Marine Sterols. XVI.[1] Polyhydroxysterols of the Soft Corals of the Andaman and Nicobar Coasts. (1). Isolation of (24*S*)-24-Methylcholest-5-ene-3β,25ξ,26-triol and (24*S*)-24-Methylcholestane-3β,5β,6α,25-tetrol

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Nine polyhydroxysterols were isolated from the lipid extract of two *Sclerophytum* sp. soft corals collected in the Andaman and Nicobar Islands. Of these, seven compounds (1, 4a—6c) had previously been isolated from southern Japan soft coral *Sarcophyton glaucum*. The structures of the two new steroids 2 and 3 were determined as (24*S*)-24-methylcholest-5-ene-3β,25ξ,26-triol and (24*S*)-24-methylcholestane-3β,5β,6α,25-tetrol, respectively, by means of spectroscopic analyses, and by correlation with the known compounds.

Keywords coelenterata; soft coral; *Sclerophytum* sp.; polyhydroxysterol; 24-methylcholest-5-ene-3β,25ξ,26-triol; 24-methylcholestane-3β,5β,6α,25-tetrol

Soft corals contain a diversity of 3β-monohydroxysterols, including some having a biogenetically modified C-17 side chain.[2,3] The predominant sterols are usually the biogenetically normal 24-methylcholestane-type sterols, as exemplified by brassicasterol with (24*S*) configuration.[3a] They often occur in soft corals as the polyhydroxy derivatives, oxygenated at various sites in both the steroid nucleus and its C-17 side chain.[2] *Sarcophyton glaucum*, one of the most typical species found in Indo-Pacific coral reefs, collected at Ishigaki Island, Okinawa, have been shown to contain eight polyhydroxysterols of which all but one compound, androstane-1β,3β,5α,6β-tetrol-17-one,[4] were derivatives of (24*S*)-24-methylcholestane.[5] As a continuation of our studies of the steroids of soft corals, we have started to investigate those of the soft corals in the Andaman and Nicobar Seas of the Indian Ocean. Of the nine soft corals collected, eight organisms were identified at the genus level as *Sclerophytum* sp. and one as an *Alcyonium* sp., by courtesy of the Zoological Survey of India, Calcutta, but further definition was not possible. Extraction and separation of these materials resulted in the isolation of various polyhydroxysterols. The present paper deals with the steroids of two *Sclerophytum* sp., code names MF-CBR-06 and MF-CBR-14, which are morphologically different but show a common steroid pattern.

Repeated chromatography of the lipid extracts of these two corals afforded polyhydroxysterols **3**, **4a** and **4b** from MF-CBR-06, and **1**, **2**, **4a**, **4b**, **5**, **6a**, **6b** and **6c**, from MF-CBR-14. Of these, compounds **2** and **3** are new, but the others were found to be known compounds previously isolated from *S. glaucum*.

The high-resolution mass spectrum of compound **2** showed the molecular formula $C_{28}H_{48}O_3$. The major ions at *m/z* 273, 271, 255, 231 and 213 in the mass spectrum (MS) of **2** are common ions of the 3β-monohydroxy-Δ^5 sterols of the soft corals,[3] derived by cleavage of the side chain with and without loss of 2H, followed by concurrent loss of H_2O and cleavage of the D-ring.[6] This indicates compound **2** to bear a dihydroxylated C-17 side chain, while the chemical shifts of signals in its proton nuclear magnetic resonance (^{1}H-NMR) spectrum (in pyridine-d_5), especially those of H-3, 18, 19 (H-3, δ 3.80—3.93, br; H-18, δ 0.68, 3H, s; H-19, δ 1.07, 3H, s), and an olefinic proton at δ 5.41—5.60,

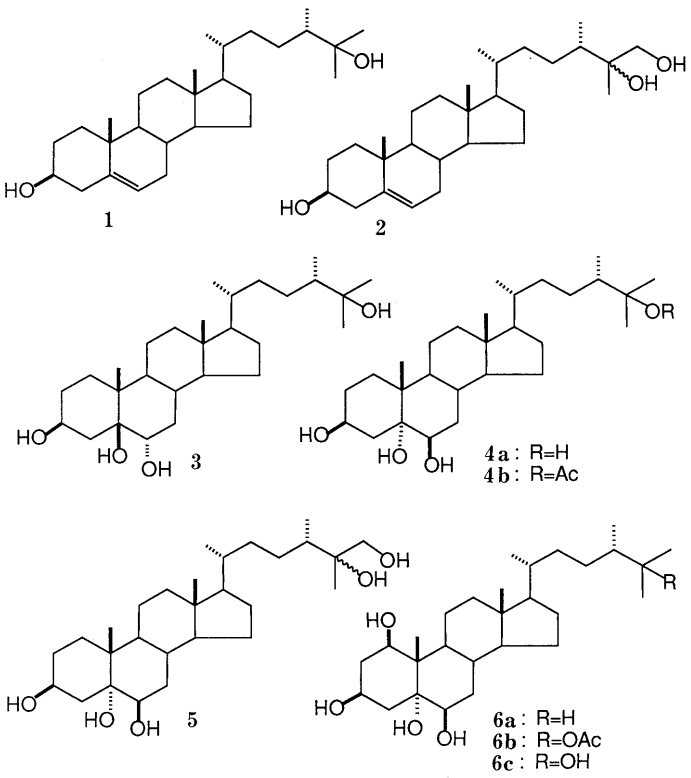

Chart 1

suggest it to be a 3β-hydroxy-Δ^5-steroid derivative.[3] The ^{1}H-NMR showed the signals of a secondary methyl at δ 1.27 (d, J=7.0 Hz), and a methyl which is adjacent to an oxygen atom at δ 1.46 (s), and two doublet proton signals at δ 3.96 and 4.02 (J=10.5 Hz). These results indicate that **2** is oxygenated at C-25 and C-26 as found in the known compound (24*S*)-24-methylcholestane-3β,5α,6β,25ξ,26-pentol (**5**) isolated from *S. glaucum* previously,[5c] and from MF-CBR-14 in the present study. Glycolation of the C-5 double bond of **2** with hydrogen peroxide and formic acid followed by hydrolysis gave a product which was identical with **5**. Compound **2** was thus shown to be (24*S*)-24-methylcholest-5-ene-3β,25ξ,26-triol, a possible biogenetic precursor of **5**.

The other unknown compound **3** was a polyhydroxysterol

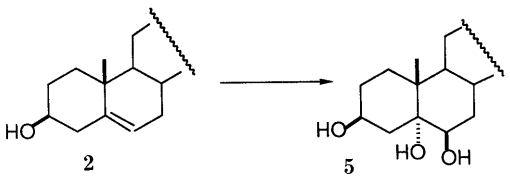

Chart 2

whose MS pattern was almost indistinguishable from that of the known compound **4a**. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **3** supported the structural similarity with **4a** and showed common signals (see Experimental) as regards the carbons in the side chain and C- and D-rings of the steroid nucleus.[3,5] In contrast, the ^{1}H-NMR spectra of **3** and **4a** showed significant discrepancies. In **4a** and its derivatives having axially oriented 5α- and 6β-hydroxyl groups, the protons at 3α and 4β and the angular methyl group at C-10 have a 1,3-diaxial disposition with respect to one of these hydroxyl groups. As a result they suffer significant pyridine-induced deshielding,[7] and their ^{1}H-NMR signals occur at unusually lowfield (H-3, *ca.* δ 5.0; H-4β, *ca.* δ 3.0; H-19, *ca.* δ 1.8).[4,5] These effects were absent in the spectrum of **3** except for a signal at δ 2.49 (br d, J=14.5 Hz) which was supposed to be due to H-4β. Comparison of the ^{1}H-NMR chemical shifts (in $CDCl_3$) of two hydroxy methine protons (δ 3.78, dd, J=12.0, 5.0 Hz; δ 4.27, br s, half width 8 Hz) and two angular methyl groups (δ 0.66 and 0.93) of **3** with those reported for the four diastereomers of 3β-hydroxycholestane-5,6-diol[8] revealed that these chemical shifts were virtually the same as those of cholestane-3β,5β,6α-triol (H-3, δ 4.25, br s; H-6, δ 3.78, dd, J=12, 4 Hz; H-18, δ 0.65; H-19, δ 0.93). The other three isomers show distinctly different patterns either in their chemical shifts or their coupling patterns as regards these signals. The ^{13}C-NMR chemical shifts of **3** at C-1 (δ 26.4) and C-4 (δ 30.8) were significantly different from those of 5α,6β-glycol derivatives, *e.g.* **4a** (C-1, δ 32.2, C-4, δ 42.9). The calculated values of the chemical shifts of 3β,5β,6α-trihydroxysterol, derived from those of 5β-cholestan-3β-ol (C-1, δ 30.0, C-4, δ 33.6),[9] by application of the semiempirical derivation of ^{13}C-NMR chemical shifts reported by Beierbeck *et al.*,[10] are δ 24.2 (C-1) and δ 32.3 (C-4) respectively. Pyridinium chlorochromate (PCC) oxidation of **3** and **4a** gave the C-5 isomeric diketones **7** and **8**. Dehydration of these two compounds with thionyl chloride in pyridine afforded the same Δ^4-3,6-diketo derivative **9**. These results unequivocally indicate compound **3** to be (24*S*)-24-methylcholestane-3β,5β, 6α,25-tetrol. The structures of new polyhydroxysterols isolated from other soft coral samples are under investigation.

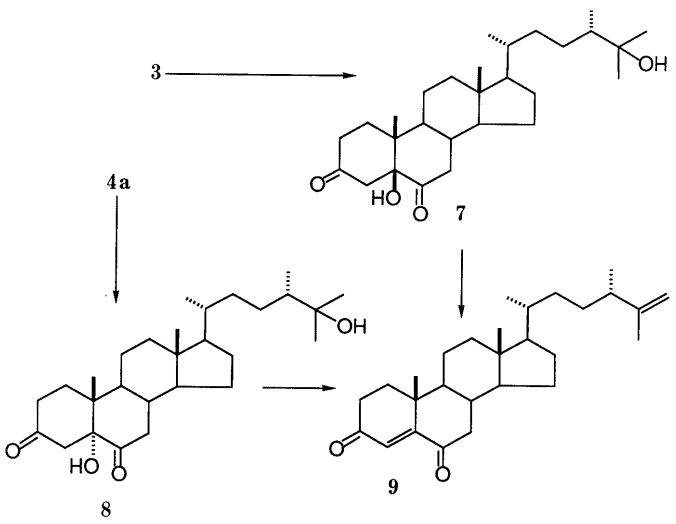

Chart 3

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. NMR spectra were determined on a JEOL JNM GX-270 spectrometer at 270 MHz (^{1}H) and on a JEOL JNM FX-90Q spectrometer at 22.5 MHz (^{13}C) with tetramethylsilane as an internal standard. MS were determined on a JEOL JMS D 300 mass spectrometer.

Materials The locations of collection and the code numbers of the soft corals and the individual polyhydroxysterols isolated are as follows. 92°43′E, 11°41′N: MF-CBR-06 (sterols, MF-CBR-06-06 to MF-CBR-06-08); MF-CBR-13 (sterols, MF-CBR-13-01 and MF-CBR-13-02); MF-CBR 14 (sterols, MF-CBR-14-01 to MF-CBR-14-09); MF-CBR-25 (sterols, MF-CBR-25-01 to MF-CBR-25-02). 91°31′E, 10°30′N: MF-CBR-27 (sterols, MF-CBR-27-01 to MF-CBR-27-05); MF-CBR-30 (sterols, MF-CBR-30-01 to MF-CBR-30-04); MF-CBR-33 (sterols, MF-CBR-33-01 to MF-CBR-33-03); MF-CBR-38 (sterols, MF-CBR-38-01 to MF-CBR-38-07); MF-CBR-41 (sterols, MF-CBR-41-01 to MF-CBR-41-03). The soft corals were freed from other organisms and algae as thoroughly as possible, washed with water, cut into thin slices and preserved in EtOH. The extraction was carried out using EtOH by percolation, every 2 d. The process was repeated 6 to 8 times. The solvent was evaporated off by distillation under reduced pressure and the dark-colored slimy residue was extracted into ethyl acetate several times. The residues obtained after extraction were air-dried and extracted with ethyl acetate in a Soxhlet apparatus. The combined crude extracts were then chromatographed over Sephadex LH-20, using $CHCl_3$–MeOH–hexane (1:1:3) and the fractions that were free from coloring matter and showed distinct spots over silica gel layers were combined. This materials was subjected to extensive chromatography over silica gel columns with mixtures of ethyl acetate–petroleum ether (5:95 to 70:30) and MeOH–$CHCl_3$ (1:9). The amounts of the soft coral materials, other than MF-CBR-06 and MF-CBR-14, and the yields of the individual polyhydroxysterols isolated witll be presented elsewhere.

Polyhydroxysterols of MF-CBR-06 and MF-CBR-14 After the extraction and separation as described above, the soft coral materials MF-CBR-06 (1.1 kg after extraction) gave the polyhydroxysterols MF-CBR-06-06 (150 mg), MF-CBR-06-07 (95 mg), and MF-CBR-06-08 (168 mg). Similarly the soft coral MF-CBR-14 (1.8 kg) gave the polyhydroxysterols MF-CBR-14-01 (52 mg), MF-CBR-14-02 (70 mg), MF-CBR-14-03 (310 mg), MF-CBR-14-04 (10 mg), MF-CBR-14-05 (8 mg), MF-CBR-14-06 (7 mg), MF-CBR-14-07 (8 mg), MF-CBR-14-08 (30 mg), and MF-CBR-14-09 (8 mg). The known compounds were identified from the ^{1}H-NMR and MS spectra, and by thin-layer chromatography (TLC) with authentic specimens, as follows: MF-CBR-06-06, (24*S*)-24-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate (**4b**): MF-CBR-06-08, (24*S*)-24-methylcholestane-3β,5α,6β,25-tetrol (**4a**); MF-CBR-14-01, (24*S*)-24-methylcholest-5-ene-3β,25-diol (**1**); MF-CBR-14-02, **4b**; MF-CBR-14-03, mixture of (24*S*)-24-methylcholestane-1β,3β,5α,6β-tetrol (**6a**) and (24*S*)-24-methylcholestane-1β,3β,5α,6β,25-pentol 25-monoacetate (**6b**); MF-CBR-14-04, **4a**; MF-CBR-14-06, (24*S*)-24-methylcholestane-3β,5α,6β,25ξ,26-pentol (**5**); MF-CBR-14-07, (24*S*)-24-methylcholestane-1β,3β,5α,6β,25-pentol (**6c**); MF-CBR-14-08 and MF-CBR-14-09, unknown.

(24*S*)-24-Methylcholest-5-ene-3β,25ξ,26-triol (MF-CBR-14-05, 2) mp 230—234 °C (from MeOH), $[\alpha]_D^{24}$ 0° (c=0.03, MeOH). ^{1}H-NMR pyridine-d_5) δ: 0.68 (3H, s, H-18), 1.01 (3H, d, J=6.5 Hz, H-21), 1.07 (3H, s, H-19), 1.27 (3H, d, J=7.0 Hz, H-28), 1.46 (3H, s, H-27), 3.96, 4.02 (each 1H, d, J=10.5 Hz, H-26), 3.80—3.93 (1H, m, H-3), 5.41—5.60 (1H, m, H-6). MS m/z: 432 (M^+), 414, 401, 399, 396, 383, 381, 365, 340, 329, 303, 273, 271, 255, 231, 213, 75 (base peak). High-resolution MS [Found (Calcd)] m/z: $C_{28}H_{48}O_3$ (M^+) 432.3619 (432.3603).

(24*S*)-24-Methylcholestane-3β,5β,6α,25-tetrol (MF-CBR-06-07, 3) mp 226—227 °C ($CHCl_3$–MeOH), $[\alpha]_D^{29}$ +16° (c=0.52, MeOH). ^{1}H-NMR (in

pyridine-d_5) δ: 0.67 (3H, s, H-18), 1.02 (3H, d, J=6.5 Hz), 1.08 (3H, d, J=7.0 Hz), 1.15 (3H, s, H-19), 1.35, 1.36 (each 3H, s, H-26, 27), 2.21 (1H, td, J=13.5, 4.5 Hz), 2.49 (1H, br d, J=14.5 Hz), 4.12 (1H, dd, J=12.0, 5.0 Hz, H-6), 4.48 (1H, br s, half width 7.5 Hz, H-3). ^{1}H-NMR (in $CDCl_3$) δ: 0.66 (3H, s, H-18), 0.93 (3H, s, H-19), 0.89, 0.92 (each 3H, d, J=6.5 Hz, H-21, 28), 1.15, 1.17 (each 3H, s, H-26, 27), 3.78 (1H, dd, J=12.0, 5.0 Hz, H-6), 4.27 (1H, br s, half width 8 Hz, H-3). ^{13}C-NMR (in pyridine-d_5) δ: C-1 (26.4), C-2 (28.1), C-3 (67.1), C-4 (30.8), C-5 (78.5), C-6 (71.7), C-7 (35.3), C-8 (34.2), C-9 (43.3), C-10 (41.6), C-11 (22.1), C-12 (40.2), C-13 (42.8), C-14, 17 (56.0, 56.4), C-15 (24.4), C-16, 23 (28.1, 28.4), C-18 (12.2), C-19 (17.7), C-20 (36.6), C-21 (19.2), C-22 (36.4), C-24 (45.9), C-25 (72.2), C-26, 27 (26.4, 28.1), C-28 (15.4). MS m/z: 450 (M^+), 432, 414, 399, 396, 378, 374, 356, 341, 320, 305, 289, 271, 59 (base peak). High-resolution MS [Found (Calcd)] m/z: $C_{28}H_{50}O_4$ (M^+), 450.3726 (450.3709).

Glycolation of 2 A solution of **2** (4.9 mg) in tetrahydrofuran (THF) (0.15 ml) was treated with 0.15 ml of a mixture of H_2O_2 and HCOOH (1 : 10) at 0 °C. The mixture was stirred at room temperature for 2.5 h. After addition of 10% Na_2SO_3 (1.6 ml), most of the solvents were evaporated off. The mixture was extracted with Et_2O, and washed with H_2O and saturated NaCl solution, then the solvent was evaporated off. The residue was dissolved in a mixture of MeOH (0.15 ml) and 25% NaOH solution (10 μl), and refluxed for 1 h. The mixture was diluted with Et_2O, and washed with 5% HCl, H_2O and saturated NaCl solution, then the solvent was evaporated off. Column chromatography of the residue with MeOH–$CHCl_3$ (1 : 9) gave 3.5 mg of **5**, mp 258—259 °C (lit., mp 262—264 °C), which was identical with **5** isolated from *S. glaucum*,[5c] by comparison of their ^{1}H-NMR and MS, and TLC behavior.

PCC Oxidation of 3 and 4a (a) A solution of **4a** (200 mg) in CH_2Cl_2 (10 ml) was treated with 350 mg of PCC at 0 °C. The mixture was stirred at room temperature for another 6 h. The mixture was extracted with Et_2O and the Et_2O solution was evaporated. The residue was submitted to column chromatography, eluting with Et_2O–CH_2Cl_2 (1 : 19) to give 55.5 mg of **8**, mp 267–269 °C, $[\alpha]_D^{28}$ −15° (c=0.33, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.67 (3H, s), 0.89 (3H, d, J=7.0 Hz), 0.94 (3H, d, J=6.5 Hz), 1.00 (3H, s), 1.14 (3H, s), 1.16 (3H, s), 2.72 (1H, dd, J=13.0, 12.5 Hz), 2.92 (1H, d, J=15.5 Hz). MS m/z: 410 (M^+-2H_2O), 370, 285, 257. High-resolution MS [Found (Calcd)] m/z: $C_{28}H_{42}O_2$ (M^+), 410.3185 (410.3185), (b) A solution of **3** (16.6 mg) was treated with 59 mg of PCC as described in (a). Purification of the mixture as in (a) gave 7.7 mg of **7** as an oil, $[\alpha]_D^{27}$ −2.3° (c=1.38, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.70 (3H, s), 0.81 (3H, s), 0.90, 0.96 (each 3H, d, J=6.5 Hz), 1.15, 1.17 (each 3H, s), 3.02 (1H, d, J=15.0 Hz). ^{13}C-NMR (in $CDCl_3$) δ: C-1 (31.5), C-2 (37.0), C-3, 6 (207.1, 210.2), C-4 (48.1), C-5 (83.8), C-7 (41.4), C-8 (37.4), C-9 (43.7), C-10 (44.4), C-11 (22.1), C-12 (39.5), C-13 (43.2), C-14, 17 (55.8, 56.9), C-15 (24.0), C-16, 23 (28.0), C-18 (12.1), C-19 (16.2), C-20 (36.3), C-21 (19.0), C-22 (34.8), C-24 (45.2), C-25 (73.7), C-26 (26.2), C-27 (27.4), C-28 (14.9). MS m/z: 410 (M^+-2H_2O), 395, 370, 355, 341, 327, 326, 311, 283, 270, 257.

Dehydration of 7 and 8 (a) A solution of **8** (19.7 mg) in pyridine (0.2 ml) was treated with 16 μl of $SOCl_2$. After 5 min the mixture was diluted with Et_2O, and washed with 5% HCl, H_2O and saturated NaCl solution, then the solvent was evaporated off. Chromatography of the residue over a column of 7.5% silver nitrate-impregnated silica gel with ethyl acetate–hexane (1 : 9) gave 1.7 mg of **9** as an oil, $[\alpha]_D^{26}$ −15° (c=0.12, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.72 (3H, s), 0.92 (3H, d, J=6.5 Hz), 1.00 (3H, d, J=6.5 Hz), 1.16 (3H, s), 1.64 (3H, s), 4.67 (2H, br s), 6.17 (1H, s). MS m/z: 410 (M^+), 395, 375, 341, 326, 311, 283, 270, 257. UV λ_{max}^{EtOH} nm (ε): 253 (5400). High-resolution MS [Found (Calcd)] m/z: $C_{28}H_{42}O_2$ (M^+), 410.3156 (410.3174). (b) Compound **7** (6.9 mg) in pyridine (0.07 ml) was treated with $SOCl_2$ (5 μl), worked up, and subjected to chromatography as described in (a), giving 0.5 mg of the enedione, which was identical with **9** on the basis of comparisons of their ^{1}H-NMR and MS spectra, and TLC behavior.

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