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I. Introduction

Indole is a reactive nucleus prone to dimerization when it is isolated or a part of tryptamine or tryptophan, which themselves are reactive toward many functionalities. For these reasons, bisindoles, the majority of which are of iridoid origin, are frequently isolated in nature; this field has been the subject of several reviews (1). Besides these “dimers,” there exists a growing class of noniridoid bisindoles found in the marine environment, in microorganisms, and in plant species, many of which display interesting biological activities. It is the purpose of this chapter to review the field.
in order to try to promote research on a series of compounds worthy of attention.

II. Bisindoles from the Marine Environment

Marine environments are a rich source of indole and bisindole alkaloids of great structural variety. In the absence of traditional use, the chemical constituents of marine organisms are separated following bioassay-guided fractionation, and, consequently, contrary to compounds from terrestrial sources, many of the structures are associated with significant biological activity.

A. THE SIMPLE BROMOINDOLE DIMERS

An Australian blue–green alga, *Rivularia fima* Womersley, is the source of two major (1 and 2) and four minor polybrominated dimers (3–6) (2). The structures of these metabolites were determined on the basis of $^1$H- and $^{13}$C-NMR analyses and their $^1$H–$^{13}$C-NMR coupling constants. $^{13}$C relaxation data were used to identify brominated carbons. The observed optical activity
for compounds 2–6 is due to restricted rotation around the bond connecting the two indole halves (atropisomerism). The Okinawan red alga Laurencia brongniartii contained a sulfur bromoindole dimer (7), whose symmetric nature was deduced from NMR data (nine signals in the $^{13}$C-NMR spectrum) and confirmed by X-ray analysis (3).

Antimicrobial and cytotoxic activities associated with extracts from the Fijian tunicate Polycitotrella mariae led to the isolation of citorellamine (4). The original structure—a monomer—was revised to 8 on the basis of total syntheses of the monomer and dimer and on comparison of the spectra (5). 6-Bromotryptamine (9) is often a recurring structural motif of marine natural products. 2,2-Bis(6′-bromo-3′-indolyl)ethylamine (10), isolated from the tunicate Didemnum candidum, is a simple dimer derived from 9 and 6-bromindole (6).

![Chemical structures](image)

**B. THE DIMERS WITH “SPACERS” BETWEEN THE INDOLE NUCLEI**

Structurally more elaborated dimers have a spacer unit between the two indole nuclei. Such is the case for dragmacidin (11) (7), a cytotoxic metabolite of the deep-water marine sponge, Dragmacidon sp. Hallman 1017, which inhibits the growth of P388, A549, and HCT-8 cancer cell lines in *in vitro* assays. This dimer contains a 6-bromotryptamine and a
dibromohydroxytryptamine unit as shown by $^1$H-NMR and mass spectral fragments at $m/z$ 289, 291, and 293 for the dibromohydroxyindole (C$_8$H$_5$NOBr$_2$). The location of the substituents was facilitated by the analysis of the spectra of tridebromodragmacidin (12). Coupling constant measurements and H–C COSY experiments suggested substitution at C-4' or C-7' atoms with a phenolic hydroxy. The final confirmation was obtained on calculation of chemical shifts, which were in agreement with a 4-hydroxyindole nucleus. NOE experiments and coupling constants ($J_{2,3} = 10.3$ and $J_{5,6} = 11.3$ Hz) favored a chair conformation with the two bromoindole appendages in equatorial positions on the piperazine ring.

Two other closely related dimers, dragmacidon A (13) and dragmacidon B (14), have been isolated as minor constituents of the deep-water sponge Hexadella sp., collected off the coast of British Columbia (8). The aromatic region of the $^1$H-NMR spectrum of 13 displayed signals for two 6-bromoindol-3-yl residues. The remaining aliphatic protons consisted of a singlet (N–CH$_3$ at $\delta$ 2.09 ppm) and two separated three-spin systems, each corresponding to a methylene, adjacent to a methine, as found in the piperazine ring of 11. Dragmacidon B (14) had an additional methyl group

![Chemical structures](image)
3. NONIRIDOID BISINDOLE ALKALOIDS

on the piperazine ring. Consequently, the molecule contained a twofold axis of symmetry similar to that of the simplest demethyl-dragmacidon A (15), isolated from the tunicate Didemnum candidum (6). Total synthesis of dragmacidon B (14) has been achieved (Scheme 1) by bromination of 1,4-dimethyl piperazine-2,5-dione (16) followed by coupling with 6-bromoindole (9). Dragmacidon A (13) showed in vitro cytotoxicity in the L1210 assay.

Dragmacidon d (17) has been isolated from a deep-water sponge of the genus Spongosorites (10). It exhibited a broad spectrum of biological activities: growth inhibition of feline leukemia virus, of P388 murine, and of A549 human lung tumor cell lines. Based on detailed NMR experiments, the two indole rings were identified as a 6-bromoindol-3-yl unit and a 7-hydroxy-3,4-dialkylated indole unit. Observation of carbon resonances at $\delta$ 147.9 (C-2"), 131.4 (C-5"), and 108.9 (C-4") ppm and of four exchangeable protons at $\delta$ 7.34 (2H), 11.87 (1H), and 11.88 (1H) ppm allowed the identification of a protonated 2-aminoimidazole appendage linked to the phenol containing ring via a 1,1-disubstituted ethyl moiety ($\delta$ 4.35 ppm (q, $J = 6.8$ Hz, H-6") and $\delta$ 1.50 ppm (d, $J = 6.8$ Hz, CH$_2$-CH)). $^{13}$C-NMR (e.g., $\delta$ 155.1 ppm for CO) and IR (1680 cm$^{-1}$) data suggested the presence of a 2-ketopiperazine ring as a spacer.

Topsentins A (18), B (19), and C (20) are the main active compounds of a Mediterranean shallow-water sponge, Topsentia genitrix (11). Topsentins B and C were independently isolated from Caribbean deep-sea sponges of the family Halichondriidae, under the names of topsentin (19) and bromotopsentin (20), respectively (12). Bromotopsentin (20) is an aromatic bromo derivative of 19 as confirmed by catalytic debromination. Mass spectral fragmentation was characterized by intense m/z 133 and 161 ions, assigned to hydroxyindole and a hydroxyindole substituted at the 3 position by a carbonyl group ($\delta$ 173–176 ppm for CO-s). Location of bromine, hydroxyl, or both groups at the C-6' and C-6" positions was suggested by comparison of the chemical shifts of the protons on the benzene rings with

![Scheme 1](image)

**Scheme 1.** Cava's synthesis of dragmacidon B. Reagents: (i) NBS, AIBN, CCl$_4$; (ii) 6-bromoindole, DMF; and (iii) B$_2$H$_6$, THF.
those of known bromo- and hydroxyindoles. The presence of an imidazole spacer was demonstrated by \(^1\text{H}-\text{C}-\text{NMR}\) correlation techniques and confirmed by synthesis. After analysis of the structure for hidden symmetries, two short syntheses of topsentin A were produced (Scheme 2): thermolysis of the quaternary hydrazine derivative (21) gave 18 in a single step by self-condensation of the presumed intermediate (22) whereas the same result was obtained by condensation of glyoxalylindole (23) with ammonia (13).
3. NONIRIDOID BISINDOLE ALKALOIDS

\[ \text{179} \]

\section*{A. H (21) \[ \text{18} \]}

\textbf{Scheme 2. Preparation of topsentin derivatives. Reagents: (i) 1,1-dimethylhydrazine; (ii) PrOH, reflux, 36 h; and (iii) Cu(OAc)\textsubscript{2}, NH\textsubscript{4}OH, C\textsubscript{2}H\textsubscript{5}OH.}

4,5-Dihydro-6'-deoxybromotopsentin (24) was isolated as a minor compound of a sponge tentatively identified as \textit{Spongosorites} sp. (12). In addition to the resonances attributed to unsubstituted and monosubstituted benzene rings, significant signals were observed for a deshielded ABX system, corresponding to an N-CH\textsubscript{2}-CH-N sequence. The COSY spectrum placed the methine group at the 3 position of the bromoindole in accordance with the proposed structure (24).

All of the natural topsentins showed activity as antiviral and antitumor agents, and, consequently, several C-5\textsuperscript{m} analogues, named “neotopsentins” (25–27), were prepared to establish structure–activity relationships. Topsentin and bromotopsentin were active against the \textit{Herpes simplex} virus (HSV-1) and corona virus A-59. Topsentin had \textit{in vitro} activity against P388 and human tumor (HCT-8, A549) cells and had \textit{in vivo} activity against P388 and B16 melanoma. As a general observation, the introduction of a
hydroxyl group was found to enhance cytotoxicity, whereas a bromine atom decreased it (12).

Bromotopsentin (20) (14) and a new compound unfortunately also named topsentin C (28) were reported for the deep-water sponge *Hexadella* sp., collected off the coast of British Columbia (8). The $^1$H-NMR spectrum of 28 displayed two sets of resonances which could be assigned to 6-bromoindol-3-yl residues by analogy with the characteristic shifts of 24. The methyl group ($\delta$ 3.05 ppm) could be placed on the N-1 atom of the dihydroimidazole ring in accordance with the strong NOEs observed with H-5 ($\delta$ 5.16 ppm).

*Spongosorites ruetzleri* Van Soest and Stentoft was the source of three novel and related cytotoxic and antifungal alkaloids, nortopsentin A (29), B (30), and C (31) (15). The lack of a carbonyl link to the imidazole spacer illustrates a new condensation process in tryptophan metabolism. Nortopsentin A (29) is derived from two 6-bromoindol-3-yl units linked to the C-2 ($\delta$ 143.8 ppm) and C-4 ($\delta$ 116.1 ppm) carbon atoms of the imidazole core. Nortopsentin B (30) and nortopsentin C (31) have indolyl and 6-bromoindol-3-yl units, the latter nucleus being attached to the C-2 carbon in 30 and to C-4 in 31.

### C. Grossularines and Eudistomin

Grossularine-1 (32) is a marine metabolite of the tunicate *Dendrodoa grossularia* from New Caledonia (16). The presence of an indole nucleus substituted on C-3 by a carbonyl was deduced from the mass spectral fragmentations at $m/z$ 144 and 116 and by the NMR data (CO at $\delta$ 186.8 ppm). On the basis of $^{13}$C-NMR, a second 2,3-disubstituted indole ring was attached to an N,N-dimethyl-guanidine core and structure 33 was proposed. It was later revised after the isolation of grossularine-2 (34), a metabolite from the same source whose structure was solved by X-ray crystallography (17,18). These molecules were reported as the first natural products with an $\alpha$-carboline moiety. Grossularine-1 displayed cytotoxicity toward L1210 leukemia cells at the $\mu$g/ml level.

Eudistomin refers to a series of $\beta$-carbolines isolated from tunicates (19). Eudistomin U (35), isolated from the Caribbean ascidian *Lissoclinum fragile* (20), is the first bisindole among these molecules ($M^+$ 283.1094 for C$_{19}$H$_{13}$N$_3$). Spectral data, especially $^1$H- and $^{13}$C-NMR data, were in good accordance with those reported for $\beta$-carboline and indole, thus supporting the proposed structure (35). Isoeudistomin U (36), from the same source, comprises indole and dihydro-$\alpha$-carboline moieties. This structure was proposed on the basis of HMBC and NOE measurements (e.g., an NOE response of H-3 to irradiation at H-2') and by comparison of $^{13}$C-NMR chemical shifts: C-9a at $\delta$ 158.6 and at $\delta$ 159.2 ppm for 36 and 32, respectively. It is
the personal opinion of the authors, however, that the structure of isoeudistomin U should be reconsidered based upon the following arguments: from a chemical standpoint, a dihydro α-carboline substituted as in 36 should not be stable and would readily be oxidized to the fully aromatic compound. The information extracted from the HMBC experiment may be explained by changing chemical shift assignments; for example, the carbon signal at
δ 20.3 ppm could be reassigned to the C-6 of a 3,4-dihydro β-carboline unit. The most puzzling data concern the above-mentioned NOE between H-2' and H-3; the published value (12%) is much too high for a molecule of this size at 400 MHz! An artifactual observation must not be excluded. Last, but not least, both compounds display the same UV spectra and once again artifacts should be looked for. Whatever their structure, 35 and 36 possess strong antibacterial activity (Agrobacterium tumefaciens).

D. FASCAPLYSIN

Fascaplysin (37) is an antimicrobial, cytotoxic pigment, isolated from the Fijian sponge Fascaplysinopsis sp. Bergquist (21), whose unique structure (pyrido(1,2-a:3,4,b')diindole) was determined by X-ray diffraction analysis. Fascaplysinopsis reticulata allowed the isolation of two alkaloids as salts of sesterterpene acids along with two other neutral alkaloids (22,23). The combination of negative FABMS experiments and of \(^1\)H- and \(^13\)C-NMR techniques demonstrated that fascaplysin A (38) and homofascaplysin A (39) had the same dehydroluffariellolide diacid anion with a one-to-one anion-to-cation ratio. Spectral analysis of the alkaloid part of 39 allowed identification of acetonyl substitution (CH$_2$-CO-CH$_3$ carbons at δ 51.0, 204.6, and 30.5 ppm, respectively) at C-13 (δ 78.2 ppm). Despite the fact that 39 was found to be optically active and that acetone was not used in the isolation process, the authors did not completely exclude the possibility of 39 being an isolation artifact. The chemical shifts of H-6 and H-7 in homofascaplysin B (41) and homofascaplysin C (42) were diagnostic of the
uncharged nature of these molecules (e.g., δ 9.01 (H-6) and 8.69 (H-7) ppm in 37 vs. 8.3 (H-6) and 7.67 (H-7) ppm in 42). Formyl (42) and α-ketoester (41) functions on the C-13 position were deduced from spectroscopic data, including comparison of NMR data with those for 37. A plausible biogenetic pathway to fascaplysin (37) involves condensation of tryptophan with tryptamine.

The total synthesis of 37 was based upon an acid-catalyzed cyclization followed by oxidation of an intermediate (43) (24). A starting diindole derivative (44) was readily prepared from indole by two routes (Scheme 3). Exposure of 44 to trifluoroacetic acid afforded a mixture (10:1) of cyclized products [(45) and (a)] which was dehydrogenated to the fully aromatic pentacycle (43). As anticipated, peracetic-acid-mediated oxidation of 43 provided fascaplysin (37) in a 65% overall yield. Fascaplysin (37) and homofascaplysin A (39) showed reverse transcriptase inhibition activity at the μg/ml level.

E. CAULERPIN

Caulerpin (47) is an orange-red pigment, originally isolated from the green algae Caulerpa spp. of the Philippines (25). The previously assigned phenazine structure (48) was corrected to 47 on the basis of chemical degradation reactions and total synthesis (Scheme 4) (26). Extracts from the green alga C. racemosa gave two new pigments (49 and 50), derivatives of 47 (27,28). Structural analogy among these compounds was established by simple hydrolysis and esterification reactions. Caulerpin is a plant growth regulator, and this is probably due to its 3-indolyl acrylic acid dimer structure (29). A large survey of the distribution of caulerpin in algae from Bermuda, Florida, and Tasmania was conducted to reveal correlations with habitat, morphology, and taxonomy (30).

F. MISCELLANEOUS DERIVATIVES

Hyrtiosin B (51) is a symmetrical phenolic dimer, found in the Okinawan marine sponge Hyrtios erecta (31). Its presumed precursor, 3-formyl-
SCHEME 3. Gribble's synthesis of fascaplysin. Reagents: (i) NaBH₃CN, AcOH, 15°C; (ii) (COCl)₂, Et₂O, 0°C; (iii) NaH, THF–DMF, rt, 1.5 h; (iv) K₂CO₃, THF, rt; (v) NaBH₃O-COCF₃, THF, reflux, 20 h; (vi) AlH₃, THF, rt, 1.5 h; (vii) MnO₂, CHCl₃, reflux, 4 h; (viii) TFA, rt, 0.5 h; (ix) Pd–C, (EtOCH₂CH₂)₂O, reflux, 6 h; and (x) CF₃COOOH, THF, 0°C, then EtOH–HCl.
Noniridoid Bisindole Alkaloids

3. NONIRIDOID BISINDOLE ALKALOIDS

5-hydroxy indole (52), exhibited cytotoxic activity (IC₅₀ 4.3 µg/ml), but the dimer did not. Red algae of the genus *Chondria* were the source of chondriamide-A (53) and -B (54), which possessed cytotoxic activity on LOVO colon cancer cells (32). They showed similar mass fragmentation pattern except for an additional phenolic hydroxyl in 54, situated at C-7, according to H- and C-NMR experiments. A 16-Hz coupling constant proved the trans configuration of the disubstituted double bonds.

Formulas 47-50.

Scheme 4. (bottom) Preparation of caulerpin. Reagent: (i) N-methylaniline, rt, 15 d. (top) Formulas 47-50.
III. Bisindoles from Microorganisms

A. AMAUROMINE

Amauromine (55), C\textsubscript{32}H\textsubscript{36}N\textsubscript{4}O\textsubscript{2} (M\textsuperscript{*} 508.283), isolated from a culture broth of Amauroascus, is a novel alkaloid with potent vasodilating activity (33). Its \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra displayed signals for 18 protons and 16 carbons as a consequence of the symmetry of the molecule. These spectra showed the presence of a dimethylallyl group, as in roquefortine (56). The fused indoline moiety with amide functions (IR,\nu 1660 cm\textsuperscript{-1}; \textsuperscript{13}C-NMR, \delta 166.1 ppm) was hydrolytically degraded (6N HCl, 110°C, 4h) to L-tryptophan. Consequently, carbons C-15a and C-7a with their exchangeable protons (Na\textsubscript{2}CO\textsubscript{3},CH\textsubscript{3}OD) were given the S absolute configuration. Irradiation of the methyl groups of the isoprenyl moiety induced NOEs at the protons on the C-5a and C-13a positions, thus proving the B/C and E/F cis ring junctions. A trans relative position between the proton at C-15a (C-7a) and that of C-5a (C-13a) was evidenced by the high-field absorption of the 15a proton (\delta 3.8 ppm), which experiences the anisotropic effect of an aromatic ring, A(G). Determination of the absolute configuration of the stereocenters was confirmed by comparison of the physical data (NMR, \alpha, CD) of the natural product (and derivatives) with those of synthetic analogues of known stereochemistry (34). A diketopiperazine metabolite isolated at the same time from Penicillium nigricans was given the name nigrifortine (35). The published structure is identical to that of amauromine, and the spectroscopic data for the two compounds are similar. Therefore, the compounds are most probably one and the same product.

Application of the Bycroft and Landon method (36) to introduce a reversed prenyl group at the 3a position of a physostigmine skeleton opened to way to synthesis of prenylated indoles such as amauromine (55) (Scheme 26).
3. NONIRIDOID BISINDOLE ALKALOIDS

\[
\begin{align*}
\text{COOH} & \\
\text{NH-Cbz} & \\
\text{S-CH}_3 & \\
\text{COOH} & \\
\text{NH-Cbz} & \\
\text{S-CH}_3 & \\
\text{COOH} & \\
\text{NH-Cbz} & \\
\text{S-CH}_3 & \\
\text{COOH} & \\
\text{NH-Cbz} & \\
\text{S-CH}_3 & \\
\text{COOH} & \\
\text{NH-Cbz} & \\
\text{S-CH}_3 & \\
\end{align*}
\]

\[\text{(58)}\]

\[\text{(59)}\]

\[\text{(57)}\]

\[\text{(60)}\]

\[\text{(61)}\]

\[\text{(55)}\]

**Scheme 5.** Synthesis of amauroamine. Reagents: (i) DMSO, HCl, rt, then CH$_3$OH–HCl; (ü) P$_5$S$_5$, pyridine reflux, 3 h; (iii) CH$_3$I, K$_2$CO$_3$, rt; (iv) aq. NaOH, CH$_3$OH–THF, rt, 12 h; (v) 30% HBr, AcOH, rt, 1 h; (vi) DCC, HO$\text{SO}_4$, rt, 12 h; (vii) 30% HBr, AcOH; (viii) NH$_3$, CH$_3$OH, 0°C–rt, 4 h; (ix) (CH$_3$)$_2$C $\equiv$ CH–CH$_2$–Br, K$_2$CO$_3$, dioxan, rt; and (x) TiCl$_4$, LiAlH$_4$. 
5) (37). The key intermediate, diketopiperazine (57), was prepared from N-benzylloxycarbonyl tryptophan by classical reactions. The methylthio function was introduced by phosphorous pentasulfide thionation and methylation. Coupling of the active ester, derived from 58 by DCC–HOSu with amine (59), afforded dipeptide (60) in 52% yield. The two 1,1-dimethyl-2-prenyl groups were introduced by the thio-Claisen rearrangement through the corresponding sulfonium salt. Converting 61 into amauromine (55) (38) while preserving the inverted prenyl group was realized by the combined use of TiCl4 and LiAlH4.

Two diketopiperazine containing dimers (62 and 63) were isolated from extracts of the sclerotia of Aspergillus ochraceus Wilhelm (39). According to the spectroscopic data, their gross structure was related to that of amauromine, but they showed different sets of NMR signals for each half of the molecule. A cis relationship among H-2, H-11, and the isoprenyl group was established by a nuclear Overhauser effect. Another set of diketopiperazines was isolated from Aspergillus sp., SC319 (ATCC74177) (40). The main compounds, named WIN 64821 (64) and WIN 64745 (65), are derived from tryptophan and from phenylalanine and valine, respectively. Compound 64 is a potent antagonist of substance P, and it was shown that the configurations of C-2 and C-3 were of paramount importance for biological activity.

![Chemical structures](image-url)
3. NONIRIDOID BISINDOLE ALKALOIDS

B. STAUCROSPORINE AND RELATED COMPOUNDS

Since the isolation in 1977 of the potent protein kinase inhibitor staurosporine (66) (41), more than 60 natural products, incorporating the indolo[2,3-a]pyrrolo[3,4-c]carbazole (or for sake of simplicity, indolo[2,3-a]carbazole) structure, have been described. Their various biological activities justified considerable interest in the synthesis of staurosporine and of its analogues, and the field has been the subject of reviews by Bergman (42), Steglich (43,44), and Gribble (45). The last reference includes an excellent survey of their biological activities.

1. Occurrence and Structure Elucidation

Staurosporine (66) was first isolated from a culture of Streptomyces staurosporeus Awaya (41) and subsequently from other Actinomycetes (46–49). Its structure, determined by X-ray crystallography (50,51), is a hexacyclic ring system and an aminohexose unit, attached by two glycosidic linkages to the indole nitrogen atoms. Complete and unambiguous \(^{1}\)H- and \(^{13}\)C-NMR assignments were obtained by one- and two-dimensional techniques, including HMQC and HMBC (52). In accordance with the X-ray solution, the six-membered pyran ring was found to be in a chair conformation with the N-methyl group in axial position (53). Its unusual shielding (\(\delta 1.54 \text{ ppm}\)) is probably due to the anisotropic effect of the adjacent aromatic ring system. Significant changes in the NMR spectrum upon protonation revealed a dominant (95%) boat conformation with an equatorial NH\(^{+}\)-CH\(_3\) group (\(\delta 2.79 \text{ ppm}\)) (54). The absolute configuration of staurosporine (66) was not directly proved until 1994, when an X-ray analysis, performed on 4'-N-methylstaurosporine methiodide and using anomalous diffusion, determined the stereocenters to be 2'S, 3'R, 4'R, and 6'R (55). As a consequence, the commonly used stereostructural presentation of 66 ought to be revised.

The absolute configurations of staurosporine derivatives 11-hydroxystaurosporine (67) (56), 7-oxostaurosporine (68) (57), and RK-286 C (69)
SAP1 AND MASSIOT

(66) staurosporine
(67) 11'-hydroxy-staurosporine
(68) 7'-oxo-staurosporine

(69) RK-286 C 3'-β-OH
(70) 3'-epi-RK-286 C 3'-α-OH

TAN-999 (71) and TAN-1030A (72) are two closely related indolo[2,3-a]carbazole alkaloids, produced by Nocardiosis dassonvillei C-71425 and Streptomyces sp. C-71799, respectively (60). They are the first compounds of the series having macrophage-activating properties. Their spectroscopic data are similar to those of staurosporine, except for an additional aromatic methoxy group (δ 3.95 ppm) on C-10 of 71 and an oxime (C-4' at δ 145.1 ppm, =N—OH at δ 10.45 ppm) replacing the aminomethyl substituent in 72 (61). Z-geometry of the oxime was deduced from the observation of an NOE between the methoxy (δ 3.43 ppm) and the C-2' methyl protons and the oxime hydrogen.

(58) were confirmed to be 2'S, 3'R, 4'R, and 6'R, whereas 3'-epi-RK-286 C (70) (59) (RK-1409 B) had the 3'S configuration (55). In the absence of definitive proof, the configurations of the other derivatives should be considered tentative.
(71) TAN-999  
R₁: OCH₃, R₂: H₂

(73) 7-hydroxy-staurosporine  
(UCN-01, UCN-02)
R₁: H, R₂: OH

(74) Bmy-41950  
R₁: H, R₂: O

(72) TAN-1030 A
R₁: OCH₃, R₂: H

(75) K-252a
R: CH₃

(76) K-252b
R: H

(77) staurosporine aglycone  
(K-252c)
R₁ = R₂: H, R₃: H₂

(87) arcyriaflavin-A
R₁ = R₂: H, R₃: O

(88) arcyriaflavin-B
R₁: OH, R₂: H, R₃: O

(89) arcyriaflavin-C
R₁ = R₂: OH, R₃: O
Epimers of 7-hydroxystaurosporine (73), UCN-01 and UCN-02, were isolated from *Streptomyces* sp. N-126 (62,63). Their structure was proved by comparison of their NMR data with those of 66 and by 2-D NMR experiments (COLOC). These epimers are in equilibrium in acidic or alkaline solution, thus impeding determination of their absolute configuration (64). The two compounds possessed protein kinase C inhibition, antimicrobial, and cytotoxic activities. Bmy-41950 (74) is an oxidized (maleimide) derivative of 73, isolated from a culture broth of *Streptomyces staurospor-ens* (65).

Other potent protein kinase C inhibitors, K-252a (75), K-252b (76), K-252c (77), and K-252d (78), were isolated from *Nocardiosis* sp. strains K-252 and K-290 (66,67) and from *Actinomadura* sp. SF-2370 (68). One- and two-dimensional NMR experiments (NOE, COLOC) (69) and single-crystal X-ray analysis of 75 (70) demonstrated that K-252a (75), K-252b (76), and staurosporine had a double-branched N-furanose moiety, whereas K-252d (78) had an \(\alpha\)-L-rhamnose unit and a free indole NH position. Sugar-free K-252c (77) is the aglycone of staurosporine. RK 286 C (69) and RK 286 D (79) are powerful protein kinase C inhibitors from the culture of *Streptomyces* sp. TK 286 (48,58); they may be regarded as shunt metabolites in the biosynthesis of staurosporine (71).

The common indolo[2,3-\(a\)]pyrrolo[3,4-\(c\)]carbazole core was present in the structures of AT-2433-A\(_1\) (80), AT-2433-A\(_2\) (81), AT-2433-B\(_1\) (82), and AT-2433-B\(_2\) (83) (72,73) and of rebeccamycin (84). The dimers (80–83)
from *Actinomadura melliaura* contain disaccharide units, linked to the N-methylated maleimide framework. Rebeccamycin (84), a product of *Nocardia aerocoligenes*, strain C-38383-RK-2, displayed promising antibiotic, antihypertensive and antitumor activity (74,75). The structural analysis of rebeccamycin was performed by combined use of spectroscopic methods and X-ray crystallography (74). A 4-O-methylglucose unit was found to be attached to a 1,11-dichloro-indolo[2,3-a]carbazole chromophore by a β-glycosidic bond. 11-Dechlororebeccamycin (85) and bromorebeccamycin (86) were isolated from the same *Nocardia aerocoligenes*, the latter molecule (86) being extracted in the presence of bromide ions (76).

Arcyriaflavin-A (87), -B (88), and -C (89) are pigments isolated from the slime mold *Arcyria denudata* (43,77), and from *Metatrichia vesparium* (88 and 89) (78). Arcyriaflavin-C (89), arcyriaflavin-D (90), and BE-13793C (91) are isomeric bisphenols, as determined from their spectroscopic data. The last two molecules were isolated from *Dictydiaethalium plumbeum* (44) and from *Streptoverticillium mobaraense* (79), respectively. BE-13793C showed inhibition of topoisomerase activity.

**a. Indolo[2,3-a]carbazoles from the Marine Animal Kingdom**  The origin of marine natural products is not always known with certainty: marine organism or symbiotic microorganisms? For this reason and because of the structural relationship with the above-mentioned compounds, derivatives containing the indolo[2,3-a]carbazole skeleton, isolated from the marine environment, are reviewed in this chapter.

A brown tunicate, *Eudistoma* sp., collected in Micronesia is the source of two highly cytotoxic and powerful protein kinase C inhibitors: 11-hydroxystaurosporine (92) and 3,11-dihydroxystaurosporine (93) (56). Comparison of the 1H- and 13C-NMR spectra of these molecules with those of staurosporine allowed the determination of the position of the phenol hydroxyl groups at C-11 (δ 144.1 ppm) and at C-3 and C-11 in 92 and 93, respectively.
The blue–green alga *Nostoc sphaericum* produces simpler indolo[2,3-a]carbazoles (94–96), with weak activity against the *Herpes simplex-2* virus and human cancer cell lines (80). Another blue–green alga, *Tolypothrix tjipanasensis* De Wild., yielded a series of indolocarbazole dimers (81), named *tjipanazoles-A1* (97), -A2 (98), -B (99), -Cl (100), -C2 (101), -C3 (102), -C4 (103), -D (104), -E (105), -F1 (106), -F2 (107), -G1 (108), -G2 (109), -I (110), and -J (111). *Tjipanazole D* has also been isolated from the blue–green alga *Fischerella ambigua* (82). A combination of proton decoupling, NOE experiments, and hydrolysis allowed the establishment of the nature and configuration of their sugar units. *Tjipanazole-D*, the aglycone of 97,98,99, and 105, was prepared from *p*-chlorophenylhydrazine and 1,2-cyclohexandione in a Fischer indolization (Scheme 6). Synthetic samples, obtained by N-glycosidation of 104 with D-glucose or L-rhamnose, were identical in all respects to *tjipanazole-E* (105) and *tjipanazole-G2* (109), respectively. *Tjipanazole-J* (111) is the only dimer of the series having an indolo[2,3-a]pyrrolo[3,4-c]carbazole skeleton (81).

b. Bisindolylmaleimides Slime molds (*Myxomycetes*) are the source of numerous bisindolylmaleimides which can be considered biogenetically close to the indolo[2,3-a]carbazoles. *Arcyriarubin A* (112), B (113), and C (114) and dihydroarcyriarubin B (115) were isolated from *Arcyria denudata* (43,77). *Arcyriaverdin C* (116) from *A. denudata* is a formal condensation product of maleimide and 6-hydroxyisatin (43). In *arcyriacyanin A* (117) and in its dihydro derivative 118 from *A. nutans* (43,44), the two indole nuclei are attached by a C-2 to C-4′ bond. In a similar fashion, an oxygen atom bridges the C-2 and C-4′ carbons of the two indole halves in *arcyriacyanin A* (117).
3. NONIRIDOID BISINDOLE ALKALOIDS

**Formulas 97-111.**

|   | tjipanazole | R₁ | R₂ | R₃ | R₄ |
|---|-------------|----|----|----|----|
| 97 | A₁         | Cl | Cl | CH₃ | H  |
| 98 | A₂         | Cl | Cl | H  | CH₃|
| 100| C₁         | Cl | H  | CH₃| H  |
| 101| C₂         | H  | Cl | CH₃| H  |
| 102| C₃         | Cl | H  | H  | CH₃|
| 103| C₄         | H  | Cl | H  | CH₃|
| 108| G₁         | H  | H  | CH₃| H  |
| 109| G₂         | H  | H  | H  | CH₃|

|   | tjipanazole | R₁ | R₂ | R₃ |
|---|-------------|----|----|----|
| 104| D          | Cl | Cl | Cl |
| 110| I          | Cl | Cl | H  |

**Scheme 6.** (bottom) Reagents: (i) EtOH-HCl, 65°C; and (ii) AcOH, 100°C, 8-12 h. (top) Formulas 97-111.

xocin A (119), arcyroxocin B (120), arcyroxocin A (121), arcyroxepin A (122), and arcyroxepin B (123) (43,44,77).

2. Biosynthetic Studies

Despite the therapeutic importance of indolo[2,3-α]carbazole derivatives, little work has been devoted to their biogenetic origin. Meksuriyen and
Cordell proposed that two tryptophan units with intact carbon side chains were the biogenetic precursor of staurosporine aglycone (77) (83). Participation of acetate in the biosynthesis was excluded on the basis of similar arguments. The following steps in the biosynthesis and the origin of the aminosugar moiety need further study.

In order to determine the biogenetic origin of rebeccamycin (84), S. aerocolonigenes cultures were fed labeled D-glucose, L-methionine, and L-tryptophan (84). The observed incorporations confirmed the involvement of these metabolites in biosynthesis, but the origin of the phthalimide nitrogen atom remains unknown.

Gill and Steglich have proposed an elegant scheme for the biosynthesis of the main Arcyria bisindolylmaleimides (43,44), some steps of which were tested in in vitro experiments (Scheme 7).
3. Synthetic Studies

Sarstedt and Winterfeldt's synthesis of staurosporine aglycone (77) used a photochemical cyclization as the key step (Scheme 8) (85). Amide (124), prepared from tryptamine and β-indolyl-acetylchloride, was transformed into diketo-amide (125) with DDQ. Selective borohydride reduction, followed by acetylation, led directly to the pentaacetyl derivative (126), which was then transformed into 127 under TiCl₃-mediated reduction conditions and after deacylation. Irradiation of 127 smoothly afforded the target molecule (77).

Magnus and Sear (86) made use of the indole-2,3-quinodimethane methodology to synthesize staurosporine aglycone (77) (Scheme 9). Nₐ,N₉-bis-2-formyltryptamine (128) was condensed with 2-amino Styrene, and, under standard conditions, the product (129) afforded the pentacyclic carbamate.
SCHEME 8. Sarstedt and Winterfeldt's (85) approach to staurosporine aglycone. Reagents:
(i) DDQ, aq. THF, 0°C→rt; (ii) NaBH₄, i-PrOH, rt; (iii) Ac₂O, DMAP, pyridine, 80°C;
(iv) TiCl₄, acetone–H₂O, reflux, 1h; (v) aq. NaHCO₃; (vi) and hv, CH₃OH.

(130) via an indolo-2,3-quinodimethane intermediate. Treatment of amine (131) with phosgene followed by TiCl₄ yielded the hexacyclic indolo[2,3-a]carbazole, which was selectively deprotected to afford 77.

Arcyriaflavin-A (87), the aglycone of rebeccamycin (84) and AT-2433-B₁, and arcyriaflavin-B₂ aglycone (132) have been prepared by double Fischer indolization of the corresponding bisphenylhydrazones, using polyphosphoric acid trimethylsilyl ester (PPSE) as the cyclization agent (Scheme 10) (87). The cyclization of bisphenylhydrazone (133) into osazones (134) with NBS opened a route to the preparation of unsymmetrical osazones and, hence, to unsymmetrical indolo[2,3-a]carbazole alkaloids (88).

The requirement of a selective bis-N-glycosylation method to transform 77 into staurosporine justified the introduction of a mobile N-protecting group on the lactam moiety. Use of N-benzylmaleimide as a dienophile seemed to provide a solution to the problem, but the sequence failed as it proved impossible to remove the N-benzyl protecting group (89). Versatility and adaptability to large-scale operations were offered by a route involving ammonolysis of the terphenyl-anhydride (136), prepared by a Diels–Alder reaction between bis-(2-nitrophenyl)butadiene (135) and methyl acetylenedicarboxylate (Scheme 11). Because of low yields, the Clemmensen reduction of the imide into the corresponding lactam (137) was replaced by a two-step-procedure involving the intermediacy of a hydroxylactam. The
lactam function was protected by a tetrahydropyranyl group (138) prior to triphenylphosphine-mediated deoxygenation and double-nitrene insertion (139) in order to avoid the formation of an extremely stable complex between the generated triphenylphosphine-oxide and the staurosporine aglycone. Arcyriaflavin-B (88) was obtained as a product of a Diels–Alder addition between the E,E-diene (140, R-OCH₃) and maleimide, followed by triphenylphosphine-assisted deoxygenation to the nitrene and demethylation (90).

Moody and Rahimtoola’s route to the unsubstituted indolocarbazole system is based on an intramolecular Diels-Alder reaction of a pyrano[4,3-
Scheme 10. The double Fischer indolization method of Bergman and Gribble (87,88). Reagents: (i) toluene, reflux, 24-48 h; (ii) ArNH-NH₂, MeOH-AcOH, reflux, 6 h; (iii) m-CPBA, THF, 0°C→rt; (iv) PPSE, CH₃NO₂, reflux, then Pd-C, diglyme, heating, 24 h; (v) O₃, CH₂Cl₂-MeOH, −78°C, then Me₂S; (vi) PhNH-NH₂, MeOH; and (vii) NBS, THF, pyridine, −10°C→rt.

$\text{b|indol-3-one, followed by cyclization of a nitrene intermediate (91). Acylation of ethyl indole-2-acetate with oxalyl chloride led to the corresponding indole-3-glyoxalyl chloride which was condensed with 2-nitrocinnamylamine to give 141 (Scheme 12). Upon heating at 110°C, the pyranoindolone 142 underwent an intramolecular Diels-Alder reaction to give the desired carbazole 143 after air oxidation. The final cyclization step was effected by}$
heating 143 in triethylphosphine to directly give staurosporine aglycone (77) in 22% overall yield from ethyl indole-2-acetate (92).

Weinreb and co-workers (93,94) prepared the aglycone and also aminosugars related to the carbohydrate fragment of staurosporine (Scheme 13). Their approach to the synthesis of the aglycone (77) was based on preliminary results from Steglich et al. (77). \(N\)-Benzyl-dibromomaleimide (144)
was treated with indolylmagnesium bromide to give diindolylmaleimide (145). Oxidative cyclization to the hexacyclic ring system (146) was effected in high yield with 4-toluene sulfonic acid and DDQ in refluxing benzene. Partial reduction of the imide to the corresponding lactam could be achieved only under Clemmensen conditions (89,90).

The absolute configuration of rebeccamycin (84), which remained unknown after the first stages of spectroscopic investigation (74), was determined by total synthesis (95). The first approach to the heteroaromatic system was based on the well-known Grignard method (77,78), followed
by photocyclization or silver-oxide-mediated oxidative thermal cyclization (Scheme 14). The second route made use of the Diels–Alder reaction of maleimide and the 2,2'-bisindole (148), prepared from 7,7'-dichloroindigo (147) by Wolff–Kishner reduction. The sugar moiety was introduced as 4-methoxy-triacetobromoglucose, prepared from D-glucose. Removal of the protecting groups by hydrogenolysis and ammonolysis gave synthetic rebeccamycin, in all respects identical to the natural product.

The above example shows that 2,2'-bindolyls are attractive starting materials for the preparation of potent PKC inhibitors via the Diels–Alder reaction. This approach relies on 2,2'-bisindoles, whose preparation has been reviewed (96,97). Bergman's method involves the conversion of indoles into 1,1'-carbonylindoles (149), followed by Pd(OAc)$_2$-assisted 2,2'-coupling (98). A later approach is based on a triethyl-phosphite-induced nitrene insertion as the key step in producing symmetrical or unsymmetrical 2,2'-bindolyls (Scheme 15) (150) (99).

Arcyriarubin-A (112) is a pigment produced by slime molds of the Myxomycetes family; its synthesis was achieved by Steglich et al. (77), using indolyl–Grignard chemistry in the crucial step (Scheme 16). The condensation of indolylmagnesium bromide and dibromomaleimide (151) gave monooindolyl (152) and diindolyl (153) compounds, whose ratio depended on the reaction solvent (200). In toluene, the bisindolyl derivative (153) was obtained, whereas in THF the monosubstituted compound (152) was the major product. The transformation of 153 into arcyriarubin-A (112) was performed in two steps: alkaline hydrolysis led to anhydride (154) which was also directly obtained from indolyl-3-glyoxylyl-chloride and indole acetic acid (101) or by iodine-promoted coupling of the trianion of indole acetic acid (102). Treatment of 154 with ammonium acetate yielded arcyriarubin-A. Maleic anhydrides are also converted into maleimides under mild conditions, using a mixture of methanol and hexamethyldisilazane (103). The monosubstituted product (152) was used in the synthesis of unsymmetrically substituted arcyriarubins (113) and arcyriaflavins (87–90) (77,94,95,102). N-boc-protected monooindolyl derivative (156) was reacted with the Grignard derivative of 6-tetrahydropyryloxyindole to afford the desired coupling product (157). Thermal deprotection, followed by the usual conversion of the methylimido group into an imide, led to arcyriarubin-B (113) (100).

A mild and flexible method for the preparation of bisindolylmaleimides makes use of the acylation of an appropriately substituted indolyl-3-acetimidate (158) by indolyl-3-glyoxylyl chloride (Scheme 17) (104). Cyclization with an excess of triethylamine, dehydration, and hydrolysis led to the bisindolylmaleimide core. This sequence allowed the preparation of a number of highly selective PKC inhibitors (105). An unexpected
Scheme 14. Synthesis of rebeccamycin. Reagents: (i) CH₃-MgI, C₆H₆-HMPA, rt; (ii) hv, CH₃OH, I₂, air, or C₆H₆, Ag₂O, reflux; (iii) acetobromosugar, reflux; (iv) Pd-C, H₂, then NH₃; (v) NH₂NH₂·H₂O, NaOH; (vi) Ac₂O; and (vii) N-benzyloxymethylmaleimide, sealed tube, 105°C, 8 days.
3. NONIRIDOID BISINDOLE ALKALOIDS

Scheme 15. Preparation of 2,2'-biindolyls. Reagents: (i) COCl₂; (ii) Pd(OAc)₂, AcOH; (iii) aldehyde, NaH, THF, rt, 12 h; (iv) (EtO)₃P, 170°C, 5–8 h.

rearrangement, discovered at Schering–Plough, gave aldehyde (159) which was transformed into 160 in a five-step sequence (Scheme 18). This compound proved to be as good an inhibitor of PCK as staurosporine (IC₅₀ 10nM) (106).

A solution to the problem of the formation of N-glycosidic linkages was proposed by Danishefsky and co-workers, who found that sodium salts of indoles opened α-1,2-anhydrosugar epoxides with an inversion of configuration at the anomeric carbon to afford the desired indole-N-glycosides (Scheme 19) (107). Application of the method to the total synthesis of rebeccamycin (84) called for the preparation of the α-1,2-anhydrosugar (161), resulting from deprotection, protection, and 2,2-dimethylidioxirane-mediated epoxidation of the secoaglycone (162). This latter compound was obtained in 60% overall yield using Kaneko et al.'s route (95). Coupling between 161 and 163 was achieved in 48% yield of the desired β-N-glycopyranoside (164) when 3 eq. of the anhydrosugar was used.
SCHEME 16. Synthesis of arcyriarubins. Reagents: (i) aq. KOH, reflux, 0.5 h, then aq. HCl; (ii) Boc₂O, DMAP, THF, 0°C, 40 min; (iii) C₂H₅MgBr, THF, 6-(tetrahydropyranyloxy)indole; (iv) 180°C, 45 min; (v) NH₄OAc, 180°C, 0.5 h; (vi) CH₂Cl₂, (C₂H₅)₃N; (vii) n-BuLi (2 eq), t-BuLi (1 eq), then I₂, H⁺; and (viii) Ac₂O.
Scheme 17. Hill et al.'s synthesis of bisindolymaleimides (104). Reagents: (i) Et₃N, rt; and (ii) p-TsOH, toluene.

Scheme 18. Synthesis of 160, an efficient PKC inhibitor. Reagents: (i) BF₃·Et₂O, CHCl₃, rt; (ii) NaBH₄, CeCl₃, THF, CH₃OH; (iii) NBS, DMF, rt; (iv) CuCN, NaI, CH₃CON(CH₃)₂, heating; (v) aq. KOH, DMSO, 80°C; and (vi) TFA, DMSO-H₂O.
Fluoride-induced desilylation, followed by photocyclization, led to benzyl-oxyrebeccamycin, whose deprotection required special precautions to avoid reductive cleavage of the chlorine atoms.

In the total synthesis of the staurosporine analogue (165) separated double glycosidation of the aglycone (166) was envisioned (108). The β-epoxysugar (167) was prepared from the corresponding glycal (168) with 2,2-dimethyldioxirane (Scheme 20). The oxazoline ring was generated from the bistrichloroacetimidate, the reaction product (169) of the glucal derivative (170), with trichloroacetonitrile, as depicted in Scheme 20. The glycosylation reaction between 167 and the sodium anion of 166 was performed in 48% yield. Despite the unfavorable orientation of the indolocarbazole moiety in the product of photocyclization (171), electrophilic cyclization was accomplished by sequential treatment with potassium-t-butoxide and iodine. Final radical deiodination led to fully functionalized core structure (165) of staurosporine (Scheme 21).

A similar synthetic route was adopted by Shankar and McCombie at Schering-Plough, where resorting to normal intramolecular glycosylation conditions with an intermediate similar to that of Danishefsky failed. The iodine-promoted nucleophilic cyclization devised by the Yale group thus
3. NONIRIDOID BISINDOLE ALKALOIDS

Scheme 20. Danishefsky's route for the preparation of the sugar moiety (108). Reagents: (i) NaH, CH₂Cl₂, 0°C, then Cl₂CCN; (ii) BF₃·Et₂O, −78°C; (iii) p-TsOH·H₂O, pyridine, 80°C; (iv) NaH, CH₂Cl₂, 0°C, then DMF, BnBr, 0°C; (v) Bu₄N⁺F⁻, THF, rt; (vi) NaH, CH₂Cl₂, 0°C then Cl-C₆H₄-p-OCH₃, 0°C→rt; and (vii) dimethyldioxirane. CH₂Cl₂, 0°C.

proved to be of paramount importance for the completion of the synthesis (109).

A monosubstituted derivative of the aglycone of staurosporine (172) was prepared by Winterfeldt and co-workers in order to circumvent the difficulties inherent in the selective glycosylation of the two indole halves (110). Base-catalyzed and photo-induced cyclizations were applied to the construction of the planar ring system (Scheme 22).

4. Biological Activities

Naturally occurring indolo[2,3-a]carbazole, bisindolylmaleimide alkaloids, and their synthetic analogues display diverse biological activities which have been the subject of a review (45). Protein kinase inhibition is the most significant biological activity of staurosporine (66) (III), of its aglycone (77) (112), and a wide range of synthetic analogues (113). Staurosporine is an inhibitor of low specificity (114,115), but the aglycone and numerous synthetic analogues (173–175) show specific inhibitions of particular protein kinases (A, G, etc.) (116,117). As an example, amine derivatives (176) have better PKC inhibitory activity than K-252a (75) (118). The 7-oxo-derivative (177) or aromatic ether analogues were also prepared (119). Surprisingly, N,N'-dialkyl-substituted aglycones (178 and 179) and bisindolyl maleimide analogues (180–184) exhibited strong, and sometimes extremely selective (180) and (184), protein kinase activities (120,121).

Cytotoxicity is the second most intensively investigated domain of activity of indolocarbazoles. Staurosporine has excellent in vitro activity against a variety of human tumor cell lines (KB, HeLaS3, EKVX, NB LA-H-5 . . . ).
Scheme 21. Danishefsky et al.'s approach to the synthesis of a staurosporine analogue (108). Reagents: (i) NaH, THF, 0°C, then anhydrosugar (167), reflux; (ii) CS₂Cl₂, DMAP, pyridine, CH₂Cl₂, reflux, then pentafluorophenol, reflux; (iii) n-Bu₃SnH, AIBN, C₆H₆, reflux; (iv) Bu₄N⁺F⁻, THF, molecular sieves, reflux; (v) hv, I₂(cat.), air, C₆H₆, rt; (vi) NaH, THF, 0°C→rt, then SECl₂, rt; (vii) DDQ, CH₂Cl₂, H₂O, rt; (viii) I₂, P(C₆H₅)₃, imidazole, CH₂Cl₂, 0°C→rt; (ix) DBU, THF, 0°C→rt; (x) Bu₄N⁺F⁻, THF, molecular sieves, reflux; and (xi) t-BuOK, THF, CH₃OH, rt, then I₂, −78→0°C.

with an IC₅₀ < 0.01 μM (122,123), and was even found to be active against vincristine-resistant cell lines (124).

Rebeccamycin (84) displays significant activity against P-388 and L-1210 leukemia and inhibits the growth of human lung adenocarcinoma cells (75). Numerous compounds, especially tertiary or quaternary amine derivatives
3. NONIRIDOID BISINDOLE ALKALOIDS

Scheme 22. Synthesis of the monosubstituted staurosporine aglycone (172). Reagents: (i) N,N'-carbonyl diimidazole, CH$_2$Cl$_2$, then Hunig's base, THF, rt, 18 h; (ii) KN(SiMe$_3$)$_2$, THF, 0°C, then SEM--Cl, rt; (iii) t-BuOK, t-BuOH, reflux; (iv) molecular sieves, DMF, TBAF, ethylenediamine, 80°C, 2 h; and (v) hv, acetone, O$_2$.

C. CHAETOMIUM DIMERS

Studies of mycotoxin production by fungi belonging to the genus Chaetomium revealed the production of several secondary metabolites, possessing (on the maleimide nitrogen), have been described and showed in vivo antitumor activities (125,126). The antitumor properties of indolocarbazoles seem to be related to their protein kinase inhibition activities (127). Among other pharmacological properties, the antihypertensive activity (128,129) of staurosporine and of several K-252a derivatives as well as antiviral (130), antimicrobial (131) and platelet aggregation inhibition activities (46,47,132), are worth mentioning.
remarkable antimicrobial and cytotoxic activities (Table I) (133). This is the case with chaetocin (185), which was isolated from Chaetomium minutum (134). Its symmetrical structure has been elucidated by chemical transformations and spectroscopy. The absolute configuration (S for C-3 and C-11a of the disulfide bridge) was determined by X-ray diffraction analysis, including sulfur anomalous diffusion, and was found to be opposite the configurations observed in gliotoxin (186) and sporidesmin (187); this was also proved by circular dichroism, showing a positive Cotton effect at 230–240 nm. Verticillin A (188) is another antimicrobial and cytotoxic diketopiperazine-type dimer, isolated from Verticillium sp. (strain TM-759) (135). Spectroscopic methods and degradation reactions demonstrated that 188 was an isomer of chaetocin with two α-oriented hydroxyl functions on C-11 and C-11'. Verticillin B (189) possessed a third secondary alcohol function, as shown by the chemical shift of the CH$_2$OH system (δ 4.52 ppm) (136). Verticillin A (188) and B (189) also displayed a positive Cotton effect at 236 nm, characteristic of the dithiodioxopiperazine appendage.

lla,11'a-Dihydroxychaetocin (190) was isolated from the fungus Verticillium tenerum (137) and from the culture of Acrostalagmus cinnabarinus var. melinacidinus (138,139), under the name of melinacidin IV. Melinacidin IV and two related derivatives from the same source, melinacidin II (191) and III (192), were shown to inhibit the growth of Gram-positive bacteria (Staphylococcus aureus, Streptococcus arouson, Bacillus subtilis).

![Chemical Structures](image)

|     | Compound       | R$_1$    | R$_2$ | R$_3$ | R$_4$    |
|-----|----------------|----------|-------|-------|----------|
| 185 | chaetocin      | CH$_2$OH | H     | H     | CH$_2$OH |
| 188 | verticillin A  | CH$_3$   | OH    | OH    | CH$_3$   |
| 189 | verticillin B  | CH$_3$   | OH    | OH    | CH$_2$OH |
| 191 | melinacidin II | CH$_3$   | H     | OH    | CH$_2$OH |
| 192 | melinacidin III| CH$_2$OH | OH    | H     | CH$_2$OH |
| 190 | melinacidin IV | CH$_2$OH | OH    | OH    | CH$_2$OH |
| 193 | chetracin A    | R:H      |       |       |          |
| 194 | chetracin A triacetate | R:Ac |       |       |
| No. | Source | Formula | mp (°C) | $[\alpha]_D^{20}$ | Reference |
|-----|--------|---------|---------|-----------------|-----------|
| 185 | *Chaetomium minutum* | $\text{C}_{30}\text{H}_{36}\text{N}_6\text{O}_8\text{S}_4$ | 240 (dec.) | +379° (pyridine) | [134] |
| 188 | Verticillium sp. (st. TM-759) | $\text{C}_{30}\text{H}_{36}\text{N}_6\text{O}_8\text{S}_4$ | 203–214 (dec.) | +703.7° (dioxan) | [135,136] |
| 189 | Verticillium sp. (st. TM-759) | $\text{C}_{30}\text{H}_{36}\text{N}_6\text{O}_8\text{S}_4$ | 230–233 (dec.) | +704.7° (dioxan) | [136] |
| 190 | *Acrostalagmus cinnabarinus* var. melinacidinum | $\text{C}_{30}\text{H}_{36}\text{N}_6\text{O}_8\text{S}_4$ | 232–234 (dec.) | +718° (CHCl₃) | [138,139] |
| 191 | *Acrostalagmus cinnabarinus* var. melinacidinum | $\text{C}_{30}\text{H}_{36}\text{N}_6\text{O}_8\text{S}_4$ | 230–233 (dec.) | +726° (CHCl₃) | [138,139] |
| 192 | *Verticillium tenenum* | $\text{C}_{30}\text{H}_{36}\text{N}_6\text{O}_8\text{S}_4$ | +776° (CHCl₃) | [138,139] |
| 193 | *Chaetomium abuense* Lodha | $\text{C}_{30}\text{H}_{36}\text{N}_6\text{O}_8\text{S}_4$ | 248–251 (dec.) | +723.5° (CHCl₃) | [140] |
| 195 | *Aspergillus flavus* (st. MIT-M25, 26, 27) | $\text{C}_{50}\text{H}_{48}\text{N}_6\text{O}_4$ | 204–205 | −33° (CH₂Cl₂) | [141] |
| 196 | *Chaetomium cochliodes* (HLX 833) | $\text{C}_{35}\text{H}_{36}\text{N}_6\text{O}_8\text{S}_4$ | 203–214 (dec.) | +257° (CHCl₃) | [142,143,145,146] |
| 197 | *Chaetomium globosum* Kinze ex. Fr. | $\text{C}_{35}\text{H}_{36}\text{N}_6\text{O}_8\text{S}_4$ | 191–193 | +174° (CHCl₃) | [148] |
Chetracin-A (193) is a metabolite from *Chaetomium abuense* Lodha and *C. retardatum* Carter & Khan exhibiting strong cytotoxic activity (140). Its absolute configuration and the conformation of the tetrasulfide bridge were established by X-ray crystallography. Despite the symmetrical nature of the molecule, it formed a triacetate (194) instead of a tetraacetate on treatment with Ac₂O and pyridine, which can be explained by steric hindrance of the C-11'-α-hydroxyl group and by hydrogen bonding between 11'-α-OH and the carbonyl group located at the C-1' carbon. This effect might also be responsible for the incomplete acetylations of melinacidin IV (190) and the verticillins under identical conditions.

Ditryptophenaline (195) is a metabolite from *Aspergillus flavus* (strains MIT-M25, -26, and -27) and did not exhibit any significant biological activities (141). Its symmetrical structure was established from spectroscopic data and X-ray diffraction studies. Formally, 195 is derived from the coupling of tryptophan, phenylalanine, and a methyl unit, followed by oxidative dimerization. Its absolute configuration was not determined.
Chetomin (196) is a toxic bisindole from *Chaetomium* species isolated for the first time in 1944 (142–144). More than 30 years later, chemical and spectroscopic studies (145) revealed the nature of the two halves of the molecule, but the linkage point and the stereochemistry remained to be established (three N-methyl groups had to be placed on six nitrogen atoms, two from indole nuclei and four from the diketopiperazines). Finally, the \(^{15}\text{N}\)- and \(^{13}\text{C}\)-NMR spectra of labeled \([^{15}\text{N}]\text{-chetomin}\), biosynthesized by *C. cochliodes*, allowed the full determination of the structure and the localization of a bond between the indole nitrogen and C-3 of the indoline moiety (146,147).

![Chemical structures of chetomin (196) and dethio-tetra(methylthio)chetomin (197)](image)

Reagents: i: CH\(_3\)I, pyridine, THF-CH\(_3\)OH, 10°C.

Dethio-tetra(methylthio)chetomin (197) (\(M^+ 770\) for C\(_{35}\)H\(_{42}\)N\(_6\)O\(_6\)S\(_4\)) and 196 were isolated from the culture of *Chaetomium globosum* Kinze ex. FR and were shown to possess antimicrobial activity against *E. coli* W 3110 and *Staphylococcus aureus* 2097 (148). Their \(^1\text{H}\)-NMR spectra were similar, except for the presence of four additional thiomethyl singlets between \(\delta 2.02\) and \(\delta 2.32\) ppm in the spectrum of 197. All ambiguities in these structures were erased by X-ray crystallographic analysis, chemical correlations, and the reductive methylation of 196 to 197.
IV. Alkaloids of Plant Origin

By far, bisindoles in plants are of iridoid origin; some families of plants, however, produce bisindolic metabolites with a different biosynthesis. This is exemplified by a series of alkaloids from the Simaroubaceae and by the pigments of the isatin type.

A. The Picrasma Dimers

Bis-\(\beta\)-carboline-type dimers have been isolated from the roots of *Picrasma quassioides* Bennet (Simaroubaceae), a plant used in Japan as a bitter stomachic. The \(\beta\)-carboline nuclei were recognized by typical UV absorptions as found in the simple monomeric substances isolated from the plant. The \(^1\)H-NMR data for 198 (149) displayed signals for two methylene groups as triplets (\(\delta\) 3.60 and 3.92 ppm), suggesting the presence of a 1,2-disubstituted ethane function as a spacer. The location of methoxyl groups at C-4 (\(\delta\) 149.9 ppm) and C-8 (\(\delta\) 146.0 ppm) was deduced from a comparison of the \(^1\)H- and \(^13\)C-NMR data for 198 with those of \(\beta\)-carbolines (199 and 200). Further support of the structure (198) was obtained from the mass
spectral fragments a, b, and c, arising from cleavages α to the carbonyl group.

Compound 201 is a second dimer of the same origin (150), which differed from 198 in the non-conjugated part. The \(^1\)H-NMR spectrum showed a doublet of doublets at δ 5.55 ppm, assigned to a methine, a supplementary methoxyl group (δ 3.51 ppm), and signals for two contiguous methylenes. The near superimposition of the aromatic parts of the \(^1\)C-NMR spectra of 201 and 198 led to the conclusion that the structural differences between the compounds belonged to the spacer chain. Structure 201 was deduced to account for these arguments and the observed mass spectrum fragmentations.
In continuation of the work on *P. quassioides*, picrasidine H (202) and R (203) were isolated from the root bark (151). They displayed a β-carboline-like UV spectrum with an end absorption at 360–370 nm, attributed to a conjugated carbonyl function (1660 cm⁻¹). The ¹H-NMR spectrum of 202 showed an exchangeable hydroxyl proton at δ 4.83 ppm. Spin-decoupling experiments allowed the determination of the structure of the hydroxyl-containing four-carbon subunit, which linked the two β-carboline moieties. Irradiation of the methoxyl groups at δ 4.26 and 4.02 ppm enhanced the intensities of H-3 and H-3', thus proving their locations at C-4 and C-4', respectively. The observation of signals for only 15 carbons in the ¹³C-NMR spectrum of picrasidine-R (203) was explained by a symmetrical structure, including one carbonyl (δ 200.8 ppm), two methoxyl (δ 55.3 and 56.1 ppm), and one methylene (δ 38.6 ppm) carbon. The similarities between the aromatic region of the spectra of picrasidine-R (203) and that of picrasidine-E suggested dimethoxy substitution in 203 at C-4 and C-8.

Picrasidines-F (204), -G (205), -S (206), and -T (207) are four closely related, optically inactive, quaternary alkaloids, isolated from the root bark. Each displayed typical β-carbolinium-type UV spectra, whose maxima were shifted by the addition of alkali following transformation into β-carboline anhydro bases. The structure of picrasidine-F (204) was confirmed by X-ray crystal structure analysis (152). Picrasidines-G (205), -S (206), and -T (207) had almost identical ¹H-NMR spectra and differed in their aromatic substituents, being proton, methoxyl, and hydroxyl, respectively (153). Methylation of 207 with diazomethane yielded 206 (154).

Picrasidines-M (208), -N (209), and -U (210) are composed of β-carboline and canthin-5,6-dione moieties. The presence of the latter common structural feature in 208 was suggested by two carbonyl functions with resonances at δ 156.3 and 169.9 ppm and by the observation of a prominent mass spectral fragment at m/z 236. The location of the methoxy substituents was determined by treatment of 208 with acetic anhydride which afforded 5-acetoxy-5-acanthin-6-one (211) and 4,8-dimethoxy-1-vinyl-β-carboline (213) (155) (Scheme 23).

The close resemblance of spectroscopic data for 208 and 210 suggested similar structures (156). The main difference between the NMR spectra of the aromatic regions of 210 and those of 208 concerned the C-4 position of the canthin-5,6-dione moiety in 210, in which the hydrogen was replaced by another methoxyl group (δ 3.89 ppm). The cleavage reaction with acetic anhydride also proved the assignment. The ¹H-NMR spectrum of picrasidine-N (209) showed that the methoxyl group at δ 64.7 ppm was located on the indole nitrogen. The compound was degraded into 4,9-dimethoxy-1-vinyl-β-carboline (213), thus supporting structure 209 (157).
(204) picrasidine-F $R_1$: OCH$_3$ $R_2$: H  
(205) picrasidine-G $R_1$=$R_2$: H  
(206) picrasidine-S $R_1$=$R_2$: OCH$_3$  
(207) picrasidine-T $R_1$=$R_2$: OH

(208) picrasidine-M $R$: H  
(210) picrasidine-U $R$: OCH$_3$

(209) picrasidine-N $R$: H

SCHEME 23. Chemical structure elucidation of picrasidines (M,U,N). Reagents: (i) (Ac)$_2$O, reflux.
Indirubin (214) was isolated from the fruits of the cannonball tree, Cour-oupita quianensis Aubl. (158), and shown to inhibit Lewis lung carcinoma and Walker carcinosarcoma. The structurally related dimer, candidine, is a violet indolic constituent from the culture of Candida lipolytica (159). Mass spectral fragmentations led to the proposal of two structures, with the correct one (214) being identified by synthesis from tryptanthrin and N-acetylindoxyl. Candidine (215) proved to be identical to compounds isolated from Baphicacanthus cusia and Isatis tinctoria and was given the name quingdainone (160,161).

\[ \text{Indirubin (214): } R: H \]
\[ R: \text{Ac} \]
\[ R': \text{Et} \]

\[ \text{Candidine (215)} \]

**NOTE ADDED IN PROOF.** The first total synthesis of staurosporine (66) and ent-staurosporine, based on intramolecular indole glycosylation by an appropriately functionalized oxazolidinone glycal, has been accomplished by Danishefsky and colleagues: J. T. Link, S. Raghavan, and S. J. Danishefsky, *J. Am. Chem. Soc.* 117, 552 (1995).
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