A New Polyhydroxy Sterol from the Soft Coral *Lobophytum crassum*[†,1]

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A new polyhydroxy sterol (**1**) and eight known sterols have been isolated from the soft coral *Lobophytum crassum* and characterized by spectral studies.

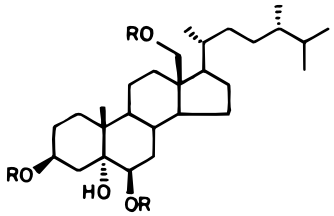
In addition to terpenoids and alkaloids, sterols also play an important role as secondary metabolites produced by marine organisms. Several excellent reviews on the structures, biosynthesis, and distribution of marine sterols have been published.[2,3] In continuation of our search for biologically active compounds from marine organisms,[4] we investigated the soft coral *Lobophytum crassum*, Vonmarenzeller 1886 (family Alcyoniidae) collected from the Mandapam coast during June 1996. A literature survey revealed that this coral yielded cembranoid diterpenes[5−7] and polyhydroxylated sterols.[8]

The 1:1 CH_2Cl_2−$MeOH_4$ extract was subjected to Si gel chromatography, eluting with *n*-hexane through hexane−Me_2CO mixtures to Me_2CO and afforded monohydroxylated and polyhydroxylated sterols. The sterol mixture fractions were acetylated using Ac_2O and pyridine and then separated by chromatography on a Silica gel column impregnated with silver nitrate. A total of eight known sterols, (22*R*,23*R*)-methylenecholesterol,[9] ergost-5,22-diene 3β-ol,[10] ergost-5,25-dien-3β-ol,[11] 24-methylenecholesterol,[12] (24*S*)-ergostane-3β,5α,6β,-25-tetrol 25-monoacetate,[13] (24*S*)-ergostane-3β,5α,6β,25-tetrol,[13] (24*S*)-ergostane-1β,3β,5α,6β-tetrol-1,3,6-triacetate,[14,15] 24-methylene cholestane-1β,3β,5α,6β-tetrol,[15] and a new polyhydroxy sterol (**1**) were isolated.

The known sterols were characterized by comparison of their ^{1}H-NMR and mass spectral data with the literature data. The presence of 3β, 5α, and 6β trihydroxy sterols have been characterized by diagnostic methine signals at δ 3.94 (1H, m) and 3.41 (1H, br s) for unacetylated sterols, and signals at δ 5.12 (1H, m) and 4.65 (1H, br s) for acetylated sterols, in their respective ^{1}H-NMR spectra.[13−15]

Compound **1a** was obtained as a viscous liquid, $[\alpha]_D$ −23° (*c* 0.2, $CHCl_3$) which analyzed for $C_{34}H_{56}O_7$. It was transparent to UV and showed absorptions in the IR spectrum at 3500 and 1735 cm^{-1}, indicating the presence of acetyl and hydroxyl groups.

The ^{1}H-NMR spectrum of **1a** contained signals for five methyls at δ 0.78 (3H, d, J = 6.5 Hz), 0.74 (3H, d, J = 6.5 Hz), 0.84 (3H, d, J = 7 Hz), 0.98 (3H, d, J = 7 Hz), and 1.12 (3H, s) and three acetoxy methyls at δ 1.98 (3H, s), 2.05 (3H, s), and 2.06 (3H, s). Further, its downfield ^{1}H-NMR spectrum displayed signals at δ 5.12 (1H, m), 4.67 (1H, br s), 4.2 (1H, d, J = 11.5 Hz), and 3.89 (1H, d, J = 11.5 Hz). The foregoing spectral data and a literature survey indicated that compound **1a** is



1 R = H

1a R = $-\overset{O}{\overset{\|}{C}}-CH_3$

a 3β,5α,6β-trihydroxy sterol with an additional hydroxyl group. The position of this additional hydroxyl group was fixed at C-18 based on two findings. The first was the absence of the characteristic shielded methyl (C-18) signal around δ 0.70 and, instead, the presence of two AB doublets at δ 4.2 and 3.89 in the ^{1}H-NMR spectrum of compound **1a**. Second, compound **1a** exhibited a signal at δ 62.9 in the ^{13}C-NMR spectrum, and the usual C-18 methyl carbon signal at δ 12.0 was absent. The stereochemistry of the C-24 methyl was assigned as *S* based on the ^{1}H-NMR chemical shifts of 26 and 27 methyls,[14,17] which resonated at δ 0.78 and 0.84, in the spectrum of compound **1a**. The structure of this peracetate was thus established as (24*S*)-ergostane-3β,5α,6β,18-tetrol-3,6,18-triacetate (**1a**).

Experimental Section

General Experimental Procedures. ^{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 are reported in parts per million, and coupling constants (J) are expressed in Hertz. Elemental analysis was carried out on a Perkin-Elmer 240-C instrument. Optical rotations were measured with a JASCO DIP-370 polarimeter. UV and IR spectra were recorded on Shimadzu and Perkin-Elmer 1310 spectrophotometers, respectively. Mass spectra were recorded on a Finnigan MAT 1020 instrument.

Animal Material. The soft coral *L. crassum* (IIC-234) was collected by skin diving at a depth of 20 ft near the Mandapam coast in the Gulf of Mannar, and a voucher specimen (IIC-234) is on deposit at the National Institute of Oceanography, Goa, India.

Extraction and Isolation. The freshly collected specimens were cut into thin slices and soaked in MeOH at the site of collection until workup. After removal of MeOH, the soft coral (700 g dry wt) was freeze-dried and extracted with MeOH-CH_2Cl_2 (1:1) (2 L × 3). After evaporation of the combined extracts under reduced pressure, the crude extract was partitioned between

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EtOAc and H_2O. Concentration of the organic layer resulted in a brownish gummy crude extract (40 g), which was subjected to Si gel chromatography, eluting with hexane, through hexane−CH_3COCH_3 mixtures to CH_3COCH_3 and MeOH. The respective monohydroxylated and polyhydroxylated sterol mixtures were acetylated and were separated on 20% $AgNO_3$-impregnated Si gel column. The 5% CH_3COCH_3 in hexane fraction afforded (22*R*,23*R*)-methylene cholesterol (500 mg),[9] ergost-5,22-dien-3β-ol (30 mg),[10] ergost-5,25-dien-3β-ol (20 mg),[11] and 24-methylenecholesterol (20 mg).[12] The 25% CH_3COCH_3 in hexane fraction yielded (24*S*)-ergostane-3β,5α,6β,25-tetrol 25-monoacetate (500 mg).[13] The 30% CH_3COCH_3 in hexane fraction contained (24*S*)-ergostane-3β,5α,6β,18-tetrol 3,6,18-triacetate (10 mg) and (24*S*)-ergostane-3β,5α,6β,25-tetrol (150 mg).[13] The 35% CH_3COCH_3 in hexane fraction yielded (24*S*)-ergostane-1β,3β,5α,6β-tetrol 1,3,6-triacetate (235 mg)[14,15] and 24-methylene cholestane-1β,3β,5α,6β-tetrol (75 mg).[15]

Compound **1a**: obtained as a colorless viscous liquid (10 mg), $[\alpha]_D$ −23°(*c* 0.2, $CHCl_3$); IR (neat) λ_{max} 3500, 1735, 1375 cm^{-1}; ^{1}H-NMR ($CDCl_3$ 200 MHz), δ 5.12 (1H, m), 4.67 (1H, br s), 4.2 (1H, d, J = 11.5 Hz), 3.89 (1H, d, J = 11.5 Hz), 1.98 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 1.12 (3H, s), 0.98 (3H, d, J = 7 Hz), 0.84 (3H, d, J = 7 Hz), 0.78 (3H, d, J = 6.5 Hz), 0.74 (3H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz), δ 31.7(C-1), 26.5(C-2), 70.5(C-3), 36.8(C-4), 74.9(C-5), 75.9(C-6), 31.5(C-7), 30.9(C-8), 45.4(C-9), 45(C-10), 23.8(C-11), 38.5(C-12), 39.14(C-13), 55.2(C-14), 27.7(C-15), 29.3(C-16), 56.3(C-17), 62.9(C-18), 15.4(C-19), 36.3(C-20), 19.1(C-21), 33.8(C-22), 29.7(C-23), 34.9(C-24), 30.27(C-25), 21.1(C-26), 21.4(C-27), 16.38(C-28); *anal.* C 70.62%, H 9.82%, calcd for $C_{34}H_{56}O_7$, C 70.79%, H 9.78%; EIMS (70 eV) *m/z* 456 (M^+ − 2 AcOH, 2), 438 (M^+ − 2 AcOH − H_2O, 2), 378 (M^+ − 3 AcOH − H_2O, 2), 257 (30).

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