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HIPPOSTEROL, A UNIQUE TRIHYDROXYLATED 5,6-SECOSTEROL FROM THE MARINE SPONGE HIPPOSPONGIA COMMUNIS

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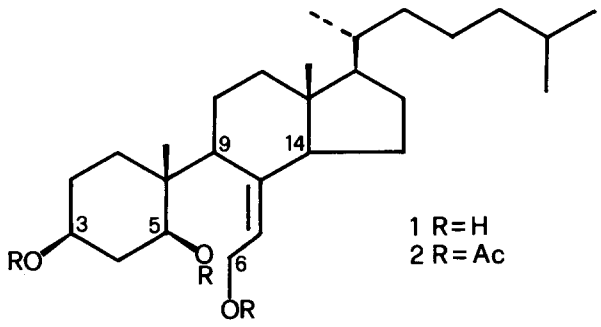
Abstract.-Hipposterol (1), a novel 5,6-secosterol, has been isolated from the marine sponge Hippospongia communis and its structure elucidated by spectral evidence and confirmed by synthesis.

As a part of our studies on polyhydroxylated sterols from sponges[1,2] we report here the isolation and the structure elucidation of a new ring B secosterol (1), named hipposterol, from the sponge H. communis (Lamarck, 1813).



The diethyl ether soluble material from the acetone and chloroform-methanol (1:1) extracts of the sponge, was chromatographed on silica gel using $CHCl_3$-MeOH mixtures as the eluant. The fraction eluted with $CHCl_3$-MeOH (97:3) was further separated by silica gel ($CHCl_3$-MeOH 93:7) and reverse phase HPLC (MeOH-H_2O 85:15) to afford hipposterol (1, 0.002 % dry weight), m.p. 85-7 °C (MeOH-H_2O 8:2), $[\alpha]_D$ +71.9 (c 0.3, $CHCl_3$).

The molecular formula of compound 1 was determined as $C_{27}H_{48}O_3$ by HRMS on the highest peak observed in the EI mass spectrum at m/z 402.3420 (M^+-H_2O, calc. for $C_{27}H_{46}O_2$ 402.3486). The IR spectrum showed hydroxyl absorption at 3400 cm^{-1} while the ^{1}H- and ^{13}C-NMR spectra immediately suggested the steroidal nature of the new metabolite. Particularly, methyl singlets at δ 0.56 (18-H_3) and 1.03 (19-H_3) and methyl doublets at δ 0.89 (J=6.3 Hz, 21-H_3) and 0.86 (6H, J=6.3 Hz, 26-H_3 and 27-H_3) in the ^{1}H-NMR spectrum of 1 were consistent with the cholestane carbon skeleton. One proton signals at δ 5.13 (dd, J=6.8 and 6.3 Hz, 7-H), 3.99 (bm, 3α-H), 3.93 (bm, 5α-H) and 4.36 and 4.23 (AB system further coupled, J_{AB}=11.7 Hz, 6-H_2) in the ^{1}H-NMR

spectrum of 1 and carbon resonances at δ 138.0 (-C-), 120.7 (-CH), 70.8 (-CH), 67.6 (-CH) and 61.9 ($-CH_2-$) in its ^{13}C-NMR spectrum indicated the presence of a trisubstituted double bond and two hydroxymethine and one hydroxymethylene groups in the molecule. Thus, 1 having four degrees of unsaturation must possess a tricyclic skeleton and, consequently, a secosterol structure. The presence of three hydroxyl groups in 1 was also supported by the mass spectrum which exhibited ions at m/z 402 (M^+-H_2O), 384 (M^+-2H_2O), 369 (M^+-2H_2O and CH_3) and 351 (M^+-3H_2O and CH_3) and confirmed by acetylation with acetic anhydride in pyridine to the corresponding triacetate 2 whose ^1H-NMR spectrum contained three acetoxymethyl resonances at δ 2.07, 2.06 and 2.02. Double resonance experiments performed on 1 and 2 gave evidence for the presence of the >C=CH-CH_2OH fragment in the molecule and showed that the two remaining hydroxyl groups were located at C-3 and C-5 and, therefore, that the scission in the steroid nucleus had occurred at the C5-C6 bond. Furthermore, the multiplicity of the 3-H proton (seven-lines multiplet at δ 4.75) and the J values of 5-H (δ 5.00, dd, J=10.7 and 4.4 Hz) in the triacetate 2, indicated the equatorial disposition of both the secondary -OAc groups. The downfield shift of the C-19 methyl resonance at δ 1.43 in the ^1H-NMR spectrum of 1 recorded in pyridine-d_5 confirmed the β-orientation of the C-5 hydroxyl function[4]. These data and the agreement of the C-18 methyl resonance (δ 0.56) with the value expected for a Δ^7-sterol prompted us to incorporate the above double bond containing fragment between C-9 and C-14, thus leading to structure 1 for the new metabolite.

In order to confirm the structure assignment we synthesized compound 1 from 5α-cholest-7-ene-3β,5,6α-triol[2] through the oxidative breaking of the C5-C6 bond by treatment with lead tetraacetate in CH_3COOH[5] for 1h, which afforded (Z)-3β-hydroxy-5-oxo-5,6-secocholest-7-en-6-al. Reduction with $LiAlH_4$ of this product yielded the synthetic compoud 1 and its 5α epimer, in the approximate ratio of 1:1, which were separated by HPLC on silica gel ($CHCl_3$-MeOH, 95:5). The chromatographic and spectroscopic properties of one of the two C-5 stereoisomers were identical with those of the natural product 1.

Hipposterol appears to be the first ring B secosterol isolated from marine sources.

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