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Topsentiasterol sulfates with novel iodinated and chlorinated side chains from the marine sponge *Topsentia* sp.

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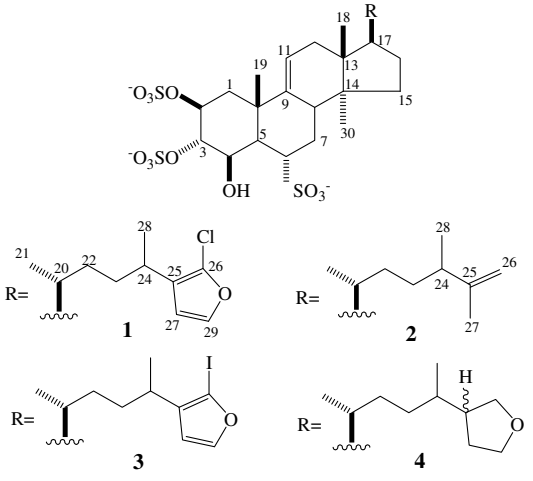
ABSTRACT

Three new marine polar steroids, the first chlorine-containing steroid sulfate (**1**), topsentiasterol sulfate F (**2**) and the first natural iodinated steroid (**3**) have been isolated from the marine sponge *Topsentia* sp. The structures of **1**–**3** were elucidated using NMR and HRESIMS as well as by chemical correlation of **1** with previously known topsentiasterol sulfate D. Compound **1** proved to be an effective inhibitor of *endo*-1,3-β-D-glucanase from the marine mollusc *Spisula sachalinensis*.

Although halogen-containing secondary metabolites are well known and are abundant in marine organisms, only a few iodinated natural products[1–5] or chlorinated steroids[6–11] are known at the present time.

In continuation of our search for new physiologically active marine natural products,[12–14] we have studied topsentiasterol sulfates isolated from a sponge belonging to the genus *Topsentia* (family Halichondriidae, class Demospongiae) collected from Vietnamese waters (depth 10–15 m, Vang Fong Bay 12°35,993N 109°18,596E, June 2007). As a result, a new chlorine-containing steroid named as chlorotopsentiasterol sulfate D (**1**)[15] along with the earlier unknown topsentiasterol sulfate F (**2**)[16] and a unique iodinated steroid (**3**)[17] were isolated and structurally elucidated. Herein we report the structures of **1–3**.

The ethanol extract of the frozen sponge (dry weight 67 g) was concentrated and subjected to column chromatography on Polychrome-1 (powdered Teflon) using H_2O, followed by silica gel chromatography ($CHCl_3$–EtOH–H_2O, 100:125:25) to obtain a crude mixture of topsentiasterol sulfates. This was further separated by preparative HPLC (YMC-ODS-A column, 80:20:1% MeOH–H_2O-1 M CH_3COONH_4) to give a fraction of halogenated topsentiasterol sulfates (38.8 mg) and **2** (0.3 mg, 0.0004% of dry weight) along with previously known topsentiasterol sulfates D[18] (23.3 mg, 0.03% of dry weight) and E[18] (3.5 mg, 0.005% of dry weight). The fraction containing halogenated topsentiasterol sulfates was twice rechromatographed by HPLC (YMC-ODS-A column, 75:25:1% MeOH–H_2O-1 M CH_3COONH_4) affording 0.5 mg of the subfraction, containing iodotopsentiasterol sulfate D (**3**), contaminated with chlorotopsentiasterol D (**1**) and pure **1** itself (15.6 mg, 0.02% of dry weight).



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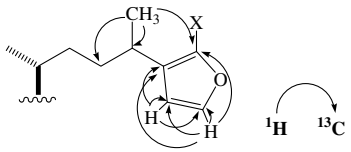
NMR data of the isolated polar steroids (Table 1) showed that these compounds belong to the 4β-hydroxy-14α-methyl steroid trisulfates, discovered by Fusetani's group from *Topsentia* sp. collected at Ishigaki Island, Okinawa.[18] Topsentiasterol sulfates A–E,[18] Sch 55867,[19] and spheciosterol sulfates A–C[20] are the only previously reported members of the 4β-hydroxy-14α-methyl sterol group isolated from sponges.[20] Similar to the related ibisterol,[21] these unusual steroid polysulfates contain a $\Delta^{9(11)}$-unsaturated steroid nucleus and sulfate groups at the 2β, 3α, and 6α positions. ^{1}H and ^{13}C NMR spectra of **1** were similar to those of other topsentiasterol sulfates,[18] including the signals of three angular methyl groups, a C-9(11)-double bond, and four oxygenated methines. These NMR spectra differed from those of topsentiasterol sulfate D mainly in the absence of a broad singlet, H-26, of a 3-substituted furan moiety. Consequently, the structure **1** was suggested as an analogue of topsentiasterol sulfate D with modification at C-26, and subsequently confirmed by analysis of COSY, DEPT, HSQC, and HMBC data (Fig. 1).

Table 1
NMR data for chlorotopsentiasterol sulfate D (**1**) and topsentiasterol sulfate F (**2**) in CD_3OD

Position	**1**			**2**	
	δ_H (mult, *J* in Hz)[a]	COSY	δ_C[b]	δ_H (mult, *J* in Hz)	COSY
1a	1.82 dd (3.7; 14.6)	H1b, H2	38.0	1.86 m	H1b, H2
1b	2.40 br d (14.6)	H1a		2.28 br d (13.9)	H1a
2	4.95 m	H1a, H1b, H3	76.3	4.94 m	H1a
3	4.74 dt (2.6; 1.0)	H2, H4, H1b	76.6	4.77 m	
4	4.48 dt (2.8; 1.2)	H3, H5, H6	69.2	4.45 m	H5
5	1.51 dd (2.9; 11.4)	H4, H6	48.6	1.46 m	H4, H6
6	4.81 dd (4.5; 11.3)	H5, H7a, H7b	77.1	4.81 m	H7a, H7b
7a	1.56 m	H6, H7b, H8	36.0	1.56 m	H6, H7b, H8
7b	2.21 dt (5.1; 11.7)	H6, H7a, H8		2.22 dt (4.3; 12.4)	H6, H7a, H8
8	2.48 m	H7a, H7b	42.0	2.49 m	H7a, H7b
9	—		147.0	—	
10	—		40.1	—	
11	5.34 m	H12a, H12b	118.2	5.35 m	H12a
12a	2.11 br d (17.2)	H12b	38.9	2.12 br d (17.2)	H12b
12b	1.97 m	H11, H12a		1.96 m	H11, H12a
13	—		46.2	—	
14	—		48.4[c]	—	
15a	1.36	H15b	35.4	1.28 m	
15b	1.42 m	H15a		1.28 m	
16a	1.25 m	H16b, H17	29.4	1.40 m	
16b	1.85 m	H15a, H15b, H16a		1.90 m	
17	1.63 m	H16a, H16b	52.8	1.65 m	
18	0.67 s		15.6	0.70 s	
19	1.43 s		26.0	1.42 s	
20	1.36 m	H17, H21	37.9	1.36 m	H21
21	0.89 d (6.5)	H20	19.6	0.90 m	H20
22a	1.03 m	H22b	35.8	0.87 m	
22b	1.37 m	H21, H22a		1.32 m	
23a	1.36 m	H22a, H23b	35.5	1.14 m	
23b	1.60 m	H23a		1.45 m	
24	2.56 m	H23a, H28	32.2	2.05 m	H28
25	—		133.5	—	
26	—	H27	126.4	4.66 s	H27
27	6.39 d (2.0)	H26	112.4	1.65 br s	H26
28	1.14 d (7.0)	H24	21.7	1.00 d (6.9)	H24
29	7.36 d (2.0)		143.5	—	
30	0.80 s	H18	19.4	0.81 s	

[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.
[c] Assignment made by HMBC.



X=H Topsentiasterol sulfate D; X=Cl Chlorotopsentiasterol sulfate D

Figure 1. Partial structure of the side chain in topsentiasterol sulfate D and **1** with selected HMBC correlations.

The molecular formula $C_{30}H_{45}O_{14}S_3Cl$ of **1** was obtained from HRESIMS measurement of the ion peaks $[M_{3H}H]$ (*m/z* 759.1575; Δ 0.8 ppm) and $[M_{3H}-H-SO_3]^2$ (*m/z* 339.0999; Δ 5.1 ppm). This steroid showed MS isotopic patterns characteristic of monochlorinated compounds ($[M_{3H}-H]^-$:$[(M_{3H}-H)+2]^-$ = 3:1). All other structural features of **1** were supported by COSY, DEPT, HSQC, and HMBC data. To confirm that **1** is a derivative of topsentiasterol sulfate D, the chemical correlation of **1** with topsentiasterol sulfate D was carried out. Both **1** and topsentiasterol sulfate D were hydrogenated over Adams' catalyst to give the same tetrahydrofuran derivative **4** identified by NMR and HRESIMS data.[22]

It is of special interest that chlorotopsentiasterol sulfate D (**1**) contains a chlorofuran fragment. This structural feature had never been found previously in natural products.[23]

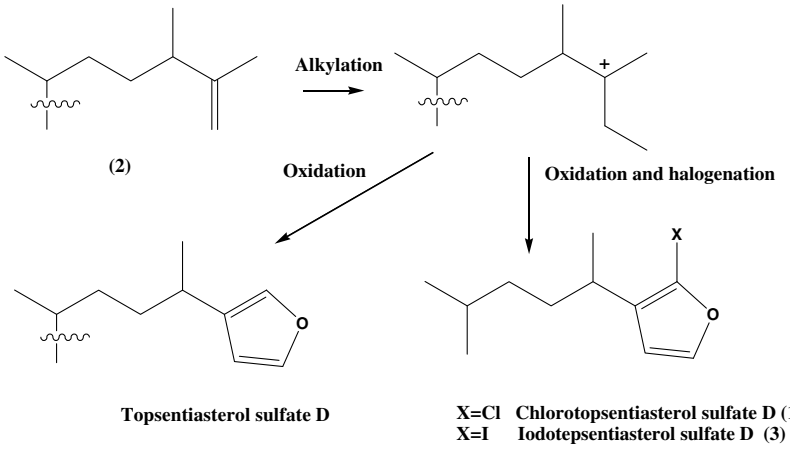
Another new steroid, named topsentiasterol sulfate F (**2**) had the molecular formula of $C_{29}H_{48}O_{13}S_3$ as determined by HRESIMS measurement of the ion peak $[M_{3H}-H]$ (*m/z* 699.2169; Δ 1.4 ppm.). Interpretation of the ^{1}H NMR and COSY spectra indicated that the steroid nucleus of **2** is identical to that of topsentiasterol sulfates. The remaining signals corresponded well with those reported for the 24-methyl-25(26)-ene side chains of codisterol from the marine alga *Codium fragila*[24] and halistanol sulfate H from the marine sponge *Pseudoaxinissa digitata*.[25] On the basis of all the above-discussed data, the structure of topsentiasterol sulfate F was established as **2**.

We have partly separated compound **3** from the fraction contaminated with **1**. This compound was identified as an analogue of topsentiasterol sulfate D with iodination at C-26 by ^{1}H NMR and MS. Compound **3** had the molecular formula $C_{30}H_{45}O_{14}S_3I$ as determined by HRESIMS measurement of the ion peaks $[M_{3H}-H]^-$ (*m/z* 851.0970; Δ 3.0 ppm.) and $[M_{3H}-H-SO_3]^2$ (*m/z* 385.0668; Δ 3.9 ppm). Its NMR spectra were closely related to those of **1** differing mainly in chemical shifts in the furan moiety. To the best of our knowledge, iodotopsentiasterol sulfate (**3**) is the first example of a natural iodine-containing steroid.

It may be that topsentiasterol sulfates with unusual side chains such as in **1**, **3**, and topsentiasterol sulfate D are biosynthesized from a sterol (or sterol polysulfate) precursor having side chains of the codisterol type, for example **2**. This biosynthesis would be realized through an additional alkylation followed by oxidation and, in the case of **1** and **3**, halogenations (Scheme 1).

Steroids related to topsentiasterol sulfates demonstrate diverse biological activities, including anti-HIV, antibacterial and antifungal properties. For example, early findings on the biological properties of topsentiasterol sulfates included antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*,[18] and antifungal activity (steroid Sch 575867 from a marine sponge of the family Astroscleridae).[19] Spheciosterol sulfates A–C from *Spheciospongia* sp. inhibited PKCζ.[20]

We have studied the effects of **1**, **2**, and topsentiasterol sulfates D and E as possible inhibitors of 1,3-β-glucanases.[26] All the substances studied did not influence the activity of exo-1,3-β-D-glucanase from the marine filamentous fungus *Chaetomium indicum*,[29] but appeared to be inhibitors of *endo*-1,3-β-D-glucanase from the marine mollusc *Spisula sachalinensis*.[30] The efficiency of *endo*-1,3-β-D-glucanase inhibition was found to depend on the



Scheme 1. Hypothetical pathways for the biosynthesis of the side chains in furan-containing topsentiasterol sulfates.

structural peculiarities of the compounds tested. Compounds **1**, **2**, and topsentiasterol sulfates D and E inhibited *endo*-1,3-β-D-glucanase from *S. sachalinensis* with IC_{50} values of 5.4, 49.6, 12.4, and 7.4 μM/L, respectively.

Thus, topsentiasterol sulfates represent a new structural group of *endo*-1,3-β-D-glucanase inhibitors. Earlier, other polyhydroxysteroidal inhibitors of these enzymes were found.[31,32] Future studies will show whether the molecular actions of these two inhibitory groups are similar.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.10.007.

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15. Chlorotopsentiasterol sulfate D (**1**): white solid; $[\alpha]_D^{20}$ +46 (*c* 0.09, MeOH); ^1H, ^{13}C NMR data, Table 1; HRESIMS *m/z* 759.1575 $[M_{3H}-H]^-$ (calcd for $C_{30}H_{44}O_{14}S_3Cl$ 759.1582).
16. Topsentiasterol sulfate F (**2**): white solid; $\{[\alpha]_D^{20}$ +40 (*c* 0.03, MeOH); ^1H NMR data, Table 1; HRESIMS *m/z* 699.2169 $[M_{3H}H]$ (calcd for $C_{29}H_{47}O_{13}S_3$ 699.21788).
17. Iodotopsentiasterol sulfate D (**3**): selected ^1H NMR data (500 MHz, CD_3OD): 0.68 (3H, s, H-18); 0.81 (3H, s, H-30), 0.89 (3H, d, *J* = 6.4, H-21), 1.13 (3H, d, *J* = 7.0, H-28), 1.43 (3H, s, H-19), 1.85 (1H, m, H-1a), 2.22 (1H, m, H-7b), 2.32 (1H, br d, *J* = 14.4, H-1b), 2.48 (1H, m, H-8), 5.34 (1H, m, H-11), 6.32 (0.8H, d, *J* = 2.0, H-27, for **3**), 6.39 (0.2H, d, *J* = 2.0, H-27, for **1**), 7.60 (0.8H, d, *J* = 2.0, H-29 for **3**), 7.36 (0.2H, d, *J* = 2.0, H-29 for **1**); HRESIMS *m/z* 851.0970 $[M_{3H}-H]^-$ (calcd for $C_{30}H_{44}O_{14}S_3I$ 851.0943).
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