Two New 19-Oxygenated Polyhydroxy Steroids from the Soft Coral *Nephthea chabroli*[1]

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Two new 19-oxygenated polyhydroxy steroids, 24-methylene cholest-5-en-1α,3*β*,19-triol (**1**) and 24-methylene cholest-5-en-3*β*,7*β*,9α,19-tetrol (**2**), and three known steroids, ergost-5,24(28)-dien-3*β*,19-diol (litosterol), cholest-5-en-3*β*,7*β*,19-triol, and ergost-5,24 (28)-dien-3*β*,7*β*,19-triol, have been isolated from the soft coral *Nephthea chabroli* and characterized through interpretation of spectral data.

The family Nephtheidae comprises many genera of which *Lemnalia*, *Paralemnalia*, *Capnella*, *Litophyton*, and *Nephthea* have received considerable attention from organic chemists.[2] A literature survey revealed that the genus *Nephthea* afforded sesquiterpenes derived from germacrene,[4] guaiane,[5] and a lone example of cadinane[6] and 19-hydroxy polyhydroxy steroids.[7−12] As part of our continuing interest in the bioactive secondary metabolites from marine organisms,[3] we have examined the soft coral *Nephthea chabroli* collected from the Mandapam coast of India in the Gulf of Mannar.

The 1:1 dichloromethane−methanol extract of the soft coral *N. chabroli* Andoum was subjected to gel filtration (Sephadex LH-20) followed by Si gel chromatography using hexane through hexanes−ethyl acetate mixtures to methanol as eluents and afforded mixtures of steroid fractions. The steroid fractions were acetylated and further purified over 20% $AgNO_3$-impregnated Si gel columns to give three known 19-oxygenated polyhydroxy steroids, ergost-5,24-(28)-dien-3*β*,19-diol-3,9-diacetate (litosterol),[13] cholest-5-en-3*β*,7*β*,19-triol-3,7,19-triacetate,[14] and ergost-5,24(28)-dien-3*β*,7*β*,19-triol-3,7,19-triacetate,[14] and two new steroids **1a** and **2a**.

Compound **1a** was obtained as a viscous liquid; $[\alpha]^{25}_D$ +2° (*c* 0.5, $CHCl_3$). It exhibited no UV absorptions above 200 nm, but showed absorptions for acetate carbonyls (1725 and 1240 cm^{-1}) and a terminal methylene (1620 and 925 cm^{-1}) in its IR spectrum. The compound was readily recognized as a 19-oxygenated polyhydroxy steroid from its ^{1}H NMR spectrum, by the conspicuous absence of a 19-methyl ^{1}H NMR signal at ca. *δ* 0.9, and showed signals for four methyl groups at *δ* 0.74 (3H, s), 0.95 (3H, d, *J* = 6.5 Hz), 1.03 (6H, d, *J* = 6.5 Hz) and three acetate methyls at *δ* 1.98 (3H, s), 2.02 (3H, s), and 2.05 (3H, s). Further, the downfield ^{1}H NMR spectrum displayed signals at *δ* 5.80 (1H, br s) for a trisubstituted double-bond proton, at *δ* 5.28 (1H, br m) and 5.23 (1H, m) for acetoxy methine protons, at *δ* 4.72 (1H, brs) and 4.61 (1H, br s) for the terminal methylene protons, and at *δ* 4.38 (1H, d, *J* = 11.5 Hz) and 4.15 (1H, d, *J* = 11.5 Hz) for a methylene-bearing acetoxy group. The foregoing spectral data is reminiscent of ergost-5,24(28)-dien-3,19-diol except for an acetoxy methine signal at *δ* 5.28 (1H, m). However, in compound **1a**, the 3*β*-acetoxy methine proton resonated at *δ* 5.23. This led us to assign the additional acetoxy group to the C-1 position in the α-orientation.[15] Due to 1,3-diaxial interactions of the 1α-acetoxy and the 3α-H, the 3α-methine proton is shifted further downfield from the normal chemical shift at ca. *δ* 4.65.[13,14] From these findings the structure of compound **1a** was assigned as 24-methylene cholest-5-en-1α,3*β*,19-triol-1,3,19-triacetate, and the corresponding naturally occurring alcohol is **1**. The structure of **1a** was further confirmed by ^{13}C NMR spectral data.

Compound **2a** was obtained as a viscous liquid, $[\alpha]^{25}_D$ +42° (*c* 0.8, $CHCl_3$). It showed no UV absorptions, and by elemental analysis was assigned a formula of $C_{34}H_{52}O_7$. The IR absorptions at 3500, 1735, 1620, and 900 cm^{-1} indicated the presence of hydroxyl, acetates, and terminal methylene groups, respectively. The ^{1}H NMR spectrum of compound **2a** resembled those of 19-hydroxy sterols.[7−12] The ^{1}H NMR spectrum of compound **2a** showed signals for the presence of three acetates at *δ* 2.07 (3H, s) and 2.05 (6H, s) and four methyls at *δ* 0.72 (3H, s), 1.05 (6H, d, *J* = 7 Hz), 0.94 (3H, d, *J* = 6.5 Hz). Further, the downfield region of the ^{1}H NMR spectrum showed signals for the presence of a trisubstituted double bond at *δ* 5.88 (1H, d, *J* = 4.5 Hz), a terminal methylene at *δ* 4.75 (1H, br s) and 4.65 (1H, br s), two acetoxy methine protons at *δ* 5.03 (1H, t, *J* = 6 Hz) and 4.72 (1H, br m), and a methylene-bearing acetoxyl group at *δ* 4.60 (1H, d, *J* = 12 Hz) and 3.98 (1H, d, *J* = 12 Hz). The above spectral data is reminiscent of cholest-5-en-3*β*,7*β*,9α,19-tetrol isolated from the black coral *Antipathes subpinnata*[14] except for the presence of terminal methylene protons at *δ* 4.75 and 4.65 (each 1H, br s). One of the characteristic signals in the ^{1}H NMR spectrum of cholest-5-en-3*β*,7*β*,9α,19-tetrol is the C-6 olefinic proton, which resonates far downfield at *δ* 5.88 (1H, d, *J* = 4.5 Hz) due to the deshielding effect of 9α-hydroxyl group. Further, the presence of a terminal methylene at C-24 in compound **2a** was confirmed by ^{13}C NMR spectral data. Thus, the structure of compound **2a** was established as 24-methylene cholest-5-en-3*β*,7*β*,9α,19-tetrol-3,7,19-triacetate, and the parent alcohol has structure **2**.

The known compounds ergost-5,24(28)-dien-3*β*,19-diol-3,9-diacetate (litosterol diacetate),[13] cholest-5-en-3*β*,7*β*,19-triol-3,7,19-triacetate,[14] and ergost-5,24(28)-dien-3*β*,7*β*,19-triol-3,7,19-triacetate[14] were characterized by comparing their spectral data with those reported in the literature.

Experimental Section

General Experimental Procedures. The ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a Varian Gemini 200 MHz spectrometer using TMS as internal standard. Chemical shifts were reported in parts per million,

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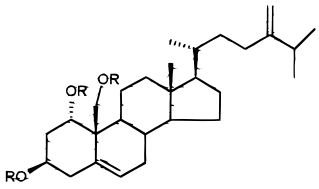

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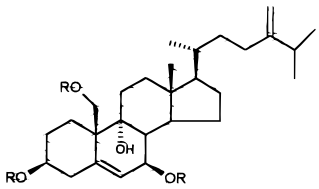
1a R = -$COCH_3$



2 R = H
2a R = -$COCH_3$

and coupling constants (*J*) were expressed in Hertz. UV and IR spectra were recorded on Shimadzu 240 and Perkin−Elmer 1310 spectrophotometers, respectively. Elemental analysis was carried out on a Perkin−Elmer 240C instrument. MS were recorded on a VG AUTOSPEC-M instrument. Optical rotations were measured on a JASCO DIP-370 polarimeter.

Animal Material. The soft coral *Nephthea chabroli* Andoum (Nephtheidae) (IIC-266) was collected during October 1997, at the Mandapam coast (N 9°18′, E 79°08′) in the Gulf of Mannar by skin diving to a 20-ft depth. The voucher specimen (IIC-266) is on deposit at the National Institute of Oceanography, Goa, India.

Extraction and Isolation. The freshly collected (2.2 kg wet wt) specimens were cut into pieces and soaked in MeOH until workup (stored at 0−5 °C for six months). The aqueous MeOH from the organism was decanted, and the coral was freeze-dried. The dried material was extracted with CH_2Cl_2−MeOH (1:1, 3 × 2.5 L). The combined extracts were concentrated under reduced pressure, and the crude extract (50 g) was partitioned between H_2O and EtOAc. Concentration of the organic layer afforded a brownish gummy crude extract (30 g). It was subjected to gel filtration (Sephadex LH-20) followed by Si gel (100−200 mesh) column chromatography using hexane through hexanes−EtOAc mixtures to MeOH as eluents. The fraction eluted with 40% EtOAc in hexane yielded ergost-5,24(28)-dien-3β,19-diol (litosterol) (30 mg). The fraction eluted with 60% EtOAc in hexane afforded cholest-5-en-3β,7β,19-triol (10 mg) and ergost-5,24(28)-dien-3β,7β,19-triol (40 mg). The fraction obtained with 80% EtOAc in hexane yielded compounds **1** and **2**. The respective steroid fractions were acetylated using Ac_2O−Pyr and separated on 20% $AgNO_3$-impregnated Si gel column as their peracetates.

Compound 1a: isolated as a viscous liquid (6 mg), $[\alpha]^{25}_D$ +2° (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 1725,1620, 1240, and 925 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.80 (1H, s), 5.28 (1H, br m), 5.23 (1H, m), 4.72 (1H, br s), 4.61 (1H, br s), 4.38 (1H, d, *J* = 11.5 Hz), 4.15 (1H, d, *J* = 11.5 Hz), 2.05 (3H, s), 2.02 (3H, s), 1.98 (3H, s), 1.03 (6H, d, *J* = 6.5 Hz), 0.95 (3H, d, *J* = 6.5 Hz), and 0.74 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.94 (s), 169.8 (s), 169.5 (s), 156.77 (s), 139.5 (s), 129.99 (d), 105.99 (t), 74.93 (d), 69.57 (d), 67.96 (t), 56.21 (d), 55.87 (d), 42.58 (d), 39.97 (s), 39.6 (d, 2C), 37.03 (s), 35.68 (d), 34.59 (t), 33.79 (t), 32.99 (t), 31.32 (t), 30. 94 (t), 29.68 (t), 28.06 (t), 24.91 (t), 24.01 (t), 21.98 (q), 21.85 (q), 21.5 (q), 21.34 (q), 18.61 (q), 14.11 (q), and12.2 (q); positive FABMS *m/z* 455 $[M + H - AcOH - C_2H_2O]^+$, 395 $[M + H - 2AcOH - C_2H_2O]^+$, and 377 $[M + H - 3AcOH]^+$; *anal.* C 73.34%, H 9.41%, calcd for $C_{34}H_{52}O_6$, C 73.35%, H 9.41%.

Compound 2a: obtained as a viscous liquid (8 mg), $[\alpha]^{25}_D$ −42°(*c* 0.8, $CHCl_3$); IR (neat) ν_{max} 3500, 1735, 1620, and 900 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.88 (1H, d, *J* = 4.5 Hz), 5.03 (1H, t, *J* = 6 Hz), 4.75 (1H, br s), 4.72 (1H, br m), 4.65 (1H, br s), 4.60 (1H, d, *J* = 12 Hz), 3.98 (1H, d, *J* = 12 Hz), 2.07 (3H, s), 2.05 (6H, s), 1.05 (6H, d, *J* = 7 Hz), 0.94 (3H, d, *J* = 6.5 Hz), and 0.72 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.52 (s), 170.49 (s, 2C), 156.74 (s), 141.42 (s), 124.69 (s), 105.93 (t), 76.99 (s), 72.70 (d), 67.81 (d), 64.5 (t), 55.71 (d), 50.01 (d), 43.33 (s), 42.4 (t), 40.23 (d), 39.17 (t), 37.78 (t), 36.95 (t), 35.72 (d), 34.6 (t), 33.78 (t), 28.1 (t), 27.59 (t), 23.93 (t), 22.68 (t), 21.99 (q), 21.85 (q), 21.32 (q), 20.98 (q), 18.69 (q), 14.12 (q), and 11.51 (q); FABMS *m/z* 513 $[M + H - AcOH]^+$; *anal.* C 71.29%, H 9.15% calcd for $C_{34}H_{52}O_7$, C 71.30%, H 9.15%.

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