prepared in 1 mL of deoxygenated D_2O buffer (pD 7, 50 mM in phosphate as buffer, 20 mM in 2,3-butynediol as ^1H NMR standard). The solubility of DMH (or DMH^{ox}) was determined by integration of the ^1H NMR peak areas of CH_3N peaks for DMH (or DMH^{ox}) and of CH_2OH peaks for 2,3-butynediol ($HOCH_2C{-}CCH_2OH$).

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Registry No. 1, 89580-95-0; 2, 131760-66-2; DMH, 131760-67-3; DMH^{ox}, 131760-68-4; DTT, 27565-41-9; DTT^{ox}, 86023-22-5; ME^{ox}, 1892-29-1; DNaseI, 9003-98-9; MeNHNHMe·2HCl, 306-37-6; $(ClCH_2CO)_2O$, 541-88-8; AcSH, 507-09-5; $HS(CH_2)_6SH$, 1191-43-1; lipoic acid, 62-46-4.

Clavudiol A and Clavirolide A, Two Marine Dolabellane Diterpenes from the Soft Coral *Clavularia viridis*

Jingyu Su,* Yongli Zhong, and Kailiang Shi

Department of Chemistry, Zhongshan University, Guangzhou, People's Republic of China

Qi Cheng and John K. Snyder*

Department of Chemistry, Boston University, 590 Commonwealth Ave., Boston, Massachusetts 02215

Shengzhi Hu and Youqing Huang

Department of Chemistry, Xiamen University, Xiamen, Fuzhou, People's Republic of China

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The structures of two new marine diterpenes, clavudiol A (1) and clavirolide A (2), isolated from the Pacific soft coral *Clavularia viridis* collected off the Xisha Islands in the South China Sea are reported. Particularly valuable in the structure assignments of these two natural products was the two-dimensional NMR FLOCK sequence for detection of long range heteronuclear (^{13}C,^1H) couplings, and the use of the QUANTA/CHARMm molecular modeling program to support the NMR conformational analysis. The absolute stereochemistry of 1 and 2 were established by circular dichroism, and the structure of 1 (relative stereochemistry) ultimately confirmed by X-ray crystallography. Both are members of the dolabellane class of diterpenes.

The Pacific soft coral *Clavularia viridis* has proven to be a rich source of intriguing natural products with structural types including the well-known antitumor[1] eicosanoids[2] such as the clavulones[3] and chlorovulone.[4] These compounds, most notably the clavulones, have been the targets of several synthetic efforts beginning with Corey's initial report in 1984.[5] Cytotoxic steroids[6] and diterpenoids of the dolabellane class[7] have also been found in *C. viridis*, with the latter class of diterpenes also reported from an unidentified species of *Clavularia*.[8] Related species of *Clavularia* are also a source of terpenoids including the biosynthetically related neodolabellanes and dolastannes.[9]

Table I. NOE and Long-Range Scalar Coupling Connectivities for Clavudiol A (1)[a]

^1H NOE's[b]	^1H–^1H long-range coupling[c]	^{13}C–^1H long-range coupling[d]
H7–H3a,H6a,H9b	H2b–H15	H10–C12 (FLOCK)
H9a–H9b	H7–H17	H15–C2,C11,C14 (FLOCK)
H10–H9a,H15,H17	H16a–H3a	H16a–C4,C5 (FLOCK)
	H16b–H5a	H16b–C3,C4 (FLOCK)
H14a–H14b	H14b–H15	H17–C7,C8 (FLOCK)
H16a–H3b		H19–C20 (FLOCK)
H16b–H5a		H20–C19 (FLOCK)
H17–H9a,H10		H9b–C7 (SINEPT)
		H19/H20–C12 (SINEPT)
		H7–C9,C17 (SINEPT)

[a] Spectra recorded in $CDCl_3$ unless otherwise noted. [b] From 2D-NOE spectrum. [c] From long-range COSY spectrum, Δ = 300 ms. [d] From 2D FLOCK and selective INEPT (SINEPT).

(1) (a) Fukushima, M.; Kato, T.; Ota, K.; Yamada, Y.; Kikuchi, H.; Kitagawa, I. *Proc. Jpn. Cancer Assoc.* **1983**, *42*, 243. (b) Honda, A.; Yamamoto, Y.; Mori, Y.; Yamada, Y.; Kikuchi, H. *Biochem. Biophys. Res. Commun.* **1985**, *130*, 515. (c) Honda, A.; Mori, Y.; Yamada, Y.; Nakaike, S.; Hayashi, H.; Otomo, S. *Res. Commun. Chem. Path. Pharm.* **1988**, *61*, 413. (d) Honda, A.; Mori, Y.; Iguchi, K.; Yamada, Y. *Prostaglandins* **1988**, *36*, 621.

(2) (a) Corey, E. J. *Experientia* **1983**, *39*, 1084. (b) Corey, E. J.; Lansbury, P. T.; Yamada, Y. *Tetrahedron Lett.* **1985**, *26*, 4174.

(3) (a) Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1982**, *23*, 5171. (b) Kobayashi, M.; Yasuzawa, T.; Yoshihara, M.; Akutsu, H.; Kyogoku, Y.; Kitagawa, I. *Tetrahedron Lett.* **1982**, *23*, 5331. (c) Kobayashi, M.; Yasuzawa, T.; Yoshihara, M.; Son, B. W.; Kyogoku, Y.; Kitagawa, I. *Chem. Pharm. Bull.* **1983**, *31*, 1440. (d) Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1983**, *24*, 1549. (e) Iguchi, K.; Yamada, Y.; Kikuchi, H.; Tsukitani, Y. *Tetrahedron Lett.* **1983**, *24*, 4433. (f) Kitagawa, I.; Kobayashi, M.; Yasuzawa, T.; Son, B. W.; Yoshihara, M.; Kyogoku, Y. *Tetrahedron* **1985**, *41*, 995.

(4) (a) Nagaoka, K.; Kaneta, S.; Mori, K.; Yamada, Y.; Honda, A.; Mori, Y. *Tetrahedron Lett.* **1985**, *26*, 5787. (b) Nagoaka, H.; Iguchi, K.; Miyakoshi, T.; Yamada, N.; Yamada, Y. *Tetrahedron Lett.* **1986**, *27*, 223. The related bromovulone and iodovulone have also been isolated from *C. viridis*: (c) Iguchi, K.; Kaneta, S.; Mori, K.; Yamata, Y.; Honda, A.; Mori, Y. *J. Chem. Soc., Chem. Commun.* **1986**, 981.

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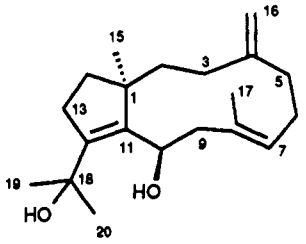
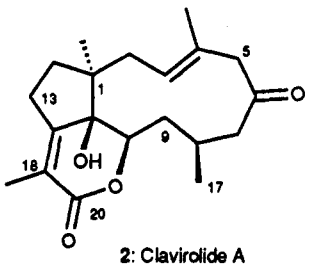
We now report the structure of two new dolabellane-type diterpenes, clavudiol A (1) and clavirolide A (2) from *C. viridis* collected off the Xisha Islands in the South China Sea. The structures of 1 and 2 were established using a variety of one and two dimensional NMR techniques in combination with molecular modeling employing the QUANTA/CHARMm program. Ultimately the structure of 1 was confirmed by X-ray crystallography. The absolute stereochemistry of 2 was assigned from the CD spectrum, while that of 1 was assigned on the basis of a common biosynthetic pathway with 2, supported by CD studies.



1: Clavudiol A



2: Clavirolide A

Results and Discussion

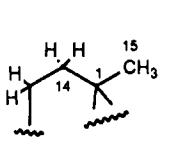
While the structure of 1 was ultimately confirmed by X-ray analysis, several spectroscopic features used to deduce the structure prior to the X-ray are worthy of note. The high-resolution mass spectrum along with the ^{13}C NMR, DEPT, and HETCOR experiments required a bicyclic skeleton with a molecular formula of $C_{20}H_{32}O_2$ bearing three olefinic double bonds. One double bond was a terminal methylene unit (152.66, s; 110.60, t), and the remaining two were tetrasubstituted (δ 141.54 and 145.78, both s), and trisubstituted (128.01, s; 130.68, d).[10] The presence of two hydroxyl groups was indicated by the O–H stretch in the IR spectrum (3420 cm^{-1}) and a broad signal in ^1H NMR spectrum (δ 3.97) integrating for two protons which exchanged with deuterium upon addition of D_2O. The two oxygen-bearing carbons (δ 66.04, s; 72.86, d) required a tertiary and a secondary alcohol group, and therefore the bicyclic skeleton which accounts for the remaining two units of unsaturation must be carbocyclic.

A double quantum filtered, phase sensitive COSY spectrum and a long-range COSY spectrum which revealed weak couplings (Table I) allowed for the mapping of the two distinct ^1H spin systems, subunits A and B, with two additional methyl singlets. The methyl group at C-1 was located in spin system A by the observation of W coupling to the H-14 proton on the opposite face of the molecule (H-14b). The exocyclic methylene group was located in the center of spin system B by allylic couplings to both the C-3 and C-5 methylene protons (Table I). The chemical shift of the C-17 vinyl methyl carbon (δ 16.84) and an NOE between H-7 and H-9b confirmed the *E* stereochemistry of the C-7/C-8 double bond.

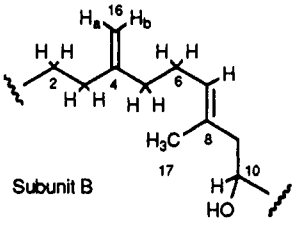
Table II. ^1H and ^{13}C NMR Data for Clavudiol A (1)[a]

position	^1H (ppm)	^{13}C (ppm)
1	–	51.22 (s)
2b	1.180 (ddd, J = 16.8, 12.9, 2.4 Hz)	
2a	1.217 (ddd, J = 16.8, 12.9, 7.2 Hz)	39.84 (t)
3a	1.807 (ddd, J = 12.9, 12.9, 2.4 Hz)	
3b	2.002 (ddd, J = 12.9, 12.9, 7.2 Hz)	31.24 (t)
4	–	152.66 (s)
5a	1.916 (m)	
5b	2.221 (m)	36.80 (t)
6a	1.901 (m)	
6b	2.168 (m)	29.35 (t)
7	5.296 (dd, J = 1.7, 3.0 Hz)	128.01 (d)
8	–	130.68 (s)
9a	2.266 (d, J = 11.5 Hz)	
9b	3.192 (dd, J = 11.5, 11.5 Hz)	49.78 (t)
10	4.145 (d, J = 11.5 Hz)	66.04 (d)
11	–	141.54 (s)
12	–	145.78 (s)
13	2.149 (m, 2 H)	34.414 (t)
14b	1.475 (m)	
14a	1.716 (m)	35.15 (t)
15	1.031 (s)	29.71 (q)
16a	4.620 (bs)	
16b	4.698 (bs)	110.60 (t)
17	1.630 (s)	16.84 (q)
18	–	72.86 (s)
19	1.370 (s)	29.92 (q)
20	1.340 (s)	31.85 (q)
OH	3.97 (br, 2 H)	

[a] Spectra recorded in $CDCl_3$. Methylene protons on the same face of the molecule as the C-15 methyl group when drawn in two dimensions are designated "a".



Subunit A



Subunit B

Examination of long-range heteronuclear $^{13}C,^1H$ couplings using the two-dimensional FLOCK[11] and one dimensional selective INEPT[12] sequences (Table I) enabled construction of the carbon skeleton. Three-bond coupling from the H-15 methyl protons to C-2, C-14, and C-11 not only located this methyl group at the bridgehead between the two spin systems, but also identified the other carbon bridging the two ^1H spin systems as an olefinic carbon belonging to the tetrasubstituted double bond. Homonuclear W coupling in the long-range COSY spectrum (Δ = 300 μs) from the H-15 protons to H-2a confirmed C-1 as the bridgehead carbon between spin systems A and B. Long-range heteronuclear couplings from H-16b to C-3 and H-16a to C-5, from H-9b to C-7, from H-19 and H-20 to

(9) From *C. inflata*: (a) Braekman, J. C.; Daloze, D.; Schubert, R.; Albericci, M.; Tursch, B.; Karlsson, R. *Tetrahedron* **1978**, *34*, 1551. (b) Bowden, B. F.; Braekman, J. C.; Coll, J. C.; Mitchell, S. J. *Aust. J. Chem.* **1980**, *33*, 927. (c) Izac, R. R.; Fenical, W.; Wright, J. M. *Tetrahedron Lett.* **1984**, *25*, 1325. From *C. koellikeri*: (d) Braekman, J. C.; Daloze, D.; Dupont, A.; Tursch, B.; DeClercq, J. P.; Germain, G.; van Meerssche, M. *Tetrahedron* **1981**, *37*, 179. (e) Endo, M.; Nakagawa, M.; Hamamoto, Y.; Nakanishi, T. *J. Chem. Soc., Chem. Commun.* **1983**, 322. Erratum: *J. Chem. Soc., Chem. Commun.* **1983**, 980. (f) Kobayashi, M.; Son, B. W.; Kido, M.; Kyogoku, Y.; Kitagawa, I. *Chem. Pharm. Bull.* **1983**, *31*, 2160. (g) Kobayashi, M.; Son, B. W.; Kyogoku, Y.; Kitagawa, I. *Chem. Pharm. Bull.* **1984**, *32*, 1667. (h) Kobayashi, M.; Son, B. W.; Fujiwara, T.; Kyogoku, Y.; Kitagawa, I. *Tetrahedron Lett.* **1984**, *25*, 5543. (i) Kobayashi, M.; Son, B. W.; Kyogoku, Y.; Kitagawa, I. *Chem. Pharm. Bull.* **1986**, *34*, 2306. From *C. violacea*: (j) Inman, W.; Crews, P. *J. Org. Chem.* **1989**, *54*, 2526. From unidentified *Clavularia* species: (k) Xia, Z.; Zhang, Z. *Jie Gou Hua Xue* **1984**, *3*, 29. (l) Huang, J.; Li, J.; Zhong, T. *Hua Kung Xue Bao* **1985**, *43*, 199.

(10) Assignment of the sp^2 nonprotonated carbons was based on heteronuclear couplings observed in the FLOCK and selective INEPT experiments. Thus, coupling from the H-17 methyl protons via two bonds to to C-8 as well as coupling via three bonds to C-7 identified the resonances for the $\Delta^{7,8}$ double bond. Two-bond couplings from both H-16a and H-16b to C-4 observed via polarization transfers in selective INEPT experiments confirmed the assignment for C-4. The two remaining sp^2 non-protonated carbons must therefore belong to the $\Delta^{11,12}$ double bond. These latter two carbons were distinguished from three bond heteronuclear couplings as indicated in Table I.

(11) Reynolds, W. F.; McLean, S.; Perpick-Dumont, M.; Enriquez, R. G. *Magn. Resn. Chem.* **1989**, *27*, 162.

(12) Bax, A. *J. Magn. Res.* **1984**, *57*, 314.

Table III. NOE and Long-Range Scalar Coupling Connectivities for Clavirolide A (2)[a]

^{1}H NOE's[b]	^{1}H–^{1}H long-range coupling[c]	^{13}C–^{1}H long-range coupling[d]
H2a–H2b	H3–H16	H3–C5 (FLOCK), C1,C16 (SINEPT)
H3–H5a,H15,H2a,H9a,H8	H3–H5a	H15–C1,C2,C11,C14 (FLOCK)
	H10–H9a	H16–C3,C4,C5 (FLOCK)
H5a–H5b,H8	H15–H2b,H14b	H19–C12,C18,C20 (FLOCK)
H7a–H7b	H17–H9a	H17–C7,C9,C8 (SINEPT)
H9a–H9b	H5a–H7a,[e]H16	H9b–C11,C17 (SINEPT)
H10–H15,H17,H8	H5b–H7b,[e]H16	H14a–C11,C12 (SINEPT)
H14a–H15		H10–C11,C20 (SINEPT)
H15–H9a		H9b–C11 (SINEPT)
H16–H5b		
H17–H7a,H7b		

[a] Spectra recorded in $CDCl_3$ unless otherwise noted. [b] From 2D-NOE spectrum. [c] From long-range COSY spectrum, Δ = 300 ms. [d] From 2D FLOCK and selective INEPT (SINEPT). [e] From spectrum recorded in $CDCl_3$ with one drop of pyridine-d_5.

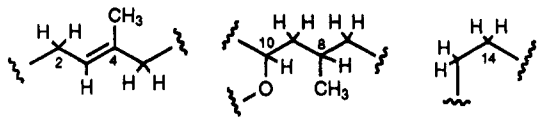
C-12, and from H-7 to C-9 and C-17 located the remaining functional groups within the individual spin systems. The relative stereochemistry of 1 was deduced by the observation of an NOE between the H-15 methyl resonance and H-10. The ^{1}H and ^{13}C chemical shift assignments for 1 are given in Table II. In this work, Reynold's FLOCK sequence[11] proved valuable in detecting long-range (two- and three-bond) heteronuclear couplings in a single spectrum, though it was not as sensitive as selective INEPT. The structure of 1 (relative stereochemistry) was ultimately resolved by X-ray crystallographic analysis.

The second compound isolated, clavirolide A (2), was also a diterpene as suggested by the high-resolution mass spectrum (332.1999, M^+, calcd for $C_{20}H_{28}O_4$ 332.1988) and ^{13}C NMR spectrum which revealed 20 carbons (four methyl, six methylene, three methine, and seven quaternary carbons from a DEPT experiment). Two of the seven units of unsaturation required by the mass spectrum were assigned to a trisubstituted double bond (^{13}C NMR δ 127.60, d, 129.40, s; ^{1}H NMR δ 5.527, bd, J = 11.5 Hz) and a ketone carbonyl group (^{13}C NMR δ 207.46; IR 1698 cm^{-1}). A lactone functionality, ultimately shown to be a δ-lactone, with a fully substituted double bond was suggested in 2 by the ^{13}C NMR (δ 165.40, 161.86, 121.74, all s) and IR (1708 cm^{-1}) spectra with the oxygen bearing methine proton (^{1}H NMR δ 4.215, dd, J = 3.8, 3.8 Hz; ^{13}C NMR δ 82.39) showing three-bond coupling to the carbonyl carbon from the selective INEPT experiment.

The δ-lactone accounted for three more units of unsaturation as well as two of the remaining three oxygens. The fourth oxygen is a tertiary alcohol (^{13}C NMR δ 78.55, s; IR ν 3440 cm^{-1}). The hydrogen of this hydroxyl group appeared as a sharp, exchangeable singlet (δ 3.610, s). The remaining two units of unsaturation must therefore result from a bicyclic carbon framework.

A double quantum filtered phase-sensitive COSY (DQCOSY), long-range COSY (Table III), and relayed coherence transfer (RCT) spectra detailed three ^{1}H spin systems, subunits A through C. Methylene proton pairs of each subunit were most easily assigned by correlation to methylene carbons (multiplicity from a DEPT experiment) in the fixed evolution HETCOR spectrum.

In subunit A, vinylic proton H-3 (δ 5.257, bd, J = 11.5 Hz) showed strong coupling to one proton of a methylene pair, H-2b (δ 2.832, dd, J = 15.1, 11.5 Hz), with much weaker coupling to the other methylene proton located at δ 1.765 (H-2a, bd, J = 15.1 Hz). Given the multiplicities of these protons, the C-2 methylene group must be adjacent to a quaternary center. Allylic coupling from H-3 to H-5b (δ 2.787, bd, J = 11.6 Hz) was observed in the long-range COSY spectra using delays of Δ = 100 and 300 μs, with much weaker coupling to H-5a (δ 3.361, d, J = 11.6 Hz) observed only using the longer delay in the spectrum run with a drop of pyridine-d_5 added. This weak coupling to H-5a was not observed in the long-range COSY spectrum (Δ = 300 μs) in $CDCl_3$ without the added pyridine. The allylic coupling between H-3 and H-5b was not observed in the COSY spectrum without the delays added to detect weaker couplings. Long-range heteronuclear coupling observed in the FLOCK spectrum from H-3 to C-5, and from the H-16 methyl protons to C-3, C-4, and C-5 confirmed the structure of subunit A. The E stereochemistry of the $\Delta^{3,4}$ double bond was established by the carbon chemical shift of the C-16 methyl group (δ 16.68) as well as NOE's between H-3 and H-5a.[13]



Subunit A Subunit B Subunit C

Subunit B spin system was clear from the DQCOSY spectrum supported by the RCT spectrum[14] which showed relayed coherences transferred via vicinally coupled partners: between the protons at C-7 and H-17, H-9a, and H-9b via H-8, between H-10 and H-8 via H-9a and H-9b, and between H-17 and H-9a and H-9b via H-8. Selective INEPT experiments also revealed coupling from the H-17 methyl doublet to two methylene carbons (which must therefore be three bond couplings), compatible with the homonuclear coupling observed in the COSY spectrum between H-8 and the methylene protons at both C-9 and C-7.

The C-14 methylene protons showed a relatively large magnetic nonequivalence (δ 1.474 and 2.068, J_{gem} = 12.8 Hz). In addition to the gem coupling, both C-14 protons showed vicinal coupling to a multiplet at δ 2.420 in an overlapped region of the spectrum integrating to three protons; H-7a of subunit B was also located in this overlapped region, though at a slightly different frequency (δ 2.468). This resonance was assumed to be the remaining methylene pair of protons located at C-13 showing magnetic equivalence or near equivalence. Correlation of this multiplet to a methylene carbon (δ 25.37) in the HETCOR spectrum confirmed this assignment. A cross-section on the carbon frequency of this signal in the HETCOR spectrum showed only a broadened singlet with no splitting, confirming the magnetic equivalence of this methylene pair. Thus an isolated pair of vicinal methylenes composed subunit C.

Detailed analysis of the long-range heteronuclear couplings observed in the two-dimensional FLOCK and selective INEPT spectra (Table III) enabled linking of the

(13) The chemical shifts of H-16 and H-2a were too close, δ 1.692 and 1.765, respectively, to unambiguously assign an observed NOE in the 2D-NOE spectrum to the C-16 methyl protons with H-2b rather than H-2a with H-2b. The overlap of the resonances for H-2b and H-5a prevented the use of a difference NOE spectrum to resolve this ambiguity since H-5a also has a potential NOE with H-16.

(14) (a) Eich, G.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* 1982, *104*, 3731. (b) Bax, A.; Drobny, G. J. *J. Magn. Reson.* 1985, *61*, 306.

subunits. Again a key observation was the long-range coupling observed from the H-15 methyl protons to C-2, C-11, and C-14, locating this methyl group (δ 0.925) at the bridgehead carbon linking subunits A and C. The tertiary alcohol functionality is also located three bonds removed from this bridgehead methyl group, with three-bond coupling between C-11 and H-14a also being observed in a selective INEPT experiment. Since the two other methyl singlets are clearly vinylic methyl groups from their chemical shift, this tertiary alcohol must also be a bridgehead carbon. (A methyl group cannot be located at this second bridgehead.) Coupling from H-10 and H-9b to C-11 observed in selective INEPT spectra linked subunit B to this bridgehead.

Long-range coupling from H-14a to the β-carbon of the α,β-unsaturated δ-lactone unit located this carbon at C-12. The methyl resonance at δ 1.737 showed long-range coupling to C-20, C-12, and C-18, thereby completing the structural details of the δ-lactone group. The coupling patterns and chemical shifts of the C-5 and C-7 methylene protons indicated that these groups are adjacent to a nonprotonated sp^2 carbon. The ketone group must by default be located at C-6, linking subunits A and B at C-5 and C-7 and identifying the dolabellane skeleton. The C-5 methylene protons, which showed a relatively large magnetic nonequivalence, appeared as a sharp double (δ 3.361) and a broadened doublet (δ 2.787, $J_{5a,5b}$ = 11.6 Hz). The broadening of the higher field proton (H-5b) due to coupling with one of the methylene protons at C-7 (δ 2.468, H-7a, visible in the DQCOSY spectrum in contrast to the allylic couplings of H-5a and H-5b with H-3 which were visible only in the long-range COSY spectra) confirmed the ketone as the link between subunits A and B.

The stereochemical assignments were accomplished by extensive NOE (both 2D-NOE and difference NOE's) and molecular modeling studies. The observation of an NOE between the H-15 methyl protons and H-10 allowed assignment of the relative stereochemistry at C-1/C-10 with the C-1 methyl and H-10 on the same face of the molecule in 1,3-diaxial fashion. A strong NOE between the H-3 vinyl proton and the H-15 methyl protons was also observed.[15] The coupling constants between the H-2 methylene protons and H-3 ($J_{2a,3}$ < 2 Hz; $J_{2b,3}$ = 11.5 Hz) required a dihedral angle between H-3 and H-2b of about 180°, an orientation that brings H-3 within 2.1 Å of the H-15 protons in accord with the observed NOE. (All distance estimates come from molecular modeling studies as discussed below.) In addition, NOE's between H-3 with H-8, H-5a and with one of the C-9 protons, as well as between H-5a and H-8, were also observed. All these geometric constraints dictated by the observed NOE's, in light of the 180° dihedral angle between H-2b and H-3, and coupling constants are incompatible with a cis-fused ring system.

Support of the stereochemistry of the ring fusion came from solvent induced shifts upon addition of a drop of pyridine-d_5 (Figure 1).[16] The largest shift, a downfield

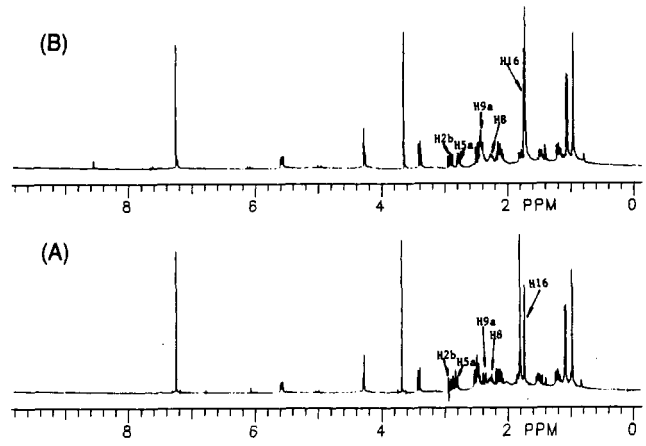


Figure 1. Pyridine-d_5-induced shifts. (A) Spectrum of **2** recorded in $CDCl_3$ with H-2b and H-5a overlapped. (B) Spectrum of **2** recorded after adding one drop of pyridine-d_5 with downfield shift of H-2b, H-9b, and H-16.

shift, was observed for H-2b ($\Delta\delta$ = 0.039 ppm) which was therefore predicted to be on the same face of the molecule as the hydroxyl group. In addition to H-2b the C-16 methyl protons and H-9a also experienced downfield shifts ($\Delta\delta$ = 0.008 and 0.037 ppm for H-16 and H-9a, respectively) upon addition of pyridine-d_5. No shift was observed for the H-15 methyl singlet which is on the opposite face. The H-2b proton was indeed predicted to be on the molecular face opposite to the C-1 methyl group based upon the large coupling with the H-3 vinyl proton ($J_{2b,3}$ = 11.5 Hz) requiring a dihedral angle of approximately 180° and on the same face as the C-16 methyl group.[17] Furthermore, H-3 was shown to be on the same molecular face as the C-1 methyl group by the NOE studies. Thus, the C-1 methyl and H-3 vinyl proton are on the face opposite of the molecule relative to the C-11 hydroxyl and C-16 methyl groups, and the H-2b proton. These observations also require a trans-fused ring system.

The stereochemistry of the C-8 methyl group was also resolved by a combination of NOE and coupling constant data supported by distance calculations using the QUANTA/CHARMm molecular mechanics program. Dipolar coupling (NOE's) between the vinyl H-3 proton with H-8 and H-9a, between H-15 and H-9a, between H-5a and H-8, and most importantly, between H-10 and H-17 were observed. Furthermore, the large coupling between H-8 and one of the C-7 protons (14.1 Hz) required a dihedral angle of 180°. These geometric constraints require the relative stereochemistry as shown. In molecular modeling studies, it proved to be impossible to simultaneously bring H-8 and H-3, as well as H-10 and H-17 within observable NOE distances in the C-8 epimer.[18] The distances between protons experiencing these NOE's in **2** all lie within 3.0 Å according to molecular modeling studies, while the distance between H-16 and H-5a was calculated to be 3.7 Å. An NOE was not observed between these two latter protons. The distance between H-3 and H-8 in the C-8 epimer is greater than 3.5 Å while the distance between

(15) Similar NOE's between a C-1 methyl group and an H-3 vinyl proton in other dolabellanes with a $\Delta^{3,4}$-*E* double bond have been observed and proven crucial in the conformational analysis of the 11-membered ring: (a) Look, S. A.; Fenical, W. *J. Org. Chem.* **1982**, *47*, 4129. (b) Matsuo, A.; Uohama, K.; Hayashi, S.; Connolly, J. D. *Chem. Lett.* **1984**, 599. (c) Rao, C. B.; Pullaiah, K. C.; Surapaneni, R. K.; Sullivan, B. W.; Albizati, K. F.; Faulkner, D. J.; He, C. H.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 2736. Conformationally analogous NOE's between a C-1 methyl group and H-3 have also been reported with an *E*-2,3-epoxide [(d) Amico, V.; Oriente, G.; Piattelli, M.; Tringali, C.; Fattorusso, E.; Magno, S.; Mayol, L. *Tetrahedron* **1980**, *36*, 1409] as well as dolabellanes with a $\Delta^{2,3}$-*E* double bond: (e) DeRosa, S.; DeStefano, S.; Macura, S.; Trivellone, E.; Zavodnik, N. *Tetrahedron* **1984**, *40*, 4991.

(16) This solvent induced shift approach is analogous to the lanthanide-induced shifts originally reported by Faulkner and Ireland to establish the trans ring fusion of dolabellanes bearing a secondary hydroxyl group at C-9; Ireland, C.; Faulkner, D. J. *J. Org. Chem.* **1977**, *42*, 3157. For a similar analysis by LIS and solvent induced shift using pyridine, see ref 15e.

(17) Bothney-by, A. A. *Adv. Magn. Resn.* **1965**, *1*, 195.

(18) We've assumed that it would be difficult to observe NOE's in our spectra across distances of more than 3.5 Å given the large number of alternative relaxation pathways available for most protons and the relative rigidity of the molecule, discussed below, resulting in correlation times dominated by isotropic tumbling.

Table IV. ^1H and ^{13}C NMR Data for Clavirolide A (2)[a]

position	^1H (ppm)	^{13}C (ppm)
1	-	50.05 (s)
2a	1.765 (bd, J = 15.1 Hz)	
2b	2.832 (dd, J = 15.1, 11.5 Hz)	33.25 (t)
3	5.527 (bd, J = 11.5 Hz)	127.60 (d)
4	-	129.40 (s)
5b	2.787 (bd, J = 11.6 Hz)	
5a	3.361 (d, J = 11.6 Hz)	54.05 (t)
6	-	207.46 (s)
7a	2.468 (dd, J = 11.2, 2.6 Hz)	
7b	2.109 (dd, J = 14.1, 11.2 Hz)	54.35 (t)
8	2.214 (m)	30.92 (d)
9a	2.331 (ddd, J = 10.5, 3.8, 3.8 Hz)	
9b	1.151 (ddd, J = 10.5, 10.5, 3.8 Hz)	37.41 (t)
10	4.215 (dd, J = 3.8, 3.8 Hz)	82.39 (d)
11	-	78.55 (s)
12	-	161.86 (s)
13	2.420 (m, 2 H)	25.37 (t)
14a	1.474 (m)	
14b	2.068 (m)	39.32 (t)
15	0.925 (s)	21.42 (q)
16	1.692 (s)	16.68 (q)
17	1.010 (d, J = 6.8 Hz)	20.74 (q)
18	-	121.74 (s)
19	1.737 (s)	12.70 (q)
20	-	165.40 (s)
OH	3.610 (s)	

[a] Spectra recorded in $CDCl_3$. Methylene protons on the same face of the molecule as the C-15 methyl group when drawn in two dimensions are designated "a". For complete designation of methylene "a" and "b" protons see Figure 2A.

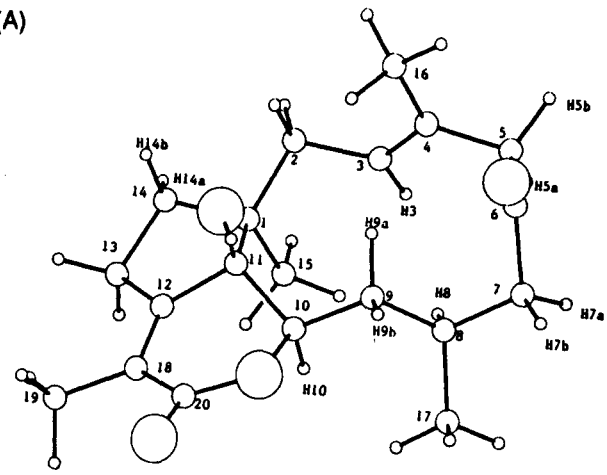
H-5a and H-8 is greater than 4 Å according to molecular mechanics calculations; NOE's were observed in these two sets of protons. This stereochemistry is in accord with the observed couplings between H-8 and the C-9 methylene protons: $J_{8,9b}$ = 10.5 Hz (dihedral angle of 165° from QUANTA/CHARMm calculations) and $J_{8,9a}$ = 3.8 Hz (calculated dihedral angle of 81°). Complete ^1H and ^{13}C NMR chemical shift assignments are given in Table IV.

The equivalent coupling between H-10 and the C-9 methylene protons (both J = 3.8 Hz) is also in accord with this stereochemical assignment, suggesting equivalent dihedral angles of either 60° or 120°. The NOE studies supported by molecular modeling calculations for a quantitative estimation of dihedral angles predict a conformation for **2** (Figure 2A) with dihedral angles between H-9a/H-10, and H-9b/H-10 of 121° and 123°, respectively. Dihedral angles of 60° are inaccessible for steric reasons. Estimated coupling constants between H-10 and the C-9 methylene protons were calculated using the equation of Osawa[19] which includes terms for relative orientation of electronegative substituents, bond length and bond angle distortions, nonbonded interactions, as well as the usual dihedral angle relationship. Using the conformation predicted in the molecular mechanics calculations for the necessary angles and distances, $J_{9a,10}$ was predicted to be 4.6 Hz, while $J_{9b,10}$ was calculated as 3.6 Hz, close to the observed values of 3.8 Hz.

Clavirolide A therefore is similar to clavulactone (**3**), which also bears a δ-lactone unit, previously reported from an unidentified species of *Clavularia*, though with a different geometry of the 3,4-double bond and a hydroxyl group located at C-11.[8b] Detailed molecular modeling studies on **2** using the QUANTA/CHARMm program revealed two minima differing only in the orientation of the C-6 carbonyl group. The conformation shown in Figure 2A, which is predicted to be 0.9 kcal/mol higher in energy than the conformation shown in Figure 2B neglecting solvent interactions, is in complete agreement with the stereochemical and conformational assignments based upon the observed NOE and coupling constant data (see below for further discussion).

(19) Imai, K.; Osawa, E. *Tetrahedron Lett.* **1989**, *30*, 4251.

(A)



NOE Distances in Angstroms

H3/H8	2.38	H8/H15	2.12
H3/H5a	2.27	H7a/H17	2.61
H3/H9b	3.04	H7b/H17	2.86
H3/H15	2.07	H9a/H10	2.88
H5a/H8	2.80	H9b/H10	2.90
H5b/H16	2.61	H10/H17	2.11

(B)

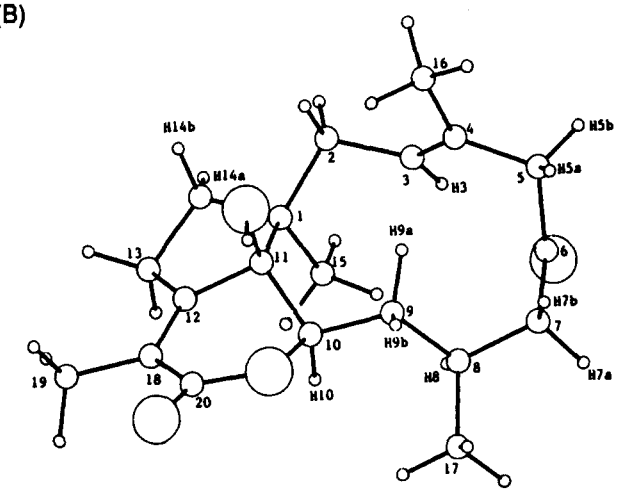
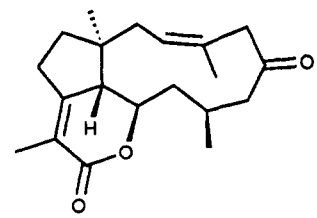


Figure 2. Conformational minima of **2** from QUANTA/CHARMm calculations. (A) Conformation in agreement with NMR studies with calculated distances between protons with observed NOE's. (B) Other carbonyl conformation found by QUANTA/CHARMm. Table lists internuclear distances calculated for conformation A between protons with observed NOE's.



3: Clavulactone

The absolute stereochemistry was assigned as shown based upon the CD spectrum, which revealed two bands:

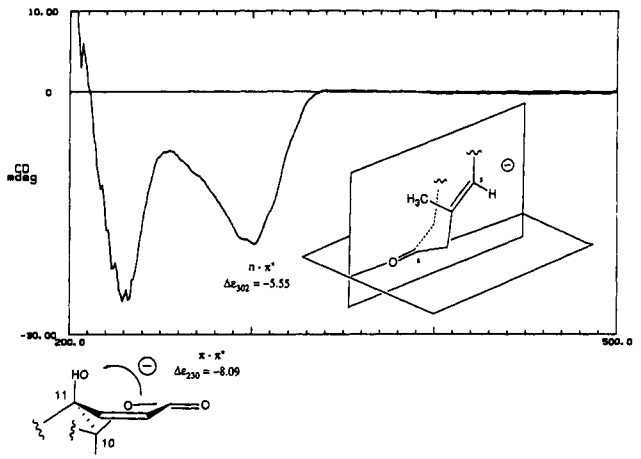
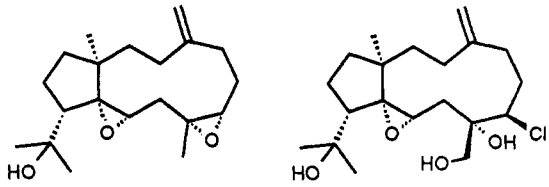


Figure 3. Circular dichroism spectrum of **2** recorded in methanol with the π-π^* band of the δ-lactone and the n-π^* band of the β,γ-unsaturated ketone.

$\Delta\epsilon_{230} = -8.09$ and $\Delta\epsilon_{302} = -5.55$ for the $\pi \rightarrow \pi^*$ transition of the δ-lactone, and the $n \rightarrow \pi^*$ transition of the β,γ-unsaturated ketone, respectively. Beecham has shown that the sign of the $\pi \rightarrow \pi^*$ transition of δ-lactones bearing an allylic oxygen is controlled by the chirality of this allylic position, and hence C-11 may be assigned as an "*R*" center (Figure 3B),[20] in agreement with the absolute stereochemistry established for **1**, also by CD.

Ireland and Faulkner have previously noted for related dolabellanes[16] that the 11-membered ring may be dominated by a single conformation. The dominance of one conformation (Figure 2A) of **2** in solution is supported by the relatively large differences in the chemical shifts, coupling constants, and NOE's of the diastereotopic methylene proton pairs (Tables III and IV).[21,22] With such conformational constraints, the $n \rightarrow \pi^*$ band in the CD may also be evaluated for the assignment of the absolute stereochemistry. Schippers and Dekker have reported that mixing of the olefinic $\pi \rightarrow \pi^*$ transition with carbonyl $n \rightarrow \pi^*$ band of β,γ-unsaturated ketones results in the chirality of the β,γ-heterodiene π-system dominating the sign of the $n \rightarrow \pi^*$ CD band.[23] The negative Cotton effect for this transition is in accord with these results for the conformation predicted by both the NMR and molecular modeling studies (Figure 2A) in light of the absolute stereochemistry predicted from the $\pi \rightarrow \pi^*$ transition of the δ-lactone (Figure 3). This conformation predicts a geometry about the ketone carbonyl that fits the observation of NOE's between H-5a and H-8 (distance calculated to be 2.8 Å). In conformation B (Figure 2B), this distance is greater than 4 Å (calculated to be 4.3 Å). Furthermore, in conformation B, both C-5 protons lie within NOE distance of the H-16 protons: H-5a/H-16 is 3.0 Å while H-5b/H-16 is 2.3 Å. However, NOE's were only observed between H-16 and H-5b (H-5b and H-5a were unambiguously distinguished as discussed above), which is actually the significantly greater distance in conformation B. Conformation A correctly predicts this distinction in H-5/H-16 distances: H-5a/H-16 is 3.7 Å while H-5b/H-16 is 2.5 Å. Finally, H-7a was shown to have only small coupling with H-8 ($J_{7a,8}$ = 2.6 Hz), in accord with the 75° calculated dihedral angle in conformation A, while the coupling between H-7b and H-8 ($J_{7b,8}$ = 14.1 Hz) requires the near 180° dihedral angle (calculated to be 164° by QUANTA/CHARMm) also observed in conformation A.

The absolute stereochemistry assigned by the CD spectrum, 1*S*,8*S*,10*R*,11*R*, is in agreement with the assigned absolute stereochemistry of the ring fusion stereocenters of other dolabellane diterpenes: clavulactone (**3**) (relative stereochemistry assigned by X-ray, absolute stereochemistry by CD spectrum),[8b] clavutriolin (**4**) (isolated from an unidentified species of *Clavularia*, absolute stereochemistry established by X-ray),[8a] and stolonidiol (**5**) (isolated from *C. viridis*, absolute stereochemistry established by X-ray on a derivative).[7] This absolute stereochemistry also follows the biosynthetic pathway proposed by Coll and co-workers on the related neodolabellane and dolastane diterpenes[9b] and is the same as deduced for dolabellanes isolated from the brown alga *Dictyota dichotoma* using Horeau's method.[15d] The antipodal dolabellane skeleton, however, has been reported from a *Dictyota* spp.[22a,24] as well as from liverworts.[15b,25]



5: Stolonidiol **4**: Clavutriolin

The absolute sterochemistry of **1** (1*R*,10*R*)[26] was assumed to be the same as **2** since **1** and **2** presumably derive via a common biosynthetic pathway. In support of this assumption, the CD spectrum of **1** showed a negative band: $\Delta\epsilon_{212} = -5.33$ assignable to the $\pi \rightarrow \pi^*$ transition of the secondary allylic alcohol unit since the remaining double bonds in **1** are relatively remote from the centers of chirality. The sign of this transition is in agreement with a C-10 "*R*" stereocenter. Thus, intramolecular hydrogen bonding between the two hydroxyl groups, indicated by the low-field broad singlet in the ^{1}H NMR spectrum which was insensitive to concentration variation (δ 3.97, 2 H) and also apparent in the X-ray structure, results in a conformational restriction for this grouping. The relatively large coupling between H-9b and H-10 (J = 11.5 Hz) and the

(20) (a) Beecham, A. F. *Tetrahedron* **1972**, *28*, 5543. For related studies on the influence of an allylic oxygen on the sign of a diene band: (b) Beecham, A. F.; Mathieson, A. McL.; Johns, S. R.; Lamberton, J. A.; Sioumis, A. A.; Batterham, T. J.; Young, I. G. *Tetrahedron* **1971**, *27*, 3725. (c) Beecham, A. F. *Tetrahedron* **1971**, *27*, 5207.

(21) See ref 9j, also: Inman, W.; Crews, P. *J. Am. Chem. Soc.* **1989**, *111*, 2822.

(22) For other conformational analyses of the 11-membered ring of the dolabellanes by NMR, see refs 15a,b,e, also: (a) Tringali, C.; Oriente, G.; Piattelli, M.; Nicolosi, G. *J. Nat. Prod.* **1984**, *47*, 615. (b) Tringali, C.; Piattelli, M.; Nicolosi, G. *Tetrahedron* **1984**, *40*, 799.

(23) Schippers, P. H.; Dekkers, H. P. J. M. *J. Am. Chem. Soc.* **1983**, *105*, 79.

(24) Tringali, C.; Nicolosi, G.; Piattelli, M.; Rocco, C. *Phytochemistry* **1984**, *23*, 1681. The antipodal skeleton was also assigned for a dolabellane isolated from the brown alga *Dilophus fasciola* (ref 15e) using Horeau's method, but the de in the esterification was only 5.1%. The assignment of the antipodal absolute stereochemistry to the dolabellanes isolated from the sea whip *Eunicea calyculata* using the relative ^{19}F chemical shifts of the diastereomeric Mosher's esters (ref 15a) has been recently corrected (ref 7).

(25) Huneck, S.; Baxter, G. A.; Cameron, A. F.; Connolly, J. D.; Harrison, L. J.; Phillips, W. R.; Rycroft, D. S.; Sim, G. A. *J. Chem. Soc., Perkin Trans. 1* **1986**, 809. The assignment of the absolute stereochemistry in ref 15b was based upon the CD spectrum of a derived allylic *p*-bromobenzoate. In this work, however, the chromophore experiencing the exciton coupling is quite remote from ring fusion stereocenters, with no evidence presented which relates the stereochemistry of the allylic stereocenter to the ring fusion sites. Thus, while the absolute stereochemistry of the allylic alcohol is quite sound, the absolute stereochemistry of the ring fusion sites were not firmly established in this work.

(26) The designation of the C-1 stereocenter of **1** as "*R*" while the C-1 center in **2** is designated as "*S*" is a consequence of the Cahn–Ingold–Prelog rules for assigning substituent priorities and not due to a differing sense of absolute chirality.

lack of coupling between H-9b and H-10, as well as the strong NOE between the H-15 methyl singlet and H-10, all help to define the conformation of the secondary allylic alcohol. Negative chirality for the $\pi \rightarrow \pi^*$ transition should be controlled by the oxygen substituent, therefore suggesting an "*R*" stereocenter at C-10.[27] Insufficient sample was in hand to confirm this conclusion by forming the *p*-bromobenzoate,[28] but the agreement between the assigned absolute stereochemistry of **1** and **2** is convincing.

Dolabellanes have also been reported from other sources, with the initial finding being in the digestive glands of the sea hare in the pioneering work of Ireland and Faulkner.[29,30] The suggestion was that these diterpenes were of dietary origin, and they have since been found in algae.[31] An algal source of related soft coral diterpenes has been suggested. Dolabellanes have since between reported in seawhips[15a] and Aplysia.[32] Clavudiol A proved to be effective in inhibiting K^+ induced contractions of blood aortic strips (ED_{35} = 3.6 μg/mL). This bioassay was not performed on **2** due to insufficient sample.

Experimental Section

General. All solvents used were analytical grade. Melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 93.94 kG (400 MHz for ^1H, 100 MHz for ^{13}C) in $CDCl_3$ unless otherwise noted using the 7.24 ppm resonance of residual $CHCl_3$ and the 77.0 ppm resonance of $^{13}CDCl_3$ as internal references for ^1H and ^{13}C, respectively. Molecular modeling was performed using the QUANTA/CHARMm program. The Boltzmann jump technique (to 3000 °C) with subsequent minimization was applied to each conformation to confirm that the structure was in a global minimum.

NMR Multipulse Sequences. NMR studies on **1**, including HETCOR[33] and FLOCK[11] experiments, were run using 8.5 mg, on **2** using 6.8 mg as these were the only quantities made available. The fixed evolution HETCOR experiment was utilized to enhance the sensitivity for detecting correlations between methylene carbons and their one bond coupled, magnetically nonequivalent protons. ^{13}C multiplicities were assigned with the DEPT experiment, and ^{13}C assignments were completed using the fixed evolution HETCOR experiment for one bond heteronuclear couplings (^1H,^{13}C), and the FLOCK and selective INEPT sequences for two and three bond heteronuclear couplings (^1H,^{13}C). The evolution time in the HETCOR experiment was fixed at 19 ms with a refocusing interval of 23.8 ms. The FLOCK sequence employed two fixed delays of Δ1 = 0.072 s and Δ2 = 0.040 s. Selective INEPT experiments were recorded with the excitation and refocusing delays optimized for different coupling constants according to the formula $\Delta 1 = {}^1/_2J$ and $\Delta 2 = {}^1/_3J$, respectively.[12] (Supplementary material is available for other acquisition parameters.)

Collection and Isolation. The soft coral *C. viridis* (Clavulariidae) was collected in the Xi Sha Island region of the South China Sea, a voucher specimen (No. 887) was preserved in the Research Center of Organic Natural Products, Zhongshan University. The sun-dried specimen (3 kg) were extracted with methanol/chloroform (10:1); the extract was evaporated in vacuo, and the residue was partitioned between ethyl acetate and water. The organic phase was was concentrated in vacuo to give a residue (120 g) which was chromatographed on silica gel, eluting with ethyl acetate/cyclohexane with increasing ethyl acetate. The fraction eluting with 9% ethyl acetate/cyclohexane (13 g) was further chromatographed on silica gel, eluting with $CHCl_3$/acetone (20:1) to give crude clavudiol A (**1**) (4.5 g), which was purified by recrystallization from acetone (2.0 g). The fraction eluting with 18% ethyl acetate/cyclohexane (15 g) was further chromatographed on silica gel, eluting with $CHCl_3$/ethyl acetate (6:1) to give crude clavirolide A (**2**). Final purification was accomplished by chromatography on alkaline alumina eluting with diethyl ether/petroleum ether (2:1) to give pure **2** (50 mg).

Clavudiol A (1): colorless needles (2.0 g, 0.067% dry wt); mp 147–149 °C; $[\alpha]_D$ −45.9° (0.031 g/mL, $CHCl_3$); CD (cyclohexane) 212 nm (Δε −5.33); IR (KBr) 3420, 2980, 2940, 2870, 1700, 1385 cm^{-1}; UV (MeOH) λ_{max} 203 (ε 23 000), 208 (20 000); HRMS (EI, 70 eV) *m/z* 304.2403 (calcd for $C_{20}H_{32}O_2$ 304.2402); ^1H and ^{13}C NMR, see Table II.

Clavirolide A (2): colorless needles (50 mg, 0.0017% dry wt); mp 188–190 °C; $[\alpha]_D$ −328.8° (0.022 g/mL, $CHCl_3$); CD (MeOH) 230 nm (Δε −8.09), 302 (−5.55); IR (KBr) 3440, 2960, 2930, 2880, 1708, 1698, 1385 cm^{-1}; UV (MeOH) λ_{max} 202 (ε 9000), 226 (23 000); HRMS (EI, 70 eV) *m/z* 332.1999 (calcd for $C_{20}H_{28}O_4$ 332.1988); ^1H and ^{13}C NMR, see Table IV.

Crystal data for 1: $C_{20}H_{32}O_2$; M_w = 304.5; orthorhombic space group $P2_12_12_1$; *a* = 8.537 (1), *b* = 10.574 (2), and *c* = 21.035 (3) Å; *Z* = 4; D_c = 1.065 g/cm^3. A colorless crystal with dimensions of 0.4 × 0.4 × 0.2 mm was selected. Data collection was carried out on an Enraf-Norius CAD-H diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å) at room temperature. The $\omega - 2\theta$ scan mode was used at a variable scan rate to $2\theta_{max}$ of 52°. The scan range was determined as a function of θ: 0.26 + 0.35(tan θ). A total of 2381 independent reflections were measured, of which 1451 ($I > 3\sigma(I)$) were used in the refinement. Empirical absorption corrections were made with the Ψ-scan technique, μ = 0.620 cm^{-1}, transmission factor 0.897–0.998.

The structure was solved by direct methods with MULTAN-82. The positional and thermal parameters of all non-hydrogen atoms were refined anisotropically, and the hydrogen atoms were subjected to isotropic refinement. All of the calculations were carried out on a PDO 11/44 computer using the SDP software package. The final refinement included 327 variables. The converged model had unweighted and weighted R agreement factors of 0.051 and 0.057, respectively, $w = [\sigma^2(I) + (0.02|F_o|)^2 + 1]^{-1}$. The final atomic coordinates, noteworthy bond distances, bond angles, and atomic thermal parameters are available in the supplementary material.

Bioassay. Clavudiol A influenced the dose–response curves of norepinephrine (NE), KCl, and $CaCl_2$ induced contractions on rabbit aortic smooth muscle:[34] PD_2 of NE was 5.30, PD_2 of KCl was 5.39, and PD_2 of $CaCl_2$ was 5.38. Effects of **1** on smooth muscle was 50 times less than verapamil. Clavudiol A also increased the level of cAMP in isolated guinea pig atria 39%.[35] The effect of 14.8 μmol/L **1** was the same as 1 μmol/L isoproterenol, possessing positive chronotropic effects.[36]

Acknowledgment. The Zhongshan University authors thank the National Foundation of the People's Republic of China for financial support and Dr. Chupu Li for identification of the soft coral. The Boston University authors thank Schering-Plough Corporation and the American Cancer Society (through the auspices of the Hubert H. Humphrey Cancer Research Center, Boston University Medical School) Grant no. IN-97L for financial support, Professor William Reynolds for copies of the FLOCK and

(27) (a) Scott, A. I.; Wrixon, A. D. *Tetrahedron* **1971**, *27*, 4787. (b) *Natural Products Chemistry*; Nakanishi, K., Goto, T., Ito, S., Natori, S., Nozoe, S., Eds.; Academic: New York, 1974; Vol. 1, p 30.

(28) Gonnella, N. C.; Nakanishi, K.; Martin, V. S.; Sharpless, K. B. *J. Am. Chem. Soc.* **1982**, *104*, 3775.

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fixed evolution HETCOR sequences (refs 6 and 7), and Professor David Coker for assistance in the molecular modeling.

Supplementary Material Available: Clavudiol (1), ^1H and ^{13}C one-dimensional spectra, DQCOSY, long-range COSY (Δ = 300 ms), HETCOR, FLOCK two-dimensional NMR spectra, and CD spectrum; clavirolide A (2) ^1H and ^{13}C one-dimensional spectra, COSY, long-range COSY (Δ = 300 ms), RCT, fixed-evolution HETCOR, and FLOCK two-dimensional spectra; tables of final atomic coordinates, noteworthy bond distances, bond angles, and atomic thermal parameters; and perspective drawing from crystallographic study of 1 (31 pages). Ordering information is given on any current masthead page.

New Briarein Diterpenes from the Caribbean Gorgonians *Erythropodium caribaeorum* and *Briareum* sp.

Evamarie O. Pordesimo, Francis J. Schmitz,* Leon S. Ciereszko, M. Bilayet Hossain, and Dick van der Helm*

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019

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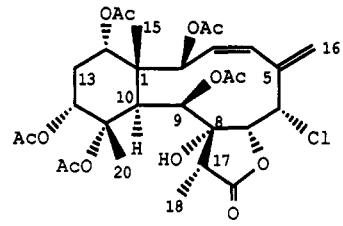
Seven new erythrolides (3–9) and the known briarein diterpenes erythrolide A (1) and erythrolide B (2) have been isolated from the gorgonian *Erythropodium caribaeorum* collected in the U.S. Virgin Islands and Jamaica. A *Briareum* sp. of gorgonian yielded nine new briarein diterpenes (10–18), which have been named briareolides. The structures of these compounds were determined by spectroscopic methods, especially one- and two-dimensional NMR. The structure and absolute stereochemistry of briareolide B (11) was determined by X-ray crystallographic analysis. Some of the briareolides have displayed antiinflammatory activity.

A large number of diterpenes that have the briarein skeleton typified by briarein A[1,2] (I) have been isolated from gorgonians,[3-13] sea pens,[14-16] and a soft coral[17] within the past 15 years. Diterpene metabolites of this type continue to intrigue investigators because of the structural novelty and complexity and interesting biological activity (e.g., insecticidal, antiinflammatory, antiviral) associated with several of these compounds.

We report here our investigation of the extracts from the gorgonians *Erythropodium caribaeorum* from the U.S. Virgin Islands and Jamaica and a species of *Briareum* from Puerto Rico. The known compounds erythrolide A[6] (1)



I

and erythrolide B[6] (2) were isolated from *E. caribaeorum* along with seven new erythrolides, compounds 3–9. The *Briareum* sp. (identified as either *B. asbestinum* or *B. polyanthes*) yielded nine new briarein type compounds 10–18, which were named briareolides.

Results and Discussion

Isolation and Structure Determination of Erythrolides from *E. caribaeorum*. The erythrolides were isolated by conventional methods as outlined in the Experimental Section. Erythrolide A (1)[6] and erythrolide B(2)[6] were identified by comparison of their ^1H NMR spectra with those reported in the literature. The ^1H NMR spectrum of 2 obtained at 20 °C in $CDCl_3$ contained some broadened signals which were sharpened considerably at 58 °C. This suggested the existence of slowly interconverting conformers for this compound. A 2D ^1H NMR homonuclear correlation experiment (COSY) allowed the assignment of all the signals in the ^1H NMR spectrum of 2, including those overlapped in the region from 5.0 to 5.7 ppm. The OH signal assignment (δ 2.71) was confirmed by exchange with CD_3OD.

Compound 3, named erythrolide C according to the nomenclature used by Look and Fenical,[6] was isolated from a fraction that was slightly less polar than the fraction containing erythrolide B (2). A molecular formula of $C_{24}H_{29}O_9Cl$, estimated from ^1H and ^{13}C NMR data, was confirmed for 3 by high-resolution FAB$^+$ mass spectrometry. The intensity of the M + 2 isotope peak observed in the low-resolution FAB$^+$ mass spectrum [(M + H +

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