112, 101, 100, 96, 92, 91, 85, 67, 57 (100); $[\alpha]^{20}_D$ -33.7° (c 0.1 g/mL, $CHCl_3$).

4(*S*)-[(*tert*-Butoxycarbonyl)amino]-2,9-dimethyl-7-[[(2-tetrahydropyranyl)oxy]methyl]-*trans*-5-decene, THP Ether 28c. Diastereoisomer I: ^1H NMR (500 MHz) δ 0.83-0.91 (6 H, m, CH_3), 1.15-1.85 (9 H, m, THP ether and $CH_2CH(CH_3)_2$), 1.42 (9 H, s, t-C_4H_9), 2.36 (1 H, m, CHC=), 3.22, 3.48, 3.58, and 3.83 (each 1 H, m, CH_2O), 4.08 (1 H, m, CHN), 5.30 (1 H, br s, NH), 4.57 (1 H, m, OCHO), 5.25-5.45 (2 H, m, CH=CH). Diastereoisomer II: ^1H NMR (500 MHz) δ 0.82-0.91 (6 H, m, CH_3), 1.1-1.9 (9 H, m, THP ether and $CH_2CH(CH_3)_2$), 1.42 (9 H, s, t-C_4H_9), 2.38 (1 H, m, CHC=), 3.26, 3.45, 3.57, and 3.85 (each 1 H, m, CH_2O), 4.09 (1 H, m, CHN), 4.32 (1 H, br s, NH), 4.55 (1 H, m, OCHO), 5.35-5.43 (2 H, m, CH=CH). Diastereoisomers I and II were virtually indistinguishable by IR and LRMS: IR (NaCl) 3340, 2960, 2875, 1700, 1510, 1468, 1452, 1386, 1368 cm^{-1}; LRMS, m/e 340, 312, 284, 283, 257, 256, 240, 227, 200, 196, 182, 166, 140, 138, 130, 123, 110, 95, 86, 85 (100); diastereoisomer I, $[\alpha]^{20}_D$ -27.6° (c 0.1 g/mL, $CHCl_3$); diastereoisomer II, $[\alpha]^{20}_D$ +0.7° (c 0.1 g/mL, $CHCl_3$).

5(*S*)-[(*tert*-Butoxycarbonyl)amino]-6-phenyl-2(*R*)-(phenylmethyl)-*trans*-3-hexenoic Acid, protected PhePhe isostere 29a: ^1H NMR (500 MHz) δ 1.40 (9 H, s, t-C_4H_9), 2.76 (3 H, m, $ArCH_2CHN$ and $ArCH_2CHCO$), 3.08 (1 H, m, $ArCH_2CHCO$), 3.29 (1 H, m, CHCOO), 4.35-4.55 (2 H, br m, CHNH), 5.44 (1 H, dd, J = 7 and 16 Hz, CH=C), 5.58 (1 H, dd, J = 10 and 16 Hz, CH=C), 7.05-7.35 (10 H, m, Ar); ^{13}C NMR (50 MHz) δ 28.3 (CH_3CO), 38.3, (CHN), 41.6 ($ArCH_2CN$), 50.5 ($ArCH_2CHCOO$), 52.7 (CHCOOH), 79.6 ($OCCH_3$), 126.4, 127.1, 127.4, 128.3, 129.1, 129.5, 130.1 (Ar and C=C), 133.5 (Ar CCH_2CN), 137.2 (CHC=C), 138.4 (Ar CCH_2CCOOH), 155.1 (O=CN), 178.4 (COOH); IR ($CHCl_3$) 3500-2500, 3440, 3040, 2980, 2940, 2860, 1705, 1685, 1600, 1492, 1453, 1369, 1286, 1165, 1075, 1031, 972, 922 cm^{-1}; LRMS, m/e 318, 317, 305, 304, 249, 248, 234, 230, 214, 204, 200, 187, 186, 170, 161, 143, 130, 129, 128, 117, 115, 105, 97, 95, 92, 91 (100), 85, 81, 77, 65, 57, 55; $[\alpha]^{20}_D$ -17.0° (c 0.1 g/mL, $CHCl_3$); HRMS (CI), m/e 395.2107 (calcd 395.2104).

5(*S*)-[(*tert*-Butoxycarbonyl)amino]-7-methyl-2(*R*)-(phenylmethyl)-*trans*-3-octenoic Acid, Protected LeuPhe Isostere 29b. Method 2, Hydrolysis/Oxidation. A solution of 0.1313 g (0.3 mmol) of the THP ether **29a** and 0.5 mg of pyridinium p-toluenesulfonate in 5 mL of CH_3OH was stirred at 25 °C for 10 h. The volatiles were removed in vacuo, and the residue was dissolved in 5 mL of acetone and cooled to 0 °C. Jones reagent (2.5 mL, 4.8 mmol, 1.92 M) was added, and the mixture was stirred 1 h at 0 °C. Ether and water were added, and the layers were separated. The aqueous layer was extracted with several portions of fresh ether, and the product was recovered by extraction of the combined etheral layers with 5% aqueous NaOH. The aqueous extracts were acidified with 10% aqueous HCl and extracted with ether. Drying ($MgSO_4$) followed by concentration in vacuo (50 °C) afforded 68.5 mg (63%) of **29b** as a pale yellow oil: ^1H NMR (500 MHz) δ 0.86 (3 H, d, J = 6 Hz, CH_3), 0.87 (3 H, d, J = 6 Hz, CH_3), 1.23 (2 H, m, CH_2-i-Pr), 1.44 (9 H, s, t-C_4H_9), 1.54 (1 H, m, $CH(CH_3)_2$), 2.81 (1 H, dd, J = 7 and 13 Hz, $ArCH_2$), 3.11 (1 H, dd, J = 7 and 13 Hz, $ArCH_2$), 3.28 (1 H, m, CHCOO), 4.05 (1 H, m, CHN), 4.28 (1 H, s, NH), 5.35 (1 H, dd, J = 6 and 15 Hz, CH=), 5.63 (1 H, dd, J = 8 and 15 Hz, CH=), 7.14-7.28 (5 H, m, Ar); ^{13}C NMR (50 MHz) δ 2.5 (CH_3CH), 24.5 ($CHCH_3$), 28.3 (CH_3CO), 38.4 (CHN), 44.4 (CH_2CHN), 50.4 ($ArCH_2$), 65.8 (CHCOOH), 79.8 ($OCCH_3$), 126.4, 126.5 (Ar para C and CHC=C), 128.3 (Ar ortho C), 129.1 (Ar meta C), 134.9 (CHC=C), 138.4 (Ar CCH_2), 155.3 (O=CN), 178.2 (COOH); IR ($CHCl_3$) 3500-2500, 3440, 3080, 3040, 2960, 2935, 2875, 1703, 1650, 1502, 1492, 1452, 1392, 1370, 1282, 1165, 1067, 972, 912, 870 cm^{-1}; LRMS, m/e 304, 260, 259, 249, 248, 244, 205, 204, 200, 161, 156, 144, 143, 130, 117, 115, 112, 105, 96, 92, 91 (100), 86, 84, 77, 65, 59, 57; $[\alpha]^{20}_D$ -32.1° (c 0.025 g/mL, $CHCl_3$); HRMS (CI), m/e 361.2222 (calcd 361.2261).

5(*S*)-[(*tert*-Butoxycarbonyl)amino]-7-methyl-2-(methylpropyl)-*trans*-3-octenoic Acid, Protected LeuLeu Isostere 29c. Diastereoisomer I: ^1H NMR (500 MHz) δ 0.87 (1.5 H, d, J = 6 Hz, CH_3), 0.90 (4.5 H, m, CH_3), 1.34 (1 H, m, $CH(CH_3)_2$), 1.43 (9 H, s, t-C_4H_9), 1.63 (2 H, m, CH_2-i-Pr), 3.09 (1 H, m, CHCOO), 4.12 (1 H, br m, CHN), 4.40 (1 H, br s, NH), 5.54 (2 H, m, CH=CH). Diastereoisomer II: ^1H NMR (500 MHz) δ 0.87 (1.5 H, d, J = 6 Hz, CH_3), 0.90 (4.5 H, m, CH_3), 1.34 (1 H, m, $CH(CH_3)_2$), 1.44 (9 H, s, t-C_4H_9), 1.62 (2 H, m, CH_2-i-Pr), 3.07 (1 H, m, CHCOO), 4.10 (1 H, br m, CHN), 4.40 (1 H, br s, NH), 5.46 (1 H, dd, J = 6 and 16 Hz, CH=), 5.56 (1 H, dd, J = 8 and 16 Hz, CH=). The ^{13}C NMR, MS, and IR spectra for both diastereoisomers were virtually indistinguishable: ^{13}C NMR (50 MHz) δ 22.4, 22.6 (2 × CH_3CH), 24.7, 25.5 (2 × $CHCH_3$), 28.4 (CH_3CO), 41.3 (CHN), 44.6 (CH_2CN), 47.0 (CH_2CCOOH), 50.2 (CHCOOH), 79.6 ($OCCH_3$), 127.7 (C=C), 134.3 (C=C), 155.3 (O=CN), 179.7 (COOH); IR ($CHCl_3$) 3500-2500, 3440, 2960, 2875, 1702, 1492, 1468, 1453, 1389, 1320, 1280, 1240; 1168, 1076, 1062, 1051, 970, 870 cm^{-1}; LRMS, m/e 272, 271, 270, 226, 225, 215, 184, 182, 170, 157, 156, 154, 152, 130, 117, 112, 109, 105, 96, 95, 91, 86, 85, 84, 82, 77, 69, 67, 57 (100); HRMS (CI), m/e 327.2409 (calcd 327.2418); diastereoisomer I, $[\alpha]^{20}_D$ +18.1° (c 0.05 g/mL, $CHCl_3$); diastereoisomer II, $[\alpha]^{20}_D$ -29.3° (c 0.02 g/mL, $CHCl_3$).

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New Spongiane Diterpenes from an Australian Nudibranch

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Nine new spongiane-type diterpenes have been isolated from a nudibranch collected in South Australia and tentatively identified as *Ceratosoma brevicaudatum* (Abraham). Variation in extent and site of oxidation distinguishes these diterpenes from others of this skeletal class. Structures were determined by detailed spectroscopic analyses with emphasis on ^1H and ^{13}C NMR data. Relayed coherence transfer and long-range COSY ^1H NMR experiments were used to identify spin systems of partial structures involving unresolved, overlapping signals.

A family of diterpenes sharing a common skeleton represented by **1**[1] and designated spongianes[2] have been reported from various sponge sources.[3,4] Metabolites with rearranged versions of this skeleton have been isolated

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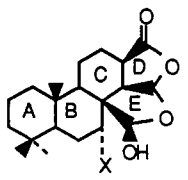
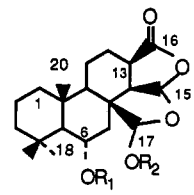
Chart I

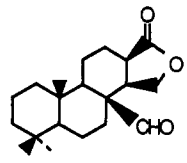


1, X = OC(O)Pr

6, X = H



	R_1	R_2
2 [13]	C(O)Pr	H , 17β
3	Ac	H , 17β
4	Ac	Ac , 17α
5	C(O)Pr	Ac , 17α



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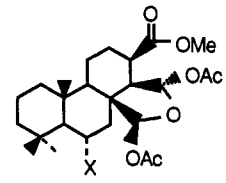
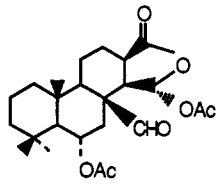


X

8	OAc
9	OC(O)Pr
10	H



11 [13]

from nudibranchs of the genus *Chromodoris* which are known to feed on sponges.[5] From a nudibranch, tentatively identified as *Ceratosoma brevicaudatum* (Abraham, 1876), collected at Stenhouse Bay, in South Australia, we have isolated nine new diterpenes of the spongiane family which differ from previously reported ones in their oxidation patterns. We reported here their structure elucidation.

Acetone and chloroform–methanol extracts of the nudibranchs were chromatographed to give nine pure compounds, **2–10** (Chart I), in <1–7-mg quantities. The formulas for these compounds were established by a combination of low-resolution mass spectrometry and ^1H and ^{13}C NMR and IR spectroscopy (see Tables I and II and Experimental Section, respectively).[6]

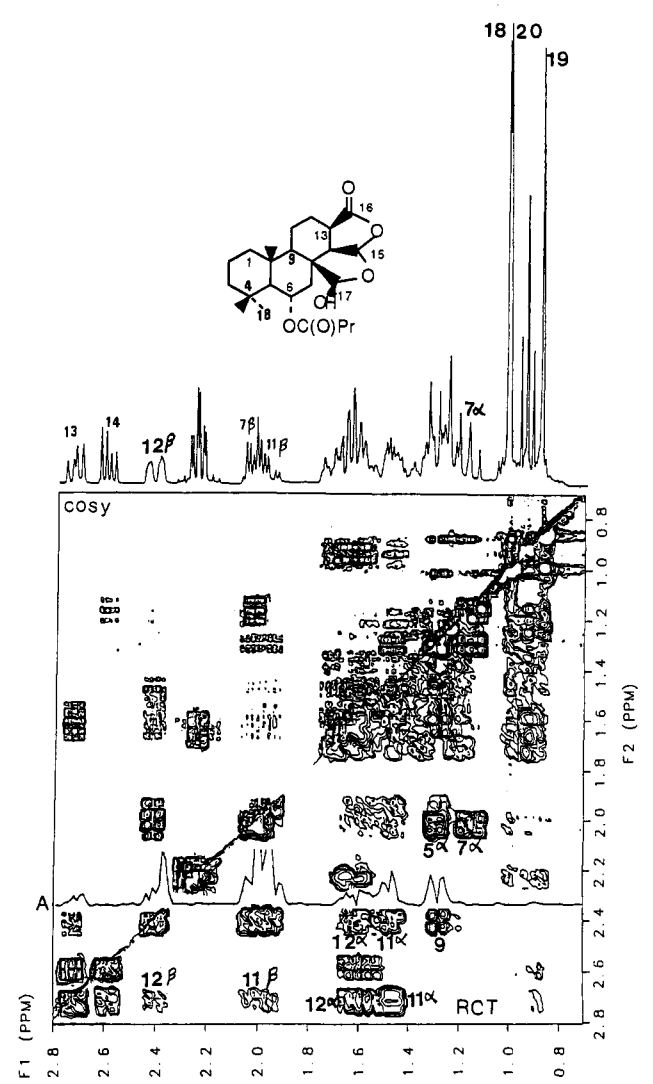


Figure 1. Combined ^1H COSY (2D correlated spectroscopy)–RCT (2D relay coherence transfer spectroscopy) plot (upfield region only) of lactone **2** in $CDCl_3$. The RCT mixing time was incremented from 25 to 37 ms; 512 FIDS, 16 scans each, were recorded for both COSY and RCT. The individual spectra were cut along the diagonal into two triangles, and the combined plot was assembled by using the upper-left triangle of COSY and the lower right triangle of RCT. A is a slice at 2.42 ppm from RCT spectrum.

Lactone **2**, $C_{24}H_{36}O_6$, showed IR and ^{13}C NMR absorptions indicative of a γ-lactone [1770 cm^{-1}; 177.1 ppm (s)], a normal ester [1730 cm^{-1}; 172.8 ppm (s)], and a hydroxyl group (3400 cm^{-1}). The acyl portion of the conventional ester was identified as a butyryl group from the isolated, coupled spin system: 2.24 (t), 1.63 (sextet), and 0.94 ppm (t). An acetal carbon and a hemiacetal carbon bonded to a common oxygen were evident from carbon signals at 104.2 (d) and 103.9 (s) ppm and long-range coupling (H/H COSY[7]) between their associated protons (see Tables I and II), 6.08 (J = 5.6 Hz) and 5.63 (br s) ppm, respectively. An exchangeable OH proton at 3.11 ppm (br s) was coupled (H/H COSY) to H-17 [5.63 ppm (br s)], and hence this hydroxyl group was fixed at position 17 in partial structure A. Beginning with the signal for the acetal proton H-15,

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(2) Kazlauskas, R.; Murphy P. T.; Wells, R. J.; Noack, K.; Oberhansli, W. E.; Schonholzer, P. *Aust. J. Chem.* **1979**, *32*, 867.

(3) Faulkner, D. J. *Nat. Prod. Rep.* **1986**, *3*, 1 and references cited therein.

(4) For recent examples, see: (a) Karuso, P.; Taylor, W. C. *Aust. J. Chem.* **1986**, *39*, 1629. (b) Karuso, P.; Bergquist, P. R.; Cambie, R. C.; Buckleton, J. S.; Clark, G. R.; Rickard, C. E. F. *Ibid.* **1986**, *39*, 1643. (c) Molinski, T. F.; Faulkner, D. J. *J. Org. Chem.* **1986**, *51*, 1144.

(5) Hochlowski, J. E.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1983**, *48*, 1141.

(6) All 1D and 2D NMR experiments were measured by using the currently available Wilmad 5-mm microcell assembly (cylindrical bulb, 529-E-5-L-20). Prior to the routine availability of this microcell we special ordered a variety of cell sizes and determined that the 20 mm length (~175 μL volume) cell with rounded top and bottom gave maximum ease in sample locking and optimum S/N, an increase of a factor of 2 compared to the conventional 5-mm tube, in the Varian XL-300 magnet. This was reported at the Varian User's Conference in Dallas/Ft. Worth, Feb 19-20, 1985, and subsequently Wilmad adopted this as one of their standard microcell sizes.

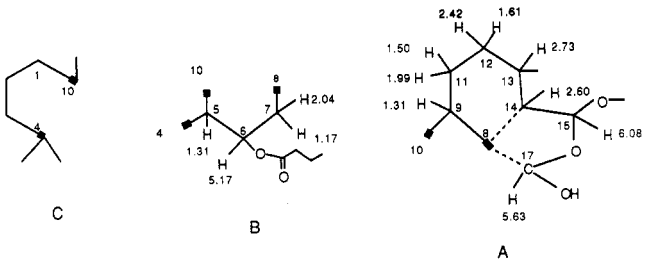
(7) (a) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *42*, 164; (b) **1981**, *44*, 542.

(8) Maudsley, A. A.; Muller, L.; Ernst, R. R. *J. Magn. Reson.* **1977**, *28*, 463. Bodenhauser, G.; Freeman, R. *Ibid.* **1977**, *28*, 471; *J. Am. Chem. Soc.* **1978**, *100*, 320.

Table I. Proton NMR Data for Compounds 1–10[a]

	δ (mult, J in Hz[b])									
H at C	1	2	3	4	5	6	7	8	9	10
5α	1.28 (dd, 14.9, 2.4)	1.31 (d, 12.4)[c]	1.32 (d, 12.4)[c]	1.13 (d, 12.5)[c]	1.14 (d, 12.5)[c]		0.90 (dd, 12.9, 2.4)[c]	1.29 (d, 12)[c]	1.29 (d, 12)[c]	0.95 (dd, 13, 3.5)[c]
6α	1.85 (dt, 14.9, 2.4, 2.4)					1.62 (m)[c]	1.57 (ddt, 12.9, 5.3, 3.3)[c]			1.58 (m)[c]
6β	1.64 (dt, 14.9, 14.9, 2.4)[c]	5.17 (dt, 4.1, 12.4)	5.17 (dt, 4.1, 12.4)	5.39 (dt, 4, 12.5)	5.38 (dt, 4, 12.5)	1.34 (dq, 3, 12.4)[c]	1.23 (dq, 3.4, 12.9)[c]	5.16 (dt, 4.5, 12)	5.17 (dt, 4, 12)	1.26 (dq, 4.5, 13)[c]
7α		1.17 (t, 12.4)[c]	1.19 (t, 12.4)[c]	1.15 (t, 12.5)[c]	1.15 (t, 12.5)[c]	1.08 (dt, 4, 12.4)[c]	0.82 (ddt, 5.3, 3.3, 12.9)[c]	1.50 (t, 12)[c]	1.50 (t, 12)[c]	1.39 (dt, 4, 13)[c]
7β	4.76 (t, 2.85)	2.04 (dd, 4.1, 12.4)	2.04 (dd, 4.1, 12.4)	2.61 (dd, 12.5, 4)	2.62 (dd, 12.5, 4)	1.87 (dt, 12.4, 2.9)[c]	2.54 (dt, 12.9, 3.4)	2.77 (dd, 4.5, 12)	2.76 (dd, 12, 4)	2.54 (dt, 13, 4)
9α		1.31 (d, 12.4)	1.31 (br d, 13)			1.46 (br d, 12.5)[c]		1.50 (br d, 13)	1.50 (br d, 13)	
11α	~1.5	1.50 (m)	1.51 (m)			1.64 (m)	1.50 (m)	1.45 (m)[d]	1.45 (m)[d]	1.46 (m)[e]
11β	1.99 (dq, 12.6, 4.1)	1.99 (dq, 4.0, 12.4)	1.97 (dq, 4.1, 12.3)			1.93 (dq, 4, 12.5)	1.81 (m)	1.70 (m)[d]	1.70 (m)[d]	1.50 (m)[e]
12α	~1.6	1.61 (m)	1.65 (m)	2.03 (m)	2.02 (m)	1.60 (m)	1.78 (m)	1.87 (br d, 13)	1.88 (br d, 13)	1.85 (br d, 13)
12β	2.38 (dm, 14.4)	2.42 (br d, 12.4)	2.43 (br d, 12.3)	1.92 (m)	1.91 (m)	2.40 (br dt, 12.5, 2)	2.50 (m)	1.70 (m)	1.70 (m)	1.71 (m)
13	2.74 (br dd, 11.6, 7.4)	2.73 (br dd, 11.4, 4.1)	2.72 (br dd, 11.4, 4.2)	2.61 (m)	2.61 (m)	2.72 (br dd, 8.3, 12)	2.67 (br dd, 7.7, 5.5)	2.83 (br td, 6.8, 13)	2.84 (ddd, 6.8, 13, 5)	2.82 (ddd, 4.5, 6, 13.5)
14	2.85 (dd, 11.6, 5.9)	2.60 (dd, 11.4, 5.6)	2.60 (dd, 11.4, 5.6)	2.82 (dd, 9, 4.5)	2.81 (dd, 9, 4.6)	2.58 (dd, 12, 6.2)	2.28 (dd, 7.7, 4.4)	2.55 (br dd, 6.8, 2)	2.55 (br d, 6.8)	2.50 (br d, 6)
15	6.04 (d, 5.9)	6.08 (d, 5.6)	6.08 (d, 5.6)	6.02 (d, 4.5)	6.02 (d, 4.6)	6.08 (d, 6.2)	4.04 (br d, 9.9) 4.09 (dd, 9.9, 4.4)	6.04 (s)	6.03 (s)	6.07 (s)
17	5.48 (s)	5.63 (br s)	5.63 (br s)	6.20 (s)	6.20 (s)	5.50 (br s)	9.96 (d, 3.3)	6.40 (s)	6.38 (s)	6.44 (s)
18	0.75	1.01 (s)	1.01 (s)	1.02 (s)	1.03 (s)	0.82 (s)[d]	0.75 (s)[d]	1.01 (s)	1.02 (s)	0.79 (s)[d]
19	0.78	0.88 (s)	0.89 (s)	0.87 (s)	0.87 (s)	0.86 (s)[d]	0.85 (s)[d]	0.85 (s)	0.85 (s)	0.86 (s)[d]
20	0.93	1.01 (s)	1.01 (s)	0.79 (s)	0.80 (s)	0.91 (s)[d]	0.69 (s)[d]	0.85 (s)	0.85 (s)	0.79 (s)
OAc			2.03 (s)	2.06, 2.28 (s)	2.27 (s)			2.03, 2.06, 2.26 (s)	2.02, 2.27 (s)	2.05, 2.13 (s)
PrCO	2.37 (t), 1.67 (sextet)[c]	2.24 (t), 1.6 (sextet)[c]			2.28 (m), 1.65 (m),[c] 0.98 (t)				2.28 (t), 1.70 (sextet)[c]	
	0.99 (t)	0.94 (t)							0.97 (t)	
OMe								3.68	3.69 (s)	3.68 (s)
OH		3.11 (br s)	2.95 (br s)							

[a] In $CDCl_3$ at 300 MHz. [b] Values given in same order as mult designation. [c] Observed by difference double resonance spectroscopy. [d,e] Assignments within a column may be interchanged for entries with the same letter.



a spin system extending to both H-11's as outlined in partial structure A was identified by H/H COSY and difference decoupling data, and this portion of the partial structure was further confirmed by proton carbon correlations (see Table II). However, the signal for the remaining proton signal at position 9 occurs in a complex, unresolved portion of the spectrum and identification of this signal was only possible by using a relayed coherence transfer (RCT) experiment[9] which is shown in Figure 1. Examination of the slices of the RCT contour map provided the chemical shift, multiplicity, and coupling constant information for H-9 (see Table I). A small long-range coupling was revealed by the H/H COSY spectrum between the hemiacetal proton at 5.63 ppm (H-17) and the methine proton at 2.60 ppm (H-14), indicating bonding as C-17→C-8→C-14 so as to result in a *W* arrangement between these two protons.

Partial structure B was readily apparent from H/H COSY and/or difference decoupling results (see Table I). A four-bond coupling as observed (H/H COSY) between signals at 2.60 (H-14) and 1.17 ppm (H-7α), providing support for the C-14, C-8, and C-7 connection.

Analysis of the carbon signals which had not been assigned to partial structures A and B by H/C COSY data revealed that they had the multiplicities and chemical shifts corresponding to partial structure C, which is found in a variety of diterpenes. Combination of partial structures A–C by superposition of the identically numbered quaternary carbons in the drawings yields structure **2**, which possesses the spongiane skeleton, cf. **1**. The chemical shifts of the ring-A carbons and C-6 in **2** were in excellent agreement with those reported[10] for cheilanthatriol 6,19-diacetate which also has a 6α-acetoxy group on a conventional A/B *trans*-decalin diterpene skeleton.

The coupling constants between H-13, H-14, and H-15 of lactone **2** are the same as those observed for **1**, and hence the stereochemistry at these centers is assumed to be the same in both compounds. The 12.4 Hz coupling of the triplet portion of the dt signal due to the H-6 proton is only appropriate for a diaxial relationship with two adjacent protons, and hence H-5 must be axial as shown in **2**. Observation of NOEs between H-17 and both H-20 and H-6 confirmed that C-17 is β-oriented and that H-17 is oriented toward H-6, leaving the hydroxyl group extending over ring C, i.e., 17*R** relative to an arbitrarily selected *S** assignment for C-5. The β-depiction for the 17-OH is thus with respect to ring E viewed in the plane of the page with the C-8/C-7 bond α to this plane.

The functionalities for lactone **3**, $C_{22}H_{32}O_6$, were found to be the same as those for **2**: a γ-lactone [1770 cm^{-1}; ^{13}C NMR 177 ppm (s)]; an ester [1730 cm^{-1}; ^{13}C NMR 170.1 ppm (s)]; an acetal carbon (104.0 ppm); a hemiacetal carbon (103.9 ppm); a hydroxyl group [3440 cm^{-1}; 2.95 ppm (br s)]. The ester was identified as an acetate by the presence of a three-proton singlet at 2.03 ppm plus ^{13}C signals at 22.0 (q) and 170.1 ppm (s). The remaining portions of the carbon spectrum of **3** and the resolved portions of the ^1H NMR spectrum, including proton *J* values and spin-decoupling results, are virtually identical with those of **2**, from which it is inferred that the two compounds have the same functionalized diterpene skeleton. Nuclear Overhauser enhancements were observed between H-20, H-17, and H-6, which confirmed that **3** and **2** have the same relative stereochemistry.

Lactone **4**, $C_{24}H_{34}O_7$, showed no hydroxyl absorption in its infrared spectrum but did exhibit ^1H and ^{13}C NMR data (see Tables I and II) which confirmed the presence of two acetate groups. Comparison of the proton and carbon NMR data of **4** with that of **3** confirmed that these two compounds have the same structure except that in the former the hydroxyl group at C-17 is acetylated, as is most distinctly indicated by the downfield shift of H-17 in **4**. Comparison of the proton chemical shifts for the acetate methyls in **3** vs. **4** reveals that in this series of compounds, an acetate at C-6 absorbs at 2.03 ppm while an acetate at C-17 absorbs at 2.26 ppm. Difference NOE experiments revealed signal enhancements between H-6β and Me-19, Me-20, and the 17-OAc group but no NOE effect between H-6β and H-17. NOE enhancement was observed between H-17 (irradiated) and Me-20, as well as between H-20 (irradiated) and H-17 and H-6β. Hence the acetoxy group at C-17 is assigned the 17α-configuration (17*S**) in contrast to **3** which has the 17β-acetoxy configuration. Acetylation of **3** with Ac_2O/Py yielded a 3:7 mixture of **4** and lactone **11**. Lactone diacetate **4** thus likely arises via prior base-catalyzed epimerization at C-17 via an aldehyde/alcohol intermediate. The 17α-acetoxy orientation in **4** apparently causes a small downfield shift of H-6β and H-7β in **4** relative to **2** and **3**. The signals for C-7, C-17, and C-20 in **4** (and **5**, see below) are shifted upfield relative to those in **2** and **3**. These changes are also consistent with the 17α-acetoxy configuration in **4** (and **5**).

The structure of **11** is based on the similarity of its spectral data with that of **2** and **7** and proton decoupling (see Experimental Section). NOE enhancements were observed between H-6β (irradiated) and H-17, H-19, and H-20 as expected for a 9β-aldehyde group. A coupling constant of near zero for H-15 indicates that this proton must be β-oriented (15*S**) so as to have a dihedral angle of ~90° with H-14.

Lactone **5**, $C_{26}H_{38}O_7$, was identified as the acetate derivative of **2** by comparison of their spectral data; the spectral differences parallel those noted for **3** and **4**. Since the proton singlet for the acetate methyl in **5** occurs at 2.27 ppm, this substituent can be assigned to C-17, and accordingly the butyrate ester must be located at C-6. Since the chemical shift of H-6β and H-7β in **5** and **4** are nearly identical, **5** is also assigned the 17α-acetoxy configuration.

The structure shown for lactone **6**, $C_{20}H_{30}O_4$, was assigned by comparing its spectral data with that of **3**. Thus **6** lacked IR and proton and carbon NMR absorptions for an acetate at C-6 but otherwise displayed spectral data very similar to that of **3**. Notable exceptions were the shift of the C-6 resonance in **6** to 20.2 ppm (t) with concomitant upfield shifts of C-5 and C-7 as expected upon replacing the acetoxy group at C-6 by a hydrogen. The stereochemistry at C-17 was assigned by analogy to **1**–**3** because of the nearly identical ^1H and ^{13}C chemical shifts, and this is confirmed by an X-ray analysis of **6** which was published just after submission of this paper.[4b,11] In the latter work

(9) Bax. A.; Drobny, G. *J. Magn. Reson.* **1985**, *61*, 306.
(10) Gupta, A. S.; Dev. S.; Sangare, M.; Septe, B. Lukacs, G. *Bull, Soc. Chim. Fr.* **1976**, 1880.
(11) Preliminary results of this work were communicated by P. Karuso prior to submission of our manuscript.

Table II. ^{13}C NMR Data for 1–10[a]

C	chem shift, ppm (mult)									
	1[b]	2[c]	3[d]	4[d]	5[d]	6[d]	7[d]	8[c]	9[d]	10[d]
1	38.9 (t)	39.0 (t)	39.0 (t)	38.8 (t)	38.7 (t)	39.1 (t)	39.0 (t)	38.8 (t)	38.8 (t)	37.9 (t)
2	18.7 (t)	18.5 (t)	18.9 (t)	18.2 (t)	18.0 (t)	18.9 (t)	18.7 (t)	18.5 (t)	18.5 (t)	18.4 (t)
3	41.9 (t)	43.1 (t)	43.1 (t)	43.3 (t)	43.2 (t)	41.5 (t)	41.8 (t)	43.2 (t)	43.2 (t)	41.8 (t)
4	32.8 (s)	33.7 (s)	33.4 (s)	33.2 (s)	e	33.3 (s)	33.3 (s)	33.0 (s)	33.0 (s)	33.1 (s)
5	49.6 (d)	58.9 (d)	58.9 (d)	58.4 (d)	58.3 (d)	55.5 (d)[f]	55.9 (d)[f]	58.5 (d)	58.5 (d)	56.3 (d)
6	24.6 (t)	70.1 (d)	70.2 (d)	70.0 (d)	69.8 (d)	20.2 (t)	18.8 (t)	70.4 (d)	70.1 (d)	20.4 (t)
7	72.7 (d)	47.3 (t)	47.3 (t)	43.7 (t)	43.6 (t)	41.9 (t)	35.7 (t)	43.9 (t)	44.0 (t)	38.9 (t)
8	50.8 (s)	47.5 (s)	47.4 (s)	47.2 (s)	e	46.9 (s)	50.8 (s)	48.2 (s)	48.3 (s)	47.6 (s)
9	48.5 (d)	54.9 (d)	54.9 (d)	54.7 (d)	54.6 (d)	56.8 (d)[f]	56.6 (d)[f]	48.8 (d)	48.3 (d)	49.2 (d)
10	39.3 (s)	39.8 (s)	39.8 (s)	39.3 (s)	e	38.1 (s)	37.8 (s)	39.5 (s)	39.5 (s)	38.0 (s)
11	16.2 (t)	16.8 (t)	16.8 (t)	17.7 (t)	17.4 (t)	16.7 (t)	16.4 (t)	16.3 (t)	16.5 (t)	15.8 (t)
12	23.3 (t)	23.9 (t)	23.9 (t)	23.6 (t)	23.6 (t)	24.0 (t)	23.1 (t)	18.1 (t)	18.2 (t)	18.8 (t)
13	37.7 (d)	37.8 (d)	37.7 (d)	35.6 (d)	35.5 (d)	37.9 (d)	38.5 (d)	37.8 (d)	37.9 (d)	37.7 (d)
14	42.2 (d)	49.5 (d)	49.5 (d)	50.2 (d)	50.4 (d)	49.6 (d)	49.0 (d)	56.8 (d)	56.9 (d)	56.9 (d)
15	104.4 (d)	104.2 (d)	104.0 (d)	102.2 (d)	103.5 (d)	104.4 (d)	66.9 (t)	99.3 (d)	99.2 (d)	99.6 (d)
16	177.3 (s)	177.1 (s)	177.0 (s)	176.2 (s)	e	177.3 (s)	177.9 (s)	173.9 (s)	173.6 (s)	174.4 (s)
17	103.6 (d)	103.9 (d)	103.9 (d)	96.6 (d)	96.8 (d)	103.9 (d)	203.8 (d)	98.4 (d)	98.3 (d)	99.2 (d)
18	33.0 (q)	36.1 (q)	36.0 (q)	36.3 (q)	36.2 (q)	33.4 (q)	33.4 (q)	36.4 (q)	36.5 (q)	33.5 (q)
19	21.2 (q)	22.0 (q)	21.9 (q)	22.0 (q)	21.9 (q)	21.5 (q)	21.4 (q)	22.2 (q)	21.2 (q)	21.6 (q)
20	15.3 (q)	17.3 (q)	17.3 (q)	14.5 (q)	14.6 (q)	16.1 (q)	14.9 (q)	14.9 (q)	15.0 (q)	14.0 (q)
OAc			170.1 (s)	169.8 (s)	e			170.5 (s)	169.8 (s)	169.9 (s)
				170.0 (s)				169.9 (s)	169.9 (s)	169.4 (s)
			22.0 (q)	22.0 (q)	20.9 (q)			169.3 (s)		
				22.1 (q)				21.1 (q)	21.3 (q)	21.2 (q)
								21.3 (q)	21.2 (q)	21.3 (q)
								21.9 (q)		
1′	173.0 (s)	172.8 (s)			e				172.4 (s)	
2′	36.7 (t)	36.9 (t)			36.9 (t)				37.1 (t)	
3′	18.7 (t)	18.3 (t)			16.6 (t)				18.6 (t)	
4′	13.8 (q)	13.7 (q)			13.7 (q)				13.7 (q)	
OMe								52.0 (q)	51.9 (q)	51.9 (q)

[a] $CDCl_3$, 75.4 MHz. [b] Data from ref 1 with some signal reassignments as per ref 4. [c] Multiplicities by DEPT experiments, carbon assignments by 2-D ^1H-^{13}C correlation experiments. [d] Multiplicities by DEPT experiments and assignments by analogy to other compounds in the table. [e] Quaternary carbon signals not observed due to small sample size. [f] Assignments within a column may be interchanged.

6 was isolated from a sponge, *Dendrilla rosea*. On the basis of the H/C correlation data for 2 obtained in our work, we believe the chemical shift assignments for C-5, C-9, and C-13 are incorrect in ref 4b.

The ^{13}C NMR data for the nonfunctionalized carbons of 7, $C_{20}H_{30}O_3$, were nearly identical with those of 6 except for minor shifts of C-6, C-7, and C-8, and hence the spongiane skeleton was also inferred for 7. Carbonyl absorption in the IR (1760 cm^{-1}) and a ^{13}C NMR signal at 177.9 ppm (s) indicated that 7 possessed a γ-lactone, but the absence of any ^{13}C NMR resonances corresponding to acetal carbons indicated functional modifications in rings D and E of 7 relative to the preceeding compounds. A doublet ^{13}C NMR signal at 203.8 ppm, a doublet ^1H NMR signal at 9.96 ppm (J = 3.3 Hz), and an IR absorption at 1710 cm^{-1} confirmed the presence of an aldehyde group. Decoupling revealed that the aldehyde proton was long-range coupled to H-7α, thus confirming that the aldehyde carbon was C-17. The triplet ^{13}C resonance at 66.9 ppm could then be assigned to the ether terminus (C-15) of a γ-lactone moiety. Proton spin decoupling also confirmed the H-11 to H-14 spin system. Lactone 7 is assigned the same relative stereochemistry as 2–6 on the basis of the similarity of ^1H and ^{13}C NMR data and observation of NOE effects between H-17 and H-20.

Metabolites 8–10 differed from all the other compounds in this series in that infrared absorption due to a γ-lactone was missing and had been replaced by IR and ^{13}C NMR data corresponding to a methyl ester [1740 cm^{-1}; 174.4 (s), 51.9 ppm (q)]. Two acetal carbon signals were observed in the ^{13}C NMR spectrum of each of these compounds [98–99 ppm (d)]. For ester 8 $C_{27}H_{40}O_9$, proton spin decoupling and H/H and H/C COSY[8] data all confirmed that the partial structure corresponding to C-11 to C-15 was present. While the spin system extending from H-14 to H-12 and one of the H-11's (1.45 ppm) was immediately clear from COSY data (see Figures 2 and 3) and difference decoupling, the second H-11 signal (1.70 ppm) was nearly coincident with H-12β, thus making it difficult to assign directly. This second H-11 resonance could be assigned by observing that it correlated with the same methylene carbon signal as the other H-11 signal at 1.45 ppm.

The chemical shift of H-9 (1.50 ppm) could also not be observed directly since two signals overlap at this position. However, one of the 1.50 ppm signals was readily assigned as H-7α by its coupling to H-6β. The remaining 1.50 ppm signal was then identified by its H/C correlation. Of the two 1.50 ppm proton signals, one correlated with a methylene carbon, which must be C-7, and the other with one of the four upfield methine carbons. Since three of these upfield methine carbons signals could easily be identified as due to C-5, C-13, and C-14 by correlation with their respective associated protons, the remaining methine carbon must be C-9, and hence H-9 absorbs at 1.50 ppm. In the RCT spectrum of 8 (see Figure 2), magnetization is transferred efficiently from H-14 to H-9 under the conditions specified (see horizontal correlation peaks at 2.55 ppm; correlation is also noted between H-13 at 2.83 ppm and H-9, but the cross-peaks do not show the multiplicity of H-9 as well). Examination of the horizontal slice at 2.55 ppm in Figure 2 showed that the H-9 signal is a broad doublet, J = 12 Hz, as expected for the spongiane skeleton. Compounds 9 and 10 were assigned this same partial structure by comparison of their corresponding proton and carbon chemical shifts and decoupling data with that of 8. For esters 8 and 9, the proton chemical shifts and coupling constant data characteristic of H-5, H-6, and H-7 in 2–5 was evident, including J values in-

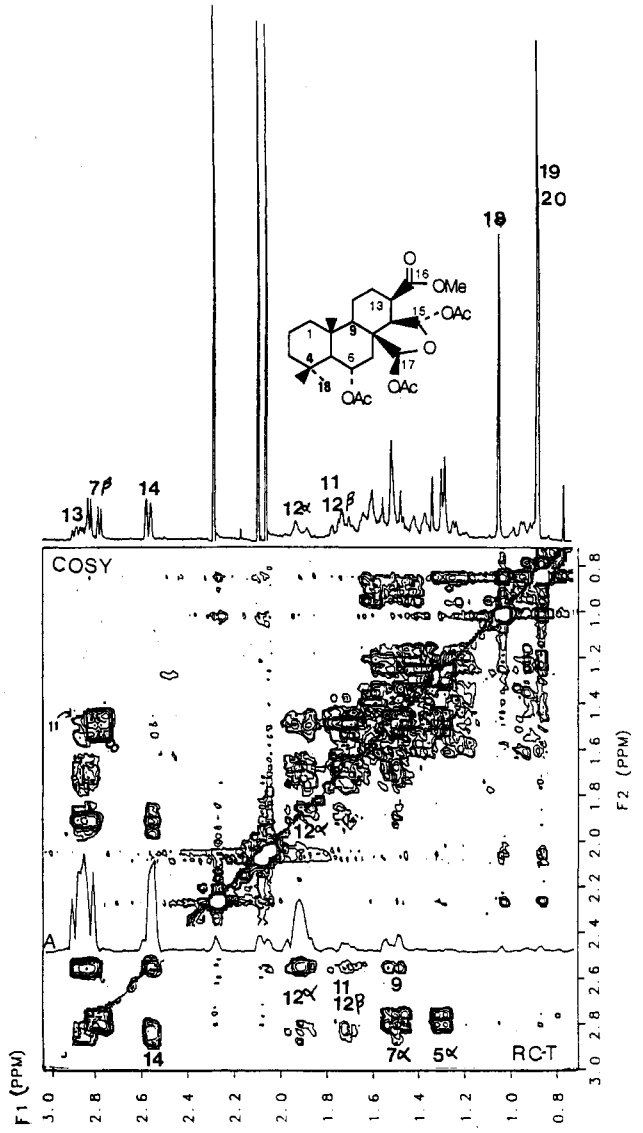


Figure 2. Combined ^{1}H COSY (2D correlated spectroscopy)–RCT (2D relay coherence transfer spectroscopy) plot (upfield region only) of lactone **8** in $CDCl_3$. The RCT mixing time was incremented from 25 to 37 ms; 512 FIDS, 16 scans each, were recorded for both COSY and RCT. The individual spectra were cut along the diagonal into two triangles, and the combined plot was assembled by using the upper left triangle of COSY and the lower right triangle of RCT. A is a slice at 2.55 ppm from RCT spectrum.

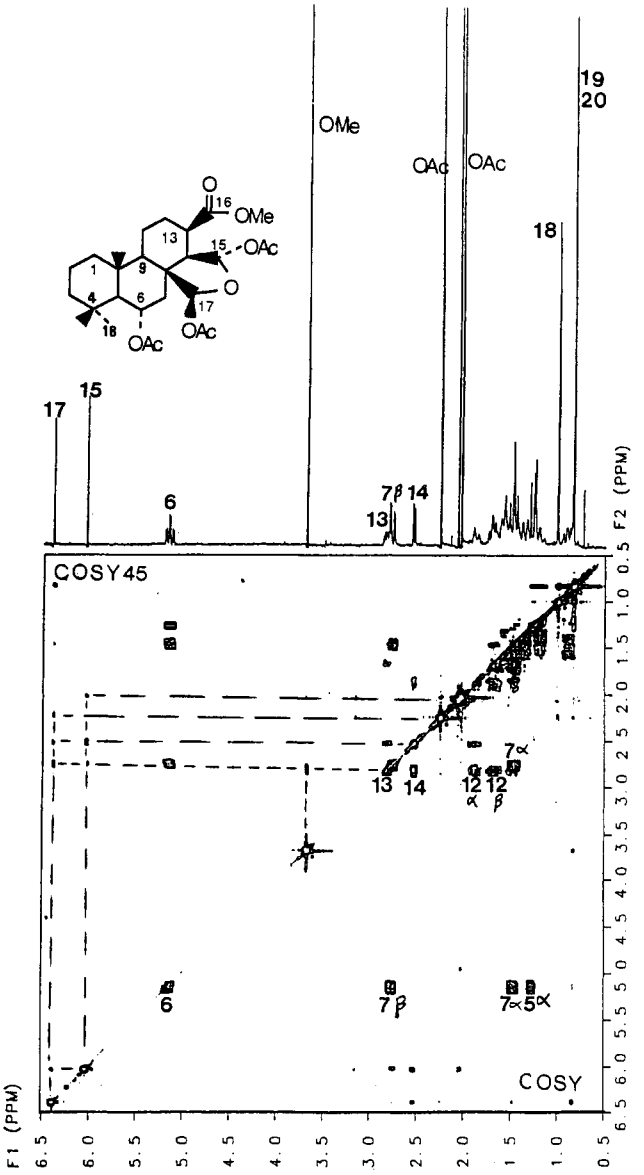


Figure 3. Combined ^{1}H COSY (2D correlated spectroscopy)–COSY 45° plot of lactone **8** in $CDCl_3$. For the COSY 45 experiment, a flip angle of 54° was used for the mixing pulse; delays $\Delta = 0.2$ before data acquisition in the t_1 and t_2 dimensions; 512 FIDS, 32 scans each. For the COSY, 512 FIDS, 16 scans each were recorded. The individual spectra were cut along the diagonal into two triangles, and the combined plot was assembled using the upper left triangle of COSY 45 and the lower right triangle of COSY.

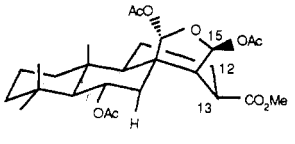
dicative of a 6α-oxygenation. Analysis of the ^{13}C NMR data for **8** and **9** revealed that they clearly match those of **2–5**, except for C-9, C-12, and C-14 (see below), providing the evidence to formulate the same spongiane skeleton for all these compounds but leaving open the question of the relative location and the stereochemistry of the ester and acetal groups.

The functionalized region of **8** was studied in detail by ^{1}H NMR spectroscopy, and the results were then applied to the analogous features of **9** and **10**. The carbomethoxy group in **8** was fixed at C-13 on the basis of a long-range coupling detected (long-range COSY = COSY 45°[7]) between the methoxy protons and H-13. In the COSY 45° spectrum (see Figure 3), coupling was also noted between H-17 and H-15, which was taken as evidence for an ether link between these two sites. Other weak correlations were observed in the long-range COSY of **8** between H-14 and H-15,-17 and between H-17 and H-7α (2.77 ppm). The acetate methyl protons at 2.26 and 2.06 ppm also showed cross-peaks with the acetal protons at H-17 and H-15, respectively. NOE's were confirmed between H-17 and H-6,-20; hence the stereochemistry at C-6, C-8, and C-17 is the same for **8** as for **2** and **3**. The acetal carbon, C-15, must also be β to allow formation of the tetrahydrofuran ring. The acetate group at C-15 is α-oriented (i.e., 15*S** relative to 5*S**), which is opposite to the C-15 configuration in **1–6**. This follows from the nearly zero coupling between H-13/-15 which indicates a nearly 90° dihedral angle between these protons. In **1–6** these protons are nearly eclipsed, resulting in a 4.5–6 Hz coupling.

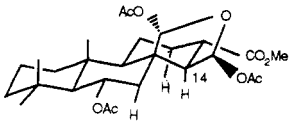
The carbon signals for **8** were unambiguously assigned with the aid of H/C COSY data. These results made it clear that relative chemical shifts of C-9 and C-14 are reversed compared to those in **1–7** (see Table II). This upfield shift of C-9 is most explicable if ring C is in a boat conformation, with C-16 being equatorially disposed and C-13 exerting a shielding effect on C-9.[12] The upfield shift

of C-12 in **8** (and **9** and **10**) is also consistent with the proposed boat conformation, since there is a γ-gauche effect between C-12 and C-15 which is not present in **1–7**. The chemical shift of C-14 in **8** relative to **2–7** is affected by several factors—opening of the lactone ring, insertion of an acetoxy group at C-15, and a ring conformational change. The net result is a downfield shift. A boat conformation for ring C in **8** is also inferred from the 13-Hz coupling between H-13 and H-12β, which indicates diaxial disposition of these protons (see stereostructure **8**). On the other hand in **1–6**, the coupling of H-13 is no greater than 4 Hz with either of the H-12 protons, in agreement with the equatorial orientation of H-13. The ~7-Hz coupling between H-13 and H-14 in **8** is compatible with the ~60° dihedral angle existing between these protons if stereoformula **8** with a boat ring C is assumed. In **1–6** the nearly eclipsed arrangement of H-13/-14 results consistently in a ~11.5-Hz coupling. A small coupling observed (COSY) between H-14 and H-12α in **8** suggests diequatorial orientation of both these protons resulting in a *W* arrangement. This is consistent with stereostructure **8**.

An alternate stereochemical possibility for **8**, namely, **8′**, with a chair ring C and α-oriented carbomethoxy group at C-13 can be ruled out because the axially disposed H-13 in **8′** would be expected to have two large couplings (10–13 Hz) with axial protons at C-12 and C-14. Only one large *J* (13 Hz) is observed for H-13 in **8**.



8



8′

Except for the changes associated with replacing an acetate with a butyrate group, all of the IR and NMR data for ester **9**, $C_{29}H_{44}O_9$, were found to be nearly identical with those of **8**, including the coupling and NOE data relating to stereochemical assignments. Hence **9** could be assigned the structure shown with the only uncertainty being the relative positions of the butyroxy and acetoxy groups. A H/H COSY experiment revealed cross-peaks between the acetate methyl signals, 2.02 and 2.27 ppm, and the acetal protons, 6.03 and 6.38 ppm, respectively, thus limiting the butyroxy group to C-6. The carbon-13 assignments were made by analogy to those of ester **8**. A boat ring-C conformation is predicted for **9** on the basis of the similarity of the presumed correspondence of the C-9 and C-14 chemical shifts between **8** and **9**.

For compound **10**, $C_{20}H_{38}O_7$, the IR and NMR data revealing the presence of a methyl ester and acetal carbons have been mentioned above. The ^{13}C NMR data for the nonfunctionalized carbons of **10** matched closely that of **6**. Combining this information with that for the functionalities outlined above leads to structure **10**. The stereochemistry depicted is based on the similarity of proton coupling constant data for the resolved protons of **10** with those of analogous protons in **8** and **9** and also the correspondence between the ^{13}C NMR data of **10** and relevant portions of **8** and **6**. A boat ring C is also predicted for **10** on the basis of the same arguments as outlined above for **9** and **8**.

Metabolites **2–10** are assumed to be sponge products, but the sponge source has not yet been identified. Following the nomenclature suggested by Kazlauskas et al. the new lactones would be named as follows:[13] **2**, 6α,17β-dihydroxy-15,17-oxidospongian-16-one 6-butyrate; **3**, 6α,17β-dihydroxy-15,17-oxidospongian-16-one 6-acetate; **4**, 6α,17α-dihydroxy-15,17-oxidospongian-16-one 6,17-diacetate; **5**, 6α,17α-dihydroxy-15,17-oxidospongian-16-one 6-butyrate 17-acetate; **6**, 17β-hydroxy-15,17-oxidospongian-16-one; **7**, spongian-17-al-16-one; **8**, methyl 15,16-dideoxy-6α,15α,17β-trihydroxy-15,17-oxidospongian-16-carboxylate 6,15,17-triacetate; **9**, methyl 15,16-dideoxy-6α,15α,17β-trihydroxy-15,17-oxidospongian-16-carboxylate 6-butyrate 15,17-diacetate; **10**, methyl 15,16-dideoxy-15α,17β-dihydroxy-15,17-oxidospongian-16-carboxylate 15,17-diacetate.

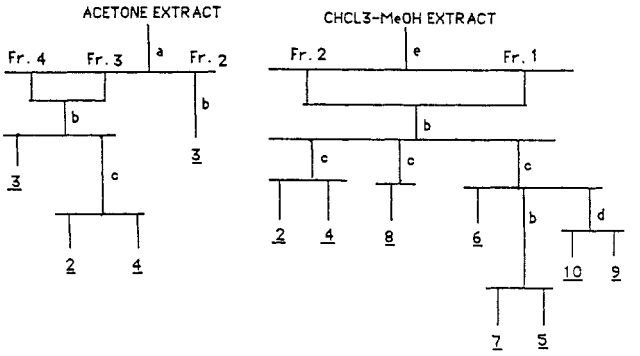


Figure 4. Fractionation of nudibranch extracts: (a) SiO_2, Sep-Pak, acetone-hexane (5:95 → 1:1) collecting five fractions (10 mL each); (b) HPLC, SiO_2, acetone–hexane (18:82); (c) HPLC, C_{18}, MeOH–H_2O (82:18); (d) HPLC, SiO_2, acetone–hexane (5:95); (e) SiO_2, Sep-Pak, acetone–hexane (2:8 → 4:6), collecting three fractions (10 mL each).

Experimental Section

Melting points were taken on an A.H. Thomas Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded at 300 MHz and ^{13}C spectra at 75.4 MHz on a Varian XL-300 spectrometer; chemical shifts are reported in parts per million (δ) downfield from internal tetramethylsilane; all 2D experiments were run by using Varian software V. 6.1c. IR spectra were measured on a Perkin-Elmer Model 298 spectrometer. Low-resolution mass spectra were recorded on a Hewlett-Packard 5985B mass spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Silica gel Sep-Pak used was Waters Associates. Altex 5 μm × 9.6 mm × 25 cm semipreprative silica gel (Li Chrosorb 60) and Adsorbosphere 5 μm × 9.6 × 25 cm reverse-phase C_{18} columns were used for HPLC separation and purification.

Extraction and Isolation Procedures. Freshly thawed specimens (four animals, dry weight, 0.23 g) were allowed to soak in acetone (100 mL) for 1 h and then in $CHCl_3$–MeOH (1:1) (250 mL) overnight. The acetone and $CHCl_3$–MeOH solutions were evaporated to dryness to obtain 20 and 18 mg of extracts, respectively. Both extracts were chromatographed to yield nine diterpenoid compounds as outlined in Figure 4.

^1H and ^{13}C NMR data for all lactones are in Tables I and II.

Lactone 2: 6.9 mg; colorless oil; IR (neat) 3400, 1770, 1730 cm^{-1}; low-resolution mass spectrum (12 eV), *m/z* (relative intensity) 332 (M^+ – $CH_3(CH_2)_2CO_2H$, 2), 314 (5), 286 (100), 271 (16), 258 (18), 229 (22), 217 (10).

Lactone 3: 3 mg; colorless oil; IR (neat) 3400, 1770, 1730 cm^{-1}; low-resolution mass spectrum (12 eV), *m/z* (relative intensity) 332 (M^+–AcOH, 2), 314 (5), 286 (100), 271 (23), 258 (23), 243 (12), 229 (34), 217 (16), 203 (18), 175 (16), 137 (16).

(12) Wehrli, F. W.; Wirthlin, T. *Interpretation of Carbon-13 NMR Spectra*; Heyden: London, 1976; Chapter 2.

(13) For clarity, the stereochemistry in ring E is also designated in the *R*/S** convention. Thus the configuration at C-17 in **2–5** and **8–10** is *R** relative to the enantiomer of **2** having the 5*S** configuration. Note that in going from **3** to **4** and **5** the priority of the groups as well as the relative configuration changes with the consequence that the specification does not change. With the same reference, **11** has the 15*S** configuration.

Lactone 4: 0.4 mg; colorless oil; IR (neat) 1770, 1730 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 375 (M^+ - OAc, 8), 332 (16), 316 (26), 314 (100), 286 (85), 258 (26), 229 (34), 203 (21), 137 (32), 124 (58).

Lactone 5: 0.2 mg; colorless oil; IR (neat) 1770, 1730 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 403 (M^+ - OAc, 6), 332 (17), 314 (100), 286 (51), 258 (9), 229 (14).

Lactone 6: 0.5 mg; white crystals ($CHCl_3$); IR (neat) 3400, 1770 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 288 (M^+ - HCOOH, 100), 273 (13), 245 (6), 207 (8), 177 (20), 149 (18), 137 (17), 123 (10).

Lactone 7: 0.8 mg; colorless oil; IR (neat) 1760, 1710 cm^{-1}; low-resolution mass spectrum (70 eV), m/z (relative intensity) 318 (M^+, 24), 290 (17), 275 (18), 218 (100), 203 (22), 167 (14), 149 (30), 137 (37), 123 (48), 109 (31), 91 (55).

Lactone 8: 5.5 mg; colorless oil; IR (neat) 1735 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 449 (M^+ - OAc, 22), 405 (19), 360 (11), 345 (5), 328 (76), 300 (100), 286 (21), 241 (13), 167 (23), 149 (52).

Lactone 9: 1 mg; colorless oil; IR (neat) 1735 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 477 (M^+ -OAc, 8), 476 (2), 434 (3), 433 (7), 328 (100), 286 (19), 241 (14).

Lactone 10: 1.5 mg; colorless oil; IR (neat) 1735 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 408 (M^+ - CH_2CO, 3), 391 (M^+ - OAc, 20), 390 (M^+ - AcOH, 3) 330 (53), 302 (100), 287 (20), 178 (18), 136 (50).

Acetylation of Lactone 3. Lactone **3** (3 mg) was reacted with acetic anhydride–pyridine (1:1) (0.4 mL) at room temperature overnight. Excess reagents were evaporated under nitrogen, and the residue was chromatographed by HPLC [H_2O/MeOH (15/85); 5 μm, C-18] to give lactone **4** and lactone **11** in a 3/7 ratio.

Lactone 11: colorless oil; IR (neat) 2730, 1780, 1740, 1715 cm^{-1}; low-resolution mass spectrum (70 eV), m/z (relative intensity) 375 (M^+ - OAc, 4), 314 (M^+ - 2AcOH, 16), 286 (100), 258 (51), 229 (33), 203 (28), 175 (32), 161 (25), 137 (88), 123 (46), 109 (75), 105 (52); ^1H NMR (300 MHz, $CDCl_3$) δ 0.77 (3H, s, H-20), 0.84 (3 H, s, H-19), 1.01 (3 H, s, H-18), 1.17 (1 H, t, J = 12.4 Hz, H-7α), 1.25 (1, H, d, J = 12.4 Hz, H-5α), 2.05 (6 H, s, OAc), 2.30 (1 H, d, J = 7.8 Hz, H-14), 2.53, (1 H, br d, J = 15 Hz, H-12), 2.87 (1 H, dd, J = 12.4, 4 Hz, H-7β), 2.98 (1 H, br t, J = 7.8 Hz, H-13), 5.17 (1 H, dt, J = 4, 12.4 Hz, H-6), 6.15 (1 H, s, H-15), 9.96 (1 H, s, H-17); ^{13}C NMR ($CDCl_3$, 75.4 MHz) (multiplicities by DEPT, assignment by analogy to other compounds in this series) δ 16.5 (t, C-11), 17.0 (q, C-20), 18.4 (t, C-2), 21.0 (q, OAc), 22.0 (q, C-19), 22.2 (q, OAc), 22.2 (t, C-12), 33.2 (s, C-4), 35.0 (d, C-13), 36.2 (q, C-18), 39.0 (t, C-1), 39.5 (s, C-10), 42.0 (t, C-3), 43.7 (t,C-7), 49.7 (s, C-8), 53.4 (d, C-14), 55.8 (d, C-9), 58.0 (d, C-5), 68.6 (d, C-6), 93.6 (d, C-15), 168.5 (s, $OCOCH_3$), 169.7 (s, $OCOCH_3$), 175.9 (s, C-16), 203.0 (d, C-17).

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Fontonamide and Anhydrohapaloxindole A, Two New Alkaloids from the Blue-Green Alga *Hapalosiphon fontinalis*

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Fontonamide (**3**) and anhydrohapaloxindole A (**4**) are two minor alkaloids that have been isolated from a cultured strain of the terrestrial blue-green alga *Hapalosiphon fontinalis*. Both compounds appear to be singlet oxygen oxidation products of hapalindole A (**1**), the major alkaloid in this cyanophyte. Hapalonamide A (**5**), the probable precursor of fontonamide, is formed along with **3** and **4** when an oxygen-aerated solution of **1** in aqueous methanol buffered at pH 8 with sodium phosphate and containing a trace of rose bengal is irradiated at room temperature. To date, however, **5** has not been identified as a constituent of *H. fontinalis*.

Hapalindole A (**1**), an unusual chlorine-containing isonitrile, is the major alkaloid in a cultured strain of the terrestrial blue-green alga *Hapalosiphon fontinalis* (Ag.) Bornet (Stigonemataceae) and is responsible in part for the antimycotic activity of this prokaryote.[1,2] Hapalindole A and the corresponding isothiocyanate **2** (hapalindole B) are isolated from a fraction resulting from flash chromatography of the lipophilic extract of *H. fontinalis* on silica gel (TLC grade) using 1:1 hexane/CH_2Cl_2 as the eluant.[1] A more polar fraction eluted with CH_2Cl_2 contains two new compounds which appear to be singlet oxygen oxidation products of hapalindole A, viz. fontonamide (**3**) and anhydrohapaloxindole A (**4**) (Chart I).

Fontonamide (**3**), mp 156–157 °C, $[\alpha]_D$ −141° (c 0.21, $CHCl_3$), is the first compound to be eluted from silica gel with dichloromethane (0.01% yield based on dried alga). The UV spectrum of **3** [λ_{max} nm (ϵ) 240 (5680), 289 (4770), 345 (1630)] indicates that the indole moiety is modified and the ^{13}C NMR spectrum shows that two carbonyls are present. One of the carbonyls has to be a conjugated ketone (singlet at δ 188.97) and the other a formamide (doublet at δ 159.75). The EI mass spectrum reveals the presence of chlorine in the molecular ion (3:1 isotopic cluster at m/z 343,345) and a high resolution mass measurement shows that the elemental composition is $C_{20}H_{22}NO_2Cl$ (obsd m/z 343.1353; calcd m/z 343.1339). The

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(2) Moore, R. E.; Cheuk, C.; Yang, X.-Q. G.; Patterson, G. M. L.; Bonjouklian, R.; Smitka, T. A.; Mynderse, J. S.; Foster, R. S.; Jones, N. D.; Swartzendruber, J. K.; Deeter, J. B. *J. Org. Chem.* **1987**, *52*, 1036.

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