An Unusual Novel C_{29} Steroid from the Soft Coral *Lobophytum crassum*[1]

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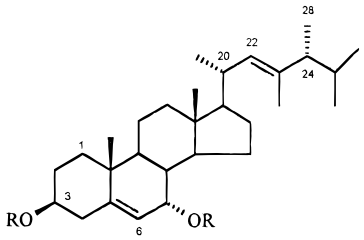
A new sterol, 23,24(*S*)-dimethylcholest-5,22-dien-3β,7α-diol (**1**), has been isolated from the soft coral *Lobophytum crassum* and characterized by interpretation of spectral data.

Marine organisms produce sterols with a remarkable variety of side chains, unconventional nuclear structures, and assorted hydroxylation patterns. Sterol patterns in marine invertebrates reflect the complexity of sterols arising through the food chain. The capability for biochemical modifications of dietary sterols makes the sterol composition even more complex. The symbiotic relationships between organisms also complicates the sterol composition.[2] In continuation of our search for biologically active secondary metabolites from marine organisms,[3,4] we have chosen the soft coral *Lobophytum crassum* Vonmarenzeller, 1886 (Alcyoniidae) collected from the Mandapam coast (N 9°18′, E 79°08′) of southern India during June 1996. Previously, we reported monohydroxy and polyhydroxylated sterols from the same species.[4] A literature survey revealed that this soft coral yielded polyhydroxylated[5] sterols and cembranoid diterpenes.[6−8]

After removal of an aqueous methanol extract from the soft coral, the organism was freeze-dried and extracted with CH_2Cl_2−MeOH (1:1, 3 × 2 L). The combined extracts were concentrated under reduced pressure, and the resultant crude extract was partitioned between H_2O and EtOAc. The organic layer was concentrated, and the resultant gummy crude extract (40 g) was subjected to gel filtration (Sephadex LH-20) followed by Si gel column chromatography using first hexane, then hexane−acetone mixtures, and finally MeOH as eluents. This afforded several monohydroxylated and polyhydroxylated sterols.[4] The 20% acetone-in-hexane fraction contained a mixture of sterols, which was acetylated (Ac_2O/Pyr) and further purified using 20% $AgNO_3$-impregnated Si gel column chromatography to afford compound **1a**.



1 R = H

1a R = Ac

Compound **1a** was obtained as a colorless, viscous liquid, $[\alpha]^{25}_D$ +44.7° (*c* 0.067, $CHCl_3$) and analyzed for $C_{33}H_{52}O_4$. It was transparent in UV light and showed IR absorptions at 1725, 1600, and 1250 cm^{-1}, indicating the presence of an acetyl carbonyl and a double bond.

The ^1H NMR spectrum ($CDCl_3$, 400 MHz) of compound **1a** exhibited signals at δ 5.60 (1H, d, J = 5.0 Hz), 4.64 (1H, m), 2.05 (3H, s), 2.02 (3H, s), 1.17 (3H, s), 0.88 (3H, d, J = 7.0 Hz), 0.86 (3H, d, J = 7.0 Hz), 0.83 (3H, d, J = 7.5 Hz), 0.78 (3H, d, J = 7.5 Hz), and 0.60 (3H, s), characteristic of (24*S*)-ergost-5-en-3β-acetate, except for the presence of an additional vinylic methyl group at δ 1.49 (3H, d, J = 1.0 Hz), an allylic acetoxy methine proton at δ 4.85 (1H, br d, J = 8.0 Hz), and a trisubstituted double-bond proton signal at δ 4.66 (1H, q, J = 1.5 and 10.0 Hz). A literature survey revealed that in 22-en-23-methyl sterols the proton signal at C-22 appears as a quartet at about δ 4.7, and the methyl signal is at δ 1.49 as a doublet with a small coupling constant.[9−11] This indicated that compound **1a** contained a 22-en-23-methyl side chain. The presence of the 22-en-23-methyl side chain was further proved by a mass spectral fragment ion at *m*/*z* 254 [$M^+ + 1 - 2AcOH$ − side chain ($C_{10}H_{19}$)]. Generally, in 3β,7α diol Δ^5 sterols the H-6 vinylic hydrogen resonates at ca. δ_H 5.60,[12] whereas in the 7β isomer it resonates at around δ_H 5.29.[13] Hence, the stereochemistry of the C-7 allylic acetoxy group was assigned as α in compound **1a**. The ^{13}C NMR signals at δ 170.16 (s), 170.09 (s), 135.78 (s), 135.26 (s), 131.6 (d), and 127.29 (d) corroborated the presence of two acetyl groups and two trisubstituted double bonds. Further, the stereochemistry of the C-24 methyl was assigned as *S*, based on the ^1H NMR chemical shifts of 26 and 27 methyls, which resonated at δ 0.78 and 0.83, in the spectrum of compound **1a.**[14,15]

Thus, the structure of compound **1a** was established as 23,24(*S*)-dimethylcholest-5,22-dien-3β,7α-diol-3,7-diacetate.

Experimental Section

General Experimental Procedures. UV and IR spectra were recorded on Shimadzu-240 and Perkin−Elmer 1310 spectrophotometers. Optical rotations were measured with a JASCO DIP-370 polarimeter. Elemental analysis was carried out on a Perkin−Elmer 240C instrument. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian Gemini 400 MHz spectrometer, using TMS as internal standard. Chemical shifts were reported in parts per million, and coupling constants (J) were expressed in Hertz. MS were recorded on a VG AUTOSPEC-M instrument.

Animal Material. The soft coral *Lobophytum crassum* Vonmarenzeller, 1886 (Alcyoniidae) was collected from the Mandapam coast (N 9°18′, E 79°08′) in the Gulf of Mannar by skin diving at 20-ft depth during June 1996. The voucher specimen (IIC-234) is on deposit at the National Institute of Oceanography Museum, Goa, India.

Extraction and Isolation. The freshly collected organism was washed with fresh water, cut into thin slices, and soaked in MeOH until workup. The aqueous MeOH from the organism was decanted, and the coral was freeze-dried. The dried

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material (700 g dry wt) was extracted with CH_2Cl_2−MeOH (1:1, 3 × 2 L). The combined extracts were concentrated under reduced pressure, the crude extract was partitioned between H_2O and EtOAc. Concentration of the organic layer afforded a brownish, gummy crude extract (40 g). It was subjected to gel filtration (Sephadex LH-20) followed by Si gel column chromatography using hexane, then hexane−Me_2CO mixtures, and finally MeOH as eluents to afford several known monohydroxylated and polyhydroxylated sterols.[4] The 20% Me_2CO-in-hexane eluate consisted of a minor sterol mixture fraction that was acetylated (Ac_2O−Pyr) and further purified over 20% $AgNO_3$-impregnated Si gel column to yield compound **1a** (5 mg).

Acetylation of the Sterol Fraction. The sterol fraction (50 mg) was dissolved in pyridine (0.5 mL) and Ac_2O (2 mL) and allowed to stand overnight at room temperature. The reaction mixture was poured into ice-cold H_2O, the resulting solution extracted with EtOAc, and the organic layer dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed over Si gel to give a mixture of acetylated sterols. The mixture of sterols was further purified using 20% $AgNO_3$-impregnated Si gel column to afford compound **1a.**

Compound 1a: obtained as a colorless viscous liquid (5 mg); $[\alpha]^{25}_D$ +44.7° (*c* 0.067, $CHCl_3$); IR (neat) ν_{max} 1725, 1600, and 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.60 (1H, d, J = 5.0 Hz, H-6), 4.85 (1H, br d, J = 8.0 Hz, H-7), 4.66 (1H, q, J = 1.5 and 10 Hz, H-22), 4.64 (1H, m, H-3), 2.05 (3H, s, Ac), 2.02 (3H, s, Ac), 1.49 (3H, d, J = 1 Hz, H_3-29), 1.17 (3H, s, H_3-19), 0.88 (3H, d, J = 7.0 Hz, H_3-28), 0.86 (3H, d, J = 7.0 Hz, H_3-21), 0.83 (3H, d, J = 7.5 Hz, H_3-26), 0.78 (3H, d, J = 7.5 Hz, H_3-27) and 0.60 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.16 (s), 170.09 (s), 135.78 (s), 135.26 (s), 131.60 (d), 127.29 (d), 78.59 (d), 69.56 (d), 56.8 (d), 56.34 (d), 50.13 (d), 49.53 (s), 41.71 (d), 39.86 (s), 38.11 (d), 34.55 (d), 33.75 (t), 32.75 (t), 31.92 (t), 31.55 (d), 30.72 (t), 29.35 (t), 27.7 (t), 24.42 (q), 23.38 (t), 22.68 (2C, q), 21.71 (q), 21.65 (q), 20.57 (q), 16.93 (q), 14.2 (q), and 12.16 (q); *anal.* C 77.30%, H 10.14%, calcd for $C_{33}H_{52}O_4$ C 77.33%, H 10.16%; positive FABMS *m*/*z* 453($M^+ + 1$ − AcOH), 393($M^+ + 1$ − 2AcOH) and 254[$M^+ + 1$ − 2AcOH − side chain ($C_{10}H_{19}$)].

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