Vol. 52, No. 3, pp. 657-659, May-Jun 1989

A NEW STEROL SULFATE FROM THE MARINE SPONGE *STYLOPUS AUSTRALIS*

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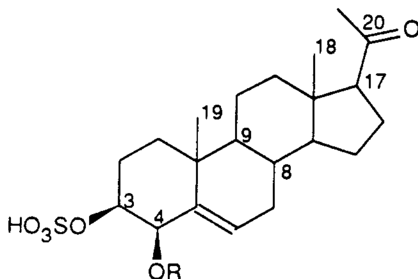
ABSTRACT.—The isolation of the new steroidal sulfate, 3β,4β-dihydroxy-pregn-5-en-20-one-3-sulfate [**1**], from the sponge *Stylopus australis*, is reported. Structural proof, including the preparation of the monoacetate derivative [**2**], is outlined, along with the identification of chimyl alcohol and the major sterols.

Although the occurrence of steroidal sulfates in a wide variety of echinoderms is well known (1–3), their occurrence in sponges is less well documented (4,5). We now report the isolation and structural elucidation of the new sterol sulfate, 3β,4β-dihydroxy-pregn-5-en-20-one-3-sulfate [**1**], obtained from the sponge *Stylopus australis* n.sp. Battershill (Hymedesmidae). This is the first report of a naturally occurring 3β,4β-dioxygenated 5-pregnene.

Partitioning of the MeOH/toluene extract from the sponge between EtOAc and H_2O, followed by reversed-phase column chromatography of the aqueous phase and subsequent Si gel column chromatography yielded the sterol sulfate **1**. The ^{1}H-nmr spectrum of **1** revealed the presence of a double-bond proton, two protons attached to oxygenated carbons, and three methyl groups, while the ^{13}C-nmr spectrum was consistent with the presence of 21 carbon atoms, including a single double bond, a ketone functionality, and two other oxygenated carbons. These data were suggestive of a steroid nucleus, and the structure of **1** was further elucidated from comparisons of its ^{13}C chemical shifts with those reported for compounds containing the 17β-acetyl function on the one hand and the Δ^5 3β,4β-diol system on the other hand (6).

The presence of a sulfate group was suggested by ms. The negative ion liquid chromatography (lc) mass spectrum exhibited a molecular ion species at *m/z* 411 $[M-H]^+$ and fragment ions at 331 $[M-HSO_3]^+$ and 313 $[M-H-H_2SO_4]^+$. The molecular formula for **1**, $C_{21}H_{32}O_6S$, was obtained from a high resolution fab mass spectrum. Strong ir absorption bands at 1215 and 1255 cm^{-1} supported the presence of a sulfate ester (2).

Confirmation of the structure was afforded by the acetylation of **1** to give the monoacetylated product **2**. The ^{1}H- and ^{13}C-nmr spectra of acetate **2** were consistent with the formation of a monoacetate at position 4, while the lc mass spectrum showed *m/z* 453 $[M-H]^+$ and 395



1 R=H
2 R=Ac

$[M - C_2H_3O_2]^+$. The stereochemistry of sterol **1** was established as 3β,4β from a consideration of H-H coupling constants. Values of 1.1, 3.3, and 3.3 Hz for the H-3 coupling constants, corresponding to couplings to the 2β, 2α, and 4α protons, respectively (7), implied the H-3 was in an axial, and therefore α, orientation. The observation of large nOe interactions between H-4α and H-6 and between H-3α, H-2α, and H-4α confirmed the stereochemical assignment. All attempts to hydrolyze the sulfate **1** to the parent diol were unsuccessful, despite a wide variety of standard methods being attempted. This is perhaps not surprising as the conditions required for hydrolysis of the sulfate would lead to elimination from the 4β allylic alcohol, as found for 4β-hydroxy-3β-*p*-tolylsulfonyloxyandrost-5-en-17-one (8).

An nmr analysis of the sterol mixture of the organic phase of the sponge extract showed the major component to be cholesterol, together with minor quantities of 24-methylene cholesterol (6,9). This was confirmed by gc-ms analysis of the trimethylsilyl ethers of the sterol mixture. The known compound, 2,3-dihydroxy-1-hexadecyloxypropane (chimyl alcohol) (10,11), was also isolated from the organic phase. Overall the sterol sulfate **1** was found to be present in the sponge at a level comparable to that of the co-occurring sterols. Investigations of two other sponges of the family Hymedesmidae, both *Hymedesmia* species, showed that **1** was not present in these sponges.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured for MeOH solutions using a Jasco J-20c automatic recording spectropolarimeter, and ir spectra were obtained using a Pye Unicam SP-300 infrared spectrophotometer. Melting points were obtained using an Electrothermal melting point apparatus and were uncorrected. ^{13}C-nmr spectra at 75 MHz and ^{1}H-nmr spectra at 300 MHz were obtained using CD_3OD or $CDCl_3$ solutions on a Varian XL-300 spectrometer. Fab mass spectra were obtained on a VG7070F spectrometer, while lc-ms data were obtained from a Finnigan TSP46 spectrometer equipped with a thermospray source. Gc-ms was performed on a Hewlett Packard HP-Ultra 2 capillary column, cross-linked 5% phenyl methyl silicone (50 m × 0.32 mm × 0.52 μm), using a Hewlett Packard 5710A gas chromatograph linked to a Hitachi M-80B mass spectrometer equipped with an M-0101 data system. Samples were run under a temperature program from 200 to 310° at 1° per min. The trimethylsilyl ethers of the sterols were obtained by standard procedures (12). Chromatography was performed using Si gel (Davisil 35-70 μ) and C-18 reversed-phase material prepared by coating the Si gel with *n*-octadecyltrichlorosilane after the method of Evans *et al.* (13).

COLLECTION AND EXTRACTION OF SPONGE.—*S. australis* (185 g wet wt) was collected by scuba diving in April 1986, from Goat Island Canyon, Leigh, off the North Island of New Zealand. A voucher specimen, 86L02-01, is held at the Department of Chemistry, University of Canterbury. The sponge was stored frozen, and then in a typical extraction, 85 g was blended with MeOH-toluene (4:1). The resulting extract (4.4 g) was partitioned between EtOAc and H_2O. The extract from the aqueous phase (3.8 g) was taken up in MeOH and filtered to remove an insoluble purple pigment.

3β,4β-DIHYDROXY-PREGN-5-EN-20-ONE 3-SULFATE [**1**].—The MeOH-soluble extract (3.2 g) was chromatographed on a reversed-phase C-18 column using an H_2O/MeOH to MeOH/CH_2Cl_2 gradient (14). Fraction 5 (182 mg) was rechromatographed on a columnn of Si gel using a CH_2Cl_2/MeOH gradient, yielding fractions 7–12 which were pure by tlc [Si gel, CH_2Cl_2-MeOH (3:1) blue-purple spot with vanillin/H_2SO_4 reagent] and by ^{13}C nmr. These were recombined to give a white solid (101 mg) recrystallized from EtOAc/MeOH as white needles: mp 120–121°; $[\alpha]^{25}D$ −100° (c = 0.1, MeOH); cd (MeOH) Δε (nm) 0 (324), +11 (284), 0 (240); ord (MeOH) θ (nm) +4.5 (302), 0 (286), −9.2 (257); ir ν max (KBr) 3600 (br OH), 2995, 1700, 1620, 1460, 1385, 1360, 1255, 1215 cm^{-1}; ms *m/z* $[M-H]^+$ 411.1833 (calcd 411.1841 for $C_{21}H_{31}O_6S$), $[M-HSO_3]^+$ 331, $[M-H-H_2SO_4]^+$ 313; ^{1}H nmr (CD_3OD) δ 4.18 (1H, dt, J = 11.1, 3.3, 3.3 Hz, H-3α), 4.38 (1H, d, J = 3.3 Hz, H-4α), 5.67 (1H, dd, J = 5.3, 3.1 Hz, H-6), 2.63 (1H, t, J = 9.0 Hz, H-17α), 0.62 (3H, s, H-18), 1.19 (3H, s, H-19), 2.11 (3H, s, H-21); ^{13}C nmr (CD_3OD), δ 39.0 (C-1), 25.8 (C-2), 81.6 C-3), 77.2 (C-4), 143.8 (C-5), 129.5 (C-6), 33.5 (C-7), 33.4 (C-8), 52.0 (C-9), 37.7 (C-10), 22.0 (C-11), 40.1 (C-12), 45.4 (C-13), 58.5 (C-14), 24.0 (C-15), 24.1 (C-16), 64.9 (C-

17), 13.9 (C-18), 21.7 (C-19), 212.8 (C-20), 32.0 (C-21).

3β,4β-DIHYDROXY-PREGN-5-EN-20-ONE 3-SULFATE 4-ACETATE [**2**].—Sulfate **1** (10 mg) was treated with Ac_2O (2 ml) and pyridine (2 ml) at room temperature overnight. H_2O (1 ml) and 1 drop of HCl (2 M) were added, and the product **2** extracted with $CHCl_3$ (3 × 0.5 ml) as a brown oil. This was recrystallized from $CHCl_3$ to yield **2** (8 mg) as a white solid: mp 122–123°; ir ν max (smear) 2995, 1735, 1705, 1635, 1460, 1380, 1235, 1195 cm^{-1}; ms *m/z* $[M-H]^+$ 453, $[M-C_2H_3O_2]^+$ 395; ^1H nmr ($CDCl_3$) δ 4.43 (1H, dt, H-3α), 5.72 (1H, d, H-4α), 5.81 (1H, dd, H-6), 2.53 (1H, t, H-17α), 0.62 (3H, s, H-18), 1.12 (3H, s, H-19), 2.00 (3H, s H-21), 2.13 (3H, s, H-23); ^{13}C nmr ($CDCl_3$), δ 38.7 (C-1), 24.4 (C-2), 77.4 (C-3), 76.7 (C-4), 141.7 (C-5), 126.9 (C-6), 31.6 (C-7), 31.6 (C-8), 50.1 (C-9), 36.1 (C-10), 21.8 (C-11), 37.0 (C-12), 44.0 (C-13), 56.9 C-14), 23.6 (C-15), 22.8 (C-16), 63.6 (C-17), 13.2 (C-18), 20.5 (C-19), 209.1 (C-20), 31.9 (C-21), 170.1 (C-22), 20.6 (C-23).

2,3-DIHYDROXY-1-HEXADECYLOXYPROPANE (CHIMYL ALCOHOL).—The organic phase from the EtOAc/H_2O partitioning of the sponge extract (432 mg) was chromatographed on Si gel using a CH_2Cl_2/MeOH gradient. The ^1H- and ^{13}C-nmr spectra of fraction 2 from this column were used to identify cholesterol and 24-methylene cholesterol by comparison with the literature data (6,9). These identifications were confirmed by subsequent gc-ms of the trimethylsilyl ethers of the sterols. Fraction 4 was rechromatographed on silica using an EtOAc/EtOH gradient to yield 5 mg of a yellow oil, the ^1H and ^{13}C nmr spectra of which corresponded to the literature data for the chimyl alcohol (10, 11).

ACKNOWLEDGMENTS

We thank Dr. Lewis K. Pannell, NIDDK, NIH, Bethesda, for obtaining the mass spectra, Dr. Chris N. Battershill, University of Canterbury, for the sponge identification, Drs. John Wetzel and Peter T.M. Kenny, SUNBOR, Osaka, for assistance with obtaining gc-ms, and the New Zealand Universities Grants Committee, the Harbor Branch Oceanographic Institution, and SeaPharm Inc. for financial support.

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Received 30 November 1988