Sclerophytins A and B. Isolation and Structures of Novel Cytotoxic Diterpenes from the Marine Coral *Sclerophytum capitalis*

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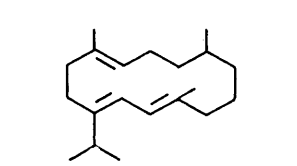
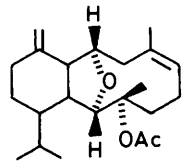
Two novel isocembrene derivatives have been isolated from the marine soft coral *Sclerophytum capitalis* and their structures determined by spectroscopic methods.

Marine invertebrates have been shown to possess a wide variety of secondary metabolites including terpenes, alkaloids, and steroids.[1] The diversity of di- and tri-terpenes encountered in marine organisms is phenomenal.[2] A soft coral of the genus *Lobophytum* has been reported to contain an isocembrene (**1**)[3] which could serve as a precursor of three novel diterpenes identified as cladiellin (**2**),[4] ophirin (**3**),[5] and eunicellin (**4**)[6] isolated from the soft corals *Cladiella* sp., *Muricella* sp., and *Eunicella stricta*, respectively. Here we report the isolation and structures of two novel diterpenes—sclerophytin A and B from the soft coral *Sclerophytum capitalis*, which we believe are derived from a deacetylcladiellin intermediate. Sclerophytin A was found to be cytotoxic against the L1210 cell line at a concentration of 0.001 μg ml^{-1}.



(1)



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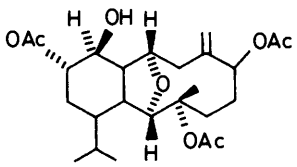

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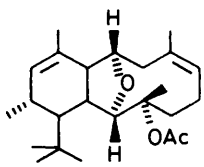

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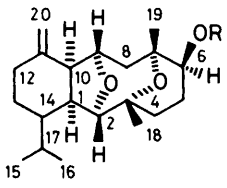
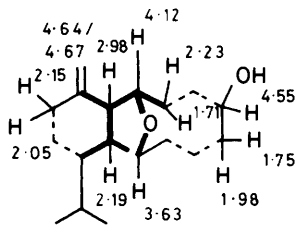
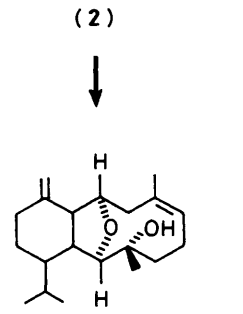


(5) R = H
(6) R = Ac



A



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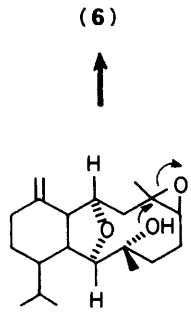

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Results and Discussion

A methanolic extract (40 l) of the coral on evaporation and lyophilization gave a residue which was triturated with hexane, chloroform, and finally with methanol. The hexane extract on repeated chromatography on silica gel 60 gave a group of fractions which were combined and evaporated to give sclerophytin B (**6**). Chromatography of the chloroform extract (2 ×) followed by h.p.l.c. gave sclerophytin A (**5**) as colourless needles, m.p. 187 °C. The ^{1}H n.m.r. spectrum in $CDCl_3$ showed the presence of an exocyclic methylene [δ 4.67 (1 H, s), 4.64 (1 H, s)], three methine protons [δ 4.55 (1 H, d, *J* 6.5 Hz), 4.12 (1 H, m), and 3.63 (1 H, s), geminal to oxygen(s)], a methine resonance [δ 2.98 (1 H, dd, *J* 13.4, 6.7 Hz)], two methyls [δ 1.20 and 1.16 (3 H, each, s)] and an isopropyl [δ 0.96 and 0.80 (3 H, each, d, *J* 6.8 Hz)]. H.r.m.s. established the molecular formula as $C_{20}H_{32}O_3$ along with the presence of a hydroxy [$\nu_{max.}$ 3 400 cm^{-1}, *m/z* 302 (*M* − H_2O, 20%)], methyl [*m/z* 305 (*M* − Me, 20%), and an isopropyl group [*m/z* 277 (*M* − Pr^i, 15%)]. The molecular formula requires five unsaturation equivalents which in conjunction with an exocyclic methylene suggests that compound (**5**) is tetracyclic in nature. The ^{13}C n.m.r. spectrum confirmed the presence of 20 carbons [3 × C, 7 × CH, 6 × CH_2, and 4 × CH_3 as determined by an attached proton test (APT)[7] and ^{1}H-^{13}C correlation (COSY) spectra] including an exocyclic methylene (147.9 and 109.0 p.p.m.) and five oxygen-bearing carbons (90.4, 79.9, 78.1, 77.0, and 74.8 p.p.m.). Of the five oxygen-bearing carbons three were methines (90.4, 79.9, and 78.1 p.p.m.) while the remaining two (77.0 and 74.8 p.p.m.) were quaternary in nature. One of the oxygens in compound (**5**) was a secondary hydroxy {as determined from the ^{1}H n.m.r. spectrum of the acetylation product [pyridine–acetic anhydride (1:1, v/v), M^+, 362, m.p. 190—191 °C]; as expected the resonance at δ 4.55 was shifted to 5.62 and a resonance appeared at δ 2.03 (3 H, s)} while the other two must be ethereal in nature. This assumption is supported by the presence of four oxygen-bearing carbons in the ^{13}C n.m.r. spectrum. A ^{1}H-^{13}C COSY spectrum was used to identify the specific resonances associated with each protonated carbon (Figure 1). A 300 MHz ^{1}H-^{1}H correlation (COSY) spectrum of compound (**5**) (Figure 2) showed that the proton at δ 4.12 was coupled to three protons at δ 2.98, 2.23, and 1.71. The protons at 2.23 and 1.71 were mutually coupled and the connectivity stopped there. The proton at δ 2.98 was coupled to a proton at δ 2.19 which in turn was coupled to a proton at δ 1.25. The proton at δ 3.63 was also coupled to that at 2.19. The above information suggests the presence of fragment A (bold lines) in (**5**).

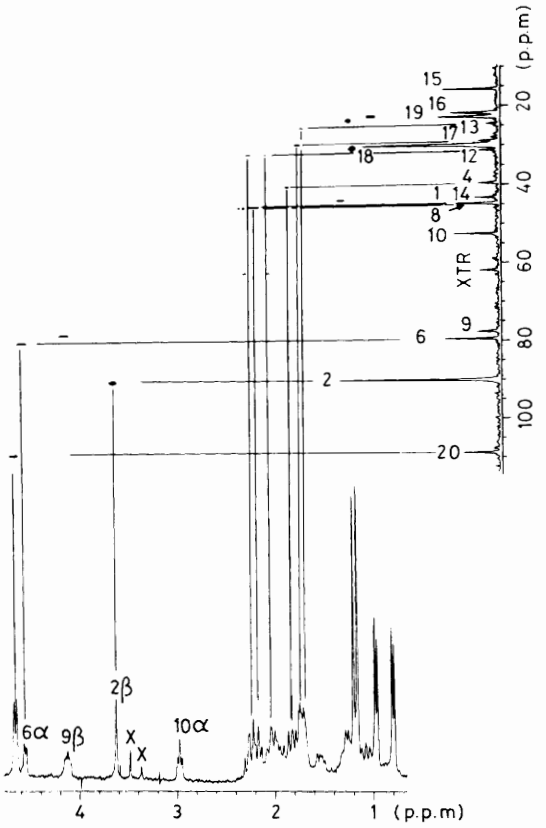

Figure 1. Two-dimensional ^{1}H-^{13}C chemical shift correlation (HETCOR) spectrum of (**1**) in $CDCl_3$ at 75.46/300.07 MHz. A normal high resolution ^{1}H spectrum is plotted along the F_1 (below) axis while the 90° projection to recover the protonated carbon spectrum is plotted along the F_2 (vertical) axis

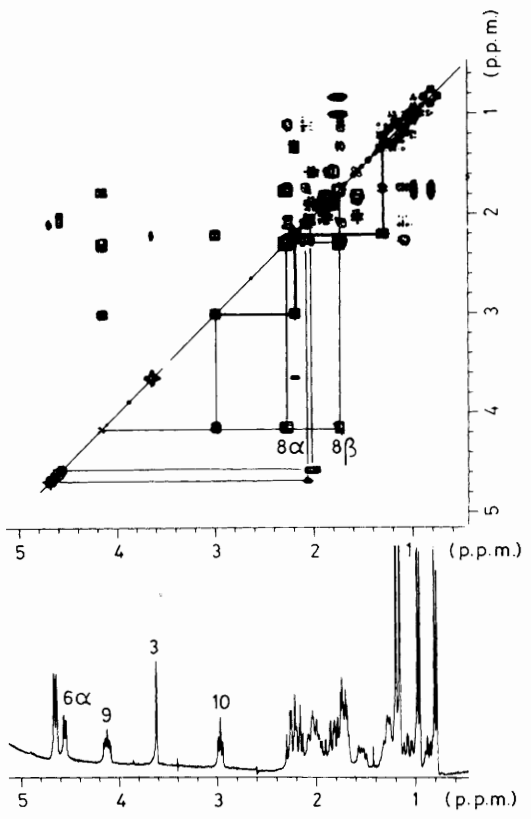

Figure 2. 300 MHz Symmetrized autocorrelated homonuclear (COSY) spectrum of (**1**) in $CDCl_3$. The normal spectrum (diagonal) is presented as a conventional high resolution spectrum (below). Cross peaks identify nuclei related by scalar coupling

A 500 MHz COSY spectrum showed the correlation between the proton at δ 4.55 and protons resonating at δ 1.98 and 1.75. Similarly one of the exocyclic methylene protons (δ 4.67) was coupled to a proton at δ 2.05, while the other proton (δ 4.64) was coupled to the protons at δ 4.12 and 3.63 suggesting that both of these protons are on the same face of (**5**) as the proton at δ 4.64. These data extend the structure of fragment A (bold face) to include the light faced part in A, leaving only one oxygen and three methylene to complete the nucleus in (**5**). One of the three methylenes (δ 24.85 p.p.m.) could be used to complete the cyclohexyl ring while the other two will complete the octyl ring as shown in A by broken lines. These spectral data strongly suggest that (**5**) is structurally related to cladiellin.[4] After considering the positions of the quaternary carbons, in the ^{13}C n.m.r. spectrum (δ 74.8 and 77.01 p.p.m.), along with the connectivities of protons at δ 3.63 (2-H), 2.23 (8-H_β), and 1.71 (8-H_α), and the multiplicities of methyls (singlets), an ether bond was fixed between C-3 and C-7. This arrangement creates a tetracyclic terpene as required by the molecular formula. The position of the isopropyl group and the final assignment of the structure was accomplished by using a long-range reverse detected heteronuclear multiple quantum[8] experiment optimized for 10 Hz coupling (Figure 3). The proton at δ 4.12 was coupled to three carbons resonating at δ 147.9, 52.5, and 90.4 p.p.m. (through a hetero atom) supporting the assignment for (**5**). As expected, the proton at δ 2.98 was coupled to carbons at δ 147.9, 109.0, 78.1, 45.3, 43.6, and 31.5 p.p.m. supporting moiety A. Similarly the exocyclic methylene protons were coupled to carbons at δ 52.5 and 31.5 p.p.m. further confirming fragment A (bold and light faced lines). The proton at δ 3.63 was coupled to carbons at δ 43.6, 45.1, 52.5, 74.8, and 78.1 p.p.m. The carbon at δ 43.6 was in turn coupled to the methyls of the isopropyl group fixing the position of the isopropyl at C-14. Coupling of the proton at δ 3.63 with carbons at δ 45.1 and 74.8 extends fragment A. Finally, the coupling of the methyls with respective carbons [proton at δ 1.20 coupled to carbons at δ 79.9, 77.0, and 45.3 p.p.m. and proton at δ 1.16 to carbons at δ 90.4, 74.8, and 39.9 p.p.m.] completed the proposed structure.

The relative stereochemistry of protons and methyls were determined: (*a*) by comparing the ^{1}H n.m.r. data with that reported for cladiellin (**2**), (*b*) from *J* values of protons, and (*c*) from a n.O.e. difference spectrum of (**5**). The positions of 2-H and 9-H and C-2 and C-9 in the ^{1}H and ^{13}C n.m.r. spectra of (**5**) were as reported for (**2**). The θ angle between 1-H and 2-H as determined from a model, was about 85°, thus exhibiting virtually no coupling between 1-H and 2-H. However, 1-H was coupled to 10-H (apparent t, could be regarded as a dd, *J* 6.7 and 13.4 Hz) which in turn was coupled to 9-H (ddd, $J_{9,10}$ 13.6 Hz) suggesting a *trans* relationship between 10-H and 9-H. A n.O.e. difference spectrum also showed negligible (1.8%) enhancement of the proton at δ 2.98 when the proton at δ 4.12 was irradiated. However, both exocyclic methylene (δ 4.67) and quaternary methyls (δ 1.20 and 1.16) experienced significant transfer (27—30% enhancement) of magnetization; similarly the proton at δ 3.63 was enhanced by 15%. Conversely, irradiation at δ 3.63 showed a magnetization transfer to methyls at C-3 and C-7. These data support the assigned β relative stereochemistry for the quaternary methyls, 2-H, 9-H, and 10-H. The proton 6-H has an α-configuration since irradiation at δ 4.55 showed less than 2% enhancement of the peaks at δ 4.12, 3.63, 1.20, and 1.16. This relative stereochemistry is also in agreement with the

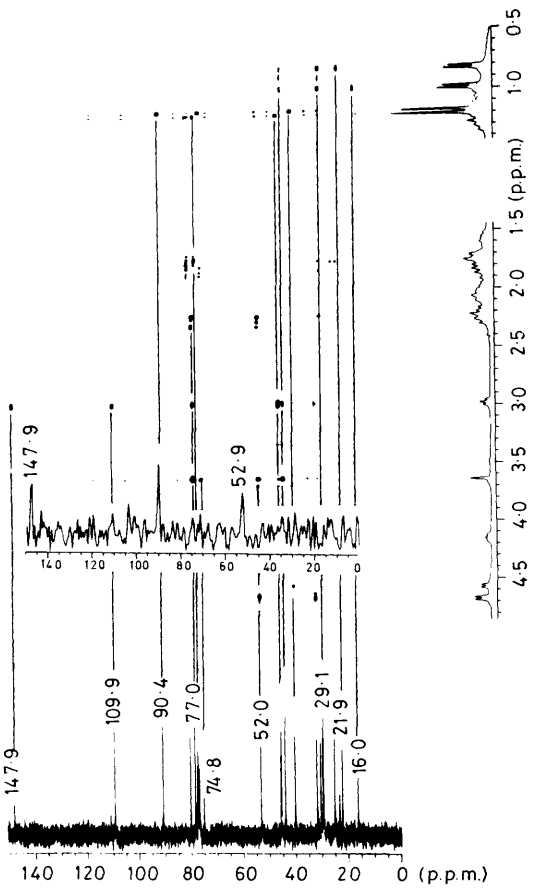

Figure 3. 300 MHz ^1H-^{13}C Long-range correlation spectrum of (**1**). A ^1H-decoupled ^{13}C spectrum is presented along the F_1 axis (below), while a high resolution ^1H spectrum is presented along the F_2 (vertical) axis

Table. Assignments of the ^{13}C- and ^1H-n.m.r. spectra of (**5**) and (**6**)

	(5)		(6)	
Carbon	δ_C	δ_H	δ_C	δ_H
1	45.16 (d)	2.19 (dd, *J* 6.7 Hz)	45.45 (d)	2.18 (dd)
2	90.49 (d)	3.63 (br s)	90.49 (d)	3.63 br s)
3	74.83 (s)		74.77 (s)	
4	39.93 (t)	1.27 (m)	39.82 (t)	
5	29.37 (t)*	1.75 and 1.98 (m)	28.99 (t)	
6	79.93 (d)	4.55 (d, *J* 6.5 Hz)	85.03 (d)	5.62 (d)
7	77.01 (d)		75.87 (d)	
8	45.38 (t)	1.71 and 2.23 (m)	45.46 (t)	1.70 and 2.23
9	78.17 (d)	4.12 (m)	77.97 (d)	4.12 (m)
10	52.59 (d)	2.98 (dd, *J* 13.4 and 6.7 Hz)	53.21 (d)	2.98 (dd)
11	147.90 (s)		147.87 (s)	
12	31.58 (t)	2.05 and 2.25 (m)	31.87 (t)	2.03 and 2.15
13	24.85 (t)*	1.70 (m)	24.79 (t)	
14	43.69 (d)	1.25 (m)	43.56 (d)	
15	16.01 (q)	0.80 (d, *J* 6.7 Hz)*	16.11 (q)	0.79* (d)
16	21.96 (q)	0.96 (d, *J* 6.7 Hz)*	21.93 (q)	0.95* (d)
17	29.13 (d)	1.78 (m)	28.07 (m)	
18	30.32 (q)	1.16 (s)	30.19 (q)	1.14 (s)
19	23.06 (q)	1.20 (s)	23.70 (q)	1.20 (s)
20	109.05 (t)	4.64 and 4.67 (s)	109.23 (t)	4.64 and 4.67 (s)
$COCH_3$			21.45 (q)	2.03
$COCH_3$			171.8 (s)	

* Can be interchanged.

biogenetic considerations. As proposed by Fennical[3] cladiellin could be derived from geranylgeraniol *via* isocembrene (**1**). Since the acetoxy at C-3 in cladiellin is α, deacetylcladiellin (**7**) will have an α-hydroxy group at C-3. Epoxidation of the double bond between C-6 and C-7 in deacetylcladiellin will result in the intermediate (**8**) which could undergo an intramolecular nucleophilic attack, from the back, at C-7 to give (**5**) with a β-hydroxy group.

Sclerophytin B (**6**) was isolated from the hexane extract by repeated column chromatography. Crystallization with acetone gave colourless needles, m.p. 191 °C. The low resolution mass spectrum gave a molecular ion at *m/z* 462 (M^+, 10%), with a fragmentation pattern suggesting the loss of methyl (347, $M-15$), acetyl (319, $M-43$), and acetic acid (302, $M-60$), but no fragment representing the loss of water. A comparison of the ^1H- and ^{13}C-n.m.r. data of (**6**) with that of (**5**) confirmed that both have the same structure except that the hydroxy group at C-6 in the former is acetylated, as indicated by the downfield shift of 6-H in (**6**) [δ 5.62 in (**6**) and 4.55 in (**5**)]. The ^1H n.m.r. and i.r. spectra of the acetylation product of (**5**) was superimposable with that of (**6**), thus confirming that (**6**) is 6-acetylsclerophytin A.

Experimental

M.p.s were recorded on a Fisher-Johns apparatus and are uncorrected. Spectra were recorded on the following instruments: i.r., Perkin-Elmer model 283; mass spectra, Finnigan model 1020 equipped with an Incos data system; 300 MHz n.m.r. spectra, Nicolet NT 300 wide bore spectrometer operating at 300.068 and 75.457 Hz for ^1H- and ^{13}C-observations, respectively. The n.m.r. instrument was controlled by a model 29C pulse programmer and was equipped with a 5 mm ^1H-^{13}C dual tuned probe. ^1H-^1H correlation and ^1H-^{13}C correlation spectra were recorded as described elsewhere.[9] The long-range reverse detected heteronuclear multiple quantum spectrum was recorded according to the method described by Bax and Summers.[8] 500 MHz ^1H and ^1H-^1H correlation spectra were recorded on a Bruker AM 500 instrument at the Colorado State University.

Sclerophytum capitalis was collected from the waters of Enewetak (Eniwetok), Micronesia and was soaked in methanol after collection. The methanolic extract (40 l) was decanted and concentrated then lyophilized to give a residue (60 g) which was triturated with hexane, chloroform, and finally with methanol. The extracts upon evaporation gave 12.52 g, 5.48 g, and 32.95 g of residue respectively.

Sclerophytin A (**5**).—The chloroform extract (2g) was subjected to chromatography on silica gel 60 (230—400 mesh, E. Merck, column 2.5 × 100 cm) using the following eluants: benzene, benzene–chloroform (1:1, v/v), chloroform, chloroform–methanol (9:1, v/v), and chloroform–methanol (1:1, v/v). Fractions 7 and 8 (100 ml each) eluted with chloroform–methanol (9:1, v/v) were combined on the basis of t.l.c. (silica gel, solvent system ethyl acetate, 100%), and evaporated to give a residue (810 mg), which was subjected to rechromatography on silica gel 60 (230—400 mesh, E. Merck, column 11.0 × 600

cm). Elution with chloroform–methanol (9:1, v/v) afforded a group of fractions (5 ml each) containing two spots (by t.l.c.) which upon evaporation gave semi-pure (**5**) (236 mg). The residue from the second chromatography was subjected to h.p.l.c. using a solvent consisting of hexane–ethyl acetate (1:1, v/v). The first of the four fractions collected by h.p.l.c. on evaporation gave a residue which was crystallized with benzene to afford (**5**) (40 mg), m.p. 187 °C; $\nu_{max.}$ 3 400 (OH), 2 980 (CH), and 1 110 cm^{-1} (COC) (Found: 320.2335. $C_{20}H_{32}O_3$ requires 320.2352); *m/z* 305 ($C_{19}H_{29}O_3$, 20%), 302 ($C_{20}H_{30}O_2$, 20), 277 ($C_{17}H_{25}O_3$, 15), 195 ($C_{12}H_{19}O_2$, 96), 179 ($C_{12}H_{19}O$, 100), and 178 ($C_{12}H_{18}O$, 70). Data for ^1H n.m.r. (500 MHz in $CDCl_3$) and ^{13}C n.m.r. in $CDCl_3$ are given in the Table.

Sclerophytin B (**6**).—In a typical experiment the hexane residue (5 g) was subjected to chromatography on silica gel 60 (230—400 mesh, E. Merck, column 2.5 × 100 cm). The column was eluted with benzene, benzene–chloroform (1:1, v/v), chloroform, and chloroform–methanol (8:2, v/v). Fractions 12—14, 25 ml each, eluted with chloroform, were combined and evaporated to dryness to yield a residue (1.5 g) which was subjected to rechromatography on a similar column using benzene and benzene–chloroform (1:1, v/v) as eluant. Fraction 3, eluted with benzene (25 ml), gave on evaporation, a white solid which was crystallized from acetone to give (**6**) as colourless needles (17 mg), m.p. 190—192 °C; *m/z* 362 (10%), 347 (5), 319 (15), 302 (21), 273 (10), and 176 (80); Data for ^1H n.m.r. (300 MHz in $CDCl_3$ and ^{13}C n.m.r. in $CDCl_3$ are given in the Table.

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