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New Cytotoxic Sesterterpenes from the Marine Sponge *Spongia* sp.

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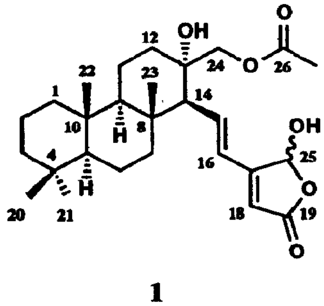
Abstract: Six new cytotoxic sesterterpene lactones, spongianolides A-F (1-6) were isolated from the extracts of the marine sponge *Spongia* sp. The structures were determined by the interpretation of spectral data.

Several natural products bearing the γ-hydroxy butenolide moiety have been isolated from marine sources,[1-6] and some of them exhibited potent anti-inflammatory[7] and anti-tumor properties.[5] In our continuing search for new bioactive substances which modulate protein kinase C (PKC),[8] we examined extracts of a sponge of the genus *Spongia*. In this paper we describe the isolation, structure elucidation and biological properties of a series of new γ-hydroxy butenolides.

The black-colored *Spongia* sp. collected in the channel between Ohio and Grassy Keys (-5 m), Florida (voucher specimen FIT 419 on deposit at Florida Institute of Technology, Melbourne, FL), was freeze-dried (480 g) and extracted with EtOH. The crude extract (7.6 g) was fractionated by gel filtration (Sephadex LH-20, MeOH), and the fractions that inhibited PKC (IC_{50}~15 μg/ml) were combined and repurified by repeated HPLC (C_{18}, MeOH-H_2O) to yield six new sesterterpene lactones, spongianolides A-F (1-6).



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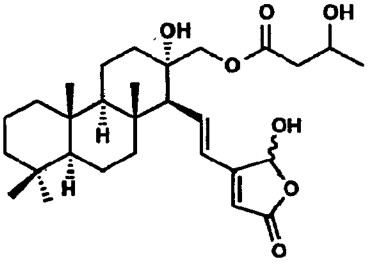

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The molecular formula of spongianolide A (1)[9] was determined to be $C_{27}H_{40}O_6$ by HRFABMS. The IR bands at 3400 and 1737 cm^{-1} indicated the presence of alcohol and ester functions. In addition to resonances for one acetate, the ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed four methyls attached to quaternary carbons, suggesting a sesterterpenoid structure. The high field signals in the ^{13}C NMR spectrum, including four methyls, five methylenes, two methines and three quaternary carbons, were very similar to those assigned to rings A and B of spongiane type diterpenes.[10] The ^{13}C resonances of two olefinic carbons at δ 163 (s,C-17) and 116 (d, C-18), a carbonyl at δ 171 (C-19) and a hemiacetal at δ 99 (C-25) indicated the presence of a γ-hydroxy butenolide moiety.[1-6] The UV absorption band at 264 nm and the ^{1}H and ^{13}C NMR chemical shifts required that the butenolide moiety be conjugated to a *trans*- disubstituted double bond (δ_H 6.69 and 6.46 J =16 Hz, δ_C 141.4 and 126.3). The resonance at 6.46 ppm of the disubstituted double bond in addition coupled to an allylic methine signal at 2.12 ppm. This signal was assigned to H-14 as demonstrated by the correlation of H_3-23 to C-14 and H-14 to C-8 and C-23 in the HMBC experiment, and thus the butenolide side chain is attached to C-14. The acetyl group is attached to a primary alcohol as indicated by the correlations of the carbonyl at δ 171 (C-26) to the methyl at δ 2.08 (H_3-27) and one of the diastereotopic protons at δ 4.39 (H_2-24). The H_2-24 in turn showed a correlation to a quaternary carbon at δ 73 (C-13) which because of its chemical shift should bear a hydroxyl group not accounted so far. Additional HMBC correlations from H_3-20 and H_3-21 to C-3, C-4 and C-5; H_3-22 to C-1, C-5, C-9 and C-10; H_3-23 to C-7, C-8, C-9 and C-14; and H-14 to C-13 supported the structure. The A/B and B/C ring junctions of **1** were confirmed as *trans* on the basis of the observed NOE from H-9 to H-5 and H-9 to H-14 (Figure 1). Likewise, the assignment of β configuration to the substituents at C-13 and C-14 is based on the observed NOE between H_3-23 and one of the H_2-24 and between H_3-23 and H-15.

Spongianolide B (**2**)[11], differed from **1** only in the nature of the ester side chain where the acetyl group is replaced by a γ-hydroxybutyryl group.

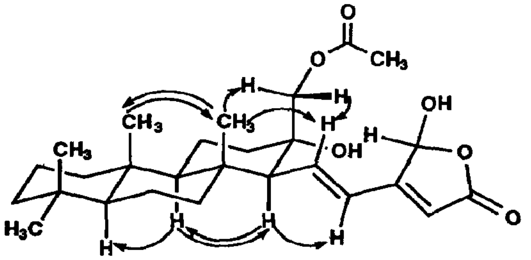
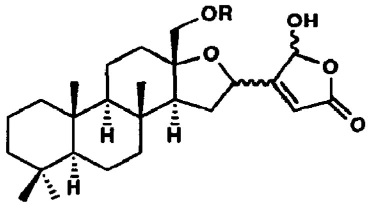


Figure 1. NOE data of spongianolide A (**1**).



3 16*R*, R = $COCH_3$
4 16*S*, R = $COCH_3$
5 16*R*, R = $COCH(OH)CH_3$
6 16*S*, R = $COCH(OH)CH_3$

Comparison of the ^{1}H and ^{13}C NMR spectra of spongianolides C (**3**)[12] and D (**4**)[13] with those of **1** revealed that the olefinic proton and carbon signals at positions 15 and 16 of **1** were replaced by aliphatic methylene and oxygenated methine signals in **3** and **4**. These signals constituted a tetrahydrofuranyl ring system as suggested by proton connectivities of H-14, H-15 and H-16 in the COSY spectra. That **3** and **4** were diastereomeric at C-16 was evident from the coupling constants between H-16 and the diastereotopic H-15 (dd, J=9, 9 Hz in **3** and br d, J=10 Hz in **4**). Biosynthetically, compounds **3** and **4** could be derived from **1** by the addition of 13-OH to C-16 to form a tetrahydrofuran ring. The configuration at C-16 for **3** and **4** was determined by NOE experiments. For **4**, irradiation of H-16 caused significant enhancements of H-24 and H-15β, and irradiation of H_3-23 caused enhancement of H-24 establishing *cis* relationship between H-15β, H-16, H-23 and H-24. On the other hand, significant NOE was observed between H-18 and H-24 in **3**. It was also observed that with 3 (16*R*) and 4 (16*S*) the measured coupling constants between H_2-15 and H-16 were very close to the corresponding values obtained from the molecular mechanics calculations.[14]

Spongianolides E (5)[15] and F (6)[16], isomeric at C-16, possessed a γ-hydroxybutyryl ester side chain instead of an acetyl ester side chain as in 3 and 4.

Lactones 1-5 inhibited PKC at IC_{50} 20-30 μM and did not inhibit the human 85 kD phospholipase A_2. Compounds 1-4 potently inhibited (IC_{50} 0.5-1.4 μM) the proliferation of the mammary tumor cell line MCF-7. After completion of this manuscript, it came to our knowledge that spongianolides C and D (3, 4) have been isolated very recently from a Caribbean sponge.[17] However, these compounds designated as lintenolides A and B were characterized only after acetylation and only partial spectroscopic data were reported for the natural products.

Table 1. ^1H NMR (500 MHz) spectral data of spongianolides A-C (1-3).

	1 (acetone-d_6)	2 (acetone-d_6)	3 ($CDCl_3$)
1	1.69 (br d, 13) 0.87 (m)	1.70 (br d, 13) 0.88 (m)	1.64 (m) 0.83 (m)
2	1.63 (m) 1.40 (m)	1.64 (m) 1.41 (m)	1.62 (m) 1.42 (br d, 13)
3	1.36 (m) 1.17 (ddd, 13.5, 13.5, 4)	1.37 (m) 1.18 (m)	1.36 (m) 1.14 (br dd, 13, 13)
5	0.88 (m)	0.89 (m)	0.86 (m)
6	1.50 (m) 1.41 (m)	1.52 (m) 1.42 (m)	1.60 (m) 1.44 (ddd, 13.5, 13.5, 4)
7	1.52 (m) 1.02 (m)	1.53 (br d, 13) 1.04 (m)	1.59 (m) 1.18 (br dd, 13, 13)
9	0.99 (dd, 13, 3.5)	1.00 (br d, 13)	1.00 (br d, 12)
11	1.62 (ddd, 13, 3.5, 3.5) 1.35 (m)	1.63 (br d, 13) 1.35 (m)	1.78 (br d, 13) 1.26 (br ddd, 13, 13, 13)
12	2.08 (m) 1.37 (m)	2.09 (m) 1.34 (m)	2.19 (br d, 12.5) 1.31 (br dd, 13.5, 13.5)
14	2.12 (d, 10.5)	2.13 (d, 11)	1.81 (m)
15[a]	6.69 (dd, 16, 10.5) 6.68	6.72 (dd, 16, 11) 6.69	2.22 (ddd, 12, 9, 4 5) 1.88 (m)
16	6.46 (d, 16)	6.44 (d, 16)	4.90 (dd, 9, 9)
18[a]	5.92 (s) 5.96	5.94 (s) 5.98	6.04 (s)
20	0.82 (3H, s)	0.83 (3H, s)	0.83 (3H, s)
21	0.85 (3H, s)	0.85 (3H, s)	0.86 (3H, s)
22	0.87 (3H, s)	0.88 (3H, s)	0.85 (3H, s)
23	1.04 (3H, s)	1.06 (3H, s)	0.87 (3H, s)
24[a]	4.39, 4.04 (AB, 11.5) 4.39, 4.00	4.40, 4.15 (AB, 11.5) 4.41, 4.08	4.45, 3.71 (AB, 11.5)
25[a]	6.37 (br s)	6.39 (br s) 6.36	6.14 (br s)
27	2.08 (3H, s)	2.47 (dd, 15, 3) 2.45 (dd, 15, 5.5)	2.10 (3H, s)
28		4.15 (m)	
29		1.17 (3H, d, 6)	

[a] Two sets of signals were observed in 1 and 2 at a ratio of ~1:1. We attribute this to either rotamerization along the C-16/C-17 bond or epimerization at C-25 of the γ-hydroxy butenolide moiety.

Table 2. ^{13}C NMR (75 MHz) spectral data of spongianolides A-F (1, 2 in acetone-d_6 and 3-6 in $CDCl_3$).

	1[a]	2	3[a]	4	5	6
1	40 7 (t)	40.6	40.0	40.0	39.9	40.0
2	19.0 (t)	18.9	18.0	18.0	18.0	17.9
3	42.9 (t)	42.9	42.1	42.0	42.0	42.0
4	34.0 (s)	33.9	33.4	33.3	33.3	33.3
5	57.5 (d)	57.4	57.1	57.0	57.1	57.0
6	19 3 (t)	19.3	18.5	18.5	18 5	18.5
7	43.5 (t)	43.4	41.4	41.2	41.3	41.1
8	39.1 (s)	39.1	37.5	37.4	37.4	37.4
9	61.3 (d)	61.2	61.3	61.3	61.2	61.1
10	38.4 (s)	38.4	36.7	36.5	36.7	36.5
11	19.6 (t)	19.5	19.2	19.0	19.2	18.9
12	38.5 (t)	38.6	34.7	34.8	34.8	34.8
13[b]	73.8 (s) 73.7 (s)	73.9 73.7	82.3	82.4	82.2	82.6
14[b]	68.15 (d) 68.10 (d)	68.00 67.97	61.9	59.6	61.9	59.6
15[b]	141.4 (d) 141.0 (d)	141.5 (d) 141.0	28.7 (t)	28.7 (t)	28.8 (t)	28.4 (t)
16[b]	126.3 (d) 126.1 (d)	126.3 126.0	74.4	72.6	74.5	72.6
17[b]	162.7 (s) 162.5 (s)	162.8 162.5	169.5	169.3	169.5	168.6
18[b]	116.3 (d) 116.1 (d)	116.2 116.0	116.9	118.1	117.0	117.3
19	171.5 (s)	171.5	170.6	170.5	171.1	170.5
20	33.8 (q)	33 7	33.4	33.3	33.3	33.3
21	21.8 (q)	21.8	21.3	21.3	21.1	21.3
22	16.8 (q)	16.8	16.0	16.0	16.0	16.0
23	17.9 (q)	17.8	16.9	16.7	17.0	16.6
24[b]	68.0 (t) 67.9 (t)	68.0 67.7	65 1	63.3	64.9	62.6
25[b]	98.70 (d) 98.65 (d)	98.6	98.7	99.4	98 6	98.1
26[b]	171.4 (s) 171.2 (s)	172.5 172.3	171.4	171.5	172.7	172.7
27[b]	20.9 (q)	44.7 (t) 44.5 (t)	21.0 (q)	21.1 (q)	43.2 (t)	43.7 (t)
28		65.1 (d)			65.0 (d)	65.2 (d)
29		23.6 (q)			22.8 (q)	22.6 (q)

[a] Assignments were confirmed by HMBC (J=6 Hz) experiments.

[b] Two sets of signals were observed in 1 and 2 at a ratio of ~1:1. We attribute this to either rotamerization along the C-16/C-17 bond or epimerization at C-25 of the γ-hydroxy butenolide moiety.

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9. **1** $[\alpha]_D$ = -31.9° (*c* 1.4, MeOH); UV (MeOH): λ_{max} 264 nm (ε = 15820); IR (neat): 3400, 2928, 1737, 1643 cm^{-1}; HRFABMS: Obsd *m/z* 461.2906. Calcd for $C_{27}H_{41}O_6$ *m/z* 461.2902; ^1H NMR and ^{13}C NMR data see Tables 1 and 2.
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11. **2** $[\alpha]_D$ = -25.7° (*c* 1.1, MeOH); UV (MeOH): λ_{max} 265 nm (ε = 14545); IR (neat): 3410, 2928, 1753, 1732, 1642 cm^{-1}; HRFABMS: Obsd *m/z* 505.3149. Calcd for $C_{29}H_{45}O_7$ *m/z* 505.3164; ^1H and ^{13}C NMR data see Tables 1 and 2.
12. **3** $[\alpha]_D$ = +38° (*c* 2.0, MeOH); UV (MeOH): λ_{max} 218 nm (ε = 9340); IR (neat): 3330, 2936, 1741 cm^{-1}; HRFABMS: Obsd *m/z* 461.2906. Cald for $C_{27}H_{41}O_6$ *m/z* 461.2902; ^1H and ^{13}C NMR data see Tables 1 and 2.
13. **4** $[\alpha]_D$ = -16.9° (*c* 1.1, MeOH); UV (MeOH): λ_{max} 218 nm (ε = 9510); IR (neat): 3330, 2931, 1741 cm^{-1}; HRFABMS: Obsd *m/z* 461.2901. Calcd for $C_{27}H_{41}O_6$ *m/z* 461.2902; ^1H NMR (500 MHz, $CDCl_3$): δ 6.19 (br, H-25), 6.03 (s, H-18), 4.99 (br d, 10, H-16), 4.35, 4.05 (AB, 11.5, H_2-24), 2.26 (br dd, 11.5, 3, Hβ-15), 2.23 (ddd, 12, 2, 2, H-12), 2.11 (3H, s, H_3-27), 1.80 (br d, 12, H-11), 1.12 (ddd, 13.5, 13.5, 3.5, H-3), 1.00 (br d, 12, H-9), 0.86 (3H, s, H_3-22), 0.85 (6H, s, H_3-21 and H_3-23), 0.82 (3H, s, H_3-20); ^{13}C NMR data see Table 2.
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15. **5** $[\alpha]_D$ = +45° (*c* 2.4, MeOH); UV (MeOH): λ_{max} 212 nm (ε = 9640); IR (neat): 3350, 2932, 1738 cm^{-1}; HRFABMS: Obsd *m/z* 505.3160. Calcd for $C_{29}H_{45}O_7$ *m/z* 505.3164; ^1H NMR (300 MHz, $CDCl_3$): δ 6.12 (s, H-25), 6.05 (s, H-18), 4.90 (dd, 8, 8, H-16), 4.45, 3.77 (AB, 11.5, H_2-24), 4.24 (tq, 7, 6.5, H-28), 2.51 (2H, d, 7, H_2-27), 1.25 (3H, d, 6.5, H_3-29), 0.98 (br d, 12, H-9), 0.86 (3H, s, H_3-23), 0.85 (3H, s, H_3-21), 0.83 (3H, s, H_3-22), 0.82 (3H, s, H_3-20); ^{13}C NMR data see Table 2.
16. **6** $[\alpha]_D$ = -9.4° (*c* 0.7, MeOH); UV (MeOH): λ_{max} 212 nm (ε = 10,030); IR (neat): 3360, 2931, 1737 cm^{-1}; HRFABMS: Obsd *m/z* 505.3165. Calcd for $C_{29}H_{45}O_7$ *m/z* 505.3164; ^1H NMR (300 MHz, $CDCl_3$): δ 6.14 (br, H-25), 5.97 (s, H-18), 4.95 (br d, 10, H-16), 4.54, 3.99 (AB, 11.5, H_2-24), 4.19 (m, H-28), 2.48 (2H, m, H_2-27), 1.24 (3H, d, 6.5, H_3-29), 0.98 (br d, 12, H-9), 0.84 (9H, s, H_3-21, H_3-22, and H_3-23), 0.82 (3H, s, H_3-20); ^{13}C NMR data see Table 2.
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