Marine Sterols. XXIV.[1] Isolation of 24-Methylenecholestane-1α,3β,5α,6β,16β-pentol from *Sinularia* sp. of Soft Coral

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The lipid extract of *Sinularia* sp. of soft coral, collected off the coast of the Andaman and Nicobar Islands, afforded a new sterol 5, together with three known compounds 2, 3 and 4, and the aglycone (1) of 2. The structure of 5 was derived by comparison of the ^{1}H- and ^{13}C-NMR data with those of 2 and 4 having the same C,D- and A,B-ring substituents, respectively.

Keywords Coelenterate; soft coral; *Sinularia* sp.; 24-methylenecholestane-1α,3β,5α,6β,16β-pentol

Soft corals (Coelenterate) contain a diversity of mono- and polyhydroxysterols, most of which are derivatives of 24β-methylcholestane-type[2] C_{28} sterols.[3,4] Two new polyhydroxysterols, **1** and **5**, have been isolated from *Sinularia* sp. of soft coral collected off the coasts of the Andaman and Nicobar Islands, Indian Ocean, together with three known compounds recently isolated from soft corals (**2** and **4**) and sponge (**3**). The present paper deals with the structure elucidation of **1** and **5**, the latter being the most heavily oxygenated sterol so far found in soft corals.

The least polar compound **1** was a diunsaturated C_{28} diol. The carbon-13 nulcear magnetic resonance (^{13}C-NMR) signals[5] due to the A,B,C-rings, and the 19-H_3 signal in the proton (^{1}H-) NMR (δ 1.02)[6] indicated it to be a common 3β-hydroxy-Δ^5 sterol (Chart 1). Other signals (Experimental) were closely related to those of the simultaneously isolated 24-methylenecholestane-type fucoside **2**,[7] which we reported recently as a constituent of a soft coral of *Alcyonium* sp. collected in the same district. In the previous work, compound **2** was subjected to pyridinium chlorochromate oxidation, giving a five-membered ring ketone, and to acid hydrolysis which caused the rearrangement of the terminal methylene bond. This time **2** was submitted to $Pb(OAc)_4$ oxidation followed by alkaline hydrolysis. The aglycone obtained was identical with compound **1** isolated from the soft coral.

Compound **3** was a trihydroxyergostane-type sterol with two double bonds, one of them a 22*E*-double bond.[6] The chemical shifts (in pyridine-d_5), corresponding to 19-H_3 (δ 1.55), 3α-H (4.85, m) and 4β-H (3.05, dd, J=11.5, 13 Hz), were strongly down-field shifted by the pyridine-induced deshielding effect (Chart 2),[8] as compared with those taken in $CDCl_3$. This and the signals of 18-H_3 (δ 0.68), which is slightly up-field shifted,[9] and a deshielded olefinic proton (5.75) indicated the compound to be 24-methylcholesta-7,22-diene-3β,5α,6β-triol. In contrast to soft corals, sponges often elaborate 24-methylsterols as both 24α- and 24β-isomers. Both isomers of **3** were reported previously from the sponge *Sponginella gracilis*.[10] Although the ^{13}C-NMR data were available only for the 24β-isomer, they agreed well, within a deviation of 0.2 ppm, with those of **3**.

Compound **4** was a tetrahydroxysterol with a 24-methylenecholestane-type side chain, like **1** and **2**. The ^{13}C-NMR (Chart 1) showed identical signals as regards the side-chain and C- and D-rings while the signals due to the A- and B-rings were typical of a 3β,5α,6β-trihydroxylated steroid

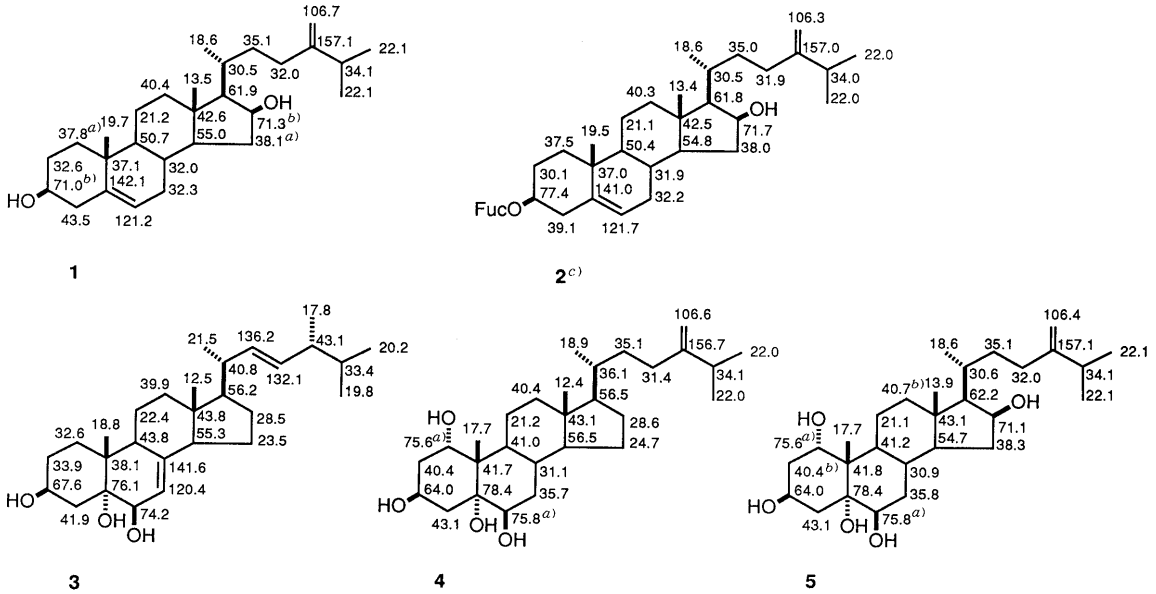

Chart 1. Structures and ^{13}C-NMR Signals (δ) of **1** to **5**

a, b) These signals may be interchanged. *c)* Taken from ref. 7.

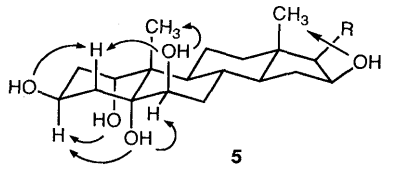

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Chart 2. Pyridine-Induced Deshielding of 1α,3β,5α,6β,16β-Hydroxyl Groups

nucleus, as in **3**.[4] In our earlier work on the polyhydroxysterols of the southern Japanese soft coral *Sarcophyton glaucum*, we pointed out the intense pyridine-induced deshielding effect of the hydroxyl groups, particularly against 19-H_3, 3α-H and 4β-H, which occupy 1,3-*syn*-periplanar positions (Chart 2).[4a] The 3α-H signal of **4** appeared 0.32 ppm down-field (δ 5.18) as compared with that of the 3β,5α,6β-triol[4a] and indicated the presence of an extra hydroxyl group at 1α. The same compound has recently been isolated from the soft coral *Sinularia numerosa* by Su *et al.*,[11] and the structure was established by X-ray crystallography. The identity was confirmed by comparison of their ^{1}H- and ^{13}C-NMR and mass spectral (MS) data (Experimental).

The ^{1}H- (Experimental) and the ^{13}C-NMR (Chart 1) data of the most polar sterol **5** indicated that it is the 16β-hydroxy derivative of **4**. The chemical shifts observed were virtually the same as those of **1** and **2**[7] regarding the C- and D-rings and side chain, and with those of **4** regarding the A- and B-rings. The pyridine-induced shift observed for 18-H_3 ($\delta_{\text{pyridine-}d_5} - \delta_{CDCl_3}$), due to 16β-OH, was +0.31 ppm, essentially identical with those observed in **1** (+0.28 ppm) and **2** (+0.26 ppm). 15β-Hydroxylation would cause the same effect,[8] but it was ruled out by the prominent β- (C-15, +13 ppm, C-17, +15 ppm) and γ-substituent effects (C-14, −1.8 ppm, C-20, −5.5 ppm) of 16-OH, as found in **1** and **2**.

Experimental

Optical rotations were determined in $CHCl_3$ on a JASCO DIP-370 digital polarimeter. NMR spectra were determined on a JEOL JMN GX-400 spectrometer at 400 MHz (^{1}H) and on a JEOL JMN FX-90Q spectrometer at 22.5 MHz (^{13}C) with tetramethylsilane (^{1}H, δ 0.00) and pyridine-d_5 (^{13}C, center peak δ 135.5) as internal standards. Mass spectra (MS) were determined on a JEOL JMS D300 mass spectrometer.

Material The soft coral, code name MF-VA-08 (dry weight 900 g), was collected in April 1991 on the coasts of the Andaman and Nicobar Islands (Maya Bunder, 12°40′N, 92°53′E). The organism was washed with fresh water, cut into slices and preserved in EtOH. The extraction was carried out using EtOH by percolation every 4 d. The process was repeated 6 times. The solvent was evaporated off by distillation under reduced pressure and the dark colored residue was extracted with ethyl acetate several times. The extract was passed over anhydrous $MgSO_4$. The extract (25 g) was chromatographed over silica gel (500 g, Acme 100—200 mesh) using solvent mixtures of petroleum ether (A)–ethyl acetate (B) as follows: A–B (9 : 1), monohydroxysterol mixture (3 g); A–B (4 : 1), fraction I (1 g); A–B (3 : 2), batyl alcohol (2 g); A–B (1 : 1 to 2 : 3), intractable gum; A–B (3 : 7), fraction II (100 mg); A–B (1 : 9), fraction III (125 mg); A–B (1 : 9) and B only, fraction IV (250 mg). Fraction I was chromatographed with a mixture of A–B (9 : 1) giving two components, pregnenolone (pregn-5-en-3β-ol-20-one), mp 182—185 °C, and **1** (50 mg). Fractions II, III and IV were chromatographed with a mixture of $CHCl_3$–MeOH giving **2** (8 mg) from II, **3** (13 mg) from III, and **4** (50 mg) and **5** (14 mg) from IV. The *Rf*s of these compounds on thin-layer chromatography (TLC) with MeOH–$CHCl_3$ (1 : 9) were 0.81 (**1**), 0.53 (**2**), 0.49 (**3**), 0.40 (**4**) and 0.28 (**5**), respectively. Compound **2** (24-methylenecholest-5-ene-3β,16β-diol-3-*O*-α-L-fucopyranoside) was identical with an authentic specimen as judged by co-TLC and ^{1}H-NMR spectral comparison.

24-Methylenecholest-5-ene-3β,16β-diol (1) Colorless needles, mp 154—156 °C, $[\alpha]_D$ +8.5° (*c* = 4.40, $CHCl_3$). ^{1}H-NMR (pyridine-d_5) δ: 1.17 (3H, s, 18-H_3), 1.08 (3H, s, 19-H_3), 1.035 and 1.040 (each 3H, d, *J* = 6.5 Hz, 26,27-H_3), 1.13 (3H, d, *J* = 7.0 Hz, 21-H_3), 3.87 (1H, m, 3α-H), 4.56 (1H, m, 16α-H), 4.84, 4.90 (each br s, 28-H_2), 5.43 (1H, m, 6-H); ($CDCl_3$) δ: 0.89 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.030 and 1.035 (each 3H, d, *J* = 6.5 Hz, 26,27-H_3), 1.02 (3H, d, *J* = 6.5 Hz, 21-H_3), 3.52 (1H, br m, 3α-H), 4.37 (1H, ddd, *J* = 7.5, 7.5, 6.5 Hz, 16α-H), 4.70, 4.75 (each br s, 28-H_2), 5.35 (1H, m, 6-H). MS *m/z*: 414 (M^+), 399, 396, 330, 315, 312, 300, 297, 285, 271, 253, 124 (base peak). High-resolution MS: 414.3490. Calcd for $C_{28}H_{46}O_2$: 414.3498.

(24*R*)-Methylcholesta-7,22-diene-3β,5α,6β-triol (3) Colorless solid, mp 221—224 °C, $[\alpha]_D$ −64° (*c* = 1.44, pyridine). The identification was made by comparison of the ^{1}H- and ^{13}C-NMR data with those reported in the literature.[10]

24-Methylenecholestane-1α,3β,5α,6β-tetrol (4) Colorless flakes, mp 292—295 °C, $[\alpha]_D$ −7.9° (*c* = 3.74, pyridine). The identification was made by comparison of the ^{1}H- (in $CDCl_3$) and ^{13}C-NMR and MS data with those reported in the literature.[11] ^{1}H-NMR (pyridine-d_5) δ: 0.73 (3H, s, 18-H_3), 0.96 (3H, d, *J* = 6.5 Hz, 21-H_3), 1.04 and 1.05 (each 3H, d, *J* = 7.0 Hz, 26,27-H_3), 1.55 (3H, s, 19-H_3), 3.04 (1H, dd, *J* = 12,0, 12.0 Hz, 4β-H), 4.09 (1H, br s, 6α-H), 4.21 (1H, br s, 1β-H), 4.83, 4.85 (each br s, 28-H_2), 5.17 (1H, m, 3α-H).

24-Methylenecholestane-1α,3β,5α,6β,16β-pentol (5) Colorless solid, mp 268—271 °C, $[\alpha]_D$ −8.0° (*c* = 1.92, pyridine). ^{1}H-NMR (pyridine-d_5) δ: 1.025 and 1.030 (each 3H, d, *J* = 6.5 Hz, 26,27-H_3), 1.11 (3H, d, *J* = 6.5 Hz, 21-H_3), 1.21 (3H, s, 18-H_3), 1.58 (3H, s, 19-H_3), 3.04 (1H, dd, *J* = 12.5, 11.5 Hz, 4β-H), 4.09 (1H, br s, 6α-H), 4.22 (1H, br s, 1β-H), 4.57 (1H, m, 16α-H), 4.82, 4.88 (each br s, 28-H_2), 5.18 (1H, m, 3α-H); ($CDCl_3$) δ: 0.90 (3H, s, 18-H_3), 1.13 (3H, s, 19-H_3), 1.01 (3H, d, *J* = 6.5 Hz, 21-H_3), 1.030 and 1.035 (each 3H, d, *J* = 6.5 Hz, 26,27-H_3), 3.50 (1H, br s, 6α-H), 3.89 (1H, br s, 1β-H), 4.37 (1H, m, 16α-H), 4.39 (1H, m, 3α-H), 4.70, 4.75 (each br s, 28-H_2). MS *m/z*: 464 (M^+), 449, 430, 412, 365, 362, 344, 321, 303, 285, 124 (base peak). High-resolution MS: 464.3510. Calcd for $C_{28}H_{48}O_5$: 464.3501.

Conversion of 2 to 1 A solution of **2** (9.2 mg) in $CHCl_3$ (1 ml) was treated, at room temperature, with $Pb(OAc)_4$ (16.7 mg) for 10 min. The mixture was diluted with Et_2O, and washed with H_2O and saturated NaCl solution. The evaporation residue was treated with 7.5% NaOH in MeOH at 50 °C for 10 min, then diluted with Et_2O. Usual work-up and silica gel column chromatography of the residue with $CHCl_3$ gave 3.7 mg of **1**. The identity was confirmed by ^{1}H-NMR and co-TLC.

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References and Notes

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