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Topsentiasterol Sulfates, Antimicrobial Sterol Sulfates Possessing Novel Side Chains, from a Marine Sponge, *Topsentia* sp.[1]

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Abstract. Five novel antimicrobial steroid sulfates, topsentiasterol sulfates A-E (**1-5**) have been isolated from a marine sponge, *Topsentia* sp. Their structures were elucidated to be 2, 3, 4, 6-tetrahydroxy-14-methyl $\Delta^{9(11)}$ sterol-2, 3, 6-trisulfates with unusual side chains. Topsentiasterol sulfates A-D have side chains terminating in furan-butenolide type functions. Topsentiasterol sulfate E is a C31 compound.

Polysulfated sterols from marine sponges have recently received considerable attention due to their anti-HIV activity.[2] With exception of ibisterol sulfate which has a $\Delta^{9(11)}$ steroid nucleus with methylation at C14,[2] all of these steroid polysulfates have normal steroid carbon skeletons possessing sulfate groups at 2β, 3α positions.[3] In our ongoing search for antifungal compounds from Japanese marine sponges, we found activity in the hydrophilic extract of *Topsentia* sp. collected at Ishigaki Island, Okinawa.[4] From this sponge we have isolated five new trisulfated sterols, topsentiasterol sulfates A-E (**1-5**), with a steroid nucleus related to that of ibisterol sulfate.[2]

The MeOH extract of the frozen sponge was partitioned between ether and water, and the aqueous phase was separated by ODS column chromatography. The antifungal fractions against *Mortierella ramannianus* were subsequently purified by ODS-HPLC to afford **1** (2.8×10^{-3} % yield based on the wet weight of the sponge), **2** (5.0×10^{-4} % yield), **3** (5.7×10^{-4} % yield), **4** (1.6×10^{-3} % yield), and **5** (5.9×10^{-3} % yield).

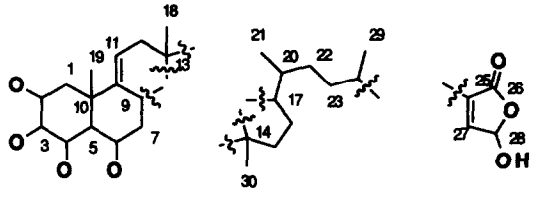
Topsentiasterol A (**1**) had a molecular formula of $C_{30}H_{43}O_{16}S_3Na_3$, which was determined by HRFABMS [$(M-Na)^-$, *m/z* 801.1488, Δ -1.0 mmu]. Intense IR bands at 3500, 1758, and 1238 cm^{-1} suggested the presence of hydroxyl, ester, and sulfate groups, respectively. The ^1H NMR spectrum (Table 1) contained three methyl singlets (0.70, 0.81, and 1.42) and two methyl doublets (0.91 and 1.15) together with a familiar oxygenated methine trio (δ4.81, 4.85, 4.96) associated with 2β, 3α, 6α-trisulfated steroids.[2] In addition, an oxygenated methine (δ 4.46, dt, *J*=1.0, 2.9 Hz) and an olefin (5.34, dt, *J*=6.1, 2.0 Hz) were observed. The ^{13}C NMR spectrum (Table 2) exhibited 5 methyls, 7 methylenes, 8 methines including 4 oxygenated methines, 3 quaternary sp^3 carbons, 2 trisubstituted double bonds, and an ester carbonyl, thereby implying the steroid nature of the molecule.

Table 1. ^{1}H NMR Data of Topsentiasterol Sulfates A-E (**1**-**5**) in CD_3OD at 303K

H	1	2[a]	3[a]	4[a]	5[a]
1α	1.85 (dd, 4.2, 14.7)	1.85	1.85	1.85	1.85
1β	2.32 (br d, 14.6)	2.36	2.35	2.32	2.36
2	4.96 (m)	4.98	4.97	4.96	4.97
3	4.81 (dd, 2.0, 2.9)	4.81	4.81	4.81	4.81
4	4.46 (dt, 1.0, 2.9)	4.47	4.47	4.46	4.47
5	1.47 (dd, 3.0, 11.3)	1.50	1.49	1.48	1.49
6	4.85 (dt, 4.5, 11.2)	4.84	4.83	4.82	4.83
7α	1.55 (q, 11.8)	1.57	1.56	1.55	1.56
7β	2.22 (dt, 11.8, 4.9)	2.23	2.23	2.22	2.23
8	2.49 (m)	2.50	2.49	2.48	2.49
11	5.34 (dt, 6.1, 2.0)	5.35	5.35	5.34	5.34
12α	2.12 (br d, 17.2)	2.13	2.12	2.10	2.11
12β	1.96 (ddd, 1.3, 5.9, 17.2)	1.97	1.96	1.95	1.94
15a	1.38 (m)	1.39	1.38	1.36	1.38
15b	1.44 (m)	1.44	1.45	1.42	1.47
16a	1.33 (m)	1.33	1.32	1.27	1.33
16b	1.92 (m)	1.95	1.91	1.85	1.91
17	1.65 (q, 9.5)	1.67	1.65	1.62	1.63
18	0.70 (s)	0.71 (s)	0.70 (s)	0.68 (s)	0.69 (s)
19	1.42 (s)	1.44 (s)	1.44 (s)	1.42 (s)	1.43 (s)
20	1.42 (m)	1.44 (m)	1.43 (m)	1.37 (m)	1.29 (m)
21	0.91 (d, 6.5)	0.93 (br d)	0.92 (d, 6.3)	0.90 (m)	0.87 (d, 7.0)
22a	1.11 (m)	1.12 (m)	1.10 (m)	1.04 (m)	0.98 (m)
22b	1.47 (m)	1.46 (m)	1.43 (m)	1.40 (m)	1.36 (m)
23a	1.47 (m)	1.48 (m)	1.53 (m)	1.42 (m)	1.09 (m)
23b	1.55 (m)	1.57 (m)	1.53 (m)	1.55 (m)	1.51 (m)
24	2.46 (qt, 7.0, 7.0)	2.58 (m)	2.46 (m)	2.55 (qt, 7.0, 7.0)	
25					1.30 (m)
26		6.10 (br)		7.22 (br s)	0.98 (d, 7.0)
27	6.91 (br s)	5.86 (s)	7.31 (q, 1.7)	6.30 (br s)	1.01 (d, 7.0)
28	6.07 (br s)		4.81 (m)	7.37 (br s)	0.67 (m)
29	1.15 (d, 6 9)	1.19 (br)	1.15 (d, 6.9)	1.16 (d, 6.9)	-0.22 (dd, 4.2, 5.4)
29					0.45 (4.2, 8.6)
30	0.81 (s)	0.82 (s)	0.81 (s)	0.80 (s)	1.09 (d, 6.4)
31					0.81 (s)

[a] Coupling constants in the steroid nucleus were almost identical with those in **1**.

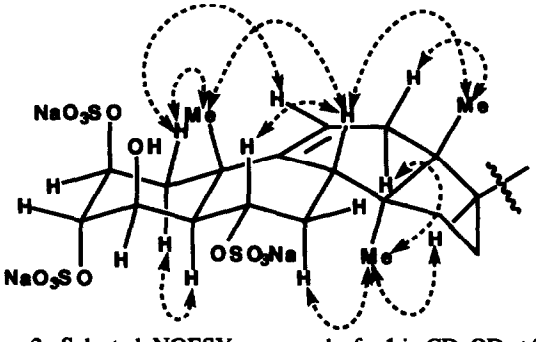
Interpretation of COSY data allowed us to assign structural units **a** and **b** (Scheme 1); W-type couplings were observed between Me19/H1α, Me18/H12β, and Me30/H15b. HMBC crosspeaks observed between Me30/C8, C13, C14, C15 and Me18/C12, C13, C14, C17 inferred the connection of units **a** and **b**, thus revealing a steroid nucleus with a methyl group at C14. The remaining ^{1}H and ^{13}C NMR signals corresponded well with those reported for 4-hydroxy-2-alkyl butenolides (unit **c**).[5,6] The HMBC spectrum confirmed the connectivity of units **b** and **c**.



unit **a** unit **b** unit **c**

Scheme 1. Structural units obtained by interpretation of COSY and ^{13}C NMR data

Stereochemistry of the steroid nucleus was established on the basis of ^{1}H-^{1}H coupling constants and NOESY data. Small coupling constants between H2 and both C1 methylene protons suggested that H2 was equatorial, whereas a conspicuous W-type coupling between H1β and H3 indicated that H3 was equatorial. A large and a small coupling constant between H5 and H6 and between H5 and H4, respectively, implied that both H5 and H6 were axial, while H4 was equatorial. Although the NOESY spectrum at 303K was not informative, a spectrum recorded at 253K gave many crosspeaks. The absence of a crosspeak between Me19 and H5 suggested a *trans*-fused A/B ring junction, while crosspeaks Me19/H8, Me18/H8, Me30/H7α, Me30/H17 led to the assignment of relative stereochemistry at C8, C13, C14, and C17 (Scheme 2).



Scheme 2. Selected NOESY cross peaks for **1** in CD_3OD at 253K

Topsentiasterol sulfate B (**2**) had the same molecular formula as **1**. Although some ^{1}H NMR signals, notably two methyl doublets, were very broad, interpretation of the HMBC spectrum using sharp ^{1}H NMR signals in the steroid nucleus demonstrated that **2** had the same steroid nucleus as **1**. The ^{1}H NMR spectrum exhibited signals for a hemiacetal (δ 6.10) and a trisubstituted olefin (δ 5.86) in the side chain, which suggested that the signal broadening was caused by an equilibrium at the hemiacetal carbon in a 3-alkyl-4-hydroxybutenolide moiety.[6,7] In fact, the broad ^{1}H NMR signals sharpened, showing two sets of signals upon

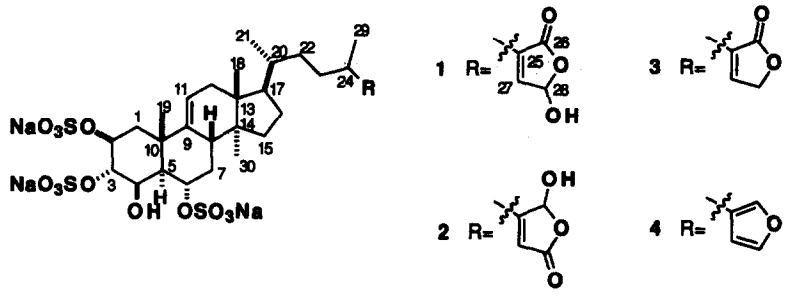


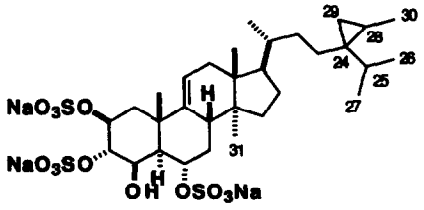
Table 2. ^{13}C NMR Data of Topsentiasterol Sulfates A-E (**1-5**) in CD_3OD at 303K

C	1	2	3	4	5	C	1	2	3	4	5
1	37.4	37.5	37.4	37.4	37.4	16	28.9	29.0	28.9	28.8	29.0
2	75.6	75.7	75.6	75.6	75.7	17	52.1	52.2	52.1	52.2	52.1
3	76.1	76.3	76.0	76.1	76.3	18	15.0	15.0	15.0	15.0	15.0
4	68.5	68.6	68.5	68.5	68.5	19	25.4	25.4	25.4	25.4	25.4
5	47.9	48.1	48.0	48.0	48.0	20	37.2	37.4	37.2	37.4	38.3
6	75.9	76.0	75.9	75.9	76.0	21	18.8	18.8	18.8	19.0	18.9
7	35.4	35.5	35.4	35.4	35.5	22	34.5	34.5	34.6	35.0	34.8
8	41.3	41.4	41.3	41.3	41.4	23	32.6	32.9	32.6	35.4	30.4
9	146.4	146.6	146.4	146.5	146.6	24	31.9	30.7	32.0	31.7	28.8
10	39.4	39.5	39.4	39.4	39.5	25	143.5	177.5	139.4	132.4	33.1
11	117.5	117.6	117.5	117.5	117.5	26	173.6	100.6	176.5	139.1	20.8
12	38.3	38.4	38.3	38.3	38.3	27	145.2	117.2	146.8	110.3	20.8
13	45.5	45.6	45.5	45.5	45.5	28	98.6	173.7	72.1	143.9	18.9
14	49.8	49.0	49.8	49.9	49.8	29	18.8	17.8	18.8	21.7	20.3
15	34.8	34.8	34.8	35.0	34.8	30	19.0	18.8	18.8	18.8	14.0
						31					18.8

cooling to 265K. The COSY spectrum measured at 265K allowed the assignment of connectivity from C20(C21) to C24(C29). Diagnostic HMBC crosspeaks H27/C25, C26, and C28 at 303K, led to placement of the 3-alkyl-4-hydroxybutenolide unit at C24. Stereochemistry of **2** was established as in case of **1**. Incidentally, the NOESY spectrum was measured at 230K for **2**, because there were few NOESY crosspeaks at 265K.

Topsentiasterol sulfate C (**3**) had a molecular formula of $C_{30}H_{43}O_{15}S_3Na_3$ as established by negative ion HRFABMS. NMR data for the steroid nucleus were almost superimposable on those of **1** and **2**. The COSY spectrum revealed connectivities from C20(C21) to C24(C29) and the terminal γ-lactone in the side chain was deduced by interpretation of HMBC data together with NMR signals for an oxymethylene (δH 4.80; δC 72.1 t).

Topsentiasterol sulfate D (**4**) had a molecular formula of $C_{30}H_{43}O_{14}S_3Na_3$ as determined by negative ion HRFABMS. ^{13}C and ^1H NMR data indicated the presence of a 3-substituted furan. Interpretation of the COSY and HMQC spectra indicated that the steroid nucleus and the C20 to C24 (C29) portion of the side chain in **4** were identical with those of topsentiasterol sulfate A (**1**). Therefore, topsentiasterol sulfate D terminate in furan instead of a hydroxybutenolide unit as in **1**.



5

The molecular formula of topsentiasterol sulfate E (**5**) was determined to be $C_{31}H_{48}O_{13}S_3Na_3$ on the basis of negative ion FABMS. Interpretation of COSY, HMQC, and HMBC data allowed assignments of all ^1H and ^{13}C signals, which readily demonstrated that **5** was a 4β-hydroxy derivative of ibisterol sulfate.[2]

Topsentiasterol sulfates A-E were antibacterial against *Pseudomonas aeruginosa* and *Escherichia coli* at a concentration of 10 μg/disk, but only topsentiasterol sulfates D and E showed antifungal activity against *Mortierella remannianus* and *Candida albicans* at 10 μg/disk. This is the first isolation of polysulfated steroids possessing a butenolide or a furan functionality at the end of the side chain.[8,9]

Experimental Section

General. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM600 NMR spectrometer in CD_3OD. FAB mass spectra were measured with a JEOL JMX-SX102 mass spectrometer with triethanolamine as a matrix. Infrared spectra were recorded on a JASCO IR-G spectrometer. Optical rotations were determined on a JASCO DIP-371 digital polarimeter in methanol.

Isolation. The frozen sponge (1.0 kg) was homogenized and extracted with MeOH (3 x 3 L), and the concentrated extract was partitioned between water and ether. The aqueous phase was chromatographed on an ODS open column (4.0 x 5.0 cm) with increasing amounts of MeOH in water. The fraction eluted with MeOH-

H_2O (75/25) (yield, 2.88 g) was subjected to reverse-phase HPLC on Cosmosil $5C_{18}$-AR [MeCN/100 mM $NaClO_4$ in H_2O (38:62)]to furnish **4** (15.9 mg). The fraction eluted with MeOH-H_2O (50/50) (yield, 0.89 g) was chromatographed on Capcellpak (2.5 x 100 cm) with [MeCN/100 mM $NaClO_4$ in H_2O (35:65)] followed by reverse-phase HPLC on an L-column [MeCN/100 mM $NaClO_4$ in H_2O (25:75)] to furnish **2** (5 mg), **1** (27.7 mg), and **3** (5.7 mg). Similarly the fraction eluted with MeOH-H_2O (30/70) was chromatographed on Capcellpak C_{18} followed by reverse-phase HPLC on an L-column [MeCN/100 mM $NaClO_4$ in H_2O (35:65)] to furnish **5** (58.8 mg).

Topsentiasterol sulfate A (1): $[\alpha]_D$ + 48.4° (*c* 0.20, MeOH); FABMS (neg) *m/z* 823 $(M-H)^-$, 801 $(M-Na)^-$, 721, 699, 601 , and 579; IR (KBr) 3500, 2940, 1760, 1645, and 1240 cm^{-1}; HRFABMS *m/z* 801.1500 [$(M-Na)^-$ $C_{30}H_{43}O_{16}S_3Na_2$, Δ -0.9 mmu]; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2.

Topsentiasterol sulfate B (2): $[\alpha]_D$ +13.1° (*c* 0.10, MeOH); FABMS (neg) *m/z* 823 $(M-H)^-$, 801 $(M-Na)^-$, 785 , 699, 579, and 563; IR (KBr) 3435, 2910, 1740, and 1220 cm^{-1}; HRFABMS *m/z* 801.1448 [$(M-Na)^-$ $C_{30}H_{43}O_{16}S_3Na_2$, Δ -6.0 mmu]; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2.

Topsentiasterol sulfate C (3):$[\alpha]_D$ +24.8° (*c* 0.10, MeOH); FABMS (neg) *m/z* 807 $(M-H)^-$, 785 $(M-Na)^-$, 761, and 683; IR (KBr) 3500, 2940, 1740, and 1220 cm^{-1}; HRFABMS *m/z* 785.1502 [$(M-Na)^-$ $C_{30}H_{43}O_{15}S_3Na_2$, Δ -5.7 mmu]; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2.

Topsentiasterol sulfate D (4): $[\alpha]_D$ +9.3° (*c* 0.10, MeOH); FABMS (neg) *m/z* 793 $(M-H)^-$, 771 $(M-Na)^-$, 669, and 567; IR (KBr) 3420, 2910, and 1220 cm^{-1}; HRFABMS *m/z* 769.1597 [$(M-Na)^-$ $C_{30}H_{43}O_{14}S_3Na_2$, Δ -1.3 mmu]; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2.

Topsentiasterol sulfate E (5): $[\alpha]_D$ +58.3 (*c* 0.10, MeOH); FABMS (neg) *m/z* 781 $(M-H)^-$, 769 $(M-Na)^-$, 753, and 667; IR (KBr) 3460, 2930, 1650, and 1220 cm^{-1}; HRFABMS *m/z* 771.2138 [$(M-Na)^-$ $C_{31}H_{49}O_{13}S_3Na_2$, Δ 1.0 mmu]; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2.

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