Variecolin, a Sesterterpenoid of Novel Skeleton from *Aspergillus variecolor* MF138

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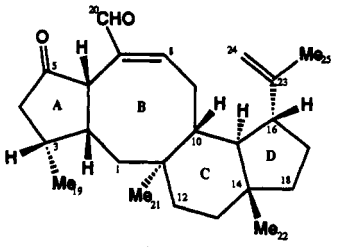
A new sesterterpenoid variecolin (1), was isolated from fermentation of *Aspergillus variecolor* MF138. It was shown to be an angiotensin II receptor binding inhibitor with an IC_{50} of 3 ± 1 μM. On the basis of spectroscopic evidence, variecolin was shown to have a novel ring skeleton, a hybrid of the ophiobolin and ceriferene class of sesterterpenoids. Conformational analysis of the tetracycle, using ^1H–^1H coupling constants and NOE's from phase-sensitive NOESY spectra, allowed determination of the conformation and relative stereochemistry of 1. A unified biogenetic scheme from geranylfarnesyl pyrophosphate, linking the ophiobolin, ceriferene, and variecolin classes of sesterterpenoids, is presented.

In our search for an angiotensin II receptor antagonist for the treatment of hypertension,[1] we discovered the receptor binding inhibitor variecolin (1) having a novel ring skeleton reminiscent, in part, of the ophiobolin and ceriferene classes of sesterpenoids.[2] We report here on the fermentation, isolation, biological activity, and, in particular, on the structure determination and stereochemical analysis of this novel tetracycle 1, primarily on the basis of 2D NMR spectroscopy.



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Results and Discussion

Fermentation and Isolation. The active constituent, variecolin was produced by solid-state fermentation of the fungus *Aspergillus variecolor* MF138 obtained from the Merck Culture Collection. The composition of the seed and production media are shown in Table I. Seed cultures of the fungus were inoculated with a source of the culture and grown on a gyratory shaker for 24 h at 27 °C. A portion of the seed culture was used to inoculate each production flask that was incubated statically at 26 °C for 14 days. The solid-state production fermentation residue was extracted with methylene chloride, and the oily residue obtained on evaporation fractionated by silica gel column chromatography. Pure variecolin was obtained on subsequent reverse-phase chromatography on a C_{18} column using gradient elution.

Structure Determination. FAB-MS of variecolin gave a molecular weight of 368 which was found by EI-HRMS to be consistent with the molecular formula $C_{25}H_{36}O_2$ [found m/z 368.2731, calcd m/z 368.2715] and supported by elemental analysis. The ^{13}C-NMR spectrum in CD_3CN (Table I) indicated 25 carbons and 36 carbon-bound protons from APT and coupled (gated) spectra, thus corroborating the molecular formula. Interpretation of the ^{13}C NMR data suggested the following carbon types: 4 CH_3, 7 CH_2, 6 CH, 2 C, 1 CH=, 1 CH_2=, 2 C=, 1 CH=O, and 1 C=O. Under forcing conditions, the compound forms a mono-TMS derivative consistent with an enolisable ketone. The molecule has eight units of unsaturation and/or

Table I. ^1H and ^{13}C NMR Spectral Assignments of Variecolin (1)

assignment	^{13}C[a]	^1H (Hz)[b,c]	^1H (Hz)[b,d]
C1 (H_β)	43.1 t	1.19 dd (1.5, 14.6)	0.93 dd (1.5, 14.7)
(H_α)		1.52 dd (11.9, 14.6)	1.36 dd (11.9, 14.6)
C2 (H_β)	39.8 d	2.76 dddd (1.5, 6.8, 10.6, 12)	2.36 dddd (1.5, 7.0, 10.4, 11.8)
C3 (H_β)	35.5 d	2.39 m	~2.00 m
C4 (H_β)	47.1 t	2.47 ddd (1.2, 8.6, 18.3)	2.26 ddd (1.1, 8.6, 18.8)
(H_α)		2.13 dt (18.3, ~1.0)	2.42 dd (1.9, 18.9)
C5	219.9 s		
C6 (H_β)	50.6 d	3.53 br d (10.6)	3.53 br d (10.3)
C7	141.0 s		
C8 (H_α)	162.4 d	6.97 m	6.02 ddd (1.5, 3.3, 6.6)
C9 (H_β)	32.4 t	2.84 br dt (16.5, 2.6)	2.54 ddt (19.7, 2.3, 2.9)
(H_α)		~2.28 m	1.85 dddd (1.1, 6.6, 12.6, 19.7)
C10 (H_β)	41.5 d	~2.25 m	~2.00 m
C11	39.9 s		
C12 (H_β)	36.3 t	~1.93 m	1.68 dt (4.7, 13.7)
(H_α)		1.00 dt (13.8, 3.4)	0.80 ddd (2.6, 4.2, 13.7)
C13 (H_α)[e]	36.0 t	~1.47 m	~1.32 m
(H_β)[e]		~1.47 m	~1.38 m
C14	44.1 s		
C15 (H_α)	49.6 d	~1.47 m	1.27 t (11.1)
C16 (H_β)	49.0 d	2.56 dt (5.2, 11.1)	2.21 dt (5.3, 11.1)
C17 (H_β)	30.8 t	~1.99 m	~1.92 m
(H_α)		~1.36 m	~1.28 m
C18 (H_β)	40.6 t	~1.40 m	~1.32 m
(H_α)		1.26 br dt (~10.3)	1.11 br dt (~11.0)
C19 (Me_α)	16.1 q	0.73 d (7.4)	0.76 d (7.4)
C20 (CHO)	194.3 d	9.13 s	8.91 s
C21 (Me_α)	22.1 q	0.93 d (0.8)	0.71 br s
C22 (Me_β)	18.3 q	0.87 d (0.8)	0.62 br
C23	152.2 s		
C24 (H_{cis})	110.8 t	4.76 br d (2.6)	4.74 br d (2.3)
(H_{trans})		4.63 dq (2.6, 1.3)	4.69 dq (2.3, 1.3)
C25 (Me)	19.3 q	1.73 t (1.3)	1.61 t (1.0)

[a] ^{13}C chemical shifts were recorded at 100 MHz at ambient temperature in CD_3CN relative to the methyl solvent peak at 1.3 ppm. The α/β designation of configuration as in steroids was adopted. [b] ^1H chemical shifts were recorded at 400 MHz at ambient temperature in CD_3CN and C_6D_6 relative to the solvent peaks at 1.93 and 7.15 ppm, respectively. Coupling constants ($J_{H,H}$) in hertz are given in parentheses. [c] In CD_3CN. [d] In C_6D_6. [e] Assignments are interchangeable.

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(1) (a) Wong, P. C.; Chiu, A. T.; Price, W. A.; Thoolen, J. M. C.; Carini, D. J.; Johnson, A. L.; Taber, R. I.; Timmermans, B. M. W. M. J. *Pharmacol. Exp. Ther.* **1988**, *247*, 1. (b) Soffer, L. R.; Sen, I. *J. Cardiovasc. Pharmacol.* **1985**, *7* (suppl. 4), S69.

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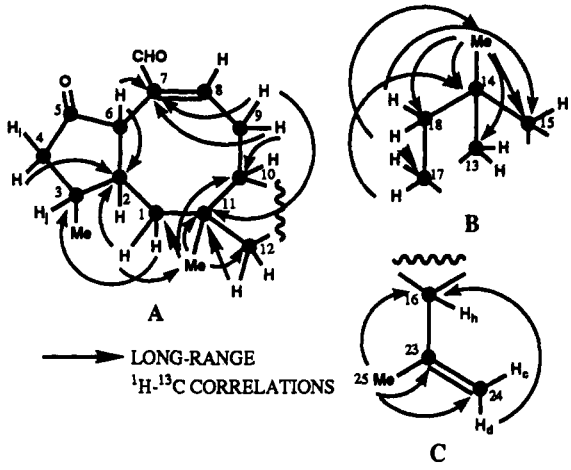
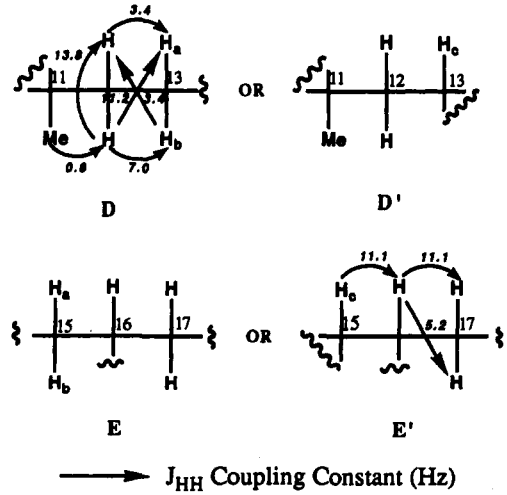
rings and, because of the presence of two double bonds and two carbonyl functions, must contain four rings. Moreover, the presence of a conjugated aldehyde (λ_{max} 241 nm), propenyl group, a five-membered ring ketone (to account for the low-field C=O position at 219.9 ppm)[3] and ^1H–^1H connectivities from COSY and decoupling experiments in CD_3CN, suggested a tetracyclic terpene containing the bicyclo[6.3.0]undecane ring system (partial structure A), characteristic of the ophiobolin class of sesterterpenoids.[2b]

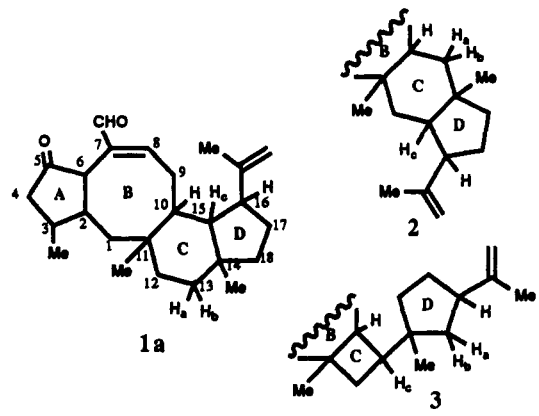


Because of the severe overlap of resonances in the 0.8–3.3 ppm region of the ^1H NMR spectrum, one-bond and long-range HETCOR experiments proved to be critical in defining the overlapping methylene and methine proton positions. Three partial structures A, B and C were deduced showing the observed two- and three-bond ^{13}C–^1H correlations which readily corroborate the bicyclo[6.3.0] ring system A. The correlation between the vinylic methyl group and the C16 methine in C is surprising and would not have been entertained from the ^1H NMR data alone, as H16 is not allylicly coupled to either the methyl group or the vinylic protons at C24. Since the coupling has a $\sin^2\theta$ angular dependence,[4] the dihedral angle between H16 and the plane of the double bond must be close to zero (see under Conformation and Stereochemistry).

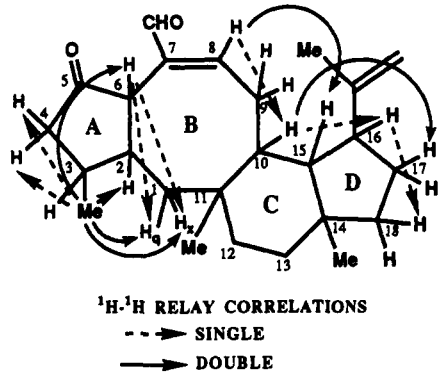


Further examination of the ^1H–^1H connectivity data suggested the partial structures D (or D′) and E (or E′).

The ambiguity between D/D′ and E/E′ results from the tight overlap of the methylene (H_a/H_b) and C15 methine (H_c) protons. This leads to three out of four possible structures (1a–3) for variecolin, since the combination of



D and E are incompatible with B. Because of the degeneracy in chemical shift of $H_a/H_b/H_c$, the single and double ^1H–^1H RELAY experiments were performed[5] which readily distinguished between the three possibilities. Using mix times of 0.02 and 0.05s, many long-range correlations were observed as shown. In particular, H10 correlates with the methine proton H16 through H15 in the single RELAY experiment which is consistent with **1a** but not with **2** or **3**. This is corroborated in the double RELAY experiment where, contrary to what one would expect for **2** and **3**, H10 is correlated with one of the methylene protons at C17 through H15 and H16.



Corroboration of structure **1a** for variecolin, and therefore, substructures D and E′, was forthcoming from the multiplet structures of the resonances H12α and H16β and decoupling studies. H12α is observed as a sharp doublet of triplets (J = 13.8, 3.4, 3.4 Hz) and suggests coupling to H12β (J = 13.8 Hz) and the methylene protons H_a/H_b at C13 in D and not to a methine proton H_c as in D′. Moreover, the multiplet structure for H16β is a sharp doublet of triplets (J = 5.2, 11.2, 11.2 Hz) as would be expected for **1a** (i.e. E′ not E). Irradiation, in turn, of H17α and H15α results in collapse of H16β to a triplet (J = 11.1 Hz) and doublet of doublets (J = 5.2, 11.1 Hz), respectively, confirming the assignment in favor of structure **1a**.

Conformation and Stereochemistry. From a Dreiding model and the ^1H–^1H coupling constants obtained from C_6D_6 spectra (see Table I), the conformation and relative stereochemistry of variecolin was determined as depicted in Figure 1. It was not possible to initially define the A/B ring junction, as the observed vicinal coupling $J_{H2,H6}$ of 10.4 Hz does not discriminate between a cis or trans orienta-

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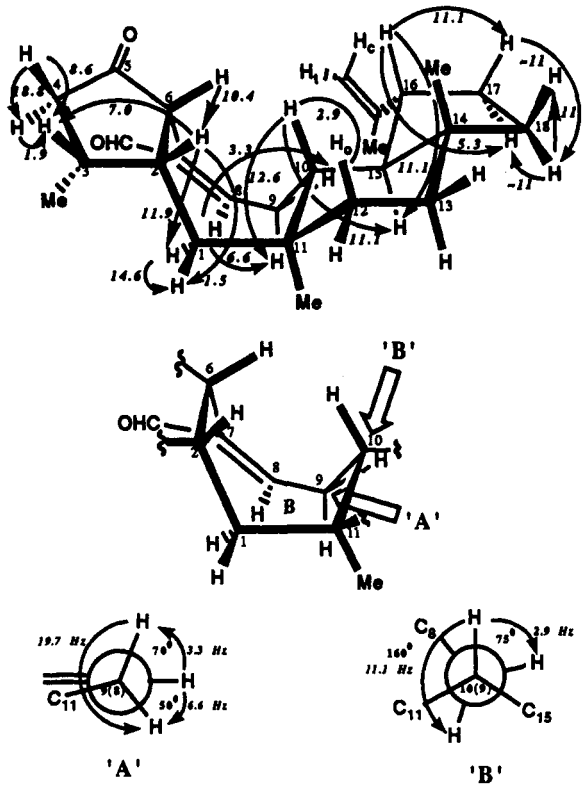

Figure 1. Conformation and relative stereochemistry of variecolin from J_{HH} coupling constants in hertz (italics) in C_6D_6 (see arrows). A and B depict Newman projections along the C9(C8) and C10(C9) bonds respectively indicating J_{HH} in hertz and the corresponding dihedral angles from Dreiding models.

tion.[6] Beginning therefore with rings C and D, the various couplings strongly suggest a conformation with a trans C/D ring junction. The couplings of the ethane fragment in ring C are consistent with a six-membered chair conformation whereas those in ring D suggest an envelope where, in particular, H15α and H16β are trans-diaxial. The stereospecific "*W*" coupling[7] between the bridgehead methyl groups at C11 and C14 with the axial protons H12β and H18α, respectively, requires them also to be axial. A trans ring junction is also implicated between rings C and D based on the large $J_{H10,H15}$ coupling of 11.1 Hz. The coupling constants were readily extractable from ^1H–^1H decoupled and 2D *J*-resolved spectra, since the first-order 5-spin system in CD_3CN, involving the protons at C8/C9/C10/C15, is no longer first-order in C_6D_6. Of the various possibilities only the conformation depicted in Figure 1 with the cis A/B ring junction, is compatible with the remaining coupling constants. In particular the Newman projections A and B along the C9(C8) and C10(C9) bonds, respectively, indicate consistency between the dihedral angles and the vicinal couplings observed. Moreover, the large J_{gem} of −19.7 Hz for the C9 methylene protons is optimal for the orientation shown in A where the geminal internuclear axis is approximately perpendicular to the nodal plane of the double bond.[8] This feature proved to be extremely diagnostic in determining the correct conformation of ring B.

Corroboration of the conformation and relative stereochemistry of variecolin, was obtained from phase-sensitive NOESY spectra. With mix times of 0.6 and 0.9 s and a

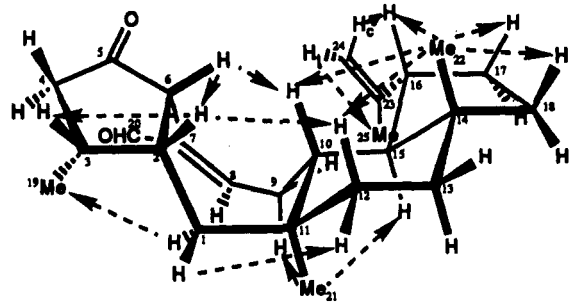

Figure 2. Conformation and relative stereochemistry of variecolin, indicating NOE's (dotted arrows) obtained from phase-sensitive NOESY spectra.

delay of 3 s between transients, positive NOE's were obtained which could be readily distinguished from the few antiphase COSY-type cross peaks resulting from zero-quantum coherences generated by the second pair of 90° pulses.[9] The NOE correlations, as shown in Figure 2, do not include those from geminal protons, all of which were readily observed. Immediate confirmatory evidence for the boat conformation of the eight-membered ring B, was obtained from the strongest observed correlation (apart from the geminal NOE's) between the "bowsprit-flagpole" protons H6β and H10β. A strong NOE was also observed between the similarly disposed H2β and H12β. The axial orientations of the bridgehead methyl groups at C11 and C14 are readily confirmed by their interactions with various axial protons. The NOE's between H2β and H3β and between the C3-Me and H1β readily support the α-configuration of the Me group at C3. Also, it is evident that the propenyl side chain at C16 has the α-configuration and has a preponderent conformation as depicted in Figure 2, in which a NOE can be observed between H16β and $H24_{cis}$. This is consistent with the observation made above that because of the near zero dihedral angle between H16β and the plane of the double bond, no coupling is observed between H16β and the geminal vinylic protons at C24.[4]

The structure and relative stereochemistry of variecolin is therefore represented as in 1. Upon completion of the structure work, it became apparent that 1 has rings C and D, including stereochemistry, in common with the X-ray structure of flocerol (**6a**), a member of the ceriferene class of sesterterpenoids,[2b] thereby establishing an important biogenetic link between the ceriferene and ophiobolin classes. The skeleton of **6a** is reminiscent of a biosynthetic intermediate of 1 expected from a blocked mutant as illustrated in Scheme I. Here, we show a unified biogenetic scheme from geranylfarnesyl pyrophosphate, based on previous biosynthetic studies of the ophiobolins[2b] and triterpenoids,[10] for the ophiobolin family [exemplified by ophiobolin C (**4a**)[11] and ceroplasteric acid (**4b**)[12]]and two groups of the ceriferene class [exemplified by floridenol (**7a**) and **6a**][2b] where intermediate **4** represents the point of divergence. It will be noted that variecolin retains the cis A/B ring junction of **4a**, the trans junction of rings B/C in **4b** and the complete stereochemistry of rings C and D in **6a**.

Biological Activity. Variecolin inhibited ^{125}I-[Sar^1,Ile^8]-angiotensin II binding in rabbit aortic or bovine

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(10) Harrison, D. M. *Nat. Prod. Rep.* **1988**, *5*, 387.
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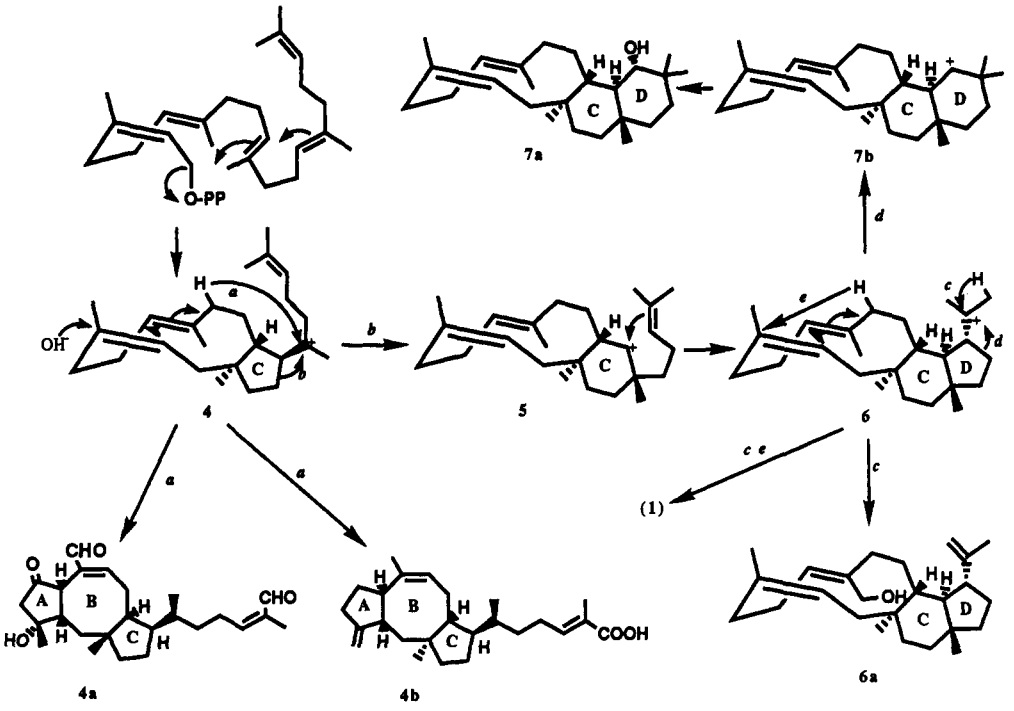
(12) Iitaka, Y.; Watanabe, I.; Harrison, I. T.; Harrison, S. *J. Am. Chem. Soc.* **1968**, *90*, 1092.

Scheme I



adrenal cortical membranes[13] with IC_{50} values of 1.1 ± 0.3 μg/mL (3.6 ± 1 μM). At 48 μM it inhibited angiotensin II (10^{-9} M) induced inositol phosphate accumulation (−52 ± 5%). However, it also inhibited carbachol (10^{-3} M) induced inositol phosphate accumulation (−80 ± 10%), indicating possible nonspecific inhibition of this angiotensin response.

Experimental Section

The IR absorption spectra were obtained with a multiple internal reflectance cell (MIR, ZnSe) on neat 10–20-μg samples. Mass spectral data were obtained by electron impact at 90 eV. Trimethylsilyl derivatives were prepared with a 1:1 mixture of BSTFA–pyridine at room temperature. Exact mass measurements were made by the peak matching method using perfluorokerosene (PFK) as internal standard.

^1H NMR chemical shifts in CD_3CN and C_6D_6 are given relative to the solvent peaks at 1.93 and 7.15 ppm, respectively. ^{13}C NMR chemical shifts in CD_3CN are given relative to the methyl solvent peak at 1.3 ppm.

Proton–proton chemical shift correlation spectra (COSY) were recorded using the standard pulse sequence of Bax et al.[14] The COSY-45 sequence was used most often with a delay of 1.0 s. Double-quantum filtered COSY spectra[15] were obtained using a delay of 2.5 s.

Single and double RELAY ^1H–^1H correlation spectra[5] were recorded using mix times of 0.02 and 0.05 s and a delay of 1 s. The 2K–2K data set was accumulated in 512 increments with 32 and 64 transients respectively for each value of t_1 for full-phase cycling (CYCLOPS).

Homonuclear (^1H) 2D *J*-resolved spectra (HOM2DJ)[16] were obtained using a delay of 3.0 s, 2K points in the chemical shift axis (f_2) and 512 increments to define the *J* coupling axis (f_1). Zero filling to 8K (f_2) and 2K (f_1) was followed by 2D transformation, the data tilted by 45° and symmetrized.

Prior to NOE experiments, the 0.02 M solution of variecolin in C_6D_6 was degassed using three freeze–thaw cycles under vacuum, flushed with dry nitrogen and tightly capped (Teflon cap). The phase-sensitive NOESY spectra (PS-NOESY) were obtained by the Hypercomplex method of States, Haberkorn, and Ruben.[9a] Two separate experiments were performed each with different phase cycles. 1K × 1K data sets were accumulated in 128 increments with 96 transients for each value of t_1. The delay time between transients was 3 s and the mix times employed were 0.6 and 0.9 s.

Proton–carbon chemical shift correlation spectra (HETCOR) were recorded in CD_3CN (11 mg/0.5 mL) using the standard pulse sequence of Bax and Morris.[17] The 512 × 2K data set was accumulated in 128 increments with 1024 transients for each value of t_1. The delay time between transients was 1.0 s and the experiment was optimized for $^1J_{CH}$ = 135 Hz. The corresponding long-range experiment was optimized for a multiple bond carbon–proton coupling constant of 9 Hz.

Fermentation of Variecolin (1). The fungus *A. variecolor* MF138 was obtained from Dr. Charles Tom, USDA, Peoria, IL, and has been deposited in the Merck Culture Collection. The composition of the seed and production media were as follows. Seed medium: corn steep liquor (Corn Products) (5.0 g/L), tomato paste (Hunt's) (40.0 g/L), oat flour (10.0 g/L), dextrose (10.0 g/L), H_3BO_3 (0.56 g/L), $(NH_4)_2Mo_7O_{24}\cdot 4H_2O$ (0.19 g/L), $ZnSO_4\cdot 7H_2O$ (2.0 g/L), $FeSO_4\cdot 7H_2O$ (10.0 mg/L), $MnSO_4\cdot H_2O$ (10.0 mg/L), $CuCl_2\cdot H_2O$ (0.25 mg/L), $CaCl_2$ (1.0 mg/L). Production medium: dextrose (150.0 g/L), urea (4.0 g/L), NZ-Amine A (Sheffield) (4.0 g/L), K_2HPO_4 (0.5 g/L), $MgSO_4\cdot 7H_2O$ (0.25 g/L), KCl (0.25 (g/L), $ZnSO_4\cdot 7H_2O$ (0.9 g/L), $CaCO_3$ (16.5 g/L).

The constituents were dissolved in distilled water and the pH adjusted to 6.8 prior to sterilization. The seed medium (54 mL) was dispensed into 250-mL Erlenmeyer flasks, protected with cotton closures and sterilized at 121 °C for 20 min. Seed cultures were inoculated with a source of the culture and grown on a gyratory shaker (220 rpm; 5.1 cm throw) for 24 h at 27 °C. A portion of the seed culture (12 mL) was used to inoculate each 2-L production flask which consisted of vermiculite (70 g) and production medium (250 mL) which was previously sterilized in 500-mL Erlenmeyer flasks for 15 min at 121 °C. Prior to mixing, the vermiculite portion of the medium was separately sterilized for 60 min at 121 °C. Production flasks (8 × 2-L total) were then incubated statically at 26 °C for 14 days.

Isolation of Variecolin (1). Solid-state production fermentation (8 flasks, each with vermiculite-based medium (70 g)) of *A. variecolor* MF138 was extracted with methylene chloride (5

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L) during 5–6 h of stirring. The extract was evaporated under vacuum, yielding an oily residue (9 g), which was redissolved in methylene chloride–methanol (19:1, 20 mL) and fractionated on a column (200 mL) of E.M. silica gel 60 (230–400 mesh) packed in hexane–methylene chloride (1:1). Elution of the column was carried out by a step gradient of methylene chloride in hexane yielding partially purified compound in the 80% methylene chloride fractions. After evaporation of the solvent under reduced pressure, the residue (600 mg) was taken up in methanol (4 mL). Upon refrigeration, a precipitate formed which was removed by filtration. The filtrate was further fractionated in two identical runs (2 mL each) on a Rainin Dynamax 60A C_{18} column (1 in. × 25 cm), eluted with a 15 mL/min gradient of acetonitrile–water (7:13 to 9:1) over 40 min. Appropriate fractions were evaporated to dryness, yielding variecolin (95 mg); the homogeneity was verified by HPLC (Whatman Partisil ODS-3) eluted with acetonitrile–H_2O (3:1, k' 5.2) and by TLC on silica gel 60 F_{254} (E. Merck) (R_f 0.40 in CH_2Cl_2 and R_f 0.52 in hexane–acetone, 4:1) and Whatman KC18 plates (R_f 0.43 in ACN–H_2O, 90:10, and R_f 0.50 in MeOH–H_2O, 95:5).

Variecolin (1): $[\alpha]_D$ −11.5° (c 0.50, ACN); EI-MS m/z 360 (M^+); IR 1735, 1687, 1626, 1455, 1404, 1382, 1228, 1208, 1192, 1141, 933, 884, 839, 808, 768, 735, 711 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 241 (12 130) and 203 (9020); ^1H NMR (CD_3CN and C_6D_6) see Table I; ^{13}C NMR (CD_3CN) see Table I. Anal. Calcd for $C_{25}H_{36}O_2$: C, 18.46; H, 9.85. Found: C, 81.53; H, 9.77.

Novel Sponge-Derived Amino Acids. 12. Tryptophan-Derived Pigments and Accompanying Sesterterpenes from *Fascaplysinopis reticulata*†

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This paper reports the bioactive constituents of *Fascaplysinopsis reticulata* collected from the Benga Lagoon of the Fiji islands. The previous literature of this genus includes two aplysinopsins (monomeric tryptophans) from *F. reticulata*, as well as fascaplysin (**5a**) (an apparent tryptophan dimer) and luffariellolide (**3**) (a sesterterpene) from *Fascaplysinopsis* sp. Our investigation of *F. reticulata* has revealed new sesterterpenes isodehydrouffariellolide (**1**) and dehydroluffariellolide diacid (**2**); unique alkaloid-sesterterpene salts fascaplysin A (**5b**) [fascaplysin cation/dehydroluffariellolide diacid anion] and homofascaplysin A cation/dehydroluffariellolide diacid anion (**6**); and novel neutral alkaloids homofascaplysin C (**7**), homofascaplysin B (**8**), and secofascaplysin A (**9**). These substances were accompanied by fascaplysin (**5a**) and the known alkaloid (+)-octopamine **4**. The most important findings in this study are (a) fascaplysin derivative **5b** is the first known salt comprised of a complex alkaloid cation and a terpene carboxylate anion, and (b) secofascaplysin A (**9**) is the first naturally occurring β-carbolinone. An amino acid biogenesis pathway is outlined for each of the above alkaloids. The biological activity profile against the HIV reverse transcriptase is reported for selected metabolites.

Nitrogen-containing metabolites are rarely observed from Dictyoceratid sponges as this group is an excellent source of di- or sesterterpenes.[1,2] A few atypical members of the Dictyoceratid family Thorectidae are sources of both sesterterpenes and amino acid derivatives.[2,3] In 1985 we collected *Fascaplysinopsis reticulata* (Thorectidae family, Dictyoceratida order) which were eye-catching because of their massive, globular, and shiny red-brown appearance.[4] This sponge was targeted for further study when the crude extracts of a 1987 collection exhibited significant bioactivity against bacteria [(inhibition zone diameter size in millimeters at 100 μg/disk) including *Staphylococcus aureus* (18), *Streptococcus pyrogenes* (11), *Candida albicans* (24), and *Trichophyton mentagrophytes* (7)] and virus [100% inhibition against reverse transcriptase at 1 mg/mL; IC_{50}'s (μg/mL), HIV on ALEX cells = 0.4, ALEX cell control = 6.2].[5]

The natural products of the genus *Fascaplysinopsis* have been the subject of prior publications. Some time ago the Roche group isolated two aplysinopsins, monomeric tryptophans, from *F. reticulata*.[6] Significantly, these were unaccompanied by terpenoids and there was no mention of biological activity properties. More recently, luffariellolide (**3**), a known sesterterpene, and fascaplysin (**5a**), a 12*H*-pyrido[1,2-*a*:3,4-*b'*]diindole, were reported by Ireland and Clardy from a Fijian collection of *Fascaplysinopsis* sp.[7a] A total synthesis of this alkaloid has just been completed by Gribble.[7b] Our comprehensive study of *F. reticulata* involved four separate Fijian collections, and its vary complex mixtures consisted of sesterterpenes,

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(1) For reviews see: (a) Crews, P.; Naylor, S. *Prog. Chem. Org. Nat. Prod.* **1985**, *48*, 203. (b) Hanson, J. R. *Nat. Prod. Rep.* **1986**, *3*, 87.

(2) For examples see: Bergquist, P. R.; Wells, R. J. In *Marine Natural Products*; Scheuer, P. J., Ed.; Academic Press: New York, 1983; Vol. V pp 35–42.

(3) Consult Table 3 in ref 1a and Tables 3 and 4 in ref 2.

(4) Our voucher collection (no. 89051) was identified by C. Diaz, UCSC Institute of Marine Sciences and Prof. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam. An underwater photo can be supplied by P.C. Particularly distinctive traits are: the thick outer ectosome layer which embeds and grains, the very sharp conules (2 mm high and 5 mm apart), and the absences of spicules. These properties and its appearance are similar to those (including the photograph) reported for *F. reticulata*: Bergquist, P. R. *New Zeal. J. Zool.* **1980**, *7*, 443.

(5) These results were provided by Dr. Tom Matthews (Syntex Research, Palo Alto, CA) and his staff. Purified cloned HIV-1 reverse transcriptase was assayed by a previously described procedure: Chen, M. S.; Oshana, S. C. *Biochem. Pharm.* **1987**, *36*, 4361.

(6) Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J. *Tetrahedron Lett.* **1977**, 61 (see ref 2, Table 4, for the correct taxonomy of this sponge).

(7) (a) Roll, D. M.; Ireland, C. M.; Lu, H. S. M.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 3276. (b) Pelcman, B.; Gribble, G. W. *Tetrahedron Lett.* **1990**, *31*, 2381.

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