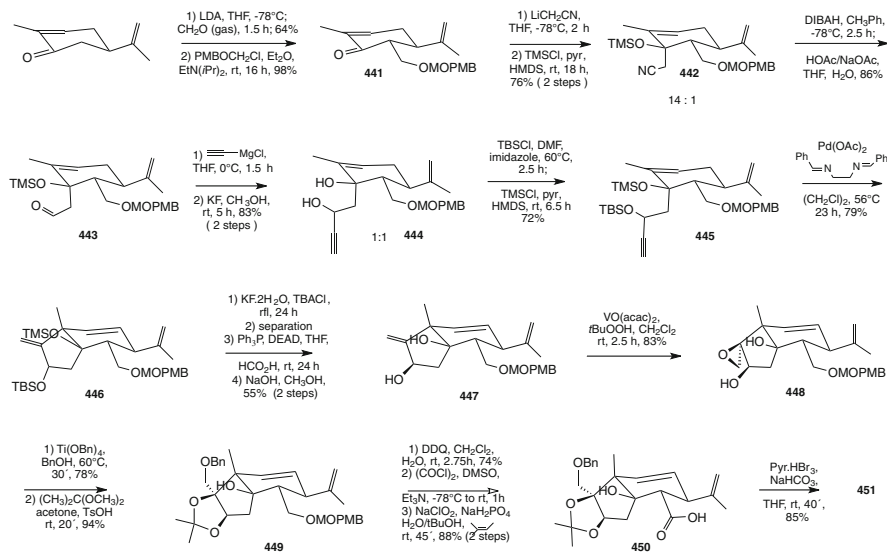


Scheme 52

epoxidation but dihydroxylation by osmium tetroxide occurred exclusively at this electron deficient double bond (Scheme 52). The dihydroxylated product **436** was converted into the desired oxirane **437** by treatment with trifluoroacetic anhydride. Avoiding nucleophilic solvents, the methoxy carbonyl group at C(5) attacks and is substituted by the tertiary hydroxy group, forming the epoxide by double inversion. The small steric as well as electronic differences between the relais substance **423** and epoxide **437** were sufficient to permit dihydroxylation of the cyclohexene double bond at higher temperatures and longer reaction times. Spontaneous lactonization afforded δ -lactone **438**. The obsolete bromoether was cleaved by zinc under carefully chosen conditions to avoid attack of the α -carbonyl epoxide. Sodium cyanide-catalyzed methanolysis of the lactone **439** was followed by intramolecular nucleophilic attack at the epoxide **440** by the alkoxylate generated with potassium hydride. This six-step sequence completed the first EPC-synthesis of methyl picrotoxate (**42**) in 21 steps and 1.6% overall yield starting with (–)-carvone.

A Variant of the Total Syntheses of Corianin by *Trost et al.*

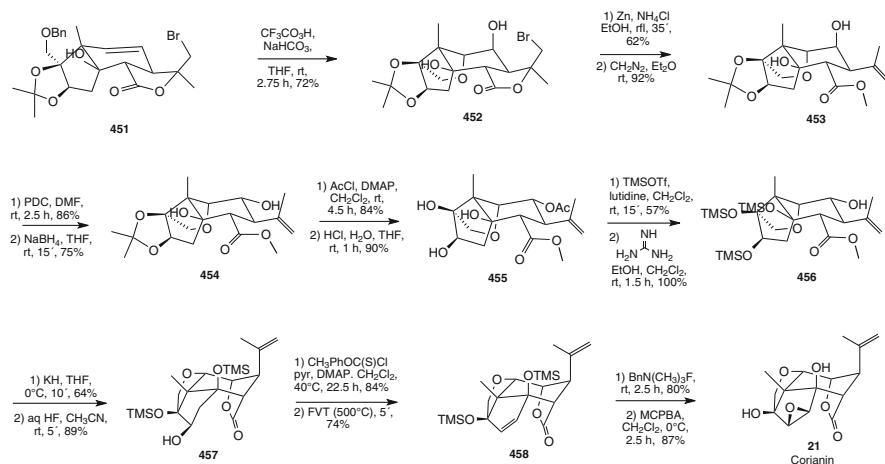
To date, the last total synthesis of a sesquiterpene picrotoxane (**132**) is a variant of the above-described picrotoxinin/corianin synthesis by *Trost et al.* Again, the synthesis started with (–)-carvone and the introduction of the C-1 unit corresponds with the earlier syntheses. The choice of the protective group was governed by the discovery that the oxygen(s) of alkoxyethyl ethers had a directing effect in the following addition of (cyanomethyl)lithium.



Scheme 53

Thus, the primary alcohol was converted quantitatively into (*p*-methoxybenzyl) methyl ether **441** (Scheme 53). Indeed, high stereoselectivity was observed in the following aldol-like reaction. The tertiary alcohol formed was protected as silyl ether **442**. Extension of the side chain was achieved by reduction of the nitrile with diisobutylaluminum hydride, hydrolysis to the aldehyde **443** and subsequent addition of acetylide. The latter reaction was accompanied by partial silyl group transfer to the newly generated propargylic alcohol. Thus, deprotection with fluoride led to the diols, **444**. The propargylic alcohol was protected as *tert*-butyl(dimethyl) silyl ether followed by a trimethylsilylation of the tertiary alcohol. The epimeric mixture (1:1) of diene **445** underwent the palladium-assisted intramolecular ene reaction in good yield. The silyl ethers of the *cis*-hydrindenes **446** were cleaved with fluoride and separated. The undesired *endo*-alcohol was inverted by *Mitsunobu* reaction and subsequent saponification. Diol **447**, when oxidized to the ketone, would allow proceeding along *Yoshikoshi*'s pathway to picrotoxinin (**1**). To proceed with the corianin synthesis, the methylene group of **447** was selectively epoxidized following *Sharpless*' protocol. The epoxide **448** was cleaved with titanium tetrabenzylate in benzyl alcohol. Subsequently the *vicinal* diol was protected as acetone **449**. Then, oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) removed the (*p*-methoxybenzyl)methyl group. The liberated primary alcohol was oxidized to the acid by *Swern* oxidation followed by *Pinnick* oxidation.

Bromolactonization of **450** enabled stereoselective attack of peracid at the endocyclic double bond of **451** (Scheme 54). Assistance of the benzyl ether led to tetrahydrofuran formation, debenzoylation, and the secondary alcohol at C(3). Subsequently, the pentacyclic bromolactone **452** was reductively cleaved with zinc and the resulting acid was protected as methylester **453**. Since lactonization by



Scheme 54

Mitsunobu reaction as well as by mesylation and subsequent base treatment failed, the secondary alcohol was inverted by oxidation with pyridinium dichromate and successive reduction with sodium borohydride. The inverted alcohol **454** was protected as an acetate and the acetonide was removed by acid treatment to enable conformational flexibility. Persilylation of triol **455** was succeeded by acetate cleavage with guanidine. Alcohol **456** was deprotonated to assist lactonization. Mild and short treatment with aqueous hydrogen fluoride allowed selective cleavage of the secondary silyl ether. Dehydration of the alcohol **457** was achieved by *Tshugaeff* reaction. The final steps toward corianin (**21**) were deprotection of the tertiary alcohols of **458** and epoxidation with peracid. This alternative corianin synthesis needed 34 steps in 0.13% overall yield.

7. Biosynthesis of Picrotoxanes

Picrotoxanes belong to the largest group of plant secondary metabolites, the isoprenoids, with over 30,000 members. Experiments to clarify the biosynthetic pathway of the picrotoxanes have been confined to the 1960s and early 1970s, however. This is long before increasing discrepancies between experimental results obtained and theory using mevalonic acid as starting unit led to the discovery of alternative biosynthetic pathways to isopentenyl diphosphate and dimethylallyl diphosphate, the central units of isoprenoid biosynthesis, by *Rohmer* (221) and *Arigoni* (222). Thus, the authors investigating the biosynthetic pathways of the picrotoxanes did not question mevalonic acid as a starting material. Indeed, all of them found incorporation of radioactively marked mevalonic acid in the chosen picrotoxanes. This is in accord with *Rohmer's* finding that most sesquiterpenes (and triterpenes), which were compounds with farnesyl diphosphate as starting material,

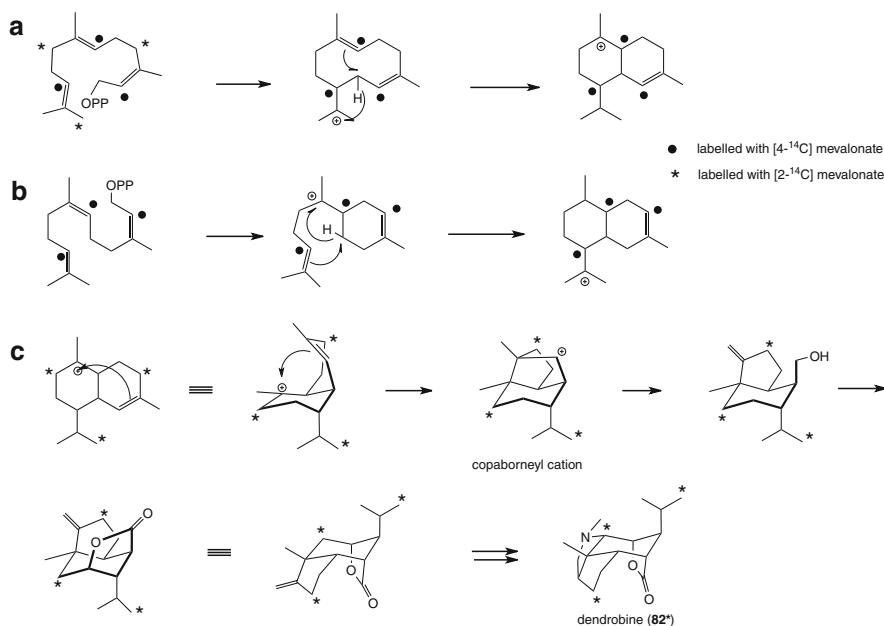
are formed in higher plants from mevalonic acid in the cytosol. PicROTOXANES cannot be formed by simple ionic olefin cyclization. Additional rearrangement is necessary to arrive at the complex structures in evidence. Early hypotheses of this pathway are summarized in several reviews (2, 3, 223).

7.1. Investigations on the Biosynthesis of Dendrobines

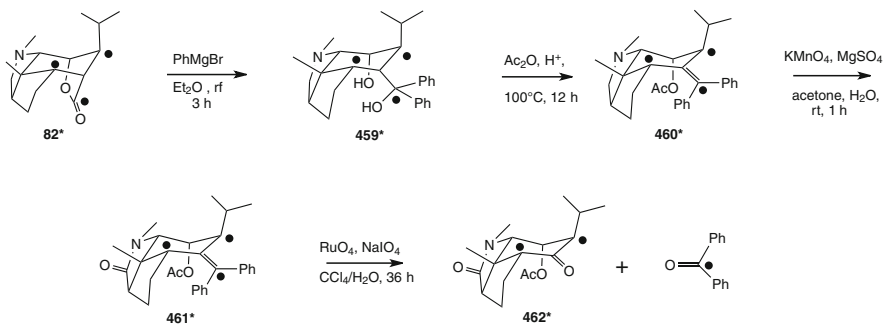
The first report of the incorporation of radioactively labelled mevalonic acid into dendrobine (**82**) was by Yamazaki *et al.* (224). Sodium [2-¹⁴C]mevalonate [(±)-**463***] was administered to stems of *D. nobile* by the cotton-wick method. After 12 days, the plants were extracted and radioactive dendrobine (**82**) was isolated by column chromatography. The total incorporation was 0.012%. Subsequent Kuhn–Roth oxidation led to acetic acid that showed the expected activity assuming the biosynthesis pathway a leading to the picROTOXANES via a cadalene as precursor (Scheme 55, pathway c).

Edwards *et al.* verified the incorporation of [2-¹⁴C]mevalonate [(±)-**463***] in *D. nobile*, recording a higher incorporation (225). To decide between two possible pathways (Scheme 55 pathway a and b) toward the *cis*-bicyclodecadiene, they chose [4-¹⁴C]mevalonate [(±)-**463***] for incorporation into dendrobine (**82**).

Again using the cotton-wick method, 0.95% incorporation in dendrobine (**82***) and 0.71% incorporation in nobilenine (**90***) were found (225). Labelling of the



Scheme 55



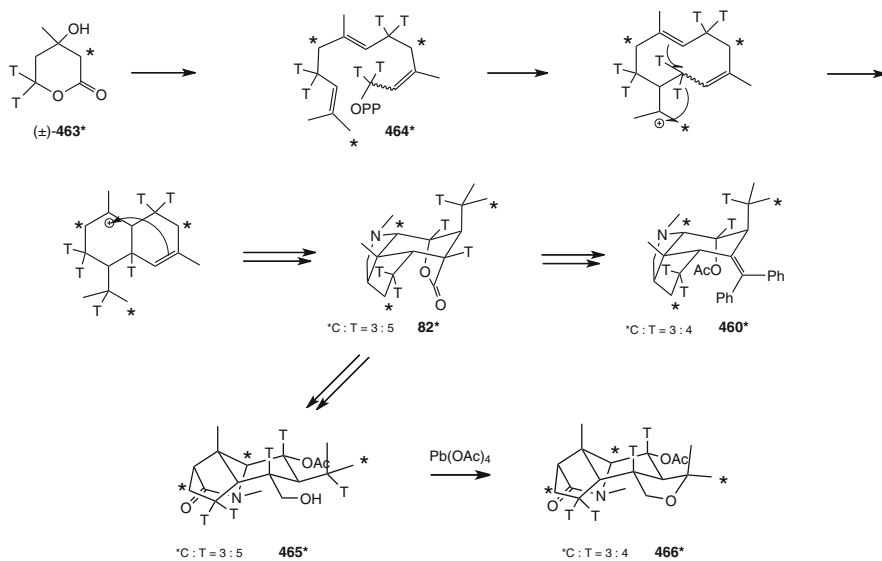
Scheme 56

lactone carbonyl was expected in only one of the two alternative pathways (Scheme 55, pathway a). Thus, isolation of this carbonyl of **82*** was attempted in various ways.

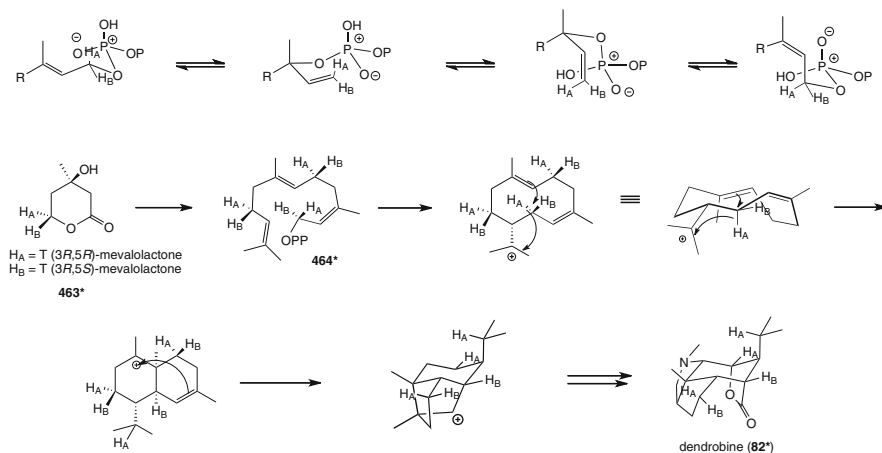
Decarboxylation methods failed. After several trials, excess phenylmagnesium bromide was used to form the tertiary alcohol, **459***, which was treated with acetic anhydride containing a trace of sulfuric acid yielding the tetrasubstituted olefin, **460*** (Scheme 56). This olefin was cleaved with ruthenium tetroxide and periodate. The resulting benzophenone was radioactive, thus the pathway to muurolene proceeds via cyclodecadiene as intermediate.

To confirm the 1,3-hydrogen shift, the prerequisite for the cyclization of the cyclodecadienyl cation to the bicyclodeceny cation (pathway a, Scheme 55), *Jommi et al.* used [2-¹⁴C, 5-³H₂]-mevalonate [(±)-**463***]. The ratio of ¹⁴C to ³H in dendrobine (**82**) and its derivatives was expected to determine the correctness of the hypothesis (226).

Feeding plants with the labelled mevalonate (±)-**463*** led to radioactive dendrobine [(±)-**82***] (incorporation 1.4%) which showed the loss of one tritium atom due to hydroxylation (Scheme 57). To determine the position of the remaining tritium atoms labelled dendrobine [(±)-**82***] was converted into known derivatives. Using *Edwards* method (225) yielded the tetrasubstituted olefin **460***, with loss of one tritium, ascertaining the position of tritium at C(5) of dendrobine (**82**). Transforming dendrobine (**82**) to the known derivative **465*** (136) allowed etherification to the tetrahydrofuran **466*** with lead tetraacetate. The functionalization of the methine group was accompanied by the loss of one tritium atom, thus ascertaining the 1,3-hydrogen shift within the biosynthetic pathway and disproving the alternative double 1,2-hydrogen shift as well as any intermediate cyclopropane formation. The question of the stereochemistry of these cyclization reactions was addressed independently by the groups of *Jommi* and *Arigoni*. *Jommi* tried to solve the questions of how and when the Δ²-double bond of (*E,E*)-farnesol is isomerized to permit the cyclization to the muurolene (227). Since both tritium atoms at position C(1) of the farnesol generated from the labelled mevalonate [(±)-**463***] were accounted for in the labelled dendrobine [(±)-**82***], isomerization by oxidation to farnesyl aldehyde and successive *trans-cis*-isomerization could not be involved.



Scheme 57



Scheme 58

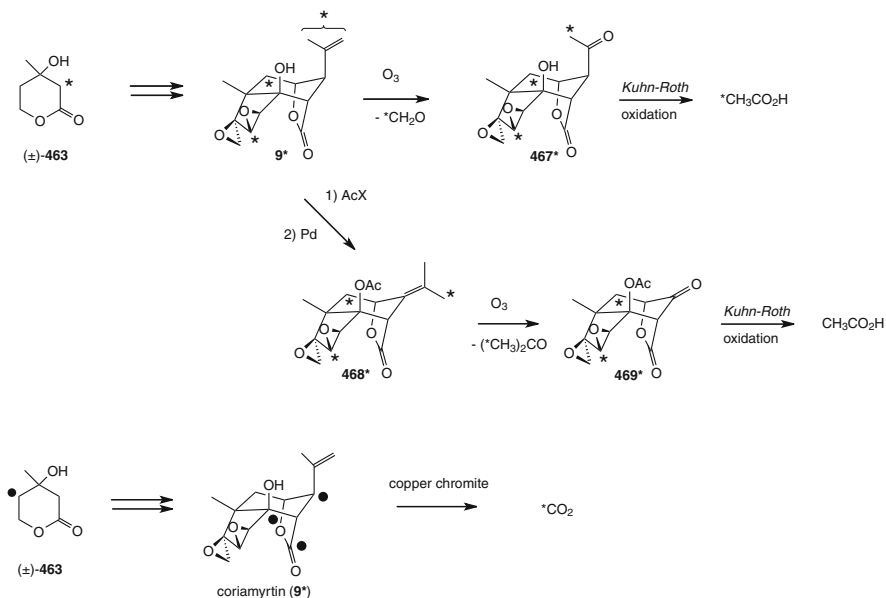
Feeding labelled (2*Z*,5*E*)-farnesol to plants of *D. nobile* failed to produce labelled dendrobine (**82***) whereas labelled (2*E*,5*E*)-farnesol was incorporated (**227**). Therefore, *Jommi* concluded that isomerization of the double bond would most likely occur with the highly strained allylic (*E,E*)-cyclodecadienyl cation. *Arigoni* pointed out that a more likely explanation would be a sigmatropic rearrangement of (*E,E*)-farnesyl diphosphate to nerolidyl diphosphate, rotation around the single bond and sigmatropic rearrangement to (2*Z*,5*E*)-farnesyl diphosphate (**228**) (Scheme 58).

The stereochemistry of the 1,3-hydrogen shift and the cyclizations involved were examined with optically pure (3*R*,5*S*)-5-[³H]-mevalonate and (3*R*,5*R*)-5-[³H]-mevalonate (228) and (1*S*,2*E*,6*E*)-1-[³H]-farnesol, respectively (227). These experiments revealed stereospecific *trans*-cyclizations as well as a 1,3-*syn*-hydrogen shift. Although more recent research supports the ionic *syn-facial* rearrangement of farnesyl diphosphate to nerolidyl diphosphate, and, after the rotation around the newly formed single bond, *trans* cyclization of the allylic cation to the distal double bond (229), it remains to the credit of *Arigoni* as to having elucidated the stereochemistry of the enzymatic farnesyl cyclization (228) and thus having solved the first of the many questions the sesquiterpene cyclases pose (229*b*).

7.2. Investigations on the Biosynthesis of Sesquiterpene Picrotoxanes

Earlier, *Jommi et al.* and *Arigoni et al.* also investigated the biosynthesis of coriamyrtin (9) and tutin (11) (96, 230–232). Again, racemic sodium [2-¹⁴C]-mevalonate [(±)-463*] was used to obtain a general idea of the biosynthesis of coriamyrtin (9) and tutin (11) in *C. japonica*. The isolated radioactive coriamyrtin (9) was degraded in various ways to ascertain the position of the radioactive atoms. As *Yamazaki* did for dendrobine (82), the authors assumed a pathway via farnesyl diphosphate, muurolenyl cation, and copaborneol (Scheme 54) for coriamyrtin (9) and tutin (11). This meant that labelling at C(2), C(9) or C(10), and C(16) was expected.

Twigs of *C. japonica* were inserted in water containing the labelled [2-¹⁴C]-mevalonate [(±)-463*] (230). Coriamyrtin (9*) and tutin (11*) were isolated albeit with low incorporation (230). Ozonization of coriamyrtin (9*) revealed the unexpected fact that the label was equally distributed between the methyl and the methylene carbon of the isopropenyl group [C(9) and C(10)] (Scheme 59). Thus, the generated formaldehyde contained 10% of the total activity and *Kuhn–Roth* oxidation of methyl ketone 467* yielded labelled acetic acid with 10% of the original activity. A double bond shift of acetylated coriamyrtin over palladium and subsequent ozonization led to a ketolactone (469*) and acetone with slightly less than 20% of the total activity. More rigorous degradation by methods developed earlier for the structure determination (2, 3) of these picrotoxanes led to aromatization of the cyclohexane moiety and oxidative cleavage of the cyclopentane. Successive decarboxylation yielded radioactive carbon dioxide, ascertaining that C(12) of coriamyrtin (9*) was labelled as expected. An interesting detail in *Biollaz's* and *Arigoni's* report (230) is the fact that incorporation of labelled mevalonic acid is uneven. The isopropenyl group contains 20% of the total activity and carbon dioxide derived from C(12) contained 60% of the total activity. This effect was not found by *Jommi et al.*, who used the more effective cotton-wick method to incorporate labelled mevalonic acid. Labelled tutin (11) yielded very similar results. Further confirmation of the proposed biosynthesis pathway was achieved by using sodium (±)-[4-¹⁴C] mevalonate [(±)-463*]. With this compound, labelling at C(4), C(6) and C(15) could



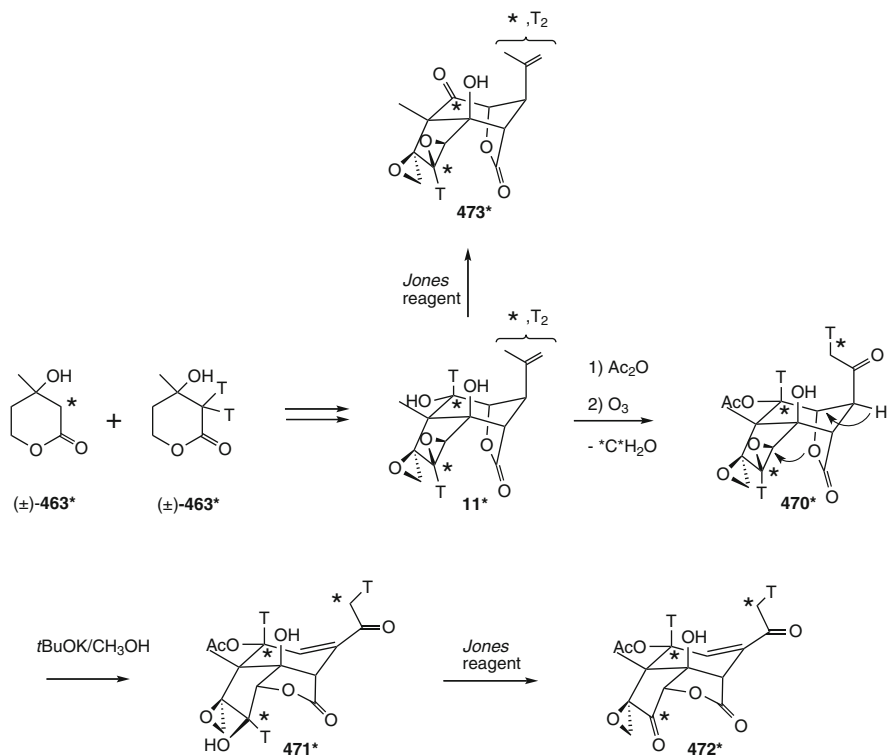
Scheme 59

be expected. Indeed, when radioactive coriamyrtin (**9***) was decarboxylated with copper chromite, evolving carbon dioxide contained 27% of the total activity (**230**) (Scheme 59). *Jommi's* simultaneous research (**96**) confirmed *Arigoni's* results.

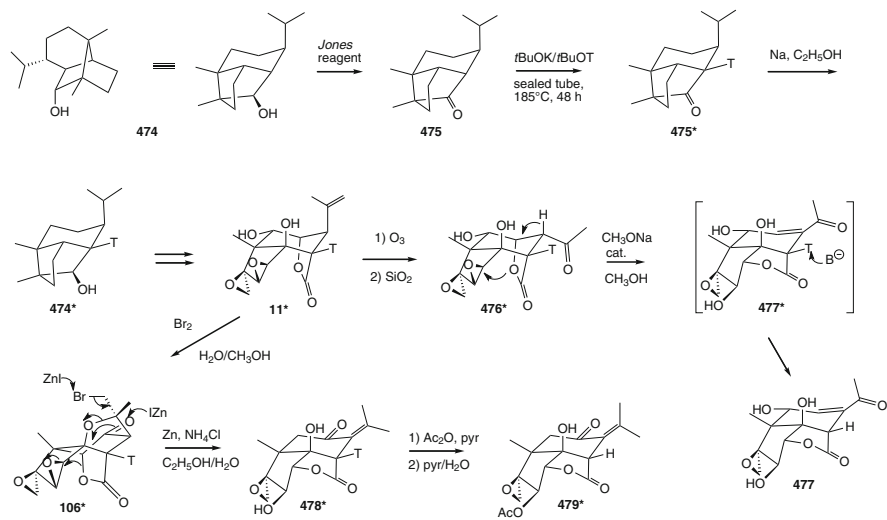
A mixture of potassium $(\pm)\text{-}[2\text{-}^{14}\text{C}]\text{-mevalonate}$ [$(\pm)\text{-463}^*$] and potassium $(\pm)\text{-}[2\text{-}^3\text{H}_2]\text{-mevalonate}$ [$(\pm)\text{-463}^*$] was administered to whole plants of *C. japonica* by the cotton-wick method (**96**). After extraction and dilution with carrier material, chromatography on silver nitrate laden silica gel, and crystallization, 0.03% incorporation into tutin (**11***) was recorded (Scheme 60). Oxidation of labelled tutin (**11***) led to **473*** with 25% loss of tritium. Acetylation and successive ozonization yielded **470*** and radioactive formaldehyde (16% ^{14}C , 18.5% T). Base treatment of the methyl ketone **470*** led to **471*** by the known rearrangement of the lactone moiety. (Experiments showed that the methyl group of the acetyl moiety is only marginally deprotonated with base!) The newly formed hydroxy group at C (12) of **471*** was oxidized to **472*** with the loss of tritium (21%). These data were in good agreement with the biosynthetic hypothesis as was the following reaction sequence: acetylation of labelled tutin (**11***), followed by palladium catalyzed double bond isomerization, and ozonization.

At this time, the postulated precursor copaborneol (**474**) was isolated as the main sesquiterpene from *Pinus sylvestris* and thus became available.

The naturally occurring copaborneol (**474**) was oxidized to ketone **475** and then labelled with tritium at the bridgehead by heating with strong base and tritiated alcohol at high temperatures (Scheme 61) (**231a**). Reduction with sodium in ethanol yielded labelled copaborneol (**474***), which was administered to *C. japonica* (**231b**).



Scheme 60

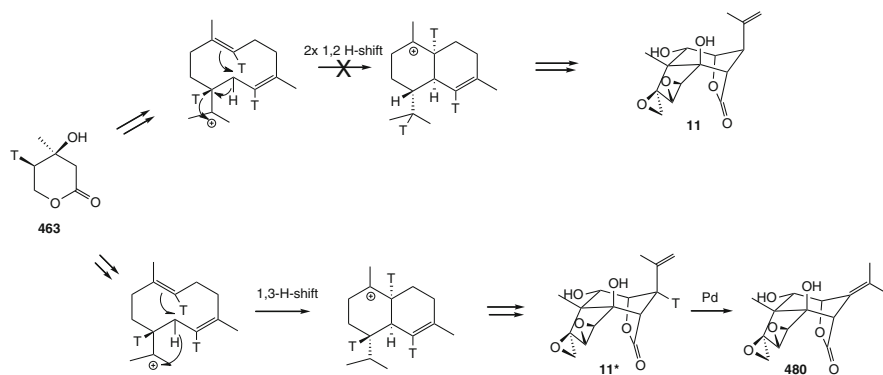


Scheme 61

The labelled tutin (**11***) isolated thereafter was ozonolyzed and subsequently epimerized at C(4) by chromatography on silica gel yielding **476*** without loss of activity. This compound was treated with methanolate in methanol, which led to the fused lactone **477*** by rearrangement of the lactone moiety. Consecutively, tritium at C(5) of the fused lactone was abstracted by base yielding unlabelled **477**. To prove that the loss of tritium in the described reaction sequence is due to its position at C(5), the inactive compound **477** was treated with a mixture of D₂O and pyridine, which led to immediate exchange of the proton at C(5) against deuterium. Alternatively, labelled tutin (**11***) was transformed into the α -bromotutinone (**106***). Careful treatment with zinc led to rearrangement of the lactone starting with reductive cleavage of the α -keto ester, which triggered the fused lactone formation by cleavage of the epoxide at C(11). A second reductive elimination led to recovery of the isopropenyl group, and subsequent isomerization of the β,γ -enone to the conjugated enone. No activity was lost in this reaction sequence to **478***. The corresponding acetate **479*** lost most of its activity when treated with wet pyridine at room temperature. These experiments proved copabornanol (**474**) as precursor of the picROTOXANES. Decades later, muurolanes and copacamphanes (copabornanes) and picROTOXANE-type sesquiterpenes with low oxidation level were found to accompany the picROTOXANES and dendrobines in *Dendrobium* species (58, 67, 68, 70, 71).

Finally, *Jommi et al.* addressed the question of the hydrogen shift following the ring closure of farnesyl diphosphate to the cyclodecadiene within the biosynthesis of tutin (**11**) (232).

A mixture of (3*S*,4*S*)-[4-³H]-mevalonate and (3*R*,4*R*)-[4-³H]-mevalonate and (\pm)-[2-¹⁴C]-mevalonate [(\pm)-**463***] was fed to whole plants of *C. japonica* by the cotton-wick method (Scheme 62). The ratio of ¹⁴C to T of the labelled tutin (**11***) showed that one of the three tritium atoms survived the biosynthetic pathway, with the other two tritium atoms lost to oxidation. For the tritium at position C(4) it is only possible for it to remain if the hydrogen transfer occurs in a one-step reaction. In the case of a double 1,2-hydrogen transfer, the tritium atom would be incorporated in the isopropyl group and vanish with the dehydrogenation to the



Scheme 62

isopropenyl group. To confirm the position of tritium at C(4), the palladium-catalyzed double bond shift was accompanied with 85% loss of tritium activity.

Although investigations of biosynthesis have become considerably more convenient by shifting to stable isotopes and nondestructive spectroscopic methods, the pioneering work was never continued. Thus, the pathway from copaborneol (**474**) to dendrobine (**82**), mono- and dilactone picrotoxanes, and norditerpene picrotoxanes is still unknown and no enzymes of this biosynthetic pathway have been defined or isolated. There is mention of genes of the dendrobine biosynthesis isolated and expressed in *Escherichia coli* (**233**).

The biosynthesis of the “norditerpene” picrotoxanes has never been investigated. *Jommi and coauthors* (**96**) suggested that the biosynthesis of this group of compounds in *H. globosa* follows the pathway to tutin (**11**)/coriamyrtin (**9**). Attack of two units of activated acetic acid or alternatively of acetoacetic acid at the *spiro*-epoxide of tutin (**11**)/coriamyrtin (**9**) permits two different ring closures. Ring closure to the *spiro*- γ -lactone would lead to pretoxin (**59**) and dendrotoxin (**60**) or to hemiacetal formation. Hemiacetal formation, dehydration, and esterification would yield capenicin (**57**), and decarboxylation prior or after hemiacetal formation would yield codendrin (**61**) and lambicin (**62**). This plausible hypothesis also accommodates the biosynthesis of the “norditerpene” picrotoxanes of *P. baccatum*. The expression “norditerpene” picrotoxanes was coined prior to the knowledge of two different pathways for isoprenoids and thus the products could have been viewed as degradation products of diterpenes. Since *Romer's* and *Arigoni's* discovery, this name is misleading, suggesting a different biosynthetic pathway as well as a different plant compartment for its biosynthesis. The names C-18 picrotoxanes and C-19 picrotoxanes, respectively would be more appropriate.

8. Physiological Activity of Picrotoxanes

Just five years after the isolation of morphine, the molecular compound picrotoxin was isolated from the fruits of *A. cocculus* (*M. cocculus*), imported from South Asia. Although no direct beneficial effects to humans were known, the fruits were used in Europe since the sixteenth century to catch fish by stunning them and to kill body lice (**2**). Thus, the most characteristic property of picrotoxin was and is its high toxicity.

8.1. Toxicity

8.1.1. Picrotoxanes Toxic for Humans and Other Mammals

Picrotoxin is described as irritating the respiratory center, exciting the vomiting center, rendering the pain center(s) more sensitive, and causing convulsions resembling epileptic seizures and death. The early findings were reviewed by *Porter* (**2**). It was *Porter* who found that the two components of the molecular compound

picrotoxin, picrotoxinin (**1**) and picrotin (**2**), differ widely in their degree of toxicity although their structures are very similar; only the isopropenyl side chain at C(4) of picrotoxinin (**1**) is formally hydrated to picrotin (**2**). Thus, picrotoxinin (**1**) is one of the most potent toxins of plant origin ($LD_{50} = 3$ mg/kg ip), whereas picrotin (**2**) is 45 times less toxic ($LD_{50} = 135$ mg/kg ip). This fact inspired Porter *et al.* to investigate the structural features necessary for the toxicity. By comparing several natural picrotoxanes and some derivatives, they found that the γ -lactone spanning the cyclohexane [C(3) to C(5)] is essential, as is the hydroxy group at C(6). Whereas the second γ -lactone has no significant effect, the epoxide does. The saturation of the isopropenyl group at C(4) has but a small effect (α -dihydropicrotoxinin (**8**) $LD_{50} = 14$ mg/kg ip) whereas the *anti* position of the γ -lactone is essential because the epimeric β -dihydropicrotoxinin is inactive up to 400 mg/kg (234)). The strong toxicity as well as the ease of isolation has made picrotoxin a very early but still invaluable research tool in neurobiology.

The only European plant containing picrotoxanes, *C. myrtifolia* (tanner's brush, mealy tree, curriers sumac) shows very similar characteristics of intoxication to picrotoxinin. The berries of this shrub are sometimes confused with edible berries (for instance, blackberries) especially by children. A recent case concerned an 8-year-old boy from southern France who developed vomiting and convulsions. His life was saved by administering diazepam repeatedly over a period of 1 day (235). Usually, the symptoms appear within two and a half hours after uptake and are mainly contributable to coriamyrtin (**9**) ($LD_{50} = 1-3$ mg/kg ip).

In 1906, Marshall reported that New Zealandean *Coriaria* species seriously hindered the raising of cattle. The animals, in not being indigenous, were drawn to the succulent leaves of these shrubs. It was found that their heart rate dropped, the body temperature fell, breathing became quicker, and the animals died of convulsions or exhaustion after feeding from shrubs the Maoris called "tutu" or "toitoi" (236). The compound mainly accountable for these effects was tutin (**11**) ($LD_{50} = 3$ mg/kg mouse, ip). As mentioned above, another animal immigrant, the Australian leaf hopper, visits these same shrubs, which was able to tolerate tutin (**11**) by partly oxidizing it to hyenanchin (**15**). These toxins are excreted unchanged within their honeydew. In years of drought, honeybees collect this honeydew without experiencing signs of intoxication themselves, but their honey is toxic to humans.

Although there are accounts of intoxications by *Coriaria* species of East Asia (28), these plants are better known for their use in traditional medicine.

Powdered fruits of *Coriaria ruscifolia* were used as poison against field rodents in Chile and serious intoxications with hepatic and gastrointestinal effects with fatal consequences were reported (38, 49). The toxicity of tutin (**11**), isolated from this Chilean *Coriaria* species, was investigated recently (237). Rats treated with dissolved tutin (**11**) showed the following effects with increasing tutin concentration: immobility, muscle spasm, seizures, and death by cardio-respiratory collapse. The main reasons for this toxicity were found by electrophysiological recordings to be antagonism against GABA and glycine in inhibitory receptors in the central nervous system (CNS) (237). *Coriaria microphylla*, growing in high altitudes of Central America and the northern part of the Andes was described as being

toxic to humans and animals. Taken in small quantities, this has hallucinogenic effects (23).

The most poisonous plant in the Cape area of South Africa is *H. globosa* (giftbom). Its scientific name is said to be connected with the use of this tree to poison wild animals, especially hyenas, and *Smith* claims that it may have been used as arrow poison (238).

Like *H. globosa* the Jamaica walnut or blackwood or bitter plum (*P. baccatum*) contains sesquiterpenes as well as “norditerpenes” of the picrotoxane type. Picrodendrin Q (74), the most toxic picrotoxane for mammals, is found in the bark of this tree. In the Dominican Republic, the tree is also called mata becerro (calf killer) and its bark is used to kill lice and bed bugs (239).

Although *Dendrobium* species are much better known as ornamental plants and for their use in traditional medicine, their alkaloids, the dendrobines, are also convulsants (2). The effects of dendrobium chloride intoxication resemble more those of strychnine intoxication than those of picrotoxinin intoxication (240). The minimal lethal dose (intravenous) in rats and mice is 20 mg/kg. The toxicity of the picrotoxanes is attributable to their ability to overcome the blood–brain barrier and to bind to inhibitory Cys-loop receptors of the postsynapses of the CNS.

8.1.2. Picrotoxanes Toxic for Invertebrates

As mentioned above, several of the picrotoxane-containing plants are used as insecticides in folklore (2, 78, 239, 241). *Li et al.* described the antifeedant and insecticidal properties of crude extracts of *C. sinica* (29). Insects share very similar inhibitory Cys-loop receptors with humans. However in the case of insects, GABA and glycine receptors are also distributed in the peripheral nervous system. Researchers looking for differences in the inhibitory Cys-loop receptors of vertebrates *versus* insects found that the combination of the five subunits of these heteropentameric membrane proteins varies from vertebrates to insects (242). Picrotoxanes had a pioneering role in these investigations and in efforts to develop more selective insecticides (*e.g.* (243–247)). The use of picrotoxanes themselves as insecticides is restricted because they pass through the mammalian blood–brain barrier. However picrotoxanes are very useful as tools in discovering differences between mammalian and insect inhibitory Cys-loop chloride channels.

Picrotoxanes also have been reported as being toxic to nematodes and *Maesobotrya floribunda* is used in Cameroon against filarial disorders (248). The nematocidal activity of 20 picrodendrins was tested against a species of the Diplogastridae (249). The researchers found that of all test compounds picrodendrin P (73) was the most potent toxin against this invertebrate being sixfold more potent than picrotoxinin (1). Functional groups that enhanced the toxicity were the γ -lactone bridging C(3) and C(5), and the ether bridging C(6) to C(8), whereas hydroxy groups at C(4) and C(8) decrease activity (249). In comparison with the avermectins, a structural group intensely investigated for use as insecticides, acaricides and helminthocides, even picrodendrin P (73) is less potent than

ivermectin, which has the additional advantage not being able to penetrate the human blood–brain barrier and to be easily degraded by soil organisms (250).

8.2. *Picrotoxanes as Therapeutics*

In Western medicine, picrotoxanes have played but a marginal role therapeutically. Picrotoxin was described as analeptic. *Porter* recorded that picrotoxin was found to be an antidote against chloral hydrate and in consequence it was used also as an antidote against barbiturate intoxication (2). It was this activity that made biomedical researchers aware of its potential as research tool in neurochemistry. Due to its high toxicity, even its use as antidote is restricted, however. Picrotoxin is employed to treat vertigo and nausea in homeopathic preparations (251).

Several picrotoxane-containing plants are used in traditional medicines. *Coriaria* species play an important role in Chinese traditional medicine, notably against mental diseases. *Coriaria nepalensis* is used against numbness, toothache, traumatic injury, and conjunctivitis (252). In the traditional medicine of Taiwan, gastrointestinal disturbances, rheumatism, and uterine cancer are treated with extracts of *Coriaria intermedia* (253). *C. sinica* is used for treatment of schizophrenia (29), and extracts of the parasitic plant on *C. nepalensis*, *L. parasiticus*, were used as shock therapy in schizophrenia (21). *Okuda et al.* point to corianin (21) as the main active component (21).

Nearly half of China's 74 *Dendrobium* species are used in Chinese traditional medicine as tonics and remedies. The main ingredient of "Jin Shi Hu" is *D. nobile* ("Chin Chai Shi Hu"), which is used to "nourish the stomach, promote the production of body fluids and reduce fever" (254, 255). Its pharmacological properties were first described by *Chen* and *Chen* as analgetic, hypertensive, and hypothermic (112). *Dendrobium* chloride itself exhibits slight analgesic and antipyretic action as well as moderate hyperglycemia (254). How much the glucosides of the picrotoxanes of *D. nobile* contribute to the beneficial effects of "Jin Shi Hu" is unknown. *In vitro* they exhibit immunomodulatory effects by stimulating the proliferation of murine T and B lymphocytes (60, 67, 71). *Dendrobium densiflorum* is the main component of the Chinese patent medicine "Mai Luo Ning" used against cerebral thrombosis and obliterate thromboangitis (57). *Dendrobium moniliforme* reduces fever and increases flow of saliva (256).

The only mention of a Picrodendraceae species as a remedy in folk medicine is the use of *Celaenodendron mexicanum* as antiseptic (48, 257). The only known Phyllanthaceae species containing picrotoxanes, *M. floribunda*, is used in Cameroon against filarial and stomach disorders (248) and its bark is used in Congo (Brazzaville) as remedy against leprosy (258).

Since most of these traditional medicines are concoctions of plant extracts that contain biologically active substances of several structural classes, the effects of the picrotoxanes are somewhat obscure.

The indirect influence that the picrotoxanes have on medicine as research tools in neurology far surpasses their therapeutic use.

8.3. *Picrotoxanes as Epileptogenic Compounds*

Picrotoxin and coriaria lactone (mainly a mixture of tutin (**11**) and coriamyrtin (**9**)) as well as many other picrotoxanes, are epileptogenic compounds and they are used to induce epilepsy in laboratory animals to test newly developed antiepileptic compounds (259). Their importance may be seen from the fact that recent estimations show that 0.4–1% of the population suffers from epileptic seizures, with one-third of the sufferers being younger than 16-years-old, and that the average duration of the disease is 12.5 to 25 years (260).

8.4. *Picrotoxanes as Tools in Neurobiological Research*

The main influence of the picrotoxanes on medicine has been their use as research tools in neurophysiology. *Porter* summarized the discoveries connected with the use of picrotoxin in neurobiology up to 1967 and noted as principal features in the action of picrotoxin its ability to produce a general and extreme central nervous system (CNS) excitation and experiments with invertebrates especially suggested “inhibition of GABA, a suspected agonist, or transmitter at inhibitory nerve synapses” (2). Shortly afterwards, *Takeuchi* and *Takeuchi* found by analyzing quantitative measurements with microelectrodes at the neuromuscular junction of crayfish (*Cambarus clarkii*) that two molecules of the neurotransmitter GABA are necessary to increase membrane conductance substantially and that picrotoxin non-competitively inhibits this conductance (261). *Kudo et al.* tested the effects of picrotoxinin (**1**), picrotin (**2**), tutin (**11**), coriamyrtin (**9**), 6-acetylpicrotoxinin, anhydropicrotin (**103**) (33), and dendrobine (**82**) (240) on the frog spinal cord. They found very similar effects for those compounds that possess a hydroxy group at C (6) (**1**, **2**, **9**, **11**), with two semisynthetic derivatives lacking activity. Dendrobine (**82**) much more closely resembled strychnine than picrotoxinin (**1**) in its activity. In the meantime, thanks to the availability of picrotoxin and an increasingly more diverse array of antagonists (243, 247) and the enormous progress in structural biology of membrane proteins (262) as well as achievements in neuropharmacology and neurochemistry (263–266), a much more differentiated picture of the effects of the picrotoxanes has emerged. Picrotoxinin (**1**) is a non-competitive antagonist of GABA at the GABA_A receptor. This ligand gated ionotropic receptor belongs to the inhibitory Cys-loop receptors (267). They consist of five protein subunits (usually closely related but not identical), which are covalently connected by disulfide bonds via oxidation of cysteines of different subunits that form loops situated between the binding and the channel domain. The extracellular domain forms β -sheets and contains the GABA binding sites (at least two). Each protein subunit forms four

α -helices (TM1–TM4) within the membrane. TM2 lines the channel, whereas TM1, TM3, and TM4 surround TM2 and shield it against the lipophilic membrane. It is thought that one of the lipophilic amino acid residues, most likely of leucine, of each TM2 helix protrudes especially deep in the channel lumen thus gating the channel (resting gate). When two GABA units, or GABA and another agonist (*e.g.* muscimol) combine with the two binding sites at the receptor, the channel of ~ 50 Å distance opens allosterically most likely by the motion of TM2 and permits chloride ion influx into the nerve cell. Charged residues flanking TM2 at both extra- and intracellular ends and in the TM3–TM4 cytoplasmic domain achieve the ion selectivity. Chloride ion influx initiates hyperpolarization and thus decreases excitatory impulses. PicROTOXIN(in) binds, strongly facilitated by ligand (GABA) binding, to a site of the receptor, which differs from that of GABA and also from that of the agonistic diazepamines. The binding of **1** prevents the influx of chloride ions and leads to undiminished excitatory impulses and thus epileptogenic effects. Although our knowledge has much expanded since *Porter's* review, many questions remain. As a result of site-directed mutagenesis, the binding site(s) of **1** is (are) thought to be located in the cytoplasmic half of the channel-forming domain of TM2 of the pentameric GABA_A receptor proteins. However, picROTOXININ (**1**) not only blocks the channel and thus prevents influx of chloride ions, it inhibits the influx allosterically also and possibly has a further binding site at TM2 (268–271). As mentioned above, structurally quite diverse antagonists (at least 100) seem to have similar effects as **1** on the GABA_A receptor and they appear to bind to the same site (272). This is surprising when compared with the fact that quite small differences in the structures of picROTOXANES (*e.g.* between picROTOXININ (**1**) and picROTIN (**2**)) lead to loss of activity. Several attempts have been published to explain the common structural, topological, and electrostatic features necessary to generate an antagonist binding at the same site as picROTOXININ (**1**) (123, 243, 244, 272, 285, 286). As described in these reports, 16 picROTOXANES (picROTOXININ (**1**), TUTIN (**11**), DIHYDROTUTIN (**12**), CORIANIN (**21**), and the PICRODENDRINS A (**63**), B (**64**), C (**17**), E (**65**), F (**66**), G (**67**), L (**69**), M (**70**), O (**72**), Q (**74**), T (**76**)) were examined and quantitative structure–activity relationships (QSAR) were investigated. Differences in the receptor-binding of the picROTOXANES in mammal and insect GABA_A receptor bindings were also investigated.

All ligand gated Cys loop receptors are closely related and have large conserved domains. Thus it is not surprising that picROTOXININ (**1**) also affects all other ligand gated chloride channels. PicROTOXIN inhibits GABA_C receptors (270, 273). Most important, picROTOXININ (**1**) is an antagonist of glycine at the mature ionotropic glycine receptors, which are mostly located in the spinal cord and the brain stem (274). Depending on the combination of very slightly differing subunits **1** can act as competitive or as non-competitive antagonist. The effect of **1** is smaller with the glycine receptor when compared with the GABA receptor and picROTIN (**2**) and dendrobine (**82**) are often more effective antagonists than **1**. Comparison of the action of picROTOXININ (**1**) and picROTIN (**2**) on GABA_A and diverse glycine receptors has led researchers to question the location of the binding site (275, 276). Contrary to the GABA_A receptor, where picROTOXIN was the earliest important research tool,

the location of the glycine receptors was mainly determined with the help of its specific antagonist, strychnine (274, 277). However, Lynch has indicated that picrotoxin is best able to distinguish between homopentameric and heteropentameric mature glycine receptors (277). Picrotoxanes not only are very useful research tools in mammalian neurophysiology but have been and will continue to be used extensively in investigating the neurobiology of invertebrates, especially that of insects. Not only do the GABA and glycine receptors differ very slightly from those of the vertebrates, where they are restricted to the central nervous system, in invertebrates these receptors are part of the peripheral nervous system too. Next to inhibition of these receptors, **1** also effectively inhibits invertebrate chloride currents gated by glutamate (278, 279), acetylcholine (280), and dopamine (281) receptors not found in vertebrates. Thus, in 2002, Dibas *et al.* reported that picrotoxin inhibits all known ligand-gated chloride channels (269). Picrotoxanes, especially picrotoxin(in), have had and most likely will have in the future an important part in clarifying the mode of action and distribution of inhibitory Cys-loop receptors. Moreover, they will contribute in allocating the information the numerous subgroups of the inhibitory Cys-loop receptors are processing.

Abbreviations

Ac	Acetyl
acac	acetylacetone
ACCN	1,1'-Azobis(cyclohexanecarbonitrile)
AIBN	2,2'-Azobisisobutyronitrile
aq	aqueous
Bn	Benzyl
BSA	<i>B</i> is(trimethylsilyl)acetamide
brsm	Based on recovered starting material
Bu	<i>n</i> -butyl
Bz	Benzoyl
cat	catalytic
CD	Circular dichroism
COLOC	Correlation spectroscopy of long range coupling
COSMIC	Force field calculation
COSY	Correlation spectroscopy
Cp	Cyclopentadienyl
CSA	Camphorsulfonic acid
Cy	Cyclohexyl
d	day(s)
dba	dibenzylidene acetone
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-Dicyclohexylcarbodiimide

DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethylazodicarboxylate
DEPT	Distortionless enhanced polarization transfer
DHF	4,5-Dihydrofuran
DIBAH	Diisobutylaluminum hydride
dicot	dicotyledon (flowering plant with two seed leaves)
diglyme	<i>Bis</i> - β -Hydroxyethyl ether
dil	diluted
DMAP	4-dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
2D-NMR	Two-dimensional nuclear magnetic resonance
% <i>ee</i>	enantiomeric excess
EPC	Enantiomerically pure compound
ESIMS	Electron spray ionization mass spectrum
Et	ethyl
exc	excess
FAB	Fast atom bombardment
FVP	Flash vacuum pyrolysis
GABA	γ -Aminobutyric acid
GLC	Gas-liquid chromatography
glyme	1,2-Dihydroxyethane
h	hours
h ν	Irradiation with light
HMBC	Heteronuclear multiple bond correlation
HMDS	Hexamethyldisilazane
HMPA	Hexamethylphosphoramide
HMQC	Heteronuclear multiple quantum correlation
HOHAHA	Homonuclear single quantum correlation
HPLC	High pressure liquid chromatography
HR-TOF-MS	High resolution time of flight-mass spectrum
HSQC	Heteronuclear single quantum correlation
IMDA	Intramolecular <i>Diels-Alder</i> reaction
<i>i</i> Pr	Isopropyl
IR	infrared (spectroscopy)
LAH	Lithium aluminum hydride
LDA	Lithium diisopropylamide
LHMDS	Lithium hexamethyldisilazide
MCPBA	<i>meta</i> -chloroperbenzoic acid
Me	Methyl
MOM	Methoxymethyl
monocot	monocotyledon (flowering plant with only one seed leaf)
Mp	Melting point

Ms	Methansulfonyl
MS	Molecular sieve
MS	Mass spectrum
MPTA Cl	(+)- α -Methoxy- α -(trifluoromethyl)phenylacetyl chloride
NBS	<i>N</i> -Bromosuccinimide
NMO	Morpholine- <i>N</i> -oxide
NMR	Nuclear magnetic resonance (spectroscopy)
NOE	Nuclear <i>Overhauser</i> effect
ODS	Octadecyl silica gel
ORD	Optical rotation dispersion (spectroscopy)
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
Ph	Phenyl
PhH	Benzene
PhCH ₃	Toluene
PMB	<i>p</i> -Methoxybenzyl
PMBOM	<i>p</i> -Methoxybenzyloxymethyl
PP	Diphosphate
PPTS	Pyridinium <i>p</i> -toluenesulfonate
pyr	pyridine
quant	quantitative yield
rfl	reflux
rt	room temperature
s.l.	<i>sensu lato</i>
<i>t</i> -Am	<i>tert</i> -amyl = 2-methylbut-2-yl
<i>t</i> -Bu	<i>tert</i> -butyl
TBAF	Tetrabutylammonium fluoride
TBS	<i>tert</i> -butyldimethylsilyl
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
Tf (OTf)	triflate=trifluoromethanesulfonate
thexyl	2,3-dimethyl-2-butyl
THF	Tetrahydrofuran, tetrahydrofuranyl
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TosMIC	Tosylmethylisocyanide = <i>p</i> -toluenesulfonylmethylisonitrile
Troc	Trichloroethoxycarbonyl
Ts	Tosyl = <i>p</i> -Toluenesulfonyl
UV	ultraviolet (spectroscopy)
Z	Benzyloxycarbonyl
Δ	High temperature
'	Minutes
''	Seconds
$[\alpha]_D$	Optical rotation at $\lambda = 589 \text{ nm}$

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References

1. Boullay PFG (1811) Nouveau Principe Immediat Cristallise, auquel la Coque du Levant doit ses Qualites Veneneuses. *Ann Chem Phys* **80**: 209
2. Porter LA (1967) Picrotoxinin and Related Substances. *Chem Rev* **67**: 441, and literature cited therein
3. Coscia CJ (1968) Picrotoxin. In: Taylor WI, Battersby AR (eds) *Cyclopentanoid Terpene Derivatives*, p. 147, and literature cited therein. Marcel Dekker, New York
4. Fischer NH, Olivier EJ, Fischer HD (1979) The Biogenesis and Chemistry of Sesquiterpene Lactones. *Prog Chem Org Nat Prod* **38**: 270, 272
5. Mackay MF, Sadek M (1983) The Crystal and Molecular Structure of Picrotoxinin. *Aust J Chem* **36**: 2111
6. Andrews PR, Brownlee RTC, Mackay MF, Poulton DB, Sadek M, Winkler DA (1983) Conformational Analysis of Picrotoxinin by N.M.R., X-Ray Crystallography, and Molecular Orbital and Classical Potential-Energy Calculations. *Aust J Chem* **36**: 2219
7. Sarma NS, Rambabu M, Anjaneyulu ASR, Rao CBS (1987) Occurrence of Picrotoxins in Marine Sponge *Spirastrella inconstans*. *Indian J Chem* **26B**: 189
8. Agarwal SK, Singh SS, Verma S, Kumar S (1999) Two Picrotoxin derivatives from *Anamirta cocculus*. *Phytochemistry* **50**: 1365
9. Perry NB, Aiyaz M, Kerr DS, Lake RJ, Leach MT (2001) NOESY on Neurotoxins: NMR and Conformational Assignment of Picrotoxins. *Phytochem Anal* **12**: 69
10. Blunt JW, Munro MHG, Swallow WH (1979) Carbon-13 NMR of Tutin and Related Substances: Application to the Identification of Minor Components of Toxic Honey. *Aust J Chem* **32**: 1339
11. Zhao WM, Ye QH, Dai JQ, Martin MT, Zhu JP (2003) *allo*-Aromadendrane- and Picrotoxane-type Sesquiterpenes from *Dendrobium moniliforme*. *Planta Med* **69**: 1136
12. Bi ZM, Wang ZT, Xu LS (2004) Chemical Constituents of *Dendrobium moniliforme*. *Acta Bot Sinica* **46**: 124
13. Gawell L, Leander K (1976) The Constitution of Aduncin, a Sesquiterpene Related to Picrotoxinin, found in *Dendrobium aduncum*. *Phytochemistry* **15**: 1991
14. Bi ZM, Mai JF, Zhu L, Wang ZT, Xu LS (2006) Study on Chemical Constituents of *Dendrobium aduncum*. *Zhongguo Yaoxue Zazhi* **41**: 1618
15. Dahmen J, Leander K (1978) Amotin and Amoenin, two Sesquiterpenes of the Picrotoxane Group from *Dendrobium amoenum*. *Phytochemistry* **17**: 1949
16. Majumder PL, Guha S, Sen S (1999) Bibenzyl Derivatives from the Orchid *Dendrobium amoenum*. *Phytochemistry* **52**: 1365
17. Morita H, Fujiwara M, Yoshida N, Kobayashi J (2000) New Picrotoxinin-type and Dendrobine-type Sesquiterpenoids from *Dendrobium* Snowflake "Red Star". *Tetrahedron* **56**: 5801
18. a) Riban J M (1864) Sur le Principe Toxique du Redoul (*Coriaria myrtifolia*). *Bull Soc Chim France* **1**: 87 b) Riban J M (1867) Sur la Coriamyrtine et ses Derives. *Bull Soc Chim France* **7**: 79
19. Okuda T, Yoshida T (1965) Establishment of the Structural Correlation between Coriamyrtin and Tutin. *Tetrahedron Lett* 4191

20. Reyes QA, Martinez JR, Bartulin J (1980) Coriamyrtin and other Metabolites of *Coriaria ruscifolia*. J Nat Prod **43**: 532
21. Okuda T, Yoshida T, Chen XM, Xie JX, Fukushima M (1987) Corianin from *Coriaria japonica* A. Gray, and Sesquiterpene Lactones from *Loranthus parasiticus* Merr. Used for Treatment of Schizophrenia. Chem Pharm Bull **35**: 182
22. Gulbis JM, Mackay MF, Wong MG (1989) Coriamyrtin and Tutin. A Crystallographic and Conformational Study. Aust J Chem **42**: 1881
23. a) Aguirre-Galviz LE, Templeton W (1990) Toxic Sesquiterpenoid Lactones from Leaves of *Coriaria microphylla*. Planta Med **56**: 244 b) Aguirre-Galviz LE (1987) Coriamyrtin from *Coriaria microphylla*. Fitoterapia **58**: 50
24. Wei H, Zeng FJ, Lu MY, Tang RJ (1998) Studies on Chemical Constituents from the Root of *Coriaria nepalensis* Wall. (*Coriaria sinica* Maxim.). Acta Pharm Sin **33**: 688; CAN 130:264785
25. Craven BM (1963) Molecular Structure of Tutin. Nature **197**: 1193
26. Mackay MF, Mathieson AMcL (1965) The Crystal Structure of α -Bromoisotutinone. Acta Crystallogr **19**: 417
27. Okuda T, Yoshida T (1965) Absolute Configuration of Tutin. Tetrahedron Lett 2137
28. Kinoshita T, Itaki N, Hikita M, Aoyagi Y, Hitozuyanagi Y, Takeya K (2005) The Isolation and Structure Elucidation of a New Sesquiterpene Lactone from the Poisonous Plant *Coriaria japonica* (Coriariaceae). Chem Pharm Bull **53**: 1040
29. Li ML, Cui J, Qin RH, Gao JM, Zhang YB, Guo XR, Zhang W (2007) Semisynthesis and Antifeedant Activity of New Acylated Derivatives of Tutin, a Sesquiterpene Lactone from *Coriaria sinica*. Heterocycles **71**: 1155
30. a) Easterfield TH, Aston BC (1900) Tutu, Part I. Proc Chem Soc **16**: 211; b) Easterfield TH, Aston BC (1901) Tutu, Part I. Tutin and Coriamyrtin. J Chem Soc **79**: 120
31. Hodges R, White EP (1966) Detection and Isolation of Tutin and Hyenanchin in Toxic Honey. NZ J Sci **9**: 233
32. Palmer-Jones T (1965) Poisonous Honey Overseas and in New Zealand. NZ Med J **64**: 631
33. Kudo Y, Niwa H, Tanaka A, Yamada K (1984) Action of Picrotoxinin and Related Compounds on the Spinal Cord: the Role of a Hydroxyl-group at the 6-Position in Antagonizing the Actions of Amino acids and Presynaptic Inhibition. Br J Pharmacol **81**: 373
34. Lowe MD, White EP (1972) Tutin in *Coriaria* Species - Identification and Estimation. NZ J Sci **15**: 303
35. Vinter JG, Davis A, Saunders MR (1987) Strategic Approaches to Drug Design. I An Integrated Software Framework for Molecular Modelling. J Comp-Aided Mol Des **1**: 31
36. Koike K, Suzuki Y, Ohmoto T (1994) Picrotoxane Terpenoids, Picrodendrins S and T, from *Picrodendron baccatum*. Phytochemistry **35**: 701
37. Okuda T, Yoshida T (1967) The Correlation of Coriamyrtin and Tutin and Their Absolute Configuration. Chem Pharm Bull **15**: 1955
38. Reyes A, Garcia-Quintana HG, Romero M, Morales D, Bartulin J (1998) Tutina y Otros Metabolitos de *Coriaria ruscifolia* L. Bol Soc Chil Quim **43**: 87
39. Chang YS, Lin MS, Jiang RL, Huang SC, Ho LK (1996) 20-Epibryonolic Acid, Phytosterols and Ellagic acid from *Coriaria intermedia*. Phytochemistry **42**: 559
40. Okuda T, Yoshida T (1971) Structure of Corianin. Tetrahedron Lett **12**: 4499
41. Guo HZ, Li JS, Wu N, Lu Y, Zheng QT (2000) Study on Constituents of the Seed from *Cuscuta japonica*. Beijing Zhongyiyao Daxue Xuebao **23**: 36
42. Biollaz M (1965) Über die Konstitution und Konfiguration einiger C₁₅-Bitterstoffe aus *Hyenanche globosa* Lambert, PhD Thesis, Nr. 3746, ETH Zürich
43. Zhao WM, private communication
44. Jommi G, Pelizzoni G, Scolastico C (1965) Correlazione tra Tutina e Ienancina. Chim Ind (Milan) **47**: 407
45. a) Jommi G, Pelizzoni F, Scolastico C (1965) Correlazione tra Tutina e Diidroisoiencina. Chim Ind (Milan) **47**: 406 b) Jommi G, Pelizzoni F, Scolastico C (1965) Costituenti della

- Hyenanche globosa*: Struttura della Sostanza D **47**: 865 c) Corbella A, Jommi G, Rindone B, Scolastico C (1967) Constituents of *Hyenanche globosa*. Structure of Substance C and Correlation Between PicROTOXININ and Tutin. *Ann Chim (Rome)* **57**: 758
46. Ohmoto T, Koike K, Fukuda H, Mitsunaga K, Ogata K, Kagei K (1989) Studies on the Constituents of *Picrodendron baccatum* Growing in Indonesia. II. Structures of Two New Sesquiterpene Lactones, PicRODENDRIN C and D. *Chem Pharm Bull* **37**: 2988
 47. Jommi G, Manitto P, Pelizzoni F, Scolastico C (1964) Costituenti amari della *Hyenanche globosa*. *Chem Ind (Milan)* **46**: 549
 48. Castaneda P, Bahena A, Garcia E, Chavez D, Mata R, Gutierrez A (1993) Secondary Metabolites from the Stem Bark of *Celaenodendron mexicanum*. *J Nat Prod* **56**: 1575
 49. Valencia E, Valenzuela E, Barros E, Aedo V, Gebauer MT, Garcia C, Gonzalez AG, Bermejo J (2001) Constituents of *Coriaria ruscifolia* Fruits. *Fitoterapia* **72**: 555
 50. a) Saika T, Tokuyama T 41st National Meeting of the Chemical Society of Japan, Higashi-Osaka, April 1980, Abstr. No. 1S14 b) Saika T, Tokuyama T 45th National Meeting of the Chemical Society of Japan, Tokyo, April 1982, Abstr. Nr. 1E44 c) Saika T, Tokuyama T, Higuchi T, Hirotsu K 47th National Meeting of the Chemical Society of Japan, Kyoto, April 1983, Abstr. Nr. 1H30
 51. Ref. 4 in Wakamatsu K, Kigoshi H, Niiyama K, Niwa H, Yamada K (1984) Stereocontrolled Total Synthesis of (+)-Asteromurin A, a PicROTOXANE Sesquiterpene Isolated from the Scale Insect *Asterococcus muratae* Kuwana. *Chem Lett* 1763
 52. Wakamatsu K, Kigoshi H, Niiyama K, Niwa H, Yamada K (1986) Stereocontrolled Total Synthesis of (+)-Tutin and (+)-Asteromurin A, Toxic PicROTOXANE Sesquiterpenes. *Tetrahedron* **42**: 5551
 53. Koike K, Ohmoto T, Kawai T, Sato T (1991) PicROTOXANE Terpenoids from *Picrodendron baccatum*. *Phytochemistry* **30**: 3353
 54. Koike K, Fukuda H, Mitsunaga K, Ohmoto T (1991) PicRODENDRINS B, G and J, New PicROTOXANE Terpenoids from *Picrodendron baccatum*. *Chem Pharm Bull* **39**: 934
 55. Suzuki Y, Koike K, Ohmoto T (1992) Eight PicROTOXANE Terpenoids, PicRODENDRINS K-R, from *Picrodendron baccatum*. *Phytochemistry* **31**: 2059
 56. Suzuki Y, Koike K, Nagahisa M, Nikaido T (2003) New PicROTOXANE Terpenoids from *Picrodendron baccatum*. *Tetrahedron* **59**: 6019
 57. Tang J, Liu QF, Dai JQ, Zhao WM (2004) A New PicROTOXANE Type Sesquiterpene from *Dendrobium densiflorum*. *Chin Chem Lett* **15**: 63
 58. Zhao CS, Liu QF, Halaweish F, Shao BP, Ye YQ, Zhao WM (2003) Copacamphane, PicROTOXANE, and Alloaromadendrane Sesquiterpene Glycosides and Phenolic Glycosides from *Dendrobium moniliforme*. *J Nat Prod* **66**: 1140
 59. Shu Y, Zhang DM, Guo SX (2004) A New Sesquiterpene Glycoside from *Dendrobium nobile* Lindl. *J Asian Nat Prod Res* **6**: 311
 60. Ye QH, Qin GW, Zhao WM (2002) Immunomodulatory Sesquiterpene Glycosides from *Dendrobium nobile*. *Phytochemistry* **61**: 885
 61. a) Conroy H (1957) PicROTOXIN: Lithium Aluminum Hydride Reductions. *Chem Ind (London)* 704–705; b) Conroy H (1957) PicROTOXIN V. Conformational analysis and problems of structure. *J Am Chem Soc* **79**: 5550
 62. Pradhan P, Mamdapur VR, Sipahimalani AT (1990) Methyl PicROTOXATE, a Minor Component of Berries of the Plant *Anamirta cocculus* and its Stereochemistry by High Resolution NMR Spectroscopy. *Indian J Chem* **29B**: 676
 63. Krische MJ, Trost BM (1998) Total Synthesis of Methyl PicROTOXATE via the Palladium Catalyzed Enyne Cycloisomerization Reaction. *Tetrahedron* **54**: 3693
 64. Tane P, Ayafor JF, Farrugia LJ, Connolly JD, Rycroft DS, Tillequin F (1996) PicROTOXIMAESIN, a Novel PicROTOXANE Sesquiterpenoid from *Maesobotrya floribunda*. *Nat Prod Lett* **9**: 39
 65. Ayafor JF, Tane P, Ngadjui BT, Connolly JD, Rycroft DS, Tillequin F (1993) PicROTOXIMAESIN, a New PicROTOXANE Sesquiterpene from *Maesa kamerunensis* Mez. *Planta Med* **59**:

- A 599; the investigated plant was subsequently identified as *Maesobotrya floribunda*, see Ref. 64
66. Okamoto T, Natsume M, Onaka T, Uchimaro F, Shimizu M (1972) Further Studies on the Alkaloidal Constituents of *Dendrobium nobile* (Orchidaceae)-Structure Determination of 4-Hydroxydendroxine and Nobilomethylene. *Chem Pharm Bull* **20**: 418
 67. Zhang X, Liu HW, Gao H, Han HY, Wang NL, Wu HM, Yao XS, Wang Z (2007) Nine New Sesquiterpenes from *Dendrobium nobile*. *Helv Chim Acta* **90**: 2386
 68. Zhang X, Tu FJ, Ju HY, Wang NL, Wang Z, Yao XS (2008) Copacamphane, Picrotoxane and Cyclocopacamphane Sesquiterpenes from *Dendrobium nobile*. *Chem Pharm Bull* **56**: 854
 69. Shen YH, Li SH, Li RT, Han QB, Zhao QS, Liang L, Sun HD, Lu Y, Cao P, Zheng QT (2004) Coriatone and Corianlactone, Two Novel Sesquiterpenes from *Coriaria nepalensis*. *Org Lett* **6**: 1593
 70. Zhao WM, Ye QH (2002) New Alloaromadendrane, Cadinene and Cyclocopacamphane Type Sesquiterpene Derivatives and Bibenzyls from *Dendrobium nobile*. *Planta Med* **68**: 723
 71. Zhao WM, Ye Q, Tan X, Jiang H, Li X, Chen K, Kinghorn AD (2001) Three New Sesquiterpene Glycosides from *Dendrobium nobile* with Immunomodulatory Activity. *J Nat Prod* **64**: 1196
 72. Jommi G, Manitto P, Pelizzoni F, Scolastico (1965) Costituenti della *Hyenanche globosa*; Struttura della Capenicina. *Chem Ind (Milan)* **47**, 1328
 73. Corbella A, Jommi G, Scolastico C (1966) Structural correlation between Capenicin and Tutin. *Tetrahedron Lett* 4819
 74. Seibl J (1967) Metastable Transitions in the Mass Spectra of Organic Compounds. *Helv Chim Acta* **50**: 263
 75. Müller B (1967) Zur Kenntnis der sesquiterpenoiden Inhaltsstoffe aus *Hyenanche globosa* Lambert, PhD Thesis Nr. 4000, ETH Zürich
 76. Corbella A, Jommi G, Rindone B, Scolastico C (1969) Structure of Pretoxin and Lambicin. *Tetrahedron* **25**: 4835
 77. Ohmoto T, Koike K, Fukuda H, Mitsunaga K, Kagei K, Kawai T, Sato T (1989) Studies on the Constituents of *Picrodendron baccatum* Growing in Indonesia: Structure of a Norditerpene Lactone, Picrodendrin A. *Chem Pharm Bull* **37**: 1805
 78. Nagahisa M, Koike K, Narita M, Ohmoto T (1994) Novel Picrotoxane Norditerpene Lactones from *Picrodendron baccatum*. *Tetrahedron* **50**: 10859
 79. a) Behr D, Leander K (1972) Studies on Orchidaceae Alkaloids XXVIII. The Absolute Configuration of the Dendrobine Alkaloids. *Acta Chem Scand* **26**: 3196 b) Behr D, Leander K (1976) Studies on Orchidaceae Alkaloids. 41. The Configuration at C-4 in δ -Nobilonine and Dihydroketopicrotoxininic Acid. *Acta Chem Scand* **B 30**: 279
 80. Hedman K, Leander K, Lüning B (1971) Studies on Orchidaceae Alkaloids XXV. N-Isopentenyl Derivatives of Dendroxin and 6-Hydroxydendroxine from *Dendrobium friedericksianum* Lindl and *Dendrobium hildebrandii* Rolfe. *Acta Chem Scand* **25**: 1142
 81. Hedman K, Leander K (1972) Studies on Orchidaceae Alkaloids XXVII. Quaternary Salts of the Dendrobine Type from *Dendrobium nobile* Lindl. *Acta Chem Scand* **26**: 3177
 82. Inubushi Y, Tsuda Y, Katarao E (1966) The Structure of Dendramine. *Chem Pharm Bull* **14**: 668
 83. Granelli I.; Leander K, Lüning B (1970) Studies on Orchidaceae Alkaloids XVI. A New Alkaloid, 2-Hydroxydendrobine, from *Dendrobium findlayianum* Par. et Rchb. f. *Acta Chem Scand* **24**: 1209
 84. Suzuki M, Yamada K, Hirata Y (1975) Synthesis of 2-Hydroxydendrobine and Nobile. *Chem Lett* 611
 85. Granelli I, Leander K (1970) Studies on Orchidaceae Alkaloids XIX. Synthesis and Absolute Configuration of Dendrine. *Acta Chem Scand* **24**: 1108
 86. Liu QF, Zhao WM, (2003) A New Dendrobine-Type Alkaloid from *Dendrobium nobile*. *Chin Chem Lett* **14**: 278

87. Elander M, Leander K (1971) Studies on Orchidaceae Alkaloids XXI. 6-Hydroxynobiline, a New Alkaloid from *Dendrobium hildebrandii* Rolfe. *Acta Chem Scand* **25**: 717
88. Liu WH, Hua YF (2007) Moniline, a New Alkaloid from *Dendrobium moniliforme*. *J Chem Res* **317**
89. Wang H, Zhao T, Che CT (1985) Dendrobine and 3-Hydroxy-2-oxodendrobine from *Dendrobium nobile*. *J Nat Prod* **48**: 796
90. Blomqvist L, Brandänge S, Gawell L, Leander K, Luning B (1973) Studies on Orchidaceae Alkaloids XXXVII. Dendrowardine, a Quaternary Alkaloid from *Dendrobium wardianum*. *Acta Chem Scand* **27**: 1439
91. The Angiosperm Phylogeny Group (AGP) (1998) An Ordinal Classification for the Families of Flowering Plants. *Ann Missouri Bot Gard* **85**: 531
92. The Angiosperm Phylogeny Group (AGP II) (2003) An Update of the Angiosperm Phylogeny Group Classification of Orders and Families of Flowering Plants. *Bot J Linn Soc* **141**: 299
93. Wink M (2006) Schriftzeichen im Logbuch des Lebens. *Molekulare Evolutionsforschung. Biol Unserer Zeit* **36**: 26
94. Hegnauer R (1962–2001) *Chemotaxonomie der Pflanzen* vols. 2, p 383; 3, pp 562, 563, 662, 674; 5, 311; 7, pp 739, 741, 743, 748; 8, pp 331, 333, 334, 452, 453, 685, 710; 9, 404, 561, 563, 564, 730 Birkhäuser Verlag, Basel and Stuttgart
95. Yokoyama J, Suzuki M, Iwatsuki K, Hasebe M (2000) Molecular Phylogeny of *Coriaria* with Special Emphasis on the Disjunct Distribution. *Mol Phylogen Evol* **14**: 11
96. Corbella A, Gariboldi P, Jommi G, Scolastico C (1969) Biosynthesis of Tutin. *J Chem Soc, Chem Commun* 634–635
97. a) Wang XK, Zhao TF, Wang M (1985) Gas Chromatographic-Mass Spectroscopic Investigation of the Alkaloids of *D. nobile* Cultivated on Eleven Trees. *Bull Chin Nat Med* **10**: 367–369 b) Wang XK, Zhao TF (1985) TLC Determination of Dendrobine in *D. nobile* Cultivated on Trees. *Acta Acad Med Sichuan* **16**: 249–253
98. Paterson CR (1947) Part IV. The Source of the Toxic Honey-Field Observations NZ J Sci Technol, Sect A **29**: 125
99. Love JL (1990) Toxic Honey - a New Zealand Story. *Anal Proc* **27**: 87
100. Sutherland MD (1992) New Zealand Toxic Honey – The Actual Story. *Anal Proc* **29**: 112
101. a) Lorence A, Nessler CL (2004) Camptothecin, over Four Decades of Surprising Findings. *Phytochemistry* **65**: 2735 b) Larsson S (2007) The New Phylogeny Has Reduced Scattering Somewhat: The “New” Chemosystematics: Phylogeny and Phytochemistry. *Phytochemistry* **68**: 2903 c) Kusari S, Zühlke S, Spiteller M (2009) An Endophytic Fungus from *Camptotheca acuminata* that Produces Camptothecin and Analogues. *J Nat Prod* **72**: 2, and literature cited therein
102. Wink M (2003) Evolution of Secondary Metabolites from an Ecological and Molecular Phylogenetic Perspective. *Phytochemistry* **64**: 3, and literature cited therein
103. a) Dunning Hotopp JC, Clark ME, Oliveira DSG, Foster GM, Fischer P, Munoz Torres MC, Giebel JD, Kumar N, Ishmael N, Wang S, Ingram J, Nene RV, Shepard J, Tomkins J, Richards S, Spiro DJ, Ghedin E, Slatko BE, Tettelin H, Werren JH (2007) Widespread Lateral Gene Transfer from Intracellular Bacteria to Multicellular Eukaryotes. *Science* **317** (5845): 1753 b) Gunatilaka AAL (2006) Natural Products from Plant-associated Microorganisms: Distribution, Structural Diversity, Bioactivity, and Implications of Their Occurrence. *J Nat Prod* **69**: 509
104. Ahimsa-Müller MA, Markert A, Hellwig S, Knoop V, Steiner U, Drewke C, Leistner E (2007) Clavicipitaceous Fungi Associated with Ergoline Alkaloid-Containing Convolvulaceae. *J Nat Prod* **70**: 1955–1960
105. Wink M, Witte L (1983) Evidence for a Widespread Occurrence of the Genes of Quinolizidine Alkaloid Biosynthesis. Induction of Alkaloid Accumulation in Cell Suspension Cultures of Alkaloid-“free” Species. *FEBS Letters* **159**: 196

106. Wu CY, Zhang G (1984) Quantitative Analysis of Sesquiterpene Lactone in *Loranthus parasiticus* (L.) Merr. Parasiting on *Coriaria sinica* Maxim, and in Seeds of *Coriaria sinica* Maxim. Acta Pharm Sin (Yaoxue Xuebao) **19**: 56
107. Henkel JB (1858) Beitrage zur Kenntniss der chemischen Bestandtheile der Früchte von *Hyenanche globosa* Lamb., Euphorbiaceae. Arch Pharm **144**: 16
108. Henry TA (1920) Hyenanchin and other Constituents of *Hyenanche globosa*. J Chem Soc **117**: 1619
109. Biollaz M, Arigoni D (1964) Structure de l'Hyenanchine, Principe Amer de l'*Hyenanche globosa* Lamb. Chimia **18**: 184
110. Suzuki H, Keimatsu I (1932) Über die Alkaloide der chinesischen Droge „Chin-shih-hu“. (II Mitteilung). Über das Dendrobin (I). J Pharm Soc Jap **52**: 1049
111. Suzuki H, Keimatsu I, Ito K (1934) Über die Alkaloide der chinesischen Droge „Chin-Shih-Hu“. (III Mitteilung). Über das Dendrobin (II). J Pharm Soc Jap **54**: 802
112. Chen KK, Chen AL (1935) The Alkaloid of “Chin-shih-hu”. J Biol Chem **111**: 653
113. Wang SY, Song XQ, Li HH (2007) Analysis of *Dendrobium nobile* Lindl Produced in Yunnan and Content of Total Alkaloids Provided by Cell Culture. Guizhou Shifan Daxue Xuebao, Ziran Kexueban **25**(2): 1.2, 16; CAN 2008: 36071
114. Inubushi Y, Ishii H, Yasui B, Konita T, Harayama T (1964) Isolation and Characterization of Alkaloids of the Chinese Drug “Chin-Shi-Hu”. Chem Pharm Bull **12**: 1175
115. Conroy H (1957) Picrotoxin. III. α -Picrotoxininic and Bromopicrotoxininic Acids; Apopicrotoxininic Dilactone. J Am Chem Soc **79**: 1726
116. Craven BM (1960) Molecular Structure and Absolute Configuration of Picrotoxinin. Tetrahedron Lett **21**
117. Craven BM (1962) The Crystal Structure of α -1-Bromopicrotoxinin. Acta Crystallogr. **15**: 387
118. Corey EJ, Pearce HL (1979) Total Synthesis of Picrotoxinin. J Am Chem Soc **101**: 5842
119. Niwa H, Wakamatsu K, Hida T, Niiyama K, Kigoshi H, Yamada M, Nagase H, Suzuki M, Yamada K (1984) Stereocontrolled Total Synthesis of (–)-Picrotoxinin and (+)-Coriamyrtin via a Common Isotwistane Intermediate. J Am Chem Soc **106**: 4547
120. Miyashita M, Suzuki T, Yoshikoshi A (1989) Selective Total Synthesis of (–)-Picrotoxinin and (–)-Picrotin. J Am Chem Soc **111**: 3728
121. Trost BM, Krische MJ (1996) General Strategy for the Asymmetric Synthesis of Picrotoxanes. J Am Chem Soc **118**: 233
122. Trost BM, Krische MJ (1999) Palladium-Catalyzed Enyne Cyclodimerization in Asymmetric Approach to the Picrotoxane Sesquiterpenes. 2. Second-Generation Total Syntheses of Corianin, Picrotoxinin, Picrotin, and Methyl Picrotoxate. J Am Chem Soc **121**: 6131
123. Schmidt TJ, Okuyama E, Fronczek FR (1999) The Molecular Structure of 2 α -Hydroxyneoisatin and Structure Activity Relationships among Convulsant Sesquiterpenes of the *seco*-Prezizaane and Picrotoxane Types. Bioorg Med Chem **7**: 2857
124. Corey EJ, Pearce HL (1980) Total Synthesis of Picrotin. Tetrahedron Lett **21**: 1823
125. Okuda T, Yoshida T (1964) Structure of Coriamyrtin. Tetrahedron Lett **9**: 439
126. Okuda T, Yoshida T (1967) Coriamyrtin X. Stereochemistry of Coriamyrtin and Problems of Derivatives. Chem Pharm Bull **15**: 1697
127. Craven BM (1964) The Crystal Structure and Absolute Configuration of α -Bromoisoetin. Acta Crystallogr **17**: 396
128. Tanaka K, Uchiyama F, Sakamoto K, Inubushi Y (1982) Stereocontrolled Total Synthesis of (\pm)-Coriamyrtin. J Am Chem Soc **104**: 4965
129. Tanaka K, Uchiyama F, Sakamoto K, Inubushi Y (1983) Synthetic Studies on a Picrotoxane Sesquiterpene, Coriamyrtin III. Completion of the Stereocontrolled Total Synthesis of Coriamyrtin. Chem Pharm Bull **31**: 1972
130. Wakamatsu K, Kigoshi H, Niiyama K, Niwa H, Yamada K (1984) Stereocontrolled Total Synthesis of (+)-Tutin. A Toxic Sesquiterpene of Picrotoxane-Type. Tetrahedron Lett **25**: 3873
131. Krische MJ, Trost BM (1998) Transformation of the Picrotoxanes: The Synthesis of Corianin and Structural Analogs from Picrotoxinin. Tetrahedron **54**: 7109

132. Trost BM, Haffner CD, Jebaratnam DJ, Krische MJ, Thomas AP (1999) The Palladium-Catalyzed Enyne Cycloisomerization Reaction in a General Approach to the Asymmetric Syntheses of the PicROTOXANE Sesquiterpenes. Part. I. First-Generation Total Synthesis of Corianin and Formal Syntheses of PicROTOXININ and PicROTOIN. *J Am Chem Soc* **121**: 6183
133. Hodges R, White EP (1964) The Structure of Mellitoxin. *Tetrahedron Lett* 371
134. Severini Ricca G, Scolastico C (1967) Complessi di Collisione e Spettri NMR.- Nota I. Spettri NMR dei Composti della Serie della Ienancina. *Gazz Chim Italiana* 97: 1905
135. Wakamatsu K, Kigoshi H, Niiyama K, Niwa H, Yamada K (1984) Stereoccontrolled Total Synthesis of (+)-Asteromurin A, a PicROTOXANE Sesquiterpene Isolated from the Scale Insect *Asterococcus muratae* Kuwana. *Chem Lett* 1763
136. a) Inubushi Y, Sasaki Y, Tsuda Y, Yasui B, Konita T, Matsumoto J, Katarao E, Nakano J (1963) Structure of Dendrobine. *Yakugaku Zasshi (J Pharm Soc Japan)* **83**: 1184 b) Inubushi Y, Sasaki Y, Tsuda Y, Yasui B, Konita T, Matsumoto J, Katarao E, Nakano J (1964) Structure of Dendrobine. *Tetrahedron* **20**: 2007
137. Onaka T, Kamata S, Maeda T, Kawazoe Y, Natsume M, Okamoto T, Uchimaru F, Shimizu M (1964) The Structure of Dendrobine. *Chem Pharm Bull* **12**: 506
138. Yamamura S, Hirata Y (1964) Structures of Nobiletine and Dendrobine. *Tetrahedron Lett* 79
139. Inubushi Y, Katarao E, Tsuda Y, Yasui B (1964) Absolute Configuration of Dendrobine. *Chem Ind (London)* 1698
140. Huang (Hwang) WK (1965) Configurations of Dendrobine and Nobiletine. *Acta Chim Sin* **31**: 333
141. Matsuda H, Tomiie Y, Nitta I (1965) 18th Annual Meeting of the Chemical Society Japan (Osaka; Abstracts of Papers, p. 215)
142. Onaka T, Kamata S, Maeda T, Kawazoe Y, Natsume M, Okamoto T, Uchimaru F, Shimizu M (1965) The Structure of Nobiletine. The Second Alkaloid from *Dendrobium nobile*. *Chem Pharm Bull* **13**: 745
143. Sha CK, Chiu RT, Yang CF, Yao NT, Tseng WH, Liao FL, Wang SL (1997) Total Synthesis of (-)-Dendrobine via α -Carbonyl Radical Cyclization. *J Am Chem Soc* **119**: 4130
144. Cassayre J, Zard S (1999) A Short Synthesis of (-)-Dendrobine. *J Am Chem Soc* **121**: 6072
145. Genovese S, Curini M, Epifano F (2009) Chemistry and Biological Activity of Azoprenylated Secondary Metabolites. *Phytochemistry* **70**: 1082
146. Yamada K, Suzuki M, Hayakawa Y, Aoki K, Nakamura H, Nagase H, Hirata Y (1972) Total Synthesis of (\pm)-Dendrobine. *J Am Chem Soc* **94**: 8278
147. Inubushi Y, Kikuchi T, Ibuka T, Tanaka K, Saji I, Tokane K (1972) Total Synthesis of the Alkaloid (\pm)-Dendrobine. *J Chem Soc, Chem Commun* 1252
148. Inubushi Y, Kikuchi T, Ibuka T, Tanaka K, Saji I, Tokane K (1974) Total Synthesis of the Alkaloid (\pm)-Dendrobine. *Chem Pharm Bull* **22**: 349
149. Brattesani DN, Heathcock CH (1975) A Synthetic Approach to the Dendrobine Skeleton. *J Org Chem* **40**: 2165
150. Yamamoto K, Kawasaki I, Kaneko T (1970) Synthesis of the Skeleton of Dendrobine. *Tetrahedron Lett* **11**: 4859
151. Kende AS, Bentley TJ, Mader RA, Ridge D (1974) A Simple Total Synthesis of (\pm)-Dendrobine. *J Am Chem Soc* **96**: 4332
152. Hu QY, Zhou G, Corey EJ (2004) Application of Chiral Cationic Catalyst to Several Classical Synthesis of Racemic Natural Products Transforms Them into Highly Enantioselective Pathways. *J Am Chem Soc* **126**: 13708
153. Borch RF, Evans AJ, Wade JJ (1975) Synthesis of 8-*epi*-Dendrobine. *J Am Chem Soc* **97**: 6282
154. Borch RF, Evans AJ, Wade JJ (1977) Synthesis of 8-*epi*-Dendrobine. *J Am Chem Soc* **99**: 1612
155. Roush WR (1978) Total Synthesis of (\pm)-Dendrobine. *J Am Chem Soc* **100**: 3599
156. Roush WR (1980) Total Synthesis of (\pm)-Dendrobine. *J Am Chem Soc* **102**: 1390
157. Roush WR, Gillis HR (1980) Improved Synthesis of the Perhydroindenone Precursor of Dendrobine. *J Org Chem* **45**: 4283

158. Martin SF, Li W (1989) General Methods for Alkaloid Synthesis via Intramolecular Diels-Alder Reactions. A Concise Formal Total Synthesis of (\pm)-Dendrobine. *J Org Chem* **54**: 268
159. Martin SF, Li W (1991) Applications of Intramolecular Diels-Alder Reactions to Alkaloid Synthesis. A Formal Total Synthesis of (\pm)-Dendrobine. *J Org Chem* **56**: 642
160. Padwa A, Dimitroff M, Liu B (2000) Formal Synthesis of (\pm)-Dendrobine: Use of the Amidofuran Cycloaddition/Rearrangement Sequence. *Org Lett* **2**: 3233
161. Padwa A, Brodney MA, Dimitroff M, Liu B, Wu T (2001) Application of Furanyl Carbamate Cycloadditions Toward the Synthesis of Hexahydroindolinone Alkaloids. *J Org Chem* **66**: 3119
162. Connolly PJ, Heathcock CH (1985) An Approach to the Total Synthesis of Dendrobine. *J Org Chem* **50**: 4135
163. Westling M, Livinghouse T (1987) Acylnitrilium Ion Cyclization in Heterocycle Synthesis. A Convergent Method for the Preparation of 2-Acylpyrrolines via the Intramolecular Acylation of Silyloxyalkenes with α -Keto Imidoyl Chlorides. *J Am Chem Soc* **109**: 590
164. Lee CH, Westling M, Livinghouse T, Williams AC (1992) Acylnitrilium Ion Initiated Heteroannulations in Alkaloid Synthesis. An Efficient, Stereocontrolled, Total Synthesis of Orchidaceae Alkaloid (\pm)-Dendrobine. *J Am Chem Soc* **114**: 4089
165. Takano S, Inomata K, Ogasawara K (1992) A Facile Approach to the Dendrobine Skeleton by Intramolecular Pauson Khand Reaction. *Chem Lett* 443
166. Cassayre J, Zard S (2001) A Short Synthesis of (–)-Dendrobine. Some Observations on the Nickel Mediated Radical Cyclisation and on the Pauson-Khand reaction. *J Organomet Chem* **624**: 316
167. Mori M, Uesaka N, Shibasaki M (1992) Novel Synthesis of Nitrogen Heterocycles Using Zirconium-Promoted Reductive Coupling. Formal Total Synthesis of Dendrobine. *J Org Chem* **57**: 3519
168. Mori M, Saitoh F, Uesaka N, Shibasaki M (1993) Formal Total Synthesis of (–)-Dendrobine Using Zirconium Promoted Cyclization. Determination of the Absolute Configuration of the Intermediary Tricyclic Ketone. *Chem Lett* 213
169. Uesaka N, Saitoh F, Mori M, Shibasaki M, Okamura K, Date T (1994) Formal Total Synthesis of (–)-Dendrobine Using Zirconium-Promoted Reductive Cyclization. *J Org Chem* **59**: 5633
170. Trost BM, Tasker AS, Rütger G, Brandes A (1991) Activation in Transition-Metal Catalysis by Catalyst Relay. A Synthetic Approach to (–)-Dendrobine. *J Am Chem Soc* **113**: 670
171. Heathcock CH, Graham SL, Pirrung MC, Plavac F, White CT (1979) Total Synthesis of Sesquiterpenes, 1970–1979. In: ApSimon J (ed) *Synthesis of Natural Products*, Vol. V, p. 510. Wiley, New York, Chichester, Brisbane, Toronto, Singapore
172. Ireland RE, Bey P (1988) Homogeneous Catalytic Hydrogenation: Dihydrocarvone [2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-]. In: *Organic Synthesis*, Coll. VI, p. 459. Wiley, New York, Chichester, Brisbane, Toronto, Singapore
173. Hayakawa Y, Nakamura H, Aoki K, Suzuki M, Yamada K, Hirata Y (1971) Synthetic Intermediate Potentially Useful for the Synthesis of Dendrobine. *Tetrahedron* **27**: 5157
174. Ruzicka L, Sternbach L (1940) Zur Kenntnis der Diterpene. Dehydrierung von Oxydationsprodukten der Abietinsäure zu 1-Methyl-oxy-phenanthren und 1,5-Dimethyl-6-oxy-naphtalin. Synthese von 1,5-Dimethyl-7-oxy- und von 1,6-Dimethyl-7-oxy-naphtalin. *Helv Chim Acta* **23**: 355
175. a) Johnson WS, Shulman S, Williamson KL, Pappo R (1962) Production of a Fused-Ring System by an Intramolecular Michael Condensation. *J Org Chem* **27**: 2015–2018 b) Barton DHR, Campos-Neves AS, Scott IA (1957) Approach to the Partial Synthesis of Aldosterone from Steroids Lacking Substitution of C-18. *J Chem Soc* 2698
176. Borch RF (1968) A New Method for the Reduction of Secondary and Tertiary Amides. *Tetrahedron Lett* 61
177. Boyce CBC, Whitehurst JC (1960) Some Preliminary Synthetical Studies with 5,6,7,8-Tetrahydro-8-methylindane-1,5-dione. *J Chem Soc* 4547; cf. Acklin W, Prelog V, Prieto

- AP (1958) Reaktionen mit Mikroorganismen. Die stereospezifische Reduktion von (\pm)- $\Delta^{4,9}$ -8-Methylhexahydroindendion-(1,5). *Helv Chim Acta* **41**: 1416
178. a) Kremers E, Wakeman N, Hixon RM (1941) Thymoquinone. In *Organic Synthesis*, Coll. I, p. 511. Wiley, New York, Chichester, Brisbane, Toronto, Singapore b) McOmie JFW, Blatchly JM (1972) Thiele-Winter Acetoxylation of Quinones. *Org React* **19**: 199
179. a) Corey EJ, Yamamoto H (1970) Modification of the Wittig Reaction to Permit the Stereospecific Synthesis of Certain Trisubstituted Olefins. Stereospecific Synthesis of α -Santalol. *J Am Chem Soc* **92**: 226 b) Corey EJ, Yamamoto H, Herron DK, Achiwa KJ (1970) New stereospecific synthetic routes to trisubstituted olefins. *J Am Chem Soc* **92**: 6635
180. Corey EJ, Gilman NW, Ganem BE (1968) New Methods for the Oxidation of Aldehydes to Carboxylic Acids and Esters. *J Am Chem Soc* **90**: 5616
181. a) Burden RS, Crombie L (1969) Amides of Vegetable Origin. New Series of 2,4-Alkadienoic Tyramine Amides from *Anacyclus pyrethrum*. *J Chem Soc* 2477 b) Bohlmann F, Zdero C (1973) New Synthesis of Cyclohexadiene Derivatives. *Chem Ber* **106**: 3779
182. Cargill RL, Jackson TE, Peet NP, Pond DM (1974) Acid-catalyzed Rearrangements of β,γ -Unsaturated Ketones. *Acc Chem Res* **7**: 106
183. Lynch VM, Li W, Martin SF, Davis BE (1990) Structure of the ABC Ring Subunit of 3-Hydroxy-2-oxodendrobine. *Acta Crystallogr, Sect C. Cryst Struct Commun* **46**: 1159
184. a) Meyers AI, Tomioka K, Fleming MP (1978) Convenient Preparation of α,β -Unsaturated Aldehydes. *J Org Chem* **43**: 3788 b) Corey EJ, Schmidt G (1980) A Simple Route to Δ^2 -Butenolides from Conjugated Aldehydes. *Tetrahedron Lett* **21**: 731
185. a) Ziegler FE, Piwinski JJ (1982) Tandem Cope-Claisen Rearrangement: Scope and Stereochemistry. *J Am Chem Soc* **104**: 7181 b) Oppolzer W, Fröstl W (1975) The Preparation of *trans*-N-Acyl, N-alkyl-1-amino-1,3-butadienes. *Helv Chim Acta* **58**: 587 c) Oppolzer W, Bieber L, Francotte (1979) An Improved, Versatile Preparation of *trans*-N-Acyl-1-Amino-1,3-Dienes. *Tetrahedron Lett* 981
186. Akhtar M, Botting NP, Cohen MA, Gani D (1987) Enantiospecific Synthesis of 3-Substituted Aspartic Acids via Enzymic Amination of Substituted Fumaric Acids. *Tetrahedron* **43**: 5899
187. Trost BM, Jebaratnam DJ (1987) Syntheses of the PicROTOXANE Skeleton via Palladium (II)-catalyzed Carbacyclization Reaction. *Tetrahedron Lett* **28**: 1611
188. Trost BM (1990) Palladium-catalyzed Cycloisomerizations of Enynes and Related Reactions. *Acc Chem Res* **23**: 34
189. Mori M, Uesaka N, Saitoh F, Shibasaki M (1994) Novel Synthesis of Nitrogen Heterocycles Using Zirconium Promoted Reductive Cyclization. *J Org Chem* **59**: 5643
190. Mori, M (2005) Development of a Novel Synthetic Method for Ring Construction Using Organometallic Complexes and Its Application to the Total Syntheses of Natural Products. *Chem Pharm Bull* **53**: 457; especially pp. 457–459
191. Mori M, Saitoh F, Uesaka N, Okamura K, Date T (1994) Synthesis and X-Ray Crystal Structures of Tricyclic Ketones Containing *trans*-Fused Azabicyclo[3.3.0]octane Units. *J Org Chem* **59**: 4993
192. Taber DF, Campell CL, Louey JP, Wang Y, Zhang W (2000) Predicting the Diastereoselectivity of Intramolecular Diene Cyclozirconation. *Curr Org Chem* **4**: 809, especially pp. 813–814
193. Sha CK, Young JJ, Jean TS (1987) Synthesis of α -Iodo Ketones. *J Org Chem* **52**: 3919
194. Sha CK, Jean TS, Wang DC (1990) Intramolecular Radical Cyclization of Silylacetylenic or Olefinic α -Iodo Ketones: Application to the Total Synthesis of (\pm)-Modhephene. *Tetrahedron Lett* **31**: 3745
195. Takazawa O, Tamura H, Kogami K, Hayashi K (1982) New Synthesis of Megastigma-4,6,8-trien-3-ones, 3-Hydroxyionol, 3-Hydroxy- β -ionone, 5,6-Epoxy-3-hydroxy- β -ionol and 3-Oxo- α -ionol. *Bull Chem Soc Jap* **55**: 1907
196. Zard SZ (1996) Iminyl Radicals: a Fresh Look at a Forgotten Species (and Some of its Relatives). *Synlett* 1148
197. Padwa A, Dimitroff M, Waterson AG, Wu T (1997) Diels-Alder Reaction of 2-Amino-Substituted Furans as a Method for Preparing Substituted Anilines. *J Org Chem* **62**: 4088

198. Padwa A, Brodney MA, Dimitroff M (1998) A New Method for the Formation of Octahydroindole Alkaloids via the Intramolecular Diels-Alder Reaction of 2-Amidofurans. *J Org Chem* **63**: 5304
199. Shioiri T, Ninomiya K, Yamada S (1972) Diphenylphosphorylazide. A New Convenient Reagent for a Modified Curtius Reaction and for the Peptide Synthesis. *J Am Chem Soc* **94**: 6203
200. Heathcock CH, Graham SL, Pirrung MC, Plavac F, White CT (1979) Total Synthesis of Sesquiterpenes, 1970–1979. In: ApSimon J (ed.), *Synthesis of Natural Products*, Vol. V, p. 330. Wiley New York, Chichester, Brisbane, Toronto, Singapore
201. Corey EJ, Cheng XM (1989) *The Logic of Chemical Synthesis*, especially pp. 86–87, 178–179. John Wiley & Son New York, Chichester, Brisbane, Toronto, Singapore
202. Corey EJ, Enders D (1976) Synthetic Routes to Polyfunctional Molecules via Metallated N, N-Dimethylhydrazones. *Tetrahedron Lett* **11**
203. Alder K, Schneider S (1936) Zur Kenntnis der Oxydation von Doppelbindungen I. Die Oxydation der endo-cis-3,6-Endomethylen-tetrahydrophthalensäure. *Liebigs Ann Chem* **524**: 189
204. Corey EJ, Gross AW (1980) Bislactonization of Unsaturated Diacids using Lead Tetraacetate. *Tetrahedron Lett* **21**: 1819
205. Tanaka K, Uchiyama F, Asada A, Furusawa Y, Inubushi Y (1983) Synthetic Studies on a Picrotoxane Sesquiterpene, Coriamyrtin. I. The Grignard Reaction of 5-(2-Methyl-1,3-dioxo-2-cyclopentyl)methyl-2,5-*H*-furanone with Isopropenyl-magnesium Bromide and Stereochemistries of the Products. *Chem Pharm Bull* **31**: 1943
206. Tanaka K, Uchiyama F, Ikeda T, Inubushi Y (1983) Synthetic Studies on a Picrotoxane Sesquiterpene, Coriamyrtin. II. An Effective Stereocontrolled Synthesis of the Picrotoxane Skeleton Except for a C₁ Unit at the C₉ Position and Functionalization of the Five-membered Ring. *Chem Pharm Bull* **31**: 1958
207. Grundmann C, Kober E (1955) An Improved Synthesis of Protoanemonin. *J Am Chem Soc* **77**: 2332
208. Niwa H, Yamada K (1986) Stereocontrolled Total Synthesis of Picrotoxane Sesquiterpenoids. *J Syn Org Chem (Japan)* **44**: 180
209. Vedejs E, Engler DA, Telschow JE (1978) Transition-Metal Peroxide Reactions. Synthesis of α -Hydroxycarbonyl Compounds from Enolates. *J Org Chem* **43**: 188
210. Johnson CR, Kirchhoff RA (1979) Synthesis of Alkenes by Reductive Elimination of β -Hydroxysulfoximines. *J Am Chem Soc* **101**: 3602
211. Dale JA, Dull DL, Mosher HS (1969) α -Methoxy- α -trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines. *J Org Chem* **34**: 2543
212. Carlsen PHJ, Katsuki T, Martin VS, Sharpless KB (1981) A Greatly Improved Procedure for Ruthenium Tetraoxide Catalyzed Oxidations of Organic Compounds. *J Org Chem* **46**: 3936
213. Corey EJ, Nicolaou KC, Shibasaki M, Machida Y, Shiner CS (1975) Superoxide Ion as a Synthetically Useful Oxygen Nucleophile. *Tetrahedron Lett* **3183**
214. Miyashita M, Suzuki T, Yoshikoshi (1985) Highly Efficient Conversion of (–)-Carvone to (+)-5 β -Hydroxycarvone. *J Org Chem* **50**: 3377
215. Gras JL (1990) Methylene Ketones and Aldehydes by Simple, Direct Methylene Transfer: 2-Methylene-1-oxo-1,2,3,4-tetrahydronaphthalene. In: *Organic Synthesis*, Coll VII., p. 332. Wiley, New York, Chichester, Brisbane, Toronto, Singapore
216. Miyashita M, Hoshino M, Yoshikoshi A (1988) Sodium Phenylseleno(triethoxy)borate, Na⁺[PhSeB(OEt)₃]: the Reactive Species Generated from (PhSe)₂ with NaBH₄ in Ethanol. *Tetrahedron Lett* **29**: 347
217. Trost BM, Lee DC, Rise F (1989) A New Palladium Catalyst for Intramolecular Carbamellation of Enynes. *Tetrahedron Lett* **30**: 651
218. Trost BM, Romero DL, Rise F (1994) Pd-Catalyzed Cycloisomerization to 1,2-Dialkylidene-cycloalkanes. 2. Alternative Catalyst System. *J Am Chem Soc* **116**: 4268

219. Eastwood FW, Harrington KJ, Josan JS, Pura JL (1970) Conversion of 2-dimethylamino-1,3-dioxolanes into alkenes. *Tetrahedron Lett* **11**: 5223
220. Sharpless KB, Umbreit MA, Nieh MT, Flood TC (1972) Lower Valent Tungsten Halides. A New Class of Reagents for Deoxygenation of Organic Molecules. *J Am Chem Soc* **94**: 6538
221. Rohmer M (2007) Diversity in Isoprene Unit Biosynthesis: The Methylerythritol Phosphate Pathway in Bacteria and Plastids. *Pure Appl Chem* **79**: 739, and literature cited therein
222. a) Giner JL, Jaun B, Arigoni D (1998) Biosynthesis of Isoprenoids in *Escherichia coli*: The Fate of the 3-H and 4-H Atoms of 1-Deoxy-D-xylose. *J Chem Soc, Chem Commun* 1857, and literature cited therein b) Eisenreich W, Bacher A, Arigoni D, Rohdich F (2004) Biosynthesis of Isoprenoids via the Non-mevalonate Pathway. *Cell Mol Life Sci* **61**: 1401
223. Cordell GA (1976) Biosynthesis of Sesquiterpenes. *Chem Rev* **76**: 425
224. Yamazaki M, Matsuo M, Arai K (1966) Biosynthesis of Dendrobine. *Chem Pharm Bull* **14**: 1058
225. Edwards OE, Douglas JL, Mootoo B (1970) Biosynthesis of Dendrobine. *Can J Chem* **48**: 2517
226. Corbella A, Gariboldi P, Jommi G (1973) Aspects of the Biosynthesis of the Terpenoid Dendrobine. *J Chem Soc, Chem Commun* 729
227. Corbella A, Gariboldi P, Jommi G, Sisti M (1975) Biosynthesis of the Terpenoid Dendrobine. Early Stages of the Pathway. *J Chem Soc, Chem Commun* 288
228. Arigoni D (1975) Stereochemical Aspects of Sesquiterpene Biosynthesis. *Pure Appl Chem* **41**: 219
229. a) Cane DE, Iyengar R, Shiao M-S (1981) Cyclonerodiol Biosynthesis and Enzymatic Conversion of Farnesyl to Nerolidyl Pyrophosphate. *J Am Chem Soc* **104**: 914 b) Cane DE (1990) Enzymatic Formation of Sesquiterpenes. *Chem Rev* **90**: 1089
230. Biollaz M, Arigoni D (1969) Biosynthesis of Coriamyrtin and Tutin. *J Chem Soc, Chem Commun* 633
231. a) Turnbull KW, Gould SJ, Arigoni D (1972) Biological Conversion of Copaborneol into Tutin. *J Chem Soc, Chem Commun* 597 b) Turnbull KW, Acklin W, Arigoni D, Corbella A, Gariboldi P, Jommi G (1972) Biological Conversion of Copaborneol into Tutin. *J Chem Soc, Chem Commun* 598
232. Corbella A, Gariboldi P, Jommi G (1972) Biosynthesis of Tutin from (4R)-[4-³H₁]Mevalononic Acid. *J Chem Soc, Chem Commun* 600
233. a) Wei XY (2005) Construction of Subtractive cDNA Library from Leaves of *Dendrobium candidum* Wall. ex Lindl by Suppression Subtractive Hybridization Technology. *Guangzhou Zhongyiyao Daxue Xuebao* **22**: 320, 326 b) Wei XY (2006) Construction and Analysis of cDNA Expression Library from Leaves of *Dendrobium candidum* Wall. ex Lindl. *Guangzhou Zhongyiyao Daxue Xuebao* **23**: 62, 68
234. Jarboe CH, Porter LA, Buckler RT (1968) Structural Aspects of Picrotoxinin Action. *J Med Chem* **11**: 729
235. Haro de L, Pommier P, Tichadou L, Hayek-Lanthois M, Arditti J (2005) Poisoning by *Coriaria myrtifolia* Linnaeus: a New Case Report and Review of the Literature. *Toxicol* **46**: 600
236. Marshall CR (1906) Physiological Action of Tutin. CA: 1906: 126934
237. Fuentelba J, Guzman L, Manriquez-Navarro P, Perez C, Silva M, Becerra J, Aguayo LG (2007) Inhibitory Effects of Tutin on Glycine Receptors in Spinal Neurons. *Eur J Pharmacol* **559**: 61
238. Smith AB (1999) Hunters and Herders in the Karoo Landscape. In: Dean WRJ, Milton SJ (eds) *The Karoo, Ecological Patterns and Processes*, p. 243. Cambridge University Press, Cambridge UK
239. Mosoco RM (1943) *Catalogus Florae Domingensis*, p. 732. L. & S. Printing Co., New York
240. Kudo Y, Tanaka A, Yamada K (1983) Dendrobine, an Antagonist of β -Alanine, Taurine and of Presynaptic Inhibition in the Frog Spinal Cord. *Br J Pharmacol* **78**: 709

241. Hayden WJ, Gillis WT, Stone DE, Broome CR, Webster GL (1984) Systematics and Palynology of *Picrodendron*: Further Evidence for Relationship with the Oldfieldioideae (Euphorbiaceae). *J Arn Arbor* **65**: 105
242. Hosie AM, Aronstein K, Sattelle DB, French-Constant RH (1997) Molecular Biology of Insect Neuronal GABA Receptors. *Trends Neurosci* **20**: 578
243. Ozoe Y, Akamatsu M (2001) Non-competitive GABA Antagonists: Probing the Mechanisms of Their Selectivity for Insect *versus* Mammalian Receptors. *Pest Manag Sci* **57**: 923
244. Schmidt TJ, Gurrath M, Ozoe Y (2004) Structure-activity Relationships of *seco*-Prezizaane and Picrotoxane/Picrodendrane Terpenoids by *Quasar* Receptor-surface Modelling. *Bioorg Med Chem* **7**: 2857
245. Hosie AM, Ozoe Y, Koike K, Ohmoto T, Nikaido T, Sattelle DB (1996) Actions of Picrodendrin Antagonists on Dieldrin-sensitive and -resistant *Drosophila* GABA Receptors. *Brit J Pharmacol* **119**: 1569, and literature cited therein
246. Ozoe Y, Akamatsu M, Higata T, Ikeda I, Mochida K, Koike K, Ohmoto T, Nikaido T (1998) Picrodendrin and Related Terpenoid Antagonists Reveal Structural Differences Between Ionotropic GABA Receptors of Mammals and Insects. *Bioorg Med Chem* **6**: 481
247. Calder JA, Wyatt JA, Frenkel DA, Casida JE (1993) CoMFA Validation of the Superposition of Six Classes of Compounds which Block GABA Receptors Non-competitively. *J Comp-Aided Mol Design* **7**: 45
248. Hutchinson J, Dalziel JM (1954) *Flora of West Tropical Africa* (revised Keay RJW) Vol.1, part 2, p. 374. HMSO, London
249. Watanabe I, Koike K, Satou T, Nikaido T (1999) Nematocidal Activity of Picrodendrins against a Species of Diplogastridae. *Biol Pharm Bull* **22**: 1310
250. Pitterna T (2007) Activators/New Natural Products (Avermectins and Milbemycins). In *Modern Crop Protection Compounds*; Krämer W, Schirmer U (eds.), vol 3, p 1069. Wiley-VCH: Weinheim
251. a) Anonymous (2001) Homöopathie in der Apotheke. *Cocculus-Reisekrankheit*. *Österr. Apotheker Zeitung*. **13** b) Morton JF (1977) *Major Medicinal Plants: Botany, Culture and Uses*, p. 93. Charles C Thomas Publishers, Springfield, IL, USA
252. Anonymous (1977) *Dictionary of Traditional Chinese Medicine*, p. 294. Shanghai People Press. Shanghai
253. Chiu NY, Chang KH (1992) *Alpine Medical Plants of Taiwan*, Vol. 3, p. 123. Southern Materials Center, Taipei
254. Tang W, Eisenbrand G (1992) *Chinese Drugs of Plant Origin*. Springer Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest
255. Zhang GN, Bi ZM, Wang ZT, Xu LS, Xu GJ (2003) *Chin. Trad. Herbal Drugs* **34**, S5 (appendix)
256. Jiangsu New Medical College (1986) *Dictionary of Chinese Herb Medicine*, p. 586. Shanghai Scientific and Technologic Press, Shanghai
257. Castaneda P, Garcia MR, Hernandez BE, Torres BA, Anaya AL, Mara R (1992) Effects of Some Compounds Isolated from *Celaenodendron mexicanum* Standl (Euphorbiaceae) on Seeds and Pathogenic Fungi. *J Chem Ecol* **18**: 1025
258. Burkill HM (1985) *The Useful Plants of West Tropical Africa*, vol 2 (softcover). Royal Botanic Gardens, Kew. Richmond, Surrey, UK
259. a) Wang Y, Zhou D, Wang B, Li HS, Chai HX, Zhou Q, Zhang SF, Stefan H (2003) A Kindling Model of Pharmacoresistent Temporal Lobe Epilepsy in Sprague-Dawley Rats Induced by *Coriaria* Lactone and Its Possible Mechanism. *Epilepsia* **44**: 475 b) Zhou H, Tang YH, Zheng Y (2006) A New Rat Model of Acute Seizure Induced by Tutin. *Brain Research* **1092**: 207
260. a) Dannhardt G, Kiefer W (2007) Antiepileptica-Wirkprinzipien und strukturelle Parameter. *Pharm Unserer Zeit* **36**: 270 b) Feuerstein TJ (2008) Antikonvulsiva, Konvulsiva-Pharmakotherapie der Epilepsien. In: K. Aktories, U. Förstermann, F. Hofmann, K. Starke (eds.)

- Allgemeine und spezielle Pharmakologie und Toxikologie; 10th edn., p. 283 Urban & Fischer, München, Jena
261. Takeuchi A, Takeuchi N (1969) A Study of the Action of PicROTOXIN on the Inhibitory Neuromuscular Junction of the Crayfish. *J Physiol* **205**: 377
 262. Smits SHJ, Schmitt L (2009) Eine Strukturbilogie für Membranproteine. *Nachr Chem* **57**: 27, and literature cited therein
 263. Hucho F (1995) Toxine als Werkzeuge in der Neurochemie. *Angew Chem* **107**: 23 (picROTOXANES are missing in this otherwise interesting publication)
 264. Dougherty DA (2008) Physical Organic Chemistry on the Brain. *J Org Chem* **73**: 3667
 265. Trauner D (guest ed) (2008) Chemical Reviews thematic Issue: Chemical Approaches to Neurobiology. *Chem Rev* **108**: 1499
 266. Narahashi T (2004) Chemicals as Tools in the Study of Excitable Membranes. *J Pharmacol Exp Ther.* **294**: 1, 282
 267. Sine SM, Engel AG (2006) Recent Advances in Cys-loop Receptor Structure and Function. *Nature* **440**: 448
 268. Wang PY, Slaughter MM (2005) Effects of GABA Receptor Antagonists on Retinal Glycine Receptors and on Homomeric Glycine Receptor Alpha Subunits. *J Neurophysiol* **93**: 3120
 269. Dibas MI, Gonzales EB, Das P, Bell-Horner CL, Dillon GH (2002) Identification of a Novel Residue within the Second Transmembrane Domain that Confers Use-facilitated Block by PicROTOXIN in Glycine α_1 -Receptors. *J Biol Chem* **277**: 9112
 270. Carland JE, Johnston GAR, Chebib M (2008) Relative Impact of Residues at the Intracellular and Extracellular Ends of the Human GABA_C $\rho 1$ Receptor M2 Domain on PicROTOXININ Activity. *Eur J Pharmacol* **580**: 27
 271. Casida JE, Tomozawa M (2008) Insecticide Interaction with γ -Aminobutyric Acid and Nicotinic Receptors: Predictive Aspects of Structural Models. *J Pestic Sci* **33**: 4
 272. Chen LG, Durkin KA, Casida JE (2006) Structural Model for γ -Aminobutyric Acid Receptor Noncompetitive Antagonist Binding: Widely Diverse Structures Fit the Same Site. *Proc Nat Acad Sci USA* **103**: 5185
 273. Qian HH, Pan Y, Zhu YJ, Khalili P (2005) PicROTOXIN Accelerates Relaxation of GABA_C Receptors. *Mol Pharmacol* **67**: 470
 274. Dresbach T, Nawrotzki R, Kremer T, Schuhmacher S, Quinones D, Kluska M, Kuhse J, Kirsch J (2008) Molecular Architecture of Glycinergic Synapses. *Histochem Cell Biol* **130**: 617
 275. Yang Z, Cromer BA, Harvey RJ, Parker MW, Lynch JW (2007) A Proposed Structural Basis for PicROTOXININ and PicROTIIN Binding in the Glycine Receptor Pore. *J Neurochem* **103**: 580
 276. Wang DS, Buckinx R, Lecorronc H, Mangin JM, Rigo JM, Legendre P (2007) Mechanisms for PicROTOXININ and PicROTIIN Blocks of α_2 Homomeric Glycine Receptors. *J Biol Chem* **282**: 16016
 277. Lynch JW (2004) Molecular Structure and Function of the Glycine Receptor Chloride Channel. *Physiol Rev* **84**: 1051
 278. Etter A, Cully DF, Liu KK, Reiss B, Vassilatis DK, Schaeffer JM, Arena JP (1999) PicROTOXIN Blockade of Invertebrate Glutamate Gated Chloride Channels: Subunit Dependence and Evidence for Binding within the Pore. *J Neurochem* **72**: 318
 279. Cleland TA (1996) Inhibitory Glutamate Receptor Channels. *Mol Neurobiol* **13**: 97
 280. Yarowsky J, Carpenter DO (1978) A Comparison of Similar Ionic Responses to γ -Aminobutyric Acid and Acetylcholine. *J Neurophysiol* **41**: 531
 281. Magoski NS, Bulloch AG (1999) Dopamine Activates two Different Receptors to Produce Variability in Sign at an Identified Synapse. *J Neurophysiol* **81**: 1330
 282. Pietra F (1995) Structurally Similar Natural Products in Phylogenetically Distant Marine Organisms, and a Comparison with Terrestrial Species. *Chem Soc Rev* **24**: 65

283. Erpenbeck D, van Soest RWM (2007) Status and Perspective of Sponge Chemosystematics. *Marine Biotechnology* **9**: 2
284. Whitham GH (1961) The Reaction of α -Pinene with Lead Tetraacetate. *J Chem Soc* 2232
285. Schmidt TJ (1999) Toxic Activities of Sesquiterpene Lactones. Structural and Biochemical Aspects. *Curr Org Chem* **3**: 577, especially pp. 599–600
286. Ju XL, Hao YL, Pei JF, Ozoe Y (2007) Investigation of Structural Requirements for Inhibitory Activity at the Rat and Housefly Picrotoxinin Binding Sites in Ionotropic GABA receptors using DISCOtech and CoMFA. *Chemosphere* **69**: 864

Combinatorial and Synthetic Biosynthesis in Actinomycetes

Marta Luzhetska, Johannes Härle, and Andreas Bechthold

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1. Introduction

Bioactive microbial metabolites represent powerful tools against both acute and degenerative diseases (96). One of the most significant fractions within these physiological active compounds is represented by the polyketides. Their pharmaceutical use takes place in a variety of applications including antibacterials, immunosuppressants, anticancer agents, antifungal drugs, cholesterol-lowering agents, and products against animal diseases. It is difficult to estimate the magnitude of their advantages, but the utility of natural products as sources of novel structures still possesses great potential. Thus, in the area of cancer, in the time frame from around the 1940s to 2006, over 155 small molecules were introduced, of which 73% were other than “synthetics”, with 47% being either natural products or directly derived there from (28). The overuse of antibiotics has caused bacteria to become resistant to common drugs. Multiple antibiotic-resistance has emerged as one of the top public health issues worldwide in the last few decades and has focused attention on the need of new antibiotics (116). Many natural products isolated from

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microorganisms or plants are growing in environmental niches (41, 54). Also marine niches have been explored and revealed new bioactive compounds (13). It seems that natural products are finely tuned for interacting with biological systems and receptor molecules. They are the result of billions of years of evolution and far surpass anything yet created by humans. For this reason they represent a superior source of drug candidates or biologically active lead compounds. However, natural products have not been optimized in evolution as clinical drugs, but rather for their biological functions useful to the producing organism (36). Due to their variable structures, natural products or derived compounds can fulfill chemical and genetic approaches for deciphering cellular processes that need not necessarily be a target for therapeutic intervention (125). One great obstacle for the discovery of new bioactive natural products includes the rediscovery of known compounds at a high frequency. Technical challenges associated with purification and structure elucidation of the metabolites from microbial fermentations are tricky and engage microbiologists and chemists. Nevertheless, due to an explosive growth of the sequenced genome number, the potential of molecular genetics tools together with high-throughput screening systems and other technical advances, natural-product drug discovery has received new support.

The advent of combinatorial chemistry as well as combinatorial biosynthesis has promised to provide a wealth of new compounds to screen for biological activity (20). However, the ability to design new target-directed structures is still not possible. The molecular-biological properties of natural products are dictated by their structures in ways that are not understood well enough. In this review we describe some of the newest highlights achieved in the area of natural product research mainly based on the application of combinatorial biosynthesis approaches. In addition, present the first results in the new field “synthetic biosynthesis”, in which synthetic genes are used for the biosynthesis, of pharmaceutically relevant compounds.

2. Combinatorial Biosynthesis and Synthetic Biosynthesis

Due to the complex structure of most natural compounds their synthesis or semi-synthesis can be quite laborious. The modification of some classes of natural products in a rational chemical manner is often associated with the practice of several protecting groups, thus the handling is strictly limited. In contrast, the ability to alter natural product structures through genetic modification of their biosynthetic machinery represents virtually endless opportunities and often might be a potential solution for avoiding problems arising using raw chemistry (126). The technology is mainly based on the genetic manipulation of genes governing secondary metabolic pathways in bacteria and other microorganisms. Polyketide synthases (PKSs), the related nonribosomal peptide synthases (NRPSs) together with post tailoring, resistance conferring and regulatory enzymes represent the players, and thus the platform of producing natural products (57, 107, 120). One remarkable feature is that, so far, 99% of all known genes involved in natural

product biosynthesis that exist are organized in gene clusters. Many gene clusters encoding natural products have been cloned and characterized. A vast amount of information has been generated by the completion of almost 700 bacterial genome sequences (www.genomesonline.org), whereof more than 20 complete genomes of prolific Actinomycetes genera (www.broadinstitute.org/annotation/genome/streptomyces_group) are included. With the online presence of the first fully sequenced genome in the genus *Streptomyces* a milestone in the area of natural product biosynthesis was gained (10). Knowledge of gene cluster regulation, pathway interactions, and metabolic flux was enhanced, which directly had a great impact on improving production and fermentation processes for the achievement of higher metabolite titers and for the selective production of a desired molecule. In this context, it is interesting to point out that in at least two industrially utilized high-producer strains, *Saccharopolyspora erythrae* (erythromycin) and *Streptomyces fradiae* (tylosin), the overproduction characteristics are mainly caused by mutations in non-PKS genes (25). Future work will show whether it will be possible to rationally design overproducer strains (52, 72, 96). Moreover, the ability to produce molecules of interest by recombinant organisms with improved large-scale fermentation probability has the positive side effect of lowering production costs and reduces environmental pollution. This is a relevant benefit compared to some conventional chemical synthesis efforts (102). Various genetic approaches have been used for the generation of several natural product-derived bioactive compounds. In the following chapter strategies in the field of combinatorial biosynthesis and metabolic engineering are listed. Additionally we want to focus attention on existing reviews presenting specific approaches in detail (9, 63, 67, 73, 74, 83, 84, 118, 121).

2.1. Achievements in Combinatorial Biosynthesis

As described above genetic methods to alter the structure of natural products have been developed in the last 10 years. Many new compounds have been generated, some of them with improved characteristics. The genetic approaches to generate novel molecules can be divided into the following categories.

1. Targeted gene disruption, involving the inactivation of selected genes, thus generating mutants that accumulate new compounds (50, 70) (Fig. 1A).
2. Over-expression of a specific gene, followed from unbalancing pathways in order to generate new metabolites (137) (Fig. 1B).
3. Heterologous gene expression (48) (Fig. 1B, C).
4. Heterologous co-expression, which involves the expression of two or several genes (55, 98) (Fig. 1B, C).
5. Tailoring modification, known as metabolic engineering, biotransformation or precursor-directed biosynthesis, involving the feeding of building blocks which are subsequently modified by a specific enzyme involved in the biosynthesis of a structurally related drug (132) (Fig. 1F).

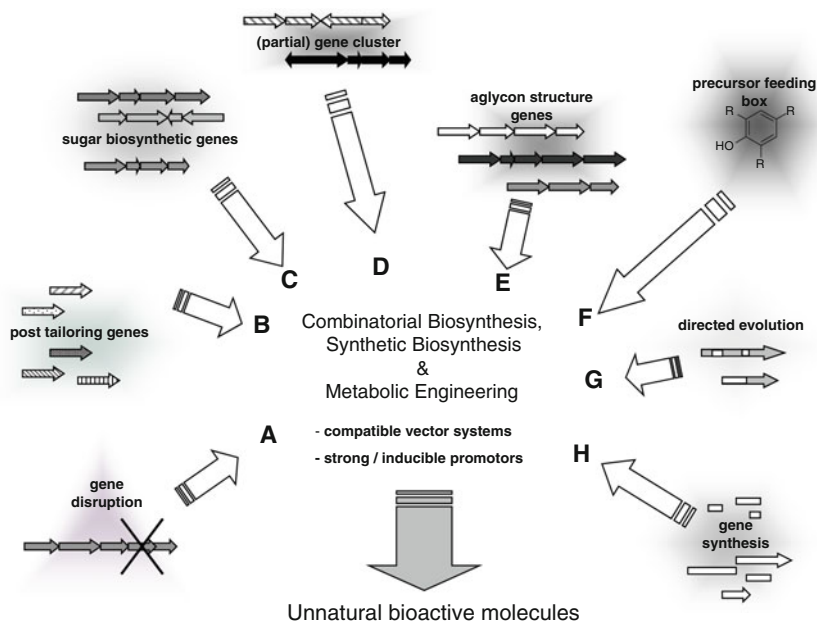


Fig. 1. Genetic approaches in the field of engineering natural products (see text for explanation)

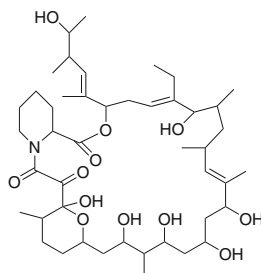
6. Mutasynthesis, also called mutational biosynthesis, involving precursor-directed biosynthesis using a mutant strain blocked in a crucial biosynthetic step, which then incorporates the alternative substrate to produce an analog of the wild-type compound (*119, 122*) (Fig. 1A, F).
7. Heterologous expression of a partial gene cluster, followed by the production of biosynthesized unnatural metabolites with altered substituents or a modified core structure (*90, 97*) (Fig. 1D).
8. Heterologous expression of an entire gene cluster to make use of the host properties like a fully sequenced genome, the with potential to influence production, improved growth properties, and well developed fermentation processes (*31, 56, 77*) (Fig. 1D).
9. Heterologous gene expression combined with targeted gene disruption (*69, 112*) (Fig. 1A, B).
10. Altering the nature of the gene *via* genetic engineering, known as directed evolution, site-directed mutagenesis, domain swapping or gene shuffling. This relatively new option leads to the possibility of improving an enzyme characteristics like thermostability, solvent tolerance, enantioselectivity, and substrate or catalytic specificity (*113, 127*) (Fig. 1G).
11. Heterologous expression of synthesized genes, involving codon optimization of the external genetic information to achieve an adequate translational quantity of the recombinant protein (*91*) (Fig. 1H).

12. Heterologous expression of pathways to enable in a host organism the production of additional precursors, and the conservation of its own metabolites or the possibility to produce new products (95) (Fig. 1D).
13. Generating synthetic organisms, involving the synthesis of a fully artificial genome and its incorporation into a bacterial coat. This procedure is still not feasible but might in future be a straightforward way of producing the products of interest (39) (Fig. 1H).

2.2. Challenges for Combinatorial Biosynthesis

2.2.1. Cloning of Biosynthetic Gene Clusters

One potential obstacle, which must be mentioned in connection with heterologous expression techniques, is that natural product biosynthetic gene clusters are often large (<100 kb) and can include repetitive pieces of DNA. From fact that the size of a DNA fragment for inserting into a cosmid is strictly limited (<40 kb), the use of bacterial artificial chromosomes (BACs) with their great loading potential (<300 kb) is more convenient. Certainly BACs are tricky to handle not just because of their genetic instability. Thus, to isolate an entire gene cluster in a single vector is still not trivial. The use of multiple, mutually compatible expression vectors, is one way to solve this problem. A few examples are: the successful cloning and adjacent heterologous expression of a 90 kb large gene cluster, encoding enzymes for the biosynthesis of the 27-membered macrolide meridamycin (1), deserves to be mentioned. It was cloned on a high copy replication BAC shuttling genes between *E. coli* and *Streptomyces* (65). In other attempts, entire gene clusters have been reconstructed using the Red/ET recombineering technology (12, 42, 123).

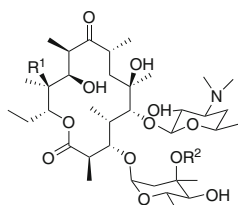


1 (Meridamycin)

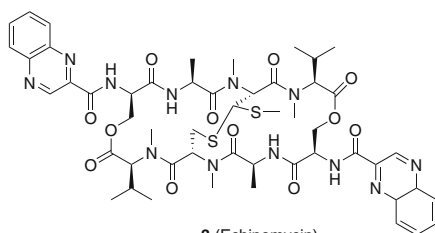
Technologies based on the site specific recombinases Cre and Flp recently were adapted for use in the Actinomycetes (32, 33). Here it was shown that these enzymes allow precise excisions of DNA between two distinct recognition target sites in the Actinomycetes. It is worthy of mention that scientists working on this group of organisms also developed a high-efficient *in vivo* random Tn5 transposon mutagenesis system allowing the identification of ORFs involved in natural product biosynthesis (91).

2.2.2. Heterologous Expression of Biosynthetic Gene Clusters

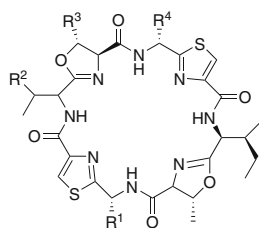
The use of *E. coli* as a host for the heterologous expression is due not only to its short cell-doubling time practicable, but offers other great advantages. Well-suited and established protocols for genetic engineering as well as efficient fermentation methods make the use of this bacterium for generating secondary metabolites more and more popular. In the interim, several natural products were generated in *E. coli*, for instance the potent antibacterial polyketide erythromycin (**2**) (89), and echinomycin (**3**), a nonribosomal peptide with antitumor activity (117), and the patellamides (**4**), which are ribosomally encoded peptides (101). However, due to genetic incompatibility, the presence of metabolic background, and the limited production amount, *E. coli* is not the universal solution and therefore alternative hosts, which are more amenable for heterologous expression of secondary metabolites, are desired. One alternative candidate might be *Pseudomonas*, a genus for which a comprehensive set of genetic tools exists. *Pseudomonas* grows rapidly and has been validated as an exceptional protein-production host. Recently, the heterologous expression of the myxochromide biosynthetic gene from *Stigmatella aurantiaca* was achieved. Genes necessary for the production of myxochromide S (**5**) were reassembled in *E. coli* by combining the >50 kbp DNA molecule out of multiple cosmids. After specific promoter exchanges, the rapid production of **5** was observed in a significantly greater amount than it was observed in the wt-strain (123). Since many of the polyketide-derived secondary metabolites require methylmalonyl-CoA for their biosynthesis, preliminary work was carried out in the genetics of *Pseudomonas putida*. A mutant containing the *tan* operon, encoding enzymes for methylmalonyl-CoA biosynthesis, can now be used for formation of complex polyketides (40).



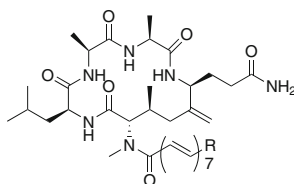
2 (Erythromycin)



3 (Echinomycin)



4 (Patellamide)

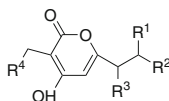
Patellamide A: R¹ = CH₂ R² = Ethyl R³ = H R⁴ = CH₂Patellamide C: R¹ = Ph R² = CH₃ R³ = CH₃ R⁴ = CH₃

5 (Myxochromide S)

Myxochromide S1: R = CH₃Myxochromide S2: R = CH₂CH₃Myxochromide S1: R = CH=CH-CH₃

2.2.3. *In Silico* Techniques to Investigate Natural Products

In comparison with the conventional procedure in which gene clusters are cloned using a specific gene probe, genome mining is a novel procedure in natural product research and is becoming more popular (20, 103). Using genome mining, a gene sufficient for germicidin (6) production in *Streptomyces* was identified. The gene was expressed in *Streptomyces venezuelae*, which was able to produce finally all five native germicidin derivatives 6a–6e (21).



6 (Germicidin)

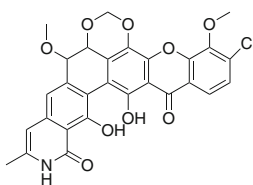
Germicidin a: R¹ = H R² = R³ = R⁴ = CH₃Germicidin b: R¹ = R² = R⁴ = CH₃ R³ = HGermicidin c: R¹ = R² = H R³ = R⁴ = CH₃Germicidin d: R¹ = R³ = H R² = R⁴ = CH₃Germicidin a: R¹ = R⁴ = H R² = R³ = CH₃

2.2.4. Investigation of Cryptic Secondary Metabolite Gene Clusters Using Computational Analysis and Combinatorial Biosynthesis

Due to whole genome sequencing projects the presence of several cryptic secondary metabolic pathways became evident. The data indicated that as much as 90% of the “chemical potential” of many organisms remained undiscovered. *Amann et al.* suggests that even less than 1% of microbial diversity has so far been sampled through laboratory-cultured organisms (4). Thus, natural product research is in the beginning of a new age as most natural products have to be detected (87). *Hopwood* and coworkers clearly demonstrated an ability to uncover genetic potential as they sequenced the whole genome of *S. coelicolor* A3. They recognized that there are many more gene clusters encoding natural product-like biosynthetic pathways than there are known natural products of this organism (10). Similar observations have now been reported for several diverse sequenced microorganisms (14, 53, 82, 85, 86, 114). Partially because of the lack of some chemical or environmental signals that might be required for the expression of the cryptic clusters, dozens of compounds are not produced under laboratory conditions, even though the biosynthetic machinery appears functional (125). Genetic methods offer a promising possibility for revealing these so-called ‘orphan’ natural product biosynthetic gene clusters (26).

2.2.5. Comparative Metabolic Profiling

The profile of metabolites in a culture containing or lacking a cryptic biosynthetic gene cluster can be compared using appropriate analytical techniques. CBS40 (7) is a novel metabolite that has been identified by such an approach (51).



7 (CBS40)

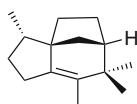
2.2.6. Preliminary Bioinformatic Analysis

To gain primary insights about structural features of a metabolic product encoded by a biosynthetic assembly line, bioinformatics analysis can be very helpful (8, 24). The procedure can allow the prediction of putative physico-chemical properties of the putative metabolic product encoded, for example, by a cryptic biosynthetic gene cluster (138). The prediction assists in choosing adequate fermentation conditions

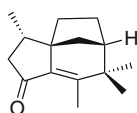
and techniques for compound isolation and characterization. Several new metabolic products of cryptic biosynthetic gene clusters have been discovered recently following this bioinformatic approach (7, 60).

2.2.7. *In Vitro* Reconstitution

Another possibility to gain the product encoded by a silent gene cluster can be achieved by a method called the “*in vitro* reconstitution” approach. A necessity is that the substrates of the pathway are predictable. The *in vitro* reconstitution of an entire biosynthetic pathway usually involves the separate overexpression of each gene, the purification of the resulting protein and the performance of enzymatic studies. Thus, the discovery of a fully elaborated metabolic product by this approach is likely to be very laborious. For example, after discovering a cryptic sesquiterpene synthase in the genome of *S. coelicolor* (64), the new epi-isozaene (8) could be produced successfully. This metabolite was shown to be an intermediate in the assembly line of the known *Streptomyces* sesquiterpene albaflavenone (9) (135).



8 (Epi-isozaene)



9 (Albaflavenone)

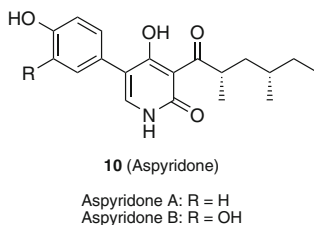
2.2.8. Comparative Profiling after Genetic Modification

A further approach for discovering new metabolic products encoded by cryptic biosynthetic pathways involves their comparative profiling. This was performed using the regulatory gene *laeA*, from *Aspergillus nidulans*. After expressing *laeA*, a few cryptic gene clusters were overexpressed (17) compared to the wild-type strain.

2.2.9. Expression by Incorporation of a Controllable Promoter

A further possibility in revealing products of silent gene clusters is the expression of putative pathway-specific activator genes upstream or within the putative cryptic biosynthetic gene clusters. This approach is based on the incorporation of an

inducible promoter. Upon addition of the inducer this strategy has been shown to cause expression of a normally silent gene cluster in *A. nidulans*. The product aspyridones (**10**) had not been described before (11). Interestingly the presence of a pyridone moiety in the aspyridone structure was not predicted via primary sequence analysis. This indicates that genetic techniques are required to accomplish the “*in silico*” approaches.



2.2.10. Metagenomics

The approach of quasi-blind cloning and expressing DNA fragments from specific environmental niches profits greatly from fast sequencing technologies. Together with optimized cultivation methods and smartly applied molecular techniques, metagenomics will reveal new insights into actinobacterial biodiversity (15, 16, 106). It has to be mentioned that this approach requires the set up for cultivation and analysis of a huge number of clones for which the complexity should not be underestimated.

2.2.11. Rational Design of PKS and NRPS Derived Natural Products

Many microbial natural products, in particular complex polyketides and nonribosomal peptides, are assembled by biosynthetic assembly lines involving modular mega-synthases and -synthetases (35). In many cases the number of modules in the assembly line directly corresponds exactly to the number of metabolic building blocks incorporated into the final product. Hence, the ability to correlate primary genetic information with protein function and chemical structure is now relatively straightforward. Several exceptions to this paradigm have emerged in recent years (45). However, studies concerning molecular interactions should provide additional insights into structure-functional relations within the multidomain enzymes (6). Despite the complexity of these catalysts, relatively early successes in exchanging or replacing modules within PKS and NRPS systems offered great promise for the biosynthesis of new natural products (27, 30, 105). Typical efforts to engineer these biosynthetic assembly lines are based on sequence alignments and the comparison of the putative secondary and tertiary structures (125). Also helpful are models that predict the stereochemical outcome due to tailoring reactions, like ketoreduction (18, 93) or adenylation as well as the transfer of each acyl-building block of the NRPS or PKS assembly line (8, 22, 44, 104). Moreover, such models

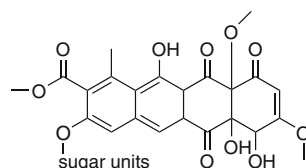
allow estimating the value of the desired compound in advance. In addition, it is necessary to take into account the complex kinetic and molecular-recognition mechanisms of multimodular PKSs. This was recognized after the failed attempt to insert additional extension modules into existing PKS frameworks (80). The specificity of domains within modular assembly lines can prevent the extension of noncognate substrates and is followed by a premature hydrolytic release of the intermediates (115). This obstacle, a “misfolding”-protective mechanism within both PKS and NRPS assembly lines, can hydrolyze misprimed carrier proteins and thus reduces or blocks production of engineered multienzymes (23, 46, 130). Recently, two thioesterases, FscTE and TyIO, involved in the biosynthesis of FR-008/candididin and tylosin, were characterized biochemically. These enzymes demonstrated a remarkable hydrolytic activity with the non-elongatable acetyl and propionyl extender units but very low activity for the native substrates malonyl and methylmalonyl thioester, respectively. Thus, these enzymes selectively remove non-elongatable residues bound to the acyl-carrier protein and so support the biosynthesis of the native polyketides (136).

2.2.12. Glycodiversification of Natural Products

Many natural products are decorated with carbohydrates essential for targeting such molecules to their biological receptors. Unglycosylated natural products are very often inactive and obtain their biological activity only after their sugar decoration. The possibility of addressing an initially detected weapon to a new target has motivated several working groups to develop *in vivo* and *in vitro* glycotecnologies for altering sugar patterns on physiologically secondary metabolites (68, 128). Adding or changing sugars can dramatically improve the parent compound's pharmacological properties, specificity and or even the molecular mechanism of action (2, 111). The chemical synthesis of natural product sugar units, due to their reactive substituents is not trivial and therefore pathways for their biosynthesis have become of great interest. This led to the development of powerful methods for manipulating sugar biosynthesis both *in vitro* and particularly *in vivo*, where the utility of sugar biosynthetic gene cassettes for NDP-deoxysugar production could be clearly demonstrated (97, 110). Thus, sugar biosynthesis plasmid-containing strains can be used together with either native or heterologously expressed glycosyltransferases (GTs) for the production of novel derivatives. Perhaps the main limitation of this approach is the substrate specificity of most of all GTs. However some GTs exhibit a broad substrate specificity and therefore are valuable for structural diversification (43).

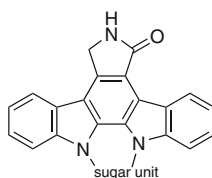
Well-known examples are novel glycosylated elloramycins (11), staurosporines (12), mithramycins (13), and steffimycins (14) generated by the group of *J. Salas*, in Oviedo, Spain (73, 99). One additional example concerns the formation of a macrolide in a mutant strain of *Saccharopolyspora erythraea*. This mutant strain lacks the erythromycin polyketide synthase gene as well as both GT genes required for the transfer of L-mycarose (*eryBV*) and D-desosamine (*eryCII*) moieties during the erythromycin biosynthesis, but still it provides the TDP-activated forms of the

two deoxy sugars. When this strain was used as host after expression of the spinosine gene cluster, several insecticide-like active spinosine analogs (**15**) were produced (**38**). Another mutant, also lacking the native sugar biosynthetic genes but equipped with several sugar gene cassettes for the co-expression of either eryCIII, tylMII, and angMII, led to novel derivatives (**100**). One further experiment is especially worthy of being mentioned. Here a strain able to generate dTDP-L-olivose (**16**), successfully converted with the aid of glycosyltransferase SpnP, the pseudoaglycone of spinosyn (**15**) to a new compound named 17-des- β -O-D-forosamine-17- α -O-L-olivosylspinosyn A (**15**) (**37**). Surprisingly, SpnP adopted the L-configuration instead of that of its natural D-configured sugar (**38**). Nevertheless, as indicated above, most GTs have very narrow substrate specificity or can tolerate only minor altered substrates (examples are NovM and GtfA) and this is a greatly limiting factor for product diversification (**3**, **66**). None of the GTs studied to date are sufficiently promiscuous for use in a truly combinatorial capacity particularly because of their co-dependence of donor/acceptor specificity (**128**). In 2005, useful knowledge was conveyed by *Minami* and *Eguchi* as they described the reversible catalytic activity of GT-B glycosyltransferases for the first time (**78**) (Fig. 2b). This newly explored feature was used for transglycosylation reactions, orienting structural diversification into a new direction (Fig. 2c, d). Moreover, this approach offers a straightforward and direct route for generating unusual NDP-deoxy sugars from readily available natural product sources (**133**). With the production of over 70 calicheamicin (**17**) analogs, the transglycosylation reaction has been presented as a powerful and exciting tool (**134**).



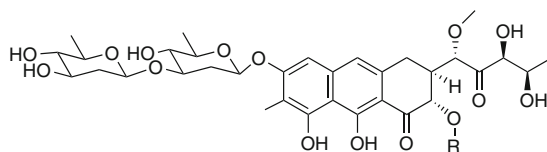
11 (Elloramycin)

sugar units : D-glucose, D-olivose, D-digitoxose, D-boivinose, D-amictose, L-rhamnose, L-olivose, L-digitoxose, L-rhodinose, L-amictose, L-mycarose, L-chromose B

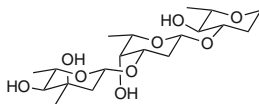


12 (Staurosporine)

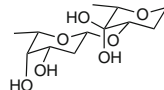
modified staurosporines: sugar unit = L-digitoxose, L-rhamnose, L-olivose.

**13 (Mithramycin)**

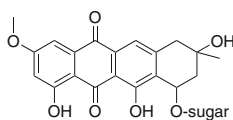
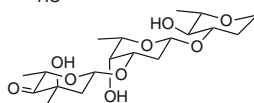
Mithramycin: R =



4C-keto-demycarosylmithramycin: R =

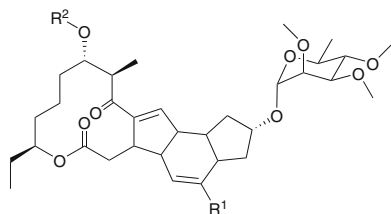


4E-keto-mithramycin: R =

**14 (Steffimycin)**

modified steffimycins:

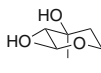
sugar units: D-boivinose, D-olivose, D-digitoxose, L-olivose, L-oleandrose, L-digitoxose, L-amicetose, L-mycarose, L-chromosome B, 3'-O-methyl-L-digitoxose, 3'-O-methyl-D-boivinose.

**15 (Spinosyn)**

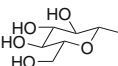
Spinosyn D: R¹ = CH₃ R² =
 Spinosyn A: R¹ = H R² =



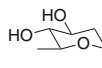
17-O-L-mycarosyl-spinosyn D: R¹ = CH₃ R² =
 17-O-L-mycarosyl-spinosyn A: R¹ = H R² =

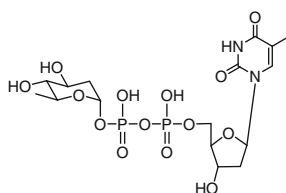


17-O-D-glucosyl-spinosyn D: R¹ = CH₃ R² =
 17-O-D-glucosyl-spinosyn A: R¹ = H R² =

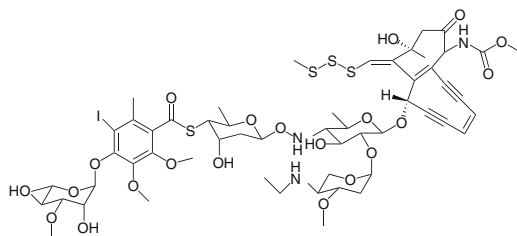


17-O-D-forosamine-17-O-L-olivosylspinisyn D: R¹ = CH₃ R² =
 17-O-D-forosamine-17-O-L-olivosylspinisyn A: R¹ = H R² =





16 (dTDP-L-Olivose)



17 (Calicheamicin)

2.3. Synthetic Biosynthesis

The speed of gene synthesis, coupled with its decreasing cost, has made synthetic genes an invaluable resource in biosynthetic engineering (94). The convenient ability to introduce restriction sites, to optimize codon usage for the efficient expression in a host organism or to engineer an enzyme towards new characteristics makes this technology irreplaceable. Although the construction of large gene clusters is still time-consuming and expensive, the first highly interesting results can be summarized. The most prominent example resulted in the synthesis of the entire set of erythromycin PKS genes (DEBS1–3), a remarkable contiguous sequence of 32 kbp, and their adjacent expression in *E. coli* (58). No less spectacular is the work, described by *Menzella* and colleagues, as they expressed heterologously 14 genes encoding type I PKS modules with differing N- and C-terminal linker variations, allowing them to be assembled into 154 possible bimodular combinations (75). After expression in *E. coli*, 72 of the 154 combinations produced the anticipated polyketide product, of which the majority were gained in the range of 0.02–10 mg dm⁻³ (Fig. 3).

Exciting results in the area of NRPS were achieved by *Baltz* and coworkers in generating novel lipopeptide derivatives like **18** (76, 81). One almost provocative alternative to re-engineering existing NRPSs is the *de novo* design of a new catalyst

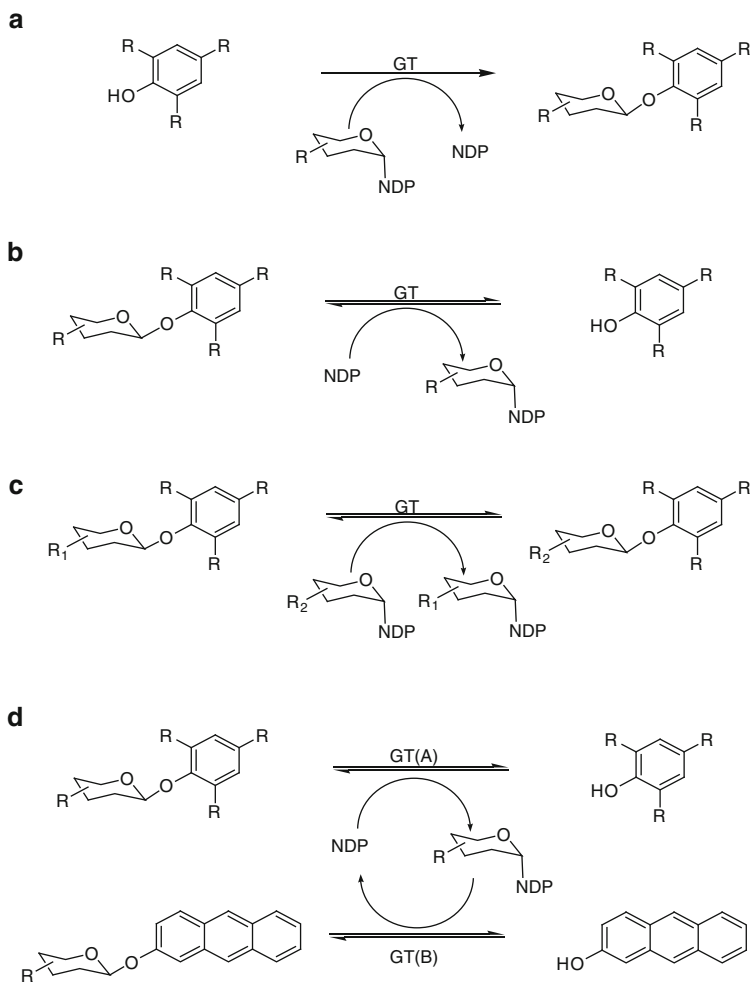


Fig. 2. GT catalyzed reactions. **(a)** The classical GT-catalyzed reaction in which a sugar is transferred from a NDP-activated donor to an aglycone acceptor, forming a glycosidic bond. **(b)** NDP sugar synthesis *via* reverse glycosyl transfer. **(c)** GT-catalyzed sugar/exchange reaction. **(d)** The aglycone/exchange strategy where GT (A) catalyzes the synthesis of a NDP sugar that gets incorporated subsequently into a second aglycone by catalysis of GT (B)

that mimics them. In this respect, a supramolecular approach based upon coiled-coil peptide mimicking NRPS active sites was recently introduced, an idea which soon might lead to great diversity in the production of unnatural natural products (62, 124).

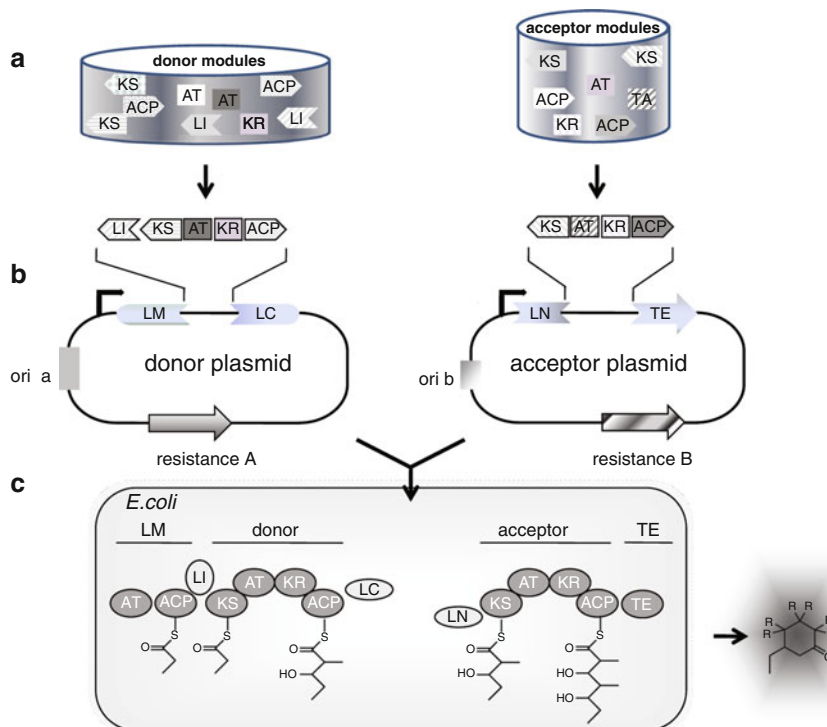
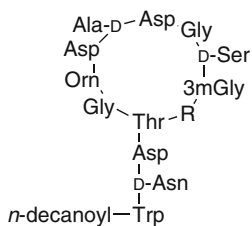


Fig. 3. Combinatorial assembly of PKS modules. **(a)** Redesign of polyketide synthase genes. Building blocks consisting of intrapeptide linker (LI), ketoreductase (KR), and the three obligate domains: ketosynthase (KS), acyl transferase (AT), and the acyl carrier protein (ACP). **(b)** Insertion of the extender modules to create the donor and acceptor plasmids. The donor plasmid provides the first PKS module flanking from the loading module (LM) and a C-terminal interpeptide linker (LC). The acceptor plasmid carries the second module flanked from the N-terminal interpeptide linker (LN) and the thioesterase (TE). The plasmids exhibit a compatible origin of replication (ori a and b) and different resistance selection marker genes (resistance A and B). **(c)** Heterologous production of the triketide lactone in *E. coli*



18 (Lipopeptide derivatives)

Daptomycin: R = Kyn
 New derivatives: R = Trp, Ile or Val

2.3.1. Structural Information for Protein Engineering

Knowledge concerning the structure of multienzyme complexes will have a strong impact on combinatorial biosynthesis in the future. The recent X-ray crystallographic structures of the modular type I mammalian and fungal FAS (71) as well as the high-resolution structure of a modular PKS didomain, comprising the ketosynthase and acyltransferase domains, offer a look at the PKS and NRPS assembly lines (108, 109) (Fig. 4). Unfortunately, the key acyl carrier protein (ACP) is absent in these structures, which is probably due to its high mobility caused by a propensity to “shuttle” significant distances, which is necessary to engage the other catalytic domains (61). For both the modular and non-modular multienzyme assembly lines, an understanding of the molecular recognition process, which guides the “shuttling” carrier proteins, will provide important input for engineering functional complexes.

2.3.2. Directed Evolution and High-Throughput Screening

The advent of directed evolution of proteins has allowed the selection of redesigned enzymes with improved properties, altered substrate specificity, and in some cases a new catalytic function (5). The identification of amino acid motifs responsible for the selectivity also within domains of multifunctional enzyme complexes is proceeding (29, 92), and might provide the ability to use site-directed mutagenesis rather than domain replacements to generate engineered PKSs for the production of new products. *Fischbach* and coworkers used both directed evolution as well as the recombining of native genes for compound diversification. After generating chimeric NRPS, the often-evident presence of impaired functionality due to reassembly was restored by directed evolution. After a few rounds of screening they were able to improve the product yields and additionally found variants that produce new derivatives (34). However, in general, the difficulties of designing assays capable of screening the variants required for the directed evolution are in most cases very laborious (Fig. 5e). An alternative and illuminating approach is the method named “homology modeling”, which is based on the solved crystal structure of a closely related protein (Fig. 5d). An example for this strategy was realized for the direct evolution of the terpene cyclase γ -humulene synthase (131). It was suggested that, through the mutation of a few key “plasticity residues” located in and around the active site, the enzyme’s promiscuity could be increased. Nineteen potential plasticity residues were identified, changed and each derivative was systematically checked for valuable combinations of amino acid substitutions using a mathematical model. Finally, seven highly active synthases were identified, targeted for investigation, and subsequently shown to operate through distinct reaction pathways to give specific products. This required the screening of 2,500 mutants, a number that can be quite adequately addressed through the applied assay. Thus, the application of an *in silico* approach to evaluate the enzyme’s “fitness” with adjacent preselecting combinations of mutations may lead to the desired outcomes (Fig. 5a).

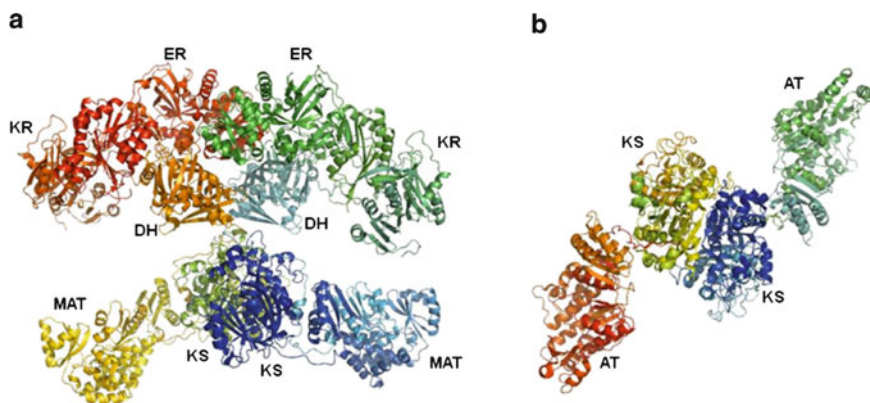


Fig. 4. X-ray determined protein crystal structures of multienzyme assembly lines. **(a)** Mammalian fatty acid synthase at 4.5 Å resolution (PDB 2cf2). Domain organization: A starter substrate, acetyl-CoA or malonyl-CoA, gets loaded onto the acyl-carrier protein (ACP/absent in the structure) via the malonyl-CoA-/acetyl-CoA-ACP transacylase (MAT). Then, the ketoacyl synthase (KS) catalyzes a decarboxylative condensation reaction and forms the β -ketoacyl-ACP. This is followed from a reduction reaction catalyzed by the β -ketoacyl reductase (KR). Subsequently, the intermediate gets dehydrated by a dehydratase (DH) and additionally reduced by a β -enoyl reductase (ER). The product gets released from the ACP by a thioesterase (absent in the structure). **(b)** Module 3 of 6-deoxyerythronolide B synthase at 2.6 Å resolution (PDB 2qo3) bound to the inhibitor cerulin. The ketosynthase (KS) – acyltransferase (AT) di-domain is part of the large homodimeric polypeptide involved in biosynthesis of erythromycin from *Saccharopolyspora erythraea*

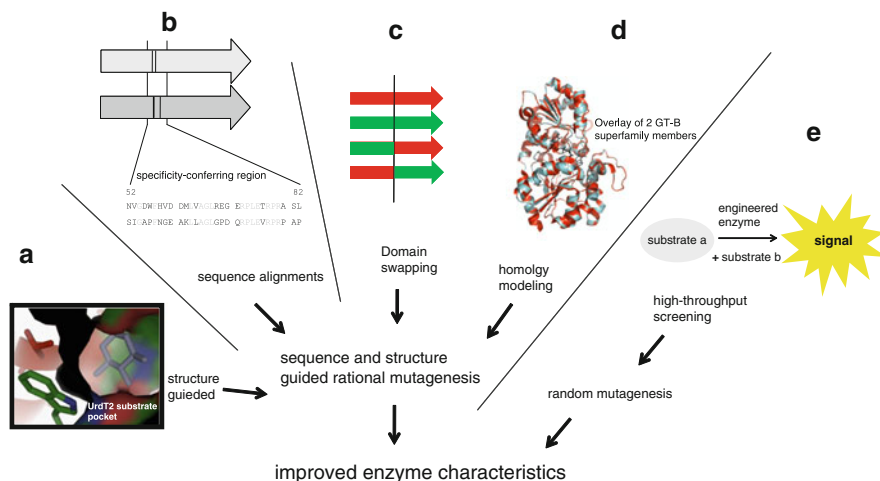
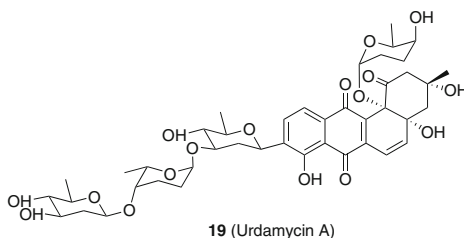


Fig. 5. Protein engineering via rational- and randomly directed strategies to alter the characteristic of an enzyme. **(a)** Structure-guided protein engineering based on the presence of a X-ray resolved crystal structure that utilizes *in silico* modeling. **(b)** Genetic engineering of a specificity-conferring region on the basis of information gained by sequence alignments. **(c)** Domain swapping experiments for combining desired N- and C-terminal characteristics. **(d)** Homology modeling, resuming structural knowledge of a closely related protein for the rational design of the enzyme of interest. **(e)** Randomly protein engineering aided by high-throughput screening

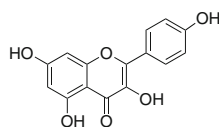
2.3.3. Engineering Enzyme Function

One dream of natural product scientists is to create new enzyme functions. The first successful attempts have been described in the glycosyltransferase field. Since nearly all GTs in natural product biosynthesis belong to the GT-B structural superfamily and possess biglobal architecture with two facing *Rossmann*-like domains, attempts to shuffle heterologous domains to create functional chimeras were performed. One of the earliest examples in producing functional hybrid GTs was presented by *Hoffmeister et al.* who engineered GTs involved in the biosynthesis of the aromatic polyketide urdamycin A (**19**) (47). In this study GTs were created with only one parental activity, with both parent activities, and, most interestingly, with a new activity capable of forming a branched saccharide chain (49). A more recent example was described by *Spencer et al.*, who successfully constructed active hybrids from the GTs, GtfA, GtfB, Orf1, and Orf10 involved in the biosynthesis of different glycopeptides. This study resulted in the conclusion that the N-terminal domain controls acceptor binding entirely (19, 59, 88); accordingly, the C-terminal domain appears to be responsible for the sugar specificity. However, in some cases this strict rule could not be confirmed, and thus additional regions within the GT-Bs can influence the acceptor and donor specificity.

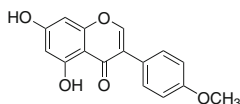


To imply directed evolution coupled with a high-throughput screening for expanding promiscuity of natural product GTs, *Aharoni* and colleagues developed a screen based on a fluorescent surrogate acceptor substrate. This was successfully applied and resulted in generating among others a glycosyltransferase of the GT-A superfamily with 400-fold higher catalytic efficiency (1). Additionally, a very similar fluorescent based screening strategy was used for generating macrolide GT derivatives of OleD able to modify a range of therapeutically important compounds (129).

The change of the acceptor affinity of a GT was demonstrated by the group of *X. Wang*. The catalytic efficiency of the GT UGT85H2 from *Medicago truncatula* towards the natural acceptor kaempferol (**20**) was increased 37-fold based on only one amino acid substitution. Another substitution dramatically improved (54-fold) the turnover rate and catalytic efficiency for biochanin A (**21**), the second natural acceptor (79). This work impressively presents how site-directed mutagenesis could lead to radical improvement of the catalytic efficiency.



20 (Kaempferol)



21 (Biochanin A)

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References

1. Aharoni A, Thieme K, Chiu CP, Buchini S, Lairson LL, Chen H, Strynadka NC, Wakarchuk WW, Withers SG (2006) High-Throughput Screening Methodology for the Directed Evolution of Glycosyltransferases. *Nat Methods* **3**: 609
2. Ahmed A, Peters NR, Fitzgerald MK, Watson JA, Hoffmann FM, Thorson JS (2006) Colchicine Glycorandomization Influences Cytotoxicity and Mechanism of Action. *J Am Chem Soc* **128**: 14224
3. Albermann C, Soriano A, Jiang J, Vollmer H, Biggins JB, Barton WA, Lesniak J, Nikolov DB, Thorson JS (2003) Substrate specificity of NovM: Implications for Novobiocin Biosynthesis and Glycorandomization. *Org Lett* **5**: 933
4. Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic Identification and In Situ Detection of Individual Microbial Cells Without Cultivation. *Microbiol Rev* **59**: 143
5. Arnold FH (2006) Fancy Footwork in the Sequence Space Shuffle. *Nat Biotechnol* **24**: 328
6. Baerga-Ortiz A, Popovic B, Siskos AP, O'Hare HM, Spitteller D, Williams MG, Campillo N, Spencer JB, Leadlay PF (2006) Directed Mutagenesis Alters the Stereochemistry of Catalysis by Isolated Ketoreductase Domains from the Erythromycin Polyketide Synthase. *Chem Biol* **13**: 277
7. Banskota AH, McAlpine JB, Sorensen D, Aouidate M, Pirae M, Alarco AM, Omura S, Shiomi K, Farnet CM, Zazopoulos E (2006) Isolation and Identification of Three New 5-Alkenyl-3,3(2H)-Furanones from Two *Streptomyces* Species Using a Genomic Screening Approach. *J Antibiotics* **59**: 168
8. Banskota AH, McAlpine JB, Sorensen D, Ibrahim A, Aouidate M, Pirae M, Alarco AM, Farnet CM, Zazopoulos E (2006) Genomic Analyses Lead to Novel Secondary Metabolites. Part 3. ECO-0501, a Novel Antibacterial of a New Class. *J Antibiotics* **59**: 533
9. Bentley R, Bennett JW (1999) Constructing Polyketides: from Collie to Combinatorial Biosynthesis. *Annu Rev Microbiol* **53**: 411
10. Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K,

- Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA (2002) Complete Genome Sequence of the Model Actinomycete *Streptomyces coelicolor* A3(2). *Nature* **417**: 141
11. Bergmann S, Schumann J, Scherlach K, Lange C, Brakhage AA, Hertweck C (2007) Genomics-Driven Discovery of PKS-NRPS Hybrid Metabolites from *Aspergillus nidulans*. *Nat Chem Biol* **3**: 213
 12. Binz TM, Wenzel SC, Schnell HJ, Bechthold A, Muller R (2008) Heterologous Expression and Genetic Engineering of the Phenalinolactone Biosynthetic Gene Cluster by Using Red/ET Recombineering. *Chembiochem* **9**: 447
 13. Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR (2009) Marine Natural Products. *Nat Prod Rep* **26**: 170
 14. Bok JW, Noordermeer D, Kale SP, Keller NP (2006) Secondary Metabolic Gene Cluster Silencing in *Aspergillus nidulans*. *Mol Microbiol* **61**: 1636
 15. Brady SF, Clardy J (2004) Palmitoylputrescine, an Antibiotic Isolated from the Heterologous Expression of DNA Extracted from Bromeliad Tank Water. *J Nat Prod* **67**: 1283
 16. Brady SF, Clardy J (2005) Cloning and Heterologous Expression of Isocyanide Biosynthetic Genes from Environmental DNA. *Angew Chem Int Ed Engl* **44**: 7063
 17. Brakhage AA, Schuemann J, Bergmann S, Scherlach K, Schroeckh V, Hertweck C (2008) Activation of Fungal Silent Gene Clusters: a New Avenue to Drug Discovery. *Prog Drug Res* **66**: 1,3
 18. Caffrey P (2003) Conserved Amino Acid Residues Correlating with Ketoreductase Stereospecificity in Modular Polyketide Synthases. *Chembiochem* **4**:654
 19. Cartwright AM, Lim EK, Kleanthous C, Bowles DJ (2008) A Kinetic Analysis of Regiospecific Glucosylation by Two Glycosyltransferases of *Arabidopsis thaliana*: Domain Swapping to Introduce New Activities. *J Biol Chem* **283**: 15724
 20. Challis GL (2008) Genome Mining for Novel Natural Product Discovery. *J Med Chem* **51**: 2618
 21. Challis GL (2008) Mining Microbial Genomes for New Natural Products and Biosynthetic Pathways. *Microbiology* **154**: 1555
 22. Challis GL, Ravel J (2000) Coelichelin, a New Peptide Siderophore Encoded by the *Streptomyces coelicolor* genome: Structure Prediction from the Sequence of its Non-Ribosomal Peptide Synthetase. *FEMS Microbiol Lett* **187**: 111
 23. Chan YA, Boyne MT, Podevels AM, Klimowicz AK, Handelsman J, Kelleher NL, Thomas MG (2006) Hydroxymalonyl-Acyl Carrier Protein (ACP) and Aminomalonyl-ACP are Two Additional Type I Polyketide Synthase Extender Units. *Proc Natl Acad Sci USA* **103**: 14349
 24. Chen AY, Cane DE, Khosla C (2007) Structure-Based Dissociation of a Type I Polyketide Synthase Module. *Chem Biol* **14**: 784
 25. Chng C, Lum AM, Vroom JA, Kao CM (2008) A Key Developmental Regulator Controls the Synthesis of the Antibiotic Erythromycin in *Saccharopolyspora erythraea*. *Proc. Natl. Acad. Sci. USA* **105**: 11346
 26. Corre C, Challis GL (2007) Heavy Tools for Genome Mining. *Chem Biol* **14**: 7
 27. Cortes J, Wiesmann KE, Roberts GA, Brown MJ, Staunton J, Leadlay PF (1995) Repositioning of a Domain in a Modular Polyketide Synthase to Promote Specific Chain Cleavage. *Science* **268**: 1487
 28. Cragg GM, Grothaus PG, Newman DJ (2009) Impact of Natural Products on Developing New Anti-Cancer Agents. *Chem Rev* **109**: 3012
 29. Del Vecchio F, Petkovic H, Kendrew SG, Low L, Wilkinson B, Lill R, Cortes J, Rudd BA, Staunton J, Leadlay PF (2003) Active-Site Residue, Domain and Module Swaps in Modular Polyketide Synthases. *J Ind Microbiol Biotechnol* **30**: 489
 30. Donadio S, Staver MJ, McAlpine JB, Swanson SJ, Katz L (1991) Modular Organization of Genes Required for Complex Polyketide Biosynthesis. *Science* **252**: 675

31. Eustáquio AS, Gust B, Galm U, Li S-M, Chater KF, Heide, L (2005) Heterologous Expression of Novobiocin and Clorobiocin Biosynthetic Gene Clusters. *Appl. Environ. Microbiol.* **71**: 2452
32. Fedoryshyn M, Petzke L, Welle E, Bechthold A, Luzhetsky A (2008) Marker Removal from Actinomycetes Genome Using Flp Recombinase. *Gene* **419**: 43
33. Fedoryshyn M, Welle E, Bechthold A, Luzhetsky A (2008) Functional Expression of the Cre Recombinase in Actinomycetes. *Appl Microbiol Biotechnol* **78**: 1065
34. Fischbach MA, Lai JR, Roche ED, Walsh CT, Liu DR (2007) Directed Evolution can Rapidly Improve the Activity of Chimeric Assembly-Line Enzymes. *Proc Natl Acad Sci USA* **104**: 11951
35. Fischbach MA, Walsh CT (2006) Assembly-Line Enzymology for Polyketide and Nonribosomal Peptide Antibiotics: Logic, Machinery, and Mechanisms. *Chem Rev* **106**: 3468
36. Floss HG (2006) Combinatorial Biosynthesis-Potential and Problems. *J. Biotechnol.* **124**: 242
37. Gaisser S, Carletti I, Schell U, Graupner PR, Sparks TC, Martin CJ, Wilkinson B (2009) Glycosylation Engineering of Spinosyn Analogues Containing an L-Olivose Moiety. *Org Biomol Chem* **7**: 1705
38. Gaisser S, Martin CJ, Wilkinson B, Sheridan RM, Lill RE, Weston AJ, Ready SJ, Waldron C, Crouse GD, Leadlay PF, Staunton J (2002) Engineered Biosynthesis of Novel Spinosyns Bearing Altered Deoxyhexose Substituents. *Chem Commun* 618
39. Gibson DG, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveri J, Stockwell TB, Brownley A, Thomas DW, Algire MA, Merryman C, Young L, Noskov VN, Glass JI, Venter JC, Hutchison CA III, Smith HO (2008) Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome. *Science* **319**: 1215
40. Gross F, Luniak N, Perlova O, Gaitatzis N, Jenke-Kodama H, Gerth K, Gottschalk D, Dittmann E, Muller R (2006) Bacterial Type III Polyketide Synthases: Phylogenetic Analysis and Potential for the Production of Novel Secondary Metabolites by Heterologous Expression in *Pseudomonads*. *Arch. Microbiol.* **185**: 28
41. Gullo VP, McAlpine J, Lam KS, Baker D, Petersen F (2006) Drug Discovery from Natural Products. *J Ind Microbiol Biotechnol* **33**: 523
42. Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-Targeted *Streptomyces* Gene Replacement Identifies a Protein Domain Needed for Biosynthesis of the Sesquiterpene Soil Odor Geosmin. *Proc Natl Acad Sci USA* **100**: 1541
43. Harle J, Bechthold A (2009) The Power of Glycosyltransferases to Generate Bioactive Natural Compounds. *Methods Enzymol* **458**: 309
44. Haydock SF, Aparicio JF, Molnar I, Schwecke T, Khaw LE, Konig A, Marsden AF, Galloway IS, Staunton J, Leadlay PF (1995) Divergent Sequence Motifs Correlated with the Substrate Specificity of (Methyl) mMalonyl-CoA:Acyl Carrier Protein Transacylase Domains in Modular Polyketide Synthases. *FEBS Lett* **374**: 246
45. Haynes SW, Challis GL (2007) Non-Linear Enzymatic Logic in Natural Product Modular Mega-Synthases and -Synthetases. *Curr Opin Drug Discov Devel* **10**: 203
46. Heathcote ML, Staunton J, Leadlay PF (2001) Role of Type II Thioesterases: Evidence for Removal of Short Acyl Chains Produced by Aberrant Decarboxylation of Chain Extender Units. *Chem Biol* **8**: 207
47. Hoffmeister D, Ichinose K, Bechthold A (2001) Two Sequence Elements of Glycosyltransferases Involved in Urdamycin Biosynthesis are Responsible for Substrate Specificity and Enzymatic Activity. *Chem Biol.* **8**: 557
48. Hoffmeister D, Weber M, Drager G, Ichinose K, Durr C, Bechthold A (2004) Rational Saccharide Extension by Using the Natural Product Glycosyltransferase LanGT4. *Chembiochem* **5**: 369
49. Hoffmeister D, Wilkinson B, Foster G, Sidebottom PJ, Ichinose K, Bechthold A (2002) Engineered Urdamycin Glycosyltransferases are Broadened and Altered in Substrate Specificity. *Chem Biol* **9**: 287

50. Hofmann C, Boll R, Heitmann B, Hauser G, Durr C, Frerich A, Weitnauer G, Glaser SJ, Bechthold A (2005) Genes Encoding Enzymes Responsible for Biosynthesis of L-Lyxose and Attachment of Eurekaate During Avilamycin Biosynthesis. *Chem Biol* **12**: 1137
51. Hornung A, Bertazzo M, Dziarnowski A, Schneider K, Welzel K, Wohlert SE, Holzenkampfer M, Nicholson GJ, Bechthold A, Sussmuth RD, Vente A, Pelzer S (2007) A Genomic Screening Approach to the Structure-Guided Identification of Drug Candidates from Natural Sources. *Chembiochem* **8**: 757
52. Hutchinson CR (1998) Combinatorial Biosynthesis for New Drug Discovery. *Curr Opin Microbiol* **1**: 319
53. Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Omura S (2003) Complete Genome Sequence and Comparative Analysis of the Industrial Microorganism *Streptomyces avermitilis*. *Nat Biotechnol* **21**: 526
54. Itokawa H, Morris-Natschke SL, Akiyama T, Lee KH (2008) Plant-Derived Natural Product Research Aimed at New Drug Discovery. *Nat Med (Tokyo)* **62**: 263
55. Kantola J, Kunnari T, Hautala A, Hakala J, Ylihonko K, Mantsala P (2000) Elucidation of Anthracyclinone Biosynthesis by Stepwise Cloning of Genes for Anthracyclines from Three Different *Streptomyces* spp. *Microbiology* **146**: 155
56. Kantola J, Kunnari T, Mantsala P, Ylihonko K (2003) Expanding the Scope of Aromatic Polyketides by Combinatorial Biosynthesis. *Comb Chem High Throughput Screen* **6**: 501
57. Khosla C, Kapur S, Cane DE (2009) Revisiting the Modularity of Modular Polyketide Synthases. *Curr Opin Chem Biol* **13**: 135
58. Kodumal SJ, Patel KG, Reid R, Menzella HG, Welch M, Santi DV (2004) Total Synthesis of Long DNA Sequences: Synthesis of a Contiguous 32-kb Polyketide Synthase Gene Cluster. *Proc Natl Acad Sci USA* **101**: 15573
59. Krauth C, Fedoryshyn M, Schleberger C, Luzhetskyy A, Bechthold A (2009) Engineering a Function into a Glycosyltransferase. *Chem Biol* **16**: 28
60. Lautru S, Deeth RJ, Bailey LM, Challis GL (2005) Discovery of a New Peptide Natural Product by *Streptomyces coelicolor* Genome Mining. *Nat Chem Biol* **1**: 265
61. Leibundgut M, Jenni S, Frick C, Ban N (2007) Structural Basis for Substrate Delivery by Acyl Carrier Protein in the Yeast Fatty Acid Synthase. *Science* **316**: 288
62. Leman LJ, Weinberger DA, Huang ZZ, Wilcoxon KM, Ghadiri MR (2007) Functional and Mechanistic Analyses of Biomimetic Aminoacyl Transfer Reactions in *de novo* Designed Coiled Coil Peptides *via* Rational Active Site Engineering. *J Am Chem Soc* **129**: 2959
63. Li S-M, Heide L (2005) New Aminocoumarin Antibiotics from Genetically Engineered *Streptomyces* Strains. *Curr Med Chem* **12**: 419
64. Lin X, Hopson R, Cane DE (2006) Genome Mining in *Streptomyces coelicolor*: Molecular Cloning and Characterization of a New Sesquiterpene Synthase. *J Am Chem Soc* **128**: 6022
65. Liu H, Jiang H, Haltli B, Kulowski K, Muszynska E, Feng X, Summers M, Young M, Graziani E, Koehn F, Carter GT, He M (2009) Rapid Cloning and Heterologous Expression of the Meridamycin Biosynthetic Gene Cluster Using a Versatile *Escherichia coli-Streptomyces* Artificial Chromosome Vector, pSBAC (perpendicular). *J Nat Prod* **72**: 389
66. Lu W, Oberthur M, Leimkuhler C, Tao J, Kahne D, Walsh CT (2004) Characterization of a Regiospecific Epivancosaminyl Transferase GtfA and Enzymatic Reconstitution of the Antibiotic Chloroeremomycin. *Proc Natl Acad Sci USA* **101**: 4390
67. Luzhetskyy A, Bechthold A (2005) It Works: Combinatorial Biosynthesis for Generating Novel Glycosylated Compounds. *Mol Microbiol* **58**: 3
68. Luzhetskyy A, Mendez C, Salas JA, Bechthold A (2008) Glycosyltransferases, Important Tools for Drug Design. *Curr Top Med Chem* **8**: 680
69. Luzhetskyy A, Taguchi T, Fedoryshyn M, Durr C, Wohlert SE, Novikov V, Bechthold A (2005) LanGT2 Catalyzes the First Glycosylation Step During Landomycin A Biosynthesis. *Chembiochem* **6**: 1406

70. Luzhetskyy A, Zhu L, Gibson M, Fedoryshyn M, Durr C, Hofmann C, Hoffmeister D, Ostash B, Mattingly C, Adams V, Fedorenko V, Rohr J, Bechthold A (2005) Generation of Novel Landomycins M and O Through Targeted Gene Disruption. *Chembiochem* **6**: 675
71. Maier T, Jenni S, Ban N (2006) Architecture of Mammalian Fatty Acid Synthase at 4.5 Å Resolution. *Science* **311**: 1258
72. McDaniel R, Thamchaipenet A, Gustafsson C, Fu H, Betlach M, Ashley G (1999) Multiple Genetic Modifications of the Erythromycin Polyketide Synthase to Produce a Library of Novel "Unnatural" Natural Products. *Proc Natl Acad Sci USA* **96**: 1846
73. Mendez C, Luzhetskyy A, Bechthold A, Salas JA (2008) Deoxysugars in Bioactive Natural Products: Development of Novel Derivatives by Altering the Sugar Pattern. *Curr Top Med Chem* **8**: 710
74. Mendez C, Weitnauer G, Bechthold A, Salas JA (2000) Structure Alteration of Polyketides by Recombinant DNA Technology in Producer Organisms-Prospects for the Generation of Novel Pharmaceutical Drugs. *Curr Pharm Biotechnol* **1**: 355
75. Menzella HG, Reid R, Carney JR, Chandran SS, Reisinger SJ, Patel KG, Hopwood DA, Santi DV (2005) Combinatorial Polyketide Biosynthesis by *de novo* Design and Rearrangement of Modular Polyketide Synthase Genes. *Nat Biotechnol* **23**: 1171
76. Miao V, Coeffet-Le Gal MF, Nguyen K, Brian P, Penn J, Whiting A, Steele J, Kau D, Martin S, Ford R, Gibson T, Bouchard M, Wrigley SK, Baltz RH (2006) Genetic Engineering in *Streptomyces roseosporus* to Produce Hybrid Lipopeptide Antibiotics. *Chem Biol* **13**: 269
77. Miao V, Coeffet-Legal MF, Brian P, Brost R, Penn J, Whiting A, Martin S, Ford R, Parr I, Bouchard M, Silva CJ, Wrigley SK, Baltz RH (2005) Daptomycin Biosynthesis in *Streptomyces roseosporus*: Cloning and Analysis of the Gene Cluster and Revision of Peptide Stereochemistry. *Microbiology* **151**: 1507
78. Minami A, Uchida R, Eguchi T, Kakinuma K (2005) Enzymatic Approach to Unnatural Glycosides with Diverse Aglycon Scaffolds Using Glycosyltransferase VinC. *J Am Chem Soc* **127**: 6148
79. Modolo LV, Escamilla-Trevino LL, Dixon RA, Wang X (2009) Single Amino Acid Mutations of Medicago Glycosyltransferase UGT85H2 Enhance Activity and Impart Reversibility. *FEBS Lett* **583**: 2131
80. Moss SJ, Martin CJ, Wilkinson B (2004) Loss of Co-Linearity by Modular Polyketide Synthases: a Mechanism for the Evolution of Chemical Diversity. *Nat Prod Rep* **21**: 575
81. Nguyen KT, Ritz D, Gu JQ, Alexander D, Chu M, Miao V, Brian P, Baltz RH (2006) Combinatorial Biosynthesis of Novel Antibiotics Related to Daptomycin. *Proc Natl Acad Sci USA* **103**: 17462
82. Nierman WC, Pain A, Anderson MJ, Wortman JR, Kim HS, Arroyo J, Berriman M, Abe K, Archer DB, Bermejo C, Bennett J, Bowyer P, Chen D, Collins M, Coulsen R, Davies R, Dyer PS, Farman M, Fedorova N, Fedorova N, Feldblyum TV, Fischer R, Fosker N, Fraser A, Garcia JL, Garcia MJ, Goble A, Goldman GH, Gomi K, Griffith-Jones S, Gwilliam R, Haas B, Haas H, Harris D, Horiuchi H, Huang J, Humphray S, Jimenez J, Keller N, Khouri H, Kitamoto K, Kobayashi T, Konzack S, Kulkarni R, Kumagai T, Lafon A, Latge JP, Li W, Lord A, Lu C, Majoros WH, May GS, Miller BL, Mohamoud Y, Molina M, Monod M, Mouyna I, Mulligan S, Murphy L, O'Neil S, Paulsen I, Penalva MA, Perteau M, Price C, Pritchard BL, Quail MA, Rabinowitz E, Rawlins N, Rajandream MA, Reichard U, Renauld H, Robson GD, Rodriguez DC, Rodriguez-Pena JM, Ronning CM, Rutter S, Salzberg SL, Sanchez M, Sanchez-Ferrero JC, Saunders D, Seeger K, Squares R, Squares S, Takeuchi M, Tekaiia F, Turner G, Vazquez de Aldana CR, Weidman J, White O, Woodward J, Yu JH, Fraser C, Galagan JE, Asai K, Machida M, Hall N, Barrell B, Denning DW (2005) Genomic Sequence of the Pathogenic and Allergenic Filamentous Fungus *Aspergillus fumigatus*. *Nature* **438**: 1151
83. Olano C, Lombo F, Mendez C, Salas JA (2008) Improving Production of Bioactive Secondary Metabolites in Actinomycetes by Metabolic Engineering. *Metab Eng* **10**: 281

84. Olano C, Mendez C, Salas JA (2009) Antitumor Compounds from Actinomycetes: from Gene Clusters to New Derivatives by Combinatorial Biosynthesis. *Nat Prod Rep* **26**: 628
85. Oliynyk M, Samborsky M, Lester JB, Mironenko T, Scott N, Dickens S, Haydock SF, Leadlay PF (2007) Complete Genome Sequence of the Erythromycin-Producing Bacterium *Saccharopolyspora erythraea* NRRL23338. *Nat Biotechnol* **25**: 447
86. Omura S, Ikeda H, Ishikawa J, Hanamoto A, Takahashi C, Shinose M, Takahashi Y, Horikawa H, Nakazawa H, Osonoe T, Kikuchi H, Shiba T, Sakaki Y, Hattori M (2001) Genome Sequence of an Industrial Microorganism *Streptomyces avermitilis*: Deducing the Ability of Producing Secondary Metabolites. *Proc Natl Acad Sci USA* **98**: 12215
87. Paradkar A, Trefzer A, Chakraborty R, Stassi D (2003) *Streptomyces* Genetics: a Genomic Perspective. *Crit Rev Biotechnol* **23**: 1
88. Park SH, Park HY, Sohng JK, Lee HC, Liou K, Yoon YJ, Kim BG (2009) Expanding Substrate Specificity of GT-B Fold Glycosyltransferase *via* Domain Swapping and High-Throughput Screening. *Biotechnol Bioeng* **102**: 988
89. Peiru S, Menzella HG, Rodriguez E, Carney J, Gramajo H (2005) Production of the Potent Antibacterial Polyketide Erythromycin C in *Escherichia coli*. *Appl Environ Microbiol* **71**: 2539
90. Perez M, Lombo F, Zhu L, Gibson M, Brana AF, Rohr J, Salas JA, Mendez C (2005) Combining Sugar Biosynthesis Genes for the Generation of L- and D-Amicetose and Formation of Two Novel Antitumor Tetracenomycins. *Chem Commun* 1604
91. Petzke L, Luzhetskyy A (2009) *In vivo* Tn5-based Transposon Mutagenesis of Streptomyces. *Appl Microbiol Biotechnol* **83**: 979
92. Reeves CD, Murlu S, Ashley GW, Piagentini M, Hutchinson CR, McDaniel R (2001) Alteration of the Substrate Specificity of a Modular Polyketide Synthase Acyltransferase Domain Through Site-Specific Mutations. *Biochemistry* **40**: 15464
93. Reid R, Piagentini M, Rodriguez E, Ashley G, Viswanathan N, Carney J, Santi DV, Hutchinson CR, McDaniel R (2003) A Model of Structure and Catalysis for Ketoreductase Domains in Modular Polyketide Synthases. *Biochemistry* **42**: 72
94. Richardson SM, Wheelan SJ, Yarrington RM, Boeke JD (2006) GeneDesign: Rapid, Automated Design of Multikilobase Synthetic Genes. *Genome Res* **16**: 550
95. Ro DK, Ouellet M, Paradise EM, Burd H, Eng D, Paddon CJ, Newman JD, Keasling JD (2008) Induction of Multiple Pleiotropic Drug Resistance Genes in Yeast Engineered to Produce an Increased Level of Anti-Malarial Drug Precursor, Artemisinic Acid. *BMC Biotechnol* **8**: 83
96. Rodriguez E, Menzella HG, Gramajo H (2009) Heterologous Production of Polyketides in Bacteria. *Meth Enzymol* **459**: 339
97. Rodriguez L, Aguirrezabalaga I, Allende N, Brana AF, Mendez C, Salas JA (2002) Engineering Deoxysugar Biosynthetic Pathways from Antibiotic-Producing Microorganisms. A Tool to Produce Novel Glycosylated Bioactive Compounds. *Chem Biol* **9**: 721
98. Salas JA, Mendez C (2005) Biosynthesis Pathways for Deoxysugars in Antibiotic-Producing Actinomycetes: Isolation, Characterization and Generation of Novel Glycosylated Derivatives. *J Mol Microbiol Biotechnol* **9**: 77
99. Sánchez C, Zhu L, Braña AF, Salas AP, Rohr J, Méndez C, Salas JA (2005) Combinatorial Biosynthesis of Antitumor Indolocarbazole Compounds. *Proc Natl Acad Sci USA* **102**: 461
100. Schell U, Haydock SF, Kaja AL, Carletti I, Lill RE, Read E, Sheehan LS, Low L, Fernandez MJ, Grolle F, McArthur HA, Sheridan RM, Leadlay PF, Wilkinson B, Gaisser S (2008) Engineered Biosynthesis of Hybrid Macrolide Polyketides Containing D-Angolosamine and D-Mycaminose Moieties. *Org Biomol Chem* **6**: 3315
101. Schmidt EW, Nelson JT, Rasko DA, Sudek S, Eisen JA, Haygood MG, Ravel J (2005) Patellamide A and C Biosynthesis by a Microcin-like Pathway in *Prochloron didemni*, the Cyanobacterial Symbiont of *Lissoclinum patella*. *Proc Natl Acad Sci USA* **102**: 7315
102. Shen B (2004) Accessing Natural Products by Combinatorial Biosynthesis. *Sci STKE* **2004**: e14.

103. Song L, Barona-Gomez F, Corre C, Xiang L, Udvary DW, Austin MB, Noel JP, Moore BS, Challis GL (2006) Type III Polyketide Synthase Beta-Ketoacyl-ACP Starter Unit and Ethylmalonyl-CoA Extender Unit Selectivity Discovered by *Streptomyces coelicolor* Genome Mining. *J Am Chem Soc* **128**: 14754
104. Stachelhaus T, Mootz HD, Marahiel MA (1999) The Specificity-Confering Code of Adenylation Domains in Nonribosomal Peptide Synthetases. *Chem Biol* **6**: 493
105. Stachelhaus T, Schneider A, Marahiel MA (1995) Rational Design of Peptide Antibiotics by Targeted Replacement of Bacterial and Fungal Domains. *Science* **269**: 69
106. Streit WR, Schmitz RA (2004) Metagenomics-the Key to the Uncultured Microbes. *Curr Opin Microbiol* **7**: 492
107. Tang L, Fu H, McDaniel R (2000) Formation of Functional Heterologous Complexes Using Subunits from the Picromycin, Erythromycin and Oleandomycin Polyketide Synthases. *Chem Biol* **7**: 77
108. Tang Y, Chen AY, Kim CY, Cane DE, Khosla C (2007) Structural and Mechanistic Analysis of Protein Interactions in Module 3 of the 6-Deoxyerythronolide B Synthase. *Chem Biol* **14**: 931
109. Tang Y, Kim CY, Mathews II, Cane DE, Khosla C (2006) The 2.7-Ångström Crystal Structure of a 194-kDa Homodimeric Fragment of the 6-Deoxyerythronolide B Synthase. *Proc Natl Acad Sci USA* **103**: 11124
110. Thibodeaux CJ, Melancon CE, Liu HW (2007) Unusual Sugar Biosynthesis and Natural Product Glycodiversification. *Nature* **446**: 1008
111. Thorson JS, Barton WA, Hoffmeister D, Albermann C, Nikolov DB (2004) Structure-based Enzyme Engineering and its Impact on *in vitro* Glycorandomization. *Chembiochem* **5**: 16
112. Trefzer A, Blanco G, Remsing L, Künzel E, Rix U, Lipata F, Braña AF, Méndez C, Rohr J, Bechthold A, Salas JA (2002) Rationally Designed Glycosylated Premithramycins: Hybrid Aromatic Polyketides Using Genes from Three Different Biosynthetic Pathways. *J Am Chem Soc* **124**: 6056
113. Trefzer A, Jungmann V, Molnar I, Botejue A, Buckel D, Frey G, Hill DS, Jorg M, Ligon JM, Mason D, Moore D, Pachlatko JP, Richardson TH, Spangenberg P, Wall MA, Zirkle R, Stege JT (2007) Biocatalytic Conversion of Avermectin to 4''-Oxo-Avermectin: Improvement of Cytochrome P450 Monooxygenase Specificity by Directed Evolution. *Appl Environ Microbiol* **73**: 4317
114. Udvary DW, Zeigler L, Asolkar RN, Singan V, Lapidus A, Fenical W, Jensen PR, Moore BS (2007) Genome Sequencing Reveals Complex Secondary Metabolome in the Marine Actinomycete *Salinispora tropica*. *Proc Natl Acad Sci USA* **104**: 10376
115. Uguru GC, Milne C, Borg M, Flett F, Smith CP, Micklefield J (2004) Active-Site Modifications of Adenylation Domains lead to Hydrolysis of Upstream Nonribosomal Peptidyl Thioester Intermediates. *J Am Chem Soc* **126**: 5032
116. Walsh CT (2002) Combinatorial Biosynthesis of Antibiotics: Challenges and Opportunities. *Chembiochem* **3**: 124
117. Watanabe K, Hotta K, Praseuth AP, Koketsu K, Migita A, Boddy CN, Wang CCC, Oguri H, Oikawa H (2006) Total Biosynthesis of Antitumor Nonribosomal Peptides in *Escherichia coli*. *Nat Chem Biol* **2**: 423
118. Weber T, Welzel K, Pelzer S, Vente A, Wohlleben W (2003) Exploiting the Genetic Potential of Polyketide Producing Streptomycetes. *J Biotechnol* **106**: 221
119. Weissman KJ (2007) Mutasynthesis - Uniting Chemistry and Genetics for Drug Discovery. *Trends Biotechnol* **25**: 139
120. Weissman KJ (2009) Introduction to Polyketide Biosynthesis. *Meth Enzymol* **459**: 3
121. Weissman KJ, Leadlay PF (2005) Combinatorial Biosynthesis of Reduced Polyketides. *Nat Rev Microbiol* **3**: 925
122. Weist S, Süßmuth RD (2005) Mutational Biosynthesis-a Tool for the Generation of Structural Diversity in the Biosynthesis of Antibiotics. *Appl Microbiol Biotechnol* **68**: 141

123. Wenzel SC, Gross F, Zhang Y, Fu J, Stewart AF, Muller R (2005) Heterologous Expression of a Myxobacterial Natural Products Assembly Line in Pseudomonads via Red/ET Recombining. *Chem Biol* **12**: 349
124. Wilcoxon KM, Leman LJ, Weinberger DA, Huang ZZ, Ghadiri MR (2007) Biomimetic Ccatalysis of Intermodular Aminoacyl Transfer. *J Am Chem Soc* **129**: 748
125. Wilkinson B, Micklefield J (2007) Mining and Engineering Natural-Product Biosynthetic Pathways. *Nat Chem Biol* **3**: 379
126. Wilkinson B, Micklefield J (2009). Biosynthesis of Nonribosomal Peptide Precursors. *Meth Enzymol* **458**: 353
127. Williams GJ, Goff RD, Zhang C, Thorson JS (2008) Optimizing Glycosyltransferase Specificity *via* “Hot Spot” Saturation Mutagenesis Presents a Catalyst for Novobiocin Glycorandomization. *Chem Biol* **15**: 393
128. Williams GJ, Thorson JS (2009) Natural Product Glycosyltransferases: Properties and Applications. *Adv Enzymol Relat Areas. Mol Biol* **76**: 55
129. Williams GJ, Zhang C, Thorson JS (2007) Expanding the Promiscuity of a Natural-Product Glycosyltransferase by Directed Evolution. *Nat Chem Biol* **3**: 657
130. Yeh E, Kohli RM, Bruner SD, Walsh CT (2004) Type II Thioesterase Restores Activity of a NRPS Module Stalled with an Aminoacyl-S-Enzyme that cannot Be Elongated. *Chembiochem* **5**: 1290
131. Yoshikuni Y, Ferrin TE, Keasling JD (2006) Designed Divergent Evolution of Enzyme Function. *Nature* **440**: 1078
132. Zhan J, Gunatilaka AAL (2006) Microbial Transformation of Amino- and Hydroxyanthraquinones by *Beauveria bassiana* ATCC 7159. *J Nat Prod* **69**: 1525
133. Zhang C, Albermann C, Fu X, Thorson JS (2006) The *in vitro* Characterization of the Iterative Avermectin Glycosyltransferase AveBI Reveals Reaction Reversibility and Sugar Nucleotide Flexibility. *J Am Chem Soc* **128**: 16420
134. Zhang C, Griffith BR, Fu Q, Albermann C, Fu X, Lee IK, Li L, Thorson JS (2006) Exploiting the Reversibility of Natural Product Glycosyltransferase-Catalyzed Reactions. *Science* **313**: 1291
135. Zhao B, Lin X, Lei L, Lamb DC, Kelly SL, Waterman MR, Cane DE (2008) Biosynthesis of the Sesquiterpene Antibiotic Alabaflavenone in *Streptomyces coelicolor* A3(2). *J Biol Chem* **283**: 8183
136. Zhou Y, Meng Q, You D, Li J, Chen S, Ding D, Zhou X, Zhou H, Deng Z (2008) Selective Removal of Aberrant Extender Units by a Type II Thioesterase for Efficient FR-008/Candicidin Biosynthesis in *Streptomyces sp.* Strain FR-008. *Appl Environ Microbiol* **23**: 7235
137. Zhu L, Luzhetskyy A, Luzhetska M, Mattingly C, Adams V, Bechthold A, Rohr J (2007) Generation of New Landomycins with Altered Saccharide Patterns Through Over-Expression of the Glycosyltransferase Gene *lanGT3* in the Biosynthetic Gene Cluster of Landomycin A in *Streptomyces cyanogenus* S-136. *Chembiochem* **8**: 83
138. Zotchev SB, Stepanchikova AV, Sergeyko AP, Sobolev BN, Filimonov DA, Poroikov VV (2006) Rational Design of Macrolides by Virtual Screening of Combinatorial Libraries Generated Through *in silico* Manipulation of Polyketide Synthases. *J Med Chem* **49**: 2077

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