A Novel 4α-Methyl Sterol from the Soft Coral *Nephthea chabroli*†

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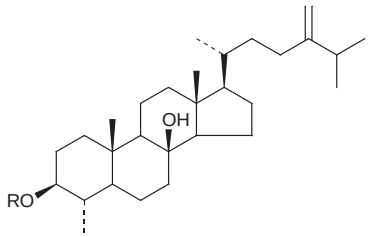
A new sterol, 4α-methyl-24-methylene-5α-cholestan-3β,8β-diol (**1**) and three known sterols 4α-methyl-24-methyl-enecholestan-3β-ol, 4α-methylcholestan-3β-ol and 24-methylenecholest-4-en-3-one are isolated from the soft coral *Nephthea chabroli* and characterized through spectral data and in the case of **1** by X-ray crystallographic analysis.

Marine organisms constitute a rich source of diverse and complex sterols; particularly among marine invertebrates the complexity of sterols arises through food chains and symbiotic relationships between organisms.[1,2] During the course of a search for biologically active secondary metabolites from marine organisms, we have examined the soft coral of the genus *Nephthea chabroli* (Nephtheidae). A literature search revealed that the genus *Nephthea* has earlier afforded sesquiterpenes, diterpenes and polyprenylated hydroquinones.[3,4] Herein, we report on the isolation of several sterols from this genus and describe the structure elucidation of a new 4α-methylsterol **1**.

A careful examination of a CH_2Cl_2–MeOH extract of the soft coral *Nephthea chabroli* collected from the Mandapam coast in southern India during October 1997 afforded three known compounds, 4α-methyl-24-methylenecholestan-3β-ol,[5] 4α-methylcholestan-3β-ol,[5] 24-methylenecholest-4-en-3-one[6] and a new sterol **1** which was subjected to structural scrutiny.



1 R = H
1a R = Ac

The new sterol **1** was obtained as a colorless crystalline solid, and analysed for $C_{29}H_{50}O_2$ (HREIMS). It was transparent in the UV region and exhibited characteristic IR bands at 3500 (OH), 1680, and 920 cm^{-1} (terminal methylene). The ^{1}H NMR spectrum exhibited signals at δ 4.70 (1 H, br s), 4.62 (1 H, br s), 3.05 (1 H, dt, $J = 4.5$ and 9.0), 1.03 (6 H, d, $J = 7.5$), 1.00 (3 H, s), 0.95 (3 H, s) and 0.90 (6 H, d, $J = 6.5$ Hz), characteristic of 24-methylenecholestan-3β-ol, except for the presence of an additional secondary methyl group. A literature survey revealed that in 4α-methyl sterols the proton at C-3 appears as a doublet of triplets at δ 3.05[5] and is shifted upfield considerably compared to that of cholestan-3-β-ol derivatives. This indicated that **1** might have a 4α-methyl group. On acetylation, **1** afforded a monoacetate (**1a**), which still retained a hydroxyl group whose tertiary nature was evident from the ^{1}H NMR spectral data. The downfield shift of the doublet of triplets signal of **1**, on conversion into **1a** (see Experimental section), implied that the C-3 OH group was acetylated. The ^{13}C NMR spectrum[7] of **1a** revealed the presence of two oxygenated carbons at δ 78.8 (s) and 73.6 (d) at tertiary and secondary centers, respectively. The position of the tertiary hydroxyl group was assigned at C-8 on the basis of the downfield shifts (δ 1.00 in **1a** as opposed to 0.82 in 24-methylenecholestan-3β-ol) of the C-19 methyl protons in **1** and **1a**.[8] From the foregoing spectral data, the structure of compound **1** was indicated as 4α-methyl-24-methylene-5α-cholestan-3β,8β-diol.

The structure of compound **1** was corroborated by X-ray crystallographic studies on the acetate **1a**. An ORTEP[9] perspective of the molecular structure and the relative stereochemistry is shown in Fig. 1. The sterol **1a** crystallizes in a non-centrosymmetric space group $P2_1$. A view of the packing down the *a* axis reveals that each molecule is

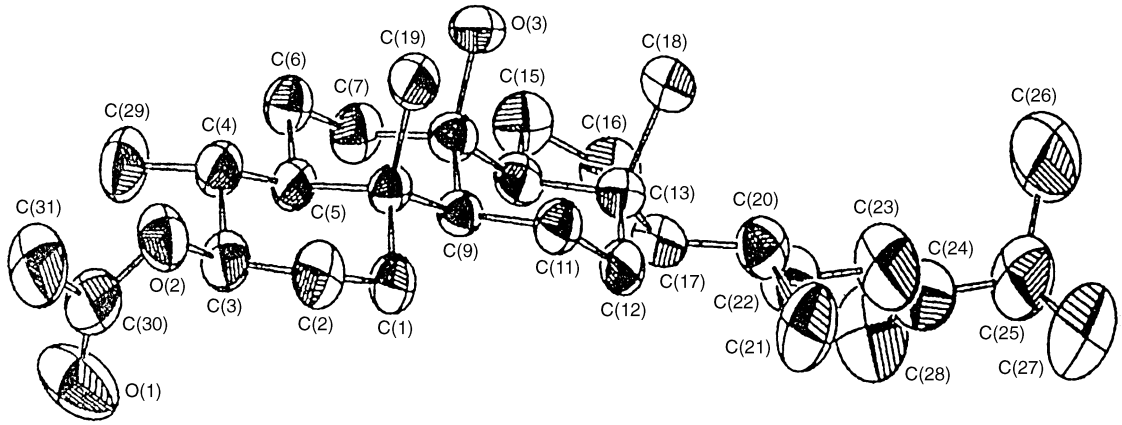


Fig. 1 Crystal structure of compound **1a**

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involved in interesting quadruple hydrogen bonding with its nearest neighbors. The proton on the C-8 hydroxyl group is hydrogen bonded to O-1 (carbonyl oxygen of the acetate moiety) of the neighboring molecule (O-3–H···O-1 with O···H distance 2.20 Å and O–H···O angle of 160.8°) along the *b* axis to generate infinite ribbons. In addition, O-1 is also C–H···O hydrogen bonded to the less acidic ring-D H-15 proton (C-15–H-15···O-1 with O–H of 2.53 Å, C···O distance 3.48 Å and C–H···O 165.9°). Thus, O-1 is involved in bifurcated hydrogen bonding and the complementary packing arrangement of the molecules in the solid state maximizes formation of hydrogen bonds.

It has been observed that 4α-methylsterols, which are intermediates in steroid biosynthesis in animals and in other divisions of the plant kingdom, are often end products of sterol biosynthesis in dinoflagellates.[5,10] They are relatively less abundant and some exhibit biological responses. For example Gunasekera *et al.*[11] have found that 4α-methylcholest-8-en-3β-ol isolated from the sponge *Agelas flabelliformis* shows strong immunosuppressive activity against murine splenocytes.

Experimental

The ^{1}H (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a Varian Gemini 200 MHz spectrometer using TMS as internal standard, UV and IR spectra on Shimadzu 240 and Perkin-Elmer 240-C instruments respectively and mass spectra on a Finnigan-MAT 1020 instrument. Melting points were measured on Buchi-510 apparatus. Optical rotations were measured on a JASCO DIP-370 polarimeter.

Extraction and Isolation.—The soft coral *Nephthea chabroli* (Nephtheidae) (IIC-266) was collected at Mandapam coast in the Gulf of Mannar in October 1997 and a voucher specimen (IIC-266) is on deposit at the National Institute of Oceanography, Goa, India. The freshly collected specimen (2.2 kg wet weight, from the Mandapam coast in southern India during October 1997) was cut into pieces and soaked in MeOH. The solvent was decanted and freeze-dried. The residue was extracted with 1 : 1 CH_2Cl_2– MeOH. After removal of the solvent *in vacuo*, the combined crude extract (30 g) was subjected to silica gel (100–200 mesh) column chromatography using hexane, hexane–ethyl acetate, and methanol, respectively, as eluents. The fraction eluting with 5% ethyl acetate in hexane yielded the known sterols 4α-methylcholestan-3β-ol, 4α-methyl-24-methylenecholestan-3β-ol and 24-methylenecholest-4-en-3-one. The fraction eluting with 10% ethyl acetate in hexane yielded 4α-methyl-24-methylene-5α-cholestan-3β,8β-diol (**1**) obtained as a colorless crystalline solid, mp 120–121 °C; $[\alpha]_D + 5.62°$ (*c* 0.2, $CHCl_3$); IR ν_{max} (KBr) 3500, 1680, 920 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.70 (1 H, br s, H-28a), 4.62 (1 H, br s, H-28b), 3.05 (1 H, dt, *J* = 4.5 and 9.0, H-3α), 1.03 (6 H, d, *J* = 7.5Hz, H-26, H-27), 1.00 (3 H, s, H-19), 0.95 (3 H, s, H-18), 0.90 (6 H, d, *J* = 6.5 Hz, H-21, H-29); EIMS (70 eV) *m/z* 430 (M^+); HREIMS *m/z* found 430.714 calc. for $C_{29}H_{50}O_2$ 430.70.

Acetylation of Compound **1**.—A solution of compound **1** (60 mg) in Ac_2O (1.5 mL) and pyridine (0.5 mL) was allowed to stand overnight at room temperature. The contents were poured into ice-cold water, extracted with ethyl acetate and the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure followed by silica gel chromatography to obtain **1a** (60 mg) as a colorless crystalline solid, mp 135 °C; $[\alpha]_D + 6.70°$ (*c* 0.2, $CHCl_3$); IR ν_{max} (KBr) 3500, 1735, 1680, 920 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.70 (1 H, br s, H_a-28), 4.62 (1 H, br s, H_b-28), 4.35 (1 H, dt, *J* = 4.5 and 9.0, H-3α), 2.05 (3 H, s, $COCH_3$), 1.03 (6 H, d, *J* = 6.5, H-26, H-27), 1.00 (3 H, s, H-19), 0.90 (3 H, s, H-18), 0.82 (6 H, d, *J* = 6.5 Hz, H-21, H-29); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.8 (s), 156.7 (s), 106.8 (t), 78.8 (s), 73.6 (d), 59.3 (d), 56.6 (d), 51.6 (d), 43.0 (d), 41.0 (d), 39.7 (s), 37.2 (d), 36.2 (d) 35.4 (d), 35.1 (d), 34.4 (t), 33.8 (t), 30.9 (d), 29.6 (d), 27.6 (t), 26.5 (t), 21.9 (t), 21.8 (t), 21.2 (q), 20.0 (q), 18.3 (q), 15.1 (q), 13.4 (q), 13.3 (q) and 18.9 (q); EIMS (70 eV), *m/z* 472 (M^+), 347 ($M^+ - C_9H_{17}$);HREIMS *m/z* found 457.3701 ($M^+ - CH_3$) calc. for $C_{30}H_{49}O_3$ 457.3681 ($M^+ - CH_3$).

Single Crystal X-Ray Analysis of Compound **1a**. *Crystal Data*.—$C_{31}H_{52}O_3$, *M* = 472.73, colourless crystals, monoclinic, space group $P2_1$, *a* = 7.937(2), *b* = 10.151(10), *c* = 18.421(3) Å, β = 102.22(2)°, *V* = 1450.4(2) Å^3, *Z* = 2, D_c = 1.082 Mg m^{-3}, *T* = 293 K, *F*(000) = 524, μ(Mo-Kα) = 0.067 mm^{-1}, crystal dimensions 0.11 × 0.09 × 0.20 mm. Data were collected on an Enraf-Nonius MACH-3 diffractometer, with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å), by the ω scan method in the range $2 \leq \theta \leq 25°$. A total of 2888 reflections were collected (+*h*, +*k*, ±*l*), with 2701 unique [R_{int} = 0.02], of which 1499 had $I > 2\sigma(I)$ and were used in all calculations. At final convergence *R*1 = 0.044, *wR*2 = 0.102 for 315 parameters and 1 restraint, goodness of fit = 1.08, $\Delta\rho_{max}$ = 0.14, $\Delta\rho_{min}$ = −0.13 e Å^{-3}. The data were reduced using XTAL (version 3.4), solved by direct methods, refined by full-matrix least squares on F^2 with the non-H atoms anisotropic and H atoms were placed in calculated positions and allowed to ride on their parent atoms.[12] However, the absolute configuration of **1a** could not be determined. Full crystallographic details, excluding structure factors, have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Instructions for Authors, *J. Chem. Research*, 1999, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 423/27.

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