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# IBISTEROL SULFATE, A NOVEL HIV-INHIBITORY SULFATED STEROL FROM THE DEEP WATER SPONGE *TOPSENTIA* SP.[1]

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**Summary:** The novel sulfated sterol ibisterol sulfate (**1**) was isolated from the deep water Caribbean sponge *Topsentia* sp. The combination of both a $\Delta^{9(11)}$ olefin and a methyl group at C-14 has not previously been reported in sponge sterols.

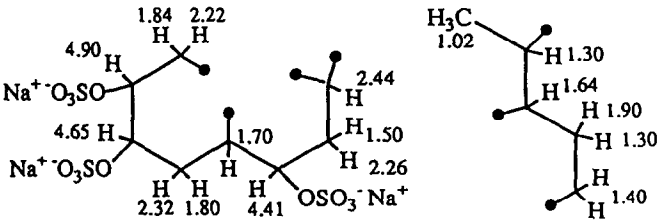
Extensive studies of sponge sterols have yielded a large number of unusual and structurally diverse sterols,[2] including numerous polyhydroxylated or sulfated compounds. Halistanol sulfate, the most common sulfated sterol isolated from sponges, was first obtained from *Halichondria* cf. *moorei*,[3] but has recently been reported from another *Halichondria* sp.[4] and from *Epipolasis* sp., along with five related sulfated sterols.[5] The weinbersterol disulfates, recently isolated from *Petrosia weinbergi*, contain an uncommon cyclopropyl group in their side chains.[6]

As part of our continuing effort to identify novel anti-HIV chemotypes, we have isolated cytoprotective sulfated sterols from several marine sponges. Halistanol sulfate was isolated from *Aaptos* sp., *Trachyopsis* sp., and two species of *Pseudaxinyssa*, while bioassay guided fractionation of a deep water collection of *Topsentia* has yielded the unique, new sulfated sterol ibisterol sulfate (**1**).[1b]

The sponge, *Topsentia* sp., was collected at a depth of 359m off Settlement Point, Grand Bahama Island, the Bahamas. Both the organic and aqueous extracts of the sponge tested positive in the NCI's primary anti-HIV screen[7] and the antiviral activity was traced to sulfated sterols. Five grams of the aqueous extract were taken through our standard precipitation procedure[8] to remove polysaccharides and other high molecular weight biopolymers. Ibisterol sulfate was isolated from the supernatant by permeation through Sephadex LH-20 ($H_2O$), followed by HPLC (cyano and $C_{18}$), to yield 12 mg (0.24%) of ibisterol sulfate. This compound was cytoprotective against HIV-1 in the NCI primary screen ($EC_{50}$ 10 $\mu$g/ml).

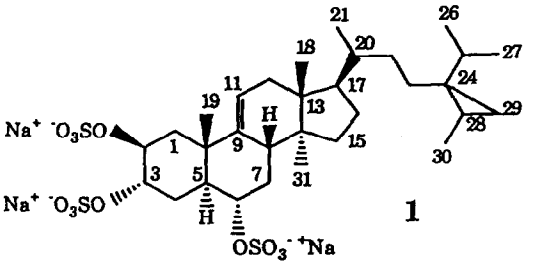
The molecular formula for **1** was established by negative ion FABMS providing a pseudomolecular ion $(M-Na)^-$ at *m/z* 755.2180 for $C_{31}H_{49}O_{12}S_3Na_2$ (calc. 755.2182). The IR spectrum contained an absorbance at 1247 $cm^{-1}$, consistent with the presence of sulfate. The $^1H$ NMR spectrum of ibisterol sulfate, with signals at 0.45 and -0.22 ppm, immediately suggested the presence a cyclopropyl group; the spectrum also contained three methyl singlets (1.20, 0.85 and 0.68 ppm) and four methyl doublets (1.09, 1.01, 0.98 and 0.87 ppm), as well as three

oxygenated methine resonances (4.90, 4.75 and 4.41 ppm) and a trisubstituted olefinic proton at 5.36 ppm. The $^{13}C$ and DEPT spectra contained only 30 signals: 10 methines, eight methylenes, seven methyls and five quaternary carbons. The remaining carbon was identified with the aid of an HMQC experiment, which established that the signal appearing at 34.80 ppm was, in fact, two methylenes. The complete $^{1}H$ and $^{13}C$ assignments are summarized in Table 1. COSY and TOCSY NMR experiments established the presence of a methylcyclopropyl group with a methine proton at 0.65 ppm (m) coupled to methylene protons at 0.44 ppm (dd,$J$=8.9, 4.4 Hz) and -0.22 ppm (br t, $J$=4.4) and to a methyl signal 1.09 ppm (d, $J$=6.3); an isolated isopropyl group 1.30 ppm (1H, m), 0.98 ppm (3H, d, $J$=6.9) and 0.87 ppm (3H, d, $J$=6.4); the olefinic proton at 5.36 ppm (br d,$J$=4.9) coupled to methylene protons at 2.11 ppm (br d, $J$=17.6) and 1.94 ppm (dd, $J$=17.1, 4.4); and two isolated methylenes at δ 1.50 (2H, m), 1.36 (1H, m) and 1.28 (1H, m), along with the partial structures shown below.




The experimental data accounted for all elements present in the molecule except the four quaternary carbons and the three singlet methyl groups. HMBC experiments established the connections between the singlet methyls and their associated quaternary carbons and linked the COSY/TOCSY spin systems leading to the proposed structure for ibisterol sulfate (**1**). For example, the C19 methyl protons (1.20 ppm) correlated with C1, C5, C9 and C10, which placed this methyl substituent on C10 (40.06 ppm, the A/B ring juncture) and located the olefin in the $\Delta^{9(11)}$ position. Correlations seen from C10 to protons on carbons 1, 5 and 11 and from C9 (145.80 ppm) to protons on carbons 7, 8, and 12 provided additional support for these assignments. In a similar manner, the quaternary carbon at δ48.09 was established at position 14 based on correlations to H12b, H15 and H18 and was found to be substituted with the methyl at δ0.85. Finally, correlations from the quaternary carbon at δ45.51 established its position as C13, substituted by the methyl group at δ0.68, and the quaternary carbon at 28.81 ppm was determined to be the third carbon of the cyclopropyl ring. This carbon (C-24) was further substituted by the methine carbon of the isopropyl group. These correlations established the side chain as identical to that described for the weinbersterols.[6]

The relative stereochemistry for ibisterol sulfate was established by a series of difference nOe experiments and coupling constant analysis. Protons on C-2 and C-3 were assigned as equatorial due to the absence of any large vicinal couplings; this placed the bulky sulfate groups in the axial position, consistent with other 2,3-disulfated sterols from sponges. Observed nOes between Me19 and H4β, H6, and H8 along with the large vicinal




1

Table 1. NMR Data for Ibisterol Sulfate[a]

| C# | $^{13}C$ δ | MULT[b] | $^{1}H$ CHEM SHIFT[c] (mult, *J* Hz) | HMBC CORREL[d] 8.3 Hz | HMBC CORREL[d] 5.5 Hz | NOE[e] |
|---|---|---|---|---|---|---|
| 1 | 37.55 | $CH_2$ | α 1.83 (dd, 14.0, 2.9) | | 10 | |
| | | | β 2.21 (br d, 14.0) | 2, 5, 10, 19 | 3 | 2, 4β, 11, 19 |
| 2 | 75.61 | CH | 4.90 (br s) | | 3 | |
| 3 | 75.39 | CH | 4.75 (br d, 2.4) | | | 2,4α |
| 4 | 25.38 | $CH_2$ | α 2.34 (br d, 14.5) | | | 3, 5 |
| | | | β 1.80 (dd, 14.5, 2.4) | | | |
| 5 | 43.50 | CH | 1.69 (dt, 10.8, 2.4) | 6, 10, 19 | 4, 6, 19 | 4α |
| 6 | 78.84 | CH | 4.41 (dt, 10.8, 4.2) | | | 7β, 19 |
| 7 | 35.34 | $CH_2$ | α 1.50 (m) | 6, 8, 31 | 6, 8 | 31 |
| | | | β 2.26 (dq, 11.7, 4.2) | 6, 9 | 5, 6, 8, 9 | |
| 8 | 41.20 | CH | 2.43 (br d, 12.7) | 15, 7 | | 18, 19 |
| 9 | 145.80 | C | ---- | | | |
| 10 | 40.06 | C | ---- | | | |
| 11 | 117.97 | CH | 5.36 (br d, 4.9) | 8, 10, 12, 13 | 8, 10, 13 | 1β |
| 12 | 38.37 | $CH_2$ | α 2.11 (br d, 17.4) | 9, 11, 13, 18 | | |
| | | | β 1.94 (dd, 17.4, 4.9) | 9, 11, 14, 18 | 9, 11, 13, 14, 18 | |
| 13 | 45.51 | C | ---- | | | |
| 14 | 48.09 | C | ---- | | | |
| 15 | 34.80 | $CH_2$ | 1.40 (m) | 14, 31 | 8, 14, 31 | |
| 16 | 28.96 | $CH_2$ | α 1.90 (m) | | 13, 18 | |
| | | | β 1.30 (m) | | 31 | |
| 17 | 52.15 | CH | 1.62 (dt, 10.3, 9.3) | 18 | 13, 18, 20 | |
| 18 | 15.01 | $CH_3$ | 0.68 (s) | 12, 13, 14, 17 | 12, 13, 14, 17, 20 | 8, 12β |
| 19 | 22.60 | $CH_3$ | 1.20 (s) | | 1, 5, 9, 10 | 1β, 4β, 6, 8 |
| 20 | 38.27 | CH | 1.30 (m) | | 17 | |
| 21 | 20.77 | $CH_3$ | 1.01 (d, 7.3) | | | |
| 22 | 34.80 | $CH_2$ | 1.36 (m) | | | |
| | | | 1.28 (m) | 20, 21, 23, 24 | 23, 24 | |
| 23 | 30.41 | $CH_2$ | 1.50 (m) | 24 | 24, 28 | |
| 24 | 28.81 | C | ---- | | | |
| 25 | 33.05 | CH | 1.30 (m) | 23, 24, 26, 27 | 23, 24, 26, 27 | |
| 26 | 18.92 | $CH_3$ | 0.98 (d, 6.9) | 24, 25, 28 | 24, 25, 28 | |
| 27 | 20.69 | $CH_3$ | 0.87 (d, 6.4) | 26 | | |
| 28 | 18.90 | CH | 0.65 (m) | | | |
| 29 | 20.28 | $CH_2$ | 0.44 (dd, 8.9, 4.4) | 28 | 23, 24, 28, 30 | |
| | | | 0.22 (br t, 4.4) | 28 | 25, 26, 28 | |
| 30 | 14.07 | $CH_3$ | 1.09 (d, 6.3) | | 28, 29 | |
| 31 | 18.75 | $CH_3$ | 0.85 (s) | | 8, 13, 14 | 7α, 12α, 17 |

[a]All spectra recorded in MeOH-$d_4$ at 500 MHz. [b]Determined by DEPT/HMQC experiments. [c]Assignments based on HMQC results. [d]Carbon number to which the proton is correlated. [e]Irradiation of this proton resulted in nOe to the protons listed.

coupling between H5 and H6 suggested a trans A/B ring juncture. This was supported by observed nOes between H5 and H4α. NOes were also observed between Me31 and H7α, H12α and H17 and between Me18 and H8, H12β; these data supported a trans C/D ring juncture. The observed nOe data are summarized in Table 1 and are consistent with the relative stereochemistry as drawn.

The proposed structure for ibisterol sulfate is a striking new addition to the large number of unusual sterols which have been isolated from sponges. The cyclopropyl bearing side chain was originally described by Kokke et al from a chrysophyte alga,[9] while the weinbersterol sulfates[6] were the second group of sterols found to contain this unusual sidechain and the first from a sponge. However, the presence of a $\Delta^{9(11)}$ double bond with methyl substitution at position 14 is more interesting from a biosynthetic point of view. This pattern is quite rare, having been observed from the marine environment only in sterols from holothurians.[10] Work by Cordeiro and Djerassi suggested that this pattern is biosynthesized in sea cucumbers directly from parkenol (the $\Delta^{9(11)}$ isomer of lanosterol), rather than from lanosterol or cycloartenol.[11] This pathway has not been observed in sponges, however, and makes this sterol an intriguing target for biosynthetic study.

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