Marine Sterols. XIX.[1] Polyhydroxysterols of the Soft Corals of the Andaman and Nicobar Coasts. (3). Isolation and Structures of Five New C_{28} Polyhydroxysterols from Two *Sclerophytum* sp. Soft Corals

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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, three compounds (7a, b, and 8) had previously been isolated from the southern Japan soft coral *Sarcophyton glaucum*. Compound 1 was identified as lobosterol having a novel 6-keto-A/B-*cis* ring juncture. The structures of the five new compounds were determined as 25-deacetyllobosterol (2), (24*S*)-24-methylcholest-7-ene-3β,5α,6β,25-tetrol 25-monoacetate (3), (24*S*)-24-methylcholest-22*E*-ene-3β,5α,6β,25-tetrol (4), (24*S*)-24-methylcholestane-3β,5α,25-triol-6-one 25-monoacetate (5a) and its C-25 deacetoxy analog (6), from the spectral data and by chemical conversion.

Keywords coelenterata; soft coral; *Sclerophytum* sp.; polyhydroxy C_{28} sterol; 25-deacetyllobosterol; 24-methylcholest-7-ene-3β,5α,6β,25-tetrol 25-monoacetate; 24-methylcholest-22*E*-ene-3β,5α,6β,25-tetrol; 24-methylcholestane-3β,5α,25-triol-6-one 25-monoacetate; 24-methylcholestane-3β,5α-diol-6-one

Soft corals contain a diversity of mono- and polyhydroxysterols, most of which are derivatives of (24*S*)-24-methylcholestane-type[2] C_{28} sterols.[3,4] As a continuation of our studies on the sterols of the soft corals of the Okinawa Islands, we have started to investigate those of the soft corals in the Andaman and Nicobar Islands in the Indian Ocean. Of the nine soft corals collected, eight organisms were identified at the genus level as *Sclerophytum* sp., which has not previously been studied chemically, and one as an *Alcyonium* sp., but further definition was not possible. Extraction and separation of their polar lipids resulted in the isolation of various known and unknown polyhydroxysterols.[5] All eight *Sclerophytum* sp. soft corals contained (24*S*)-24-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate (**7a**) or its 25-deacetyl derivative **7b**. The present paper deals with the structures of the C_{28} polyhydroxysterols, isolated from two of the *Sclerophytum* sp. soft corals, code names MF-CBR-25 and MF-CBR-27.[5a] Repeated chromatography of their polar lipid extracts afforded seven (**1**—**4**, **7a**, **b** and **8**) and three (**5a**, **6** and **7a**) polyhydroxysterols from MF-CBR-27 and MF-CBR-25, respectively. Of these, compounds **7a**, **b**, and (24*S*)-24-methylcholest-5-ene-3β,25-diol (**8**) were identical with authentic specimens that we had previously isolated from the soft coral *Sarcophyton glaucum*, collected at Ishigaki Island, Okinawa.[4]

Compound **1** was a C_{28} sterol having one ketone, one tertiary acetoxyl, one hydroxyl, and two secondary hydroxyl groups, as indicated by its proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra (Experimental). The two secondary hydroxymethine protons (δ 4.43 and 4.65) were shown to be coupled to each other with small coupling constants (J=3.0—3.5 Hz). Most of the ^{13}C-NMR signals (C-6 to C-9, and C-11 to C-28) were found to be identical with those of previously synthesized (24*S*)-24-methylcholestane-5β,25-diol-3,6-dione 25-monoacetate.[5a] Heteronuclear multiple bond correlation spectroscopy (HMBC)[6] of **1** indicated the correlation of the 7β-H (in $CDCl_3$, δ 2.48, dd) with quaternary C-5, carrying an oxygen atom (δ 85.7). The chemical shifts of the protons and calculated values of the A-ring carbons,[7] suggested that compound **1** corresponds to the known

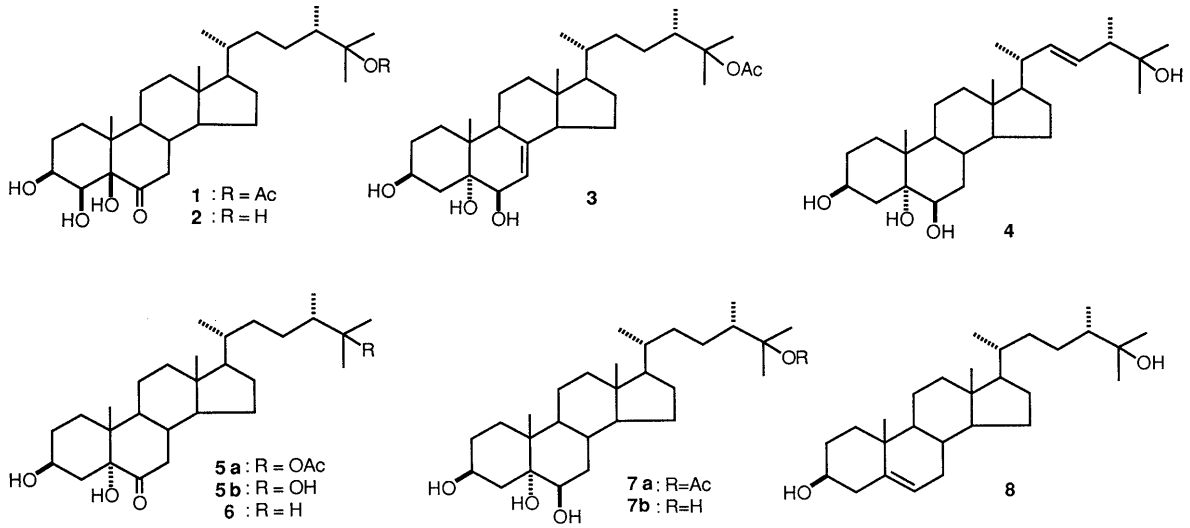

Chart 1

compound lobosterol,[8] having a 3β,4β,5β-trihydroxy-6-keto moiety. The structure of lobosterol has been established by X-ray crystallography. Direct comparison of compound **1** with an authentic specimen of lobosterol, provided by Daloze, confirmed their identity. Lobosterol is one of the earliest reported examples of marine polyhydroxysterols; its isolation from a soft coral, *Lobophytum pauciflorum*, was reported in 1976 by Tursch *et al.*, but to our knowledge isolation of this compound from other sources has not been reported since then. The characteristic feature of **1**, unlike other marine polyhydroxysterols, is that in $CDCl_3$, the ^{1}H-NMR signals of the three hydroxyl protons appear clearly (δ 2.77, d, J=11.0 Hz; δ 3.62, d, J=11.0 Hz; δ 4.49, s), possibly due to their tight internal hydrogen bondings.[9]

Compound **2** was shown to be 25-deacetyllobosterol; it had virtually the same ^{1}H- and ^{13}C-NMR chemical shifts (Experimental) as **1**, except for those of the side chain (^{1}H-NMR, δ 1.39, 1.41, each 3H, s; ^{13}C-NMR, δ 23.0, q, 23.5, q, 73.6, s). This identification was confirmed by mild alkaline hydrolysis of **1**, giving **2** as the sole product.

Compounds **3** and **4** are monounsaturated derivatives of **7a** and **7b**, respectively, having a 3β,5α,6β-trihydroxylated steroid nucleus. When studying polyhydroxysterols of *S. glaucum*,[4a] we pointed out that such a system could be readily recognized from the position and coupling pattern of the signals due to 3α-H (br m, $W_{1/2}$ = *ca.* 20 Hz), 4β-H (dd, J = *ca.* 13.0, 12.0 Hz), 6α-H (br s, $W_{1/2}$ = *ca.* 7 Hz), and 19-H_3. Because of the 1,3-*syn*-periplanar arrangement of hydroxyl groups (3α-H and 5α-OH, 4β-H and 6β-OH, 19-H_3 and 6β-OH), these protons are quite susceptible to the pyridine-induced deshielding effect,[10] and are shifted to unusually low field (**3**, 3α-H, δ 4.82, 4β-H, 3.04, 19-H_3, 1.55; **4**, 3α-H, δ 4.89, 4β-H, 2.97, 19-H_3, 1.67). This phenomenon is diagnostic for such a system and has been used subsequently by many workers in the structure elucidation of related polyhydroxysterols. The ^{1}H- and ^{13}C-NMR signals of the side chain of **3** (Experimental) were identical with those of **1** and **7a**,[11] so that the trisubstituted double bond (δ 5.75, 1H, m) was located in the steroid ring. The 18-H_3 signal (δ 0.59, taken in $CDCl_3$ solution) appeared at relatively high field, suggesting compound **3** to be a Δ^7 derivative.[12] Comparison of the ^{1}H- and ^{13}C-NMR signals with literature values revealed that they exactly coincide with those of the reference compound 24-methylcholesta-7,22-diene-3β,5α,6β-triol,[13] except for the side chain signals.

The ^{1}H- and ^{13}C-NMR signals due to the steroid nucleus of **4** (Experimental) were identical with those of **7b** reported previously,[4a] and those of the C-17 side chain were different. This indicated that the side chain is oxygenated at C-25 (δ 26.9, q, 28.7, q and 71.5, s) and bears one disubstituted double bond (δ 130.9, d and 137.2, d). From the vicinal coupling constant (15.5 Hz) of 22- and 23-H (δ 5.36 and 5.66, each 1H, dd) in the ^{1}H-NMR spectrum, the geometry at C-22 of **4** was concluded to be *E*. The mass spectrum of **4** did not give the molecular ion and the highest peak was observed at *m/z* 390. This ion could be attributed to the McLafferty-type cleavage at C-24 and C-25 with 1H transfer (Chart 2). From these results, compounds **3** and **4** were concluded to be (24*S*)-24-methylcholest-7-ene-3β,5α,6β,25-tetrol 25-monoacetate and (24*S*)-24-methylcholest-22*E*-ene-3β,5α,6β,25-tetrol, respectively. Assignment of 24*S* configuration to **4** is based only on the biogenetic analogy with other compounds simultaneously isolated.

Compounds **5a** and **6** were obtained as a crystalline mixture which was resistant to separation. The ^{1}H- and ^{13}C-NMR spectra of the mixture showed it to be composed of two compounds having 25-acetoxy-24-methylcholestane-type (major) and 24-methylcholestane-type C_{28} sterol structures, but the signals due to the steroid nucleus were common. A secondary (δ 67.3) and a tertiary hydroxyl group (δ 80.9), and one ketone moiety (δ 212.2) were present. The chemical shifts of C-12 to C-28 of the major component were identical with those of **1**, and the signals due to C-7 to C-9 and C-11 showed only small differences (less than 1.5 ppm). The ^{1}H-NMR (in $CDCl_3$) chemical shift of 3α-H (δ 3.98, m, $W_{1/2}$=20 Hz) and a signal at δ 2.72 (dd, J=13.0, 12.5 Hz), which is assignable to 7α-H, showed, in pyridine-d_5, a significant pyridine-induced deshielding effect (Δδ, 3α-H, 0.70 ppm; 7α-H, 0.42 ppm) indicating the presence of a *syn*-periplanar 5α-hydroxyl group. Partial oxidation of **7a**, using 0.8 eq of pyridinium chlorochromate (PCC), afforded 3,6-diketo-(**9**), 3-monoketo-(**10**) and 6-monoketo-(**5a**) derivatives (Chart 3). Synthetic **5a** was shown to be identical with the major component of the natural mixture from the soft coral. Alkaline hydrolysis of this mixture afforded the deacetyl derivative (**5b**) and unchanged compound **6** having a 24-methylcholestane-type side chain. The ^{1}H-NMR chemical shifts of **6** due to 28-H (δ 0.775), and 26,27-H (δ 0.782 and 0.853) corresponded to those of the reference compound (24*S*)-24-methylcholesterol (22-dihydrobrassicasterol, δ 0.775, 0.783, 0.852),[14] and the signals corresponding to those of the (24*R*) isomer (campesterol, δ 0.773, 0.802, 0.850) were not observed.[14,15]

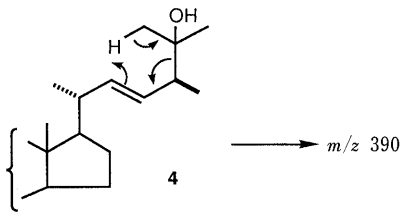

Chart 2

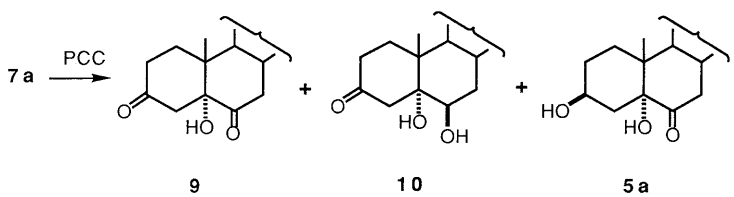

Chart 3

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. Nuclear magnetic resonance (NMR) spectra were determined on a JEOL JMS 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. Mass spectra (MS) were determined on a JEOL JMS D 300 mass spectrometer. Chromatography was done by flash column chromatography[16] using silica gel (Wako gel C-300, 200—300 mesh, Wako Pure Chemical Industries).

Materials The collection locations and the code numbers of the soft corals, and details of the individual polyhydroxysterols and the general isolation process were reported in a previous paper.[5a] One soft coral sample, code name MF-CBR-25 (2.7 kg after extraction), gave the polyhydroxysterols MF-CBR-25-01 (**7a**, 220 mg) and MF-CBR-25-02 (mixture of **5a** and **6**, 72 mg). Another soft coral sample, code name MF-CBR-27 (2.2 kg after extraction) gave seven polyhydroxysterols MF-CBR-27-01 (**1**, 31 mg), -02 (**2**, 94 mg), -03 (**8**, 11 mg), -04 (**7a**, 2100 mg), -05 (mixture of **3**, **4** and **7b**, 97 mg). Attempted purification of MF-CBR-25-02 by chromatography using several solvent systems only gave the major compound **5a** having **6** as a persistent impurity. Chromatography of MF-CBR-27-05 (*ca.* 20 mg) with MeOH–$CHCl_3$ (1 : 10) gave compounds **3** (1.9 mg), **4** (4.2 mg) and **7b** (14.7 mg). The known compounds (**1**, **7a**, **b**, and **8**) were identified from the ^{1}H-NMR and MS, and by thin-layer chromatography (TLC) with authentic specimens.[4]

(24*S*)-24-Methylcholestane-3β,4β,5β,25-tetrol-6-one 25-Monoacetate (Lobosterol) (1) mp 220—222 °C, $[\alpha]_D^{29}$ −7° (c = 1.52, $CHCl_3$). ^{1}H-NMR (pyridine-d_5) δ: 1.51, 1.52, 2.04 (each 3H, s), 0.95, 1.00 (each 3H, d, J = 6.5 Hz). Other signals, see **2**. ^{13}C-NMR ($CDCl_3$) δ: C-24 (42.1), C-25 (86.0), C-26, 27 (23.0, 23.5), C-28 (14.6), OAc (22.6, 170.5). Other signals, see **2**.

(24*S*)-24-Methylcholestane-3β,4β,5β,25-tetrol-6-one (25-Deacetyllobosterol) (2) mp 220—225 °C, $[\alpha]_D^{29}$ −8° (c = 1.68, $CHCl_3$). ^{1}H-NMR (pyridine-d_5) δ: 0.61, 0.95, 1.39, 1.41 (each 3H, s), 1.03, 1.11 (each 3H, d, J = 6.5 Hz), 2.50 (1H, dd, J = 13.5, 5.0 Hz, 7β-H), 2.58 (1H, dd, J = 13.5, 12.5 Hz, 7α-H), 4.44 (1H, br q, J = 3.0 Hz, 3α-H), 4.66 (1H, br d, J = 3.0 Hz, 4α-H). ^{13}C-NMR ($CDCl_3$) δ: C-1 (27.0), C-2 (24.3), C-3 (69.6), C-4 (70.3), C-5 (85.7), C-6 (210.6), C-7 (41.6), C-8 (38.0), C-9 (43.1), C-10 (46.6), C-11 (21.8), C-12 (39.6), C-13 (43.3), C-14 (57.0), C-15 (24.1), C-16, 23 (27.9, 28.0), C-17 (56.0), C-18 (12.1), C-19 (17.0), C-20 (36.2), C-21 (19.0), C-22 (34.8), C-24 (45.2), C-25 (73.6), C-26, 27 (26.2, 27.4), C-28 (14.9). MS m/z: 464 (M^+), 446, 431, 377. High-resolution MS [Found (Calcd)] m/z: $C_{28}H_{48}O_5$ (M^+), 464.3480 (464.3502).

(24*S*)-24-Methylcholest-7-ene-3β,5α,6β,25-tetrol 25-Monoacetate (3) mp 225—230 °C, $[\alpha]_D^{29}$ −30° (c = 0.20, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.59, 1.08, 1.97 (each 3H, s), 0.87, 0.95 (each 3H, d, J = 6.5 Hz), 1.39 (6H, s, 26, 27-H), 2.15 (1H, dd, J = 13.0, 11.5 Hz, 4β-H), 3.63 (1H, m, $W_{1/2}$ = 8 Hz, 6α-H), 4.08 (1H, m, $W_{1/2}$ = 18 Hz, 3α-H), 5.36 (1H, m, 7-H); (pyridine-d_5) δ: 0.66, 1.55, 2.02 (each 3H, s), 1.48 (6H, s), 0.92, 0.99 (each 3H, d, J = 6.5 Hz), 3.04 (1H, dd, J = 13.0, 11.5 Hz), 4.34 (1H, m), 4.82 (1H, m), 5.75 (1H, m). ^{13}C-NMR (pyridine-d_5) δ: C-1 (32.6), C-2 (33.9), C-3 (67.6), C-4 (41.9), C-5 (76.1), C-6 (74.2), C-7 (120.4), C-8 (141.6), C-9 (43.8), C-10 (38.0), C-11 (21.8), C-12 (40.0), C-13 (43.7), C-14 (55.1), C-15 (23.5), C-16, 23 (28.1), C-17 (56.3), C-18 (12.3), C-19 (18.8), C-20 (36.2), C-21 (19.3), C-22 (35.1), C-24 (42.4), C-25 (85.7), C-26, 27 (23.0, 23.5), OAc (22.5, 170.0). MS m/z: 472 ($M^+ - H_2O$), 439, 412, 394, 379, 303. High-resolution MS [Found (Calcd)] m/z: $C_{30}H_{48}O_4$ ($M^+ - H_2O$), 472.3531 (472.3553).

(24*S*)-24-Methylcholest-22*E*-ene-3β,5α,6β,25-tetrol (4) mp 241—244 °C, $[\alpha]_D^{29}$ −20° (c = 0.84, pyridine). ^{1}H-NMR (pyridine-d_5) δ: 0.76, 1.67 (each 3H, s), 1.09, 1.27 (each 3H, d, J = 6.5 Hz), 1.38, 1.42 (each 3H, s), 2.97 (1H, dd, J = 12.5, 11.5 Hz, 4β-H), 4.17 (1H, br s, $W_{1/2}$ = 7.0 Hz, 6α-H), 4.89 (1H, m, 3α-H), 5.36, 5.66 (each 1H, dd, J = 15.5, 8.5 Hz, 22,23-H). MS m/z: 390 ($M^+ - C_3H_6O$), 372, 354, 334, 316, 305, 271, 253. High-resolution MS [Found (Calcd)] m/z: $C_{25}H_{42}O_3$ ($M^+ - C_3H_6O$), 390.3146 (390.3134).

(24*S*)-24-Methylcholestane-3β,5α,25-triol-6-one 25-Monoacetate (5a) Data were derived from those of the mixture of **5a** and **6** by subtracting the data of **6**. ^{1}H-NMR (pyridine-d_5) δ: 0.65, 0.97, 1.50, 1.51 (each 3H, s), 0.84, 0.94 (each 3H, d, J = 7.0 Hz), 2.37 (1H, dd, J = 13.5, 12.0 Hz, 4β-H), 2.62 (1H, dd, J = 13.5, 4.0 Hz, 4α-H), 3.14 (1H, t, J = 12.5 Hz, 7α-H), 4.68 (1H, m, 3α-H); ($CDCl_3$) δ: 0.64, 0.81, 1.97 (each 3H, s), 1.39 (6H, s), 0.86, 0.92 (each 3H, d, J = 7.0 Hz), 2.12 (1H, dd, J = 13.0, 4.0 Hz, 4α-H), 2.72 (1H, dd, J = 13.0, 12.5 Hz, 7α-H), 3.98 (1H, m, 3α-H). MS, see synthetic **5a**. High-resolution MS [Found (Calcd)] m/z: $C_{30}H_{50}O_5$ (M^+), 490.3659 (490.3658).

Alkaline Hydrolysis of the Mixture of 5a and 6 Treatment of the mixture (*ca.* 5 mg) of **5a** and **6** with 10% KOH–MeOH, refluxing too long period (2 h), caused decomposition of 25-hydroxysterol **5b**. Column chromatography of the reaction product with ethyl acetate–hexane (1 : 1) gave **6** (1.2 mg) and a trace (0.45 mg) of **5b**.

(24*S*)-24-Methylcholestane-3β,5α,25-triol-6-one (5b) mp 257—260 °C, $[\alpha]_D^{29}$ −38° (c = 0.090, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.64, 0.79 (each 3H, s), 0.89, 0.93 (each 3H, d, J = 6.5 Hz), 1.15, 1.16 (each 3H, s), 2.75 (1H, t, J = 12.5 Hz, 7α-H), 3.96 (1H, m, 3α-H). MS m/z: 448 (M^+), 430, 415, 390, 372, 305, 303, 287. High-resolution MS [Found (Calcd)] m/z: $C_{28}H_{48}O_4$ (M^+), 448.3526 (448.3553).

(24*S*)-24-Methylcholestane-3β,5α-diol-6-one (6) mp 251—253 °C, $[\alpha]_D^{29}$ −32° (c = 0.24, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.64, 0.81 (each 3H, s), 0.775 (3H, d, J = 7.0 Hz, 28-H), 0.782, 0.853 (each 3H, d, J = 7.0 Hz, 26, 27-H), 0.912 (3H, d, J = 6.5 Hz, 21-H), 2.13 (1H, dd, J = 13.0, 4.5 Hz, 4α-H), 2.71 (1H, dd, J = 13.0, 12.0 Hz, 7α-H), 3.97 (1H, m, 3α-H). MS m/z: 432 (M^+), 414, 332, 303, 287. High-resolution MS [Found (Calcd)] m/z: $C_{28}H_{48}O_3$ (M^+), 432.3605 (432.3604).

PCC Oxidation of 7a A solution of **7a** (110 mg, 0.225 mmol) in 20 ml of CH_2Cl_2 was stirred with 26 mg (0.12 mmol) of PCC at room temperature for 20 min, then the mixture was diluted with Et_2O. The Et_2O layer was washed with H_2O and saturated NaCl solution and then the solvent was evaporated off. Column chromatography of the residue with 2.5% MeOH in $CHCl_3$ gave **9** (4.6 mg), **10** (15.1 mg) and **5a** (15.5 mg).

Compound 9 mp 255—256 °C, $[\alpha]_D^{26}$ −17° (c = 0.92, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.67, 1.01, 1.97 (each 3H, s), 0.87, 0.93 (each 3H, d, J = 6.5 Hz), 1.39 (6H, s), 2.73 (1H, t, J = 12.5 Hz, 7α-H), 2.92 (1H, d, J = 15.5 Hz, 4-H). MS m/z: 488 (M^+), 470, 428, 410, 400, 385, 370, 301. High-resolution MS [Found (Calcd)] m/z: $C_{30}H_{48}O_5$ (M^+), 488.3519 (488.3502).

Compound 10 mp 215—220 °C, $[\alpha]_D^{26}$ +1° (c = 3.02, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.71, 1.35, 1.97 (each 3H, s), 1.39 (6H, s), 0.87, 0.93 (each 3H, d, J = 6.5 Hz), 3.24 (1H, d, J = 15.0 Hz, 4-H), 3.54 (1H, br s, $W_{1/2}$ = 7 Hz, 6α-H). MS m/z: 490 (M^+), 472, 454, 430, 412, 303. High-resolution MS [Found (Calcd)] m/z: $C_{30}H_{50}O_5$ (M^+), 490.3682 (490.3658).

Compound 5a mp 240—242 °C, $[\alpha]_D^{24}$ −34° (c = 3.10, $CHCl_3$). ^{1}H-NMR: identical with natural **5a**. MS m/z: 490 (M^+), 430, 412, 397, 303. High-resolution MS [Found (Calcd)] m/z: $C_{30}H_{50}O_5$ (M^+), 490.3681 (490.3658).

Acknowledgement We are grateful to Dr. D. Daloze, Universite Libre de Bruxelles, for providing an authentic specimen of lobosterol, to the Zoological Survey of India, Calcutta for identification of the oragnisms, and to the Council of Scientific and Industrial Research, New Delhi, and the Department of Science and Technology, New Delhi, for financial support to C. B. R.

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