Marine Sterols. XVII.[1] Polyhydroxysterols of the Soft Corals of the Andaman and Nicobar Coasts. (2). Isolation and Structures of Three 16β-Hydroxy Steroidal Glycosides from an *Alcyonium* sp. Soft Coral

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3β,7β-Dihydroxy-24-methylenecholesterol (1) and three new polyhydroxysterol glycosides (2a, 3a and 4) were isolated from the lipid extract of an *Alcyonium* sp. soft coral which was collected in the Andaman and Nicobar Islands. Isolation of steroidal glycosides from soft corals is rare, if not unprecedented. Spectroscopic and chemical degradation studies indicated the new glycosides to be 24-methylenecholest-5-ene-3β,16β-diol-3-*O*-α-L-fucoside (2a) and its 7β- (3a) and 7α-hydroxy (4) derivatives.

Keywords Coelenterata; soft coral; *Alcyonium* sp.; polyhydroxysterol α-L-monofucoside; 24-methylenecholest-5-ene-3β,16β-diol; 24-methylenecholest-5-ene-3β,7β,16β-triol; 24-methylenecholest-5-ene-3β,7α,16β-triol

Soft corals (Coelenterata) are known to elaborate both 3β-monohydroxysterols and polyhydroxysterols,[2] derived mainly from a 24-methylcholestane skeleton. Polyhydroxysterols of soft corals and other marine invertebrates occur mainly in the free state or as the sulfate and examples of steroidal glycosides are rather rare, except for those found in starfishes (Echinodermata).[3] Pregnane-type acetylarabinosides have been reported from an *Alcyonium* sp. soft coral collected in Okinawa,[4] whilst, quite recently, the 3′-acetoxy monofucoside of 24-methylenecholest-5-ene 3β,16β-diol, having a spermatostatic activity, has been reported from the Sri Lankan soft coral *Sinularia crispa*.[5] To our knowledge, there are no other reports of steroidal glycoside isolated from soft corals. We have been engaged in studies of the steroidal components of the soft corals of the Andaman and Nicobar coasts, Indian Ocean. Among them, one sample was identified at the genus level as an *Alcyonium* sp. Extraction and purification of the polyhydroxysterols from this species resulted in the isolation of four compounds (**1**, **2a**, **3a**, and **4**), and three of them (**2a**, **3a**, and **4**) have now been shown to be steroidal glycosides. The present report describes the structural elucidation of these new compounds. The least polar compound **1** was a dihydroxy C_{28} sterol. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR, see Experimental) of **1** suggested the presence of a side chain with a terminal double bond and a 3,7-dihydroxy-Δ^5 steroid nucleus. The McLafferty-type cleavage ion,[6] a characteristic of Δ^{24} sterols, appeared at *m/z* 330 in the mass spectrum (MS). Its ^{1}H- and ^{13}C-NMR chemical shifts due to the steroid nucleus are virtually the same as those of synthetic cholest-5-ene-3β,7β-diol.[7] 24-Methylenecholest-5-ene-3β,7β-diol has been obtained from a finger sponge *Haliclona oculata*,[8] and its reported spectral properties showed good agreement with those of **1**.

The other three compounds (**2a**, **3a**, and **4**) were monoglycosides of C_{28} sterols. Compound **4** was purified in very small amounts from the mixture containing **3a** as

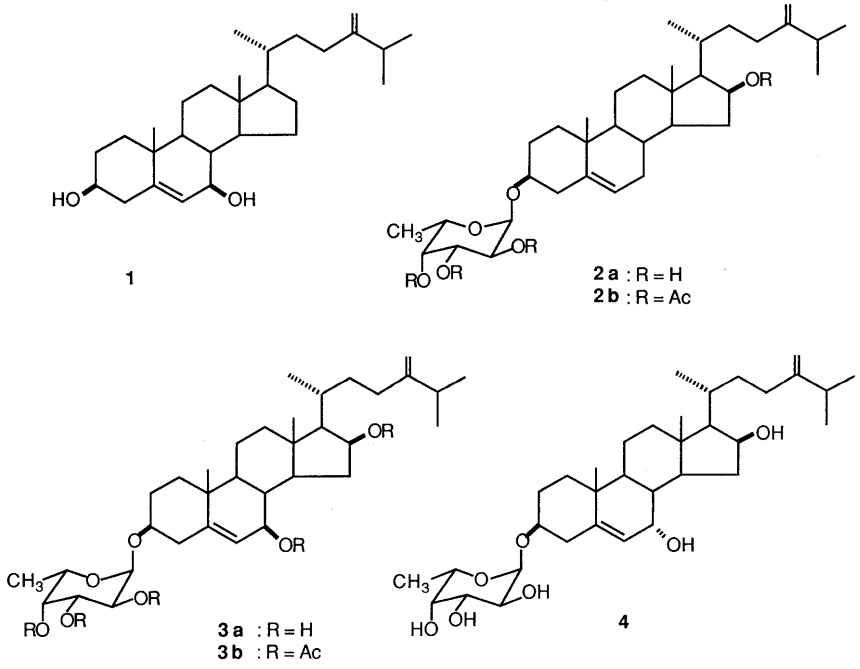
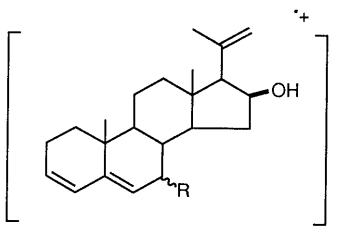


Chart 1

its major component; the two compounds have very similar mobility on chromatography. Usual acetylation gave the tetraacetate (**2b**) from **2a** and the pentaacetate **3b** from **3a** as judged from the ^{1}H-NMR spectra. The MS analyses indicated the molecular ions to be at *m/z* 560 for **2a** and *m/z* 576 for **3a** and **4**. The distributions of the fragment ions and their relative intensities in the MS of **3a** and **4** were nearly superimposable, and suggested a common substitution pattern in the steroid skeleton. Their ^{1}H- and ^{13}C-NMR spectra (Experimental)[8] indicated the presence of a normal 20*R*-24-methylenecholestane-type side chain, as in **1** (^{1}H-NMR (in pyridine-d_5), δ 4.86, 4.92 (**2a**); 4.86, 4.93 (**3a**); 4.84, 4.90 (**4**), each 1H, br s). Other common features in **2a**, **3a**, and **4** were the presence of one secondary hydroxyl group in the D-ring (^{1}H-NMR, δ 4.56, 1H, m (**2a**); 4.6—4.7, 1H, m (**3a** and **4**)), and the presence of a 6-deoxy sugar moiety (^{1}H-NMR, δ 1.60 (**2a** and **3a**), 1.59 (**4**), each 3H, d, J=6.5 Hz), as judged from their closely related chemical shifts and coupling patterns (see Experimental). Comparison of the ^{13}C-NMR chemical shifts of their sugar units with those in the literature[9] revealed that they are nearly identical, exhibiting a deviation of less than 0.5 ppm, with those of methyl-α-fucoside, except for the signal of C-1′ (**2a** and **3a**, δ 99.0; **4**, δ 99.1; methyl-α-fucoside, δ 101.6). In the MS, the ion due to the loss of the fucose unit was the major ion for all three compounds. Concurrent McLafferty-type cleavage produced the ions at *m/z* 312 from **2a** and at *m/z* 328 from **3a** and **4** (Chart 2). One secondary hydroxyl group is located at 16β, since this caused, in the ^{13}C-NMR spectrum, a significant β-hydroxy substituent effect.[10] In **2a**, the chemical shifts of C-15 (δ 38.0) and C-17 (δ 61.8) showed significant changes as compared with those of 24-methylenecholesterol acetate (C-15, δ 24.6; C-17, δ 56.4).[11] On the other hand, there was a *gauche* γ-hydroxyl interaction[10] at C-14 (δ 54.8) and C-20 (δ 30.5), with distinct upfield shifts as compared with the signals of 24-methylenecholesterol acetate (C-14, δ 56.9; C-20, δ 36.1).[11] It is known that introduction of a 16β-hydroxyl group into cholesterol (H-18, δ 0.67) causes, in the ^{1}H-NMR spectrum, deshielding of the H-18 signal (δ 0.82), which resembles that of **2a** (δ 0.89), while the introduction of a 16α-hydroxyl group has little effect at H-18.[12] Other ^{1}H-NMR chemical shifts (in $CDCl_3$) of **2a**, such as H-19 (δ 1.02)[13] and H-3α (δ 3.49, br, $W_{1/2}$=20 Hz), indicated it to be a 3β-hydroxy-Δ^5-steroid derivative. The 3α-hydroxy substitution would cause the H-3β signal to appear as a sharp multiplet ($W_{1/2}$=7 Hz).[14a] This was supported by a comparison of the chemical shifts of the carbons in the B and C rings of **2a** with those of cholesterol.[14b] The A ring carbons showed similar chemical shifts to those of cholesterol monoglucoside.[14c] These results strongly suggested **2a** to be the 3-*O*-α-monofucoside of 24-methylenecholest-5-ene-3β,16β-diol.

Compounds **3a** and **4** were monohydroxy derivatives of **2a**. Their ^{1}H- and ^{13}C-NMR properties are common with those of **2a**, except for those regarding C-5 to C-8, involving the allylic alcohol moiety. The ^{13}C-NMR (in pyridine-d_5) signals of C-6, C-7, and C-8 of **3a** appeared at δ 128.5, 72.6, and 40.6, respectively, as found in synthetic cholest-5-ene-3β,7β-diol (δ 127.9, 72.8, and 41.0).[7] In contrast, the ^{13}C-NMR chemical shifts of **4** regarding these carbons (δ 126.0, 64.7, and 38.1) showed close analogy with those of synthetic cholest-5-ene-3β,7α-diol (δ 125.5, 64.9, and 38.5).[7]

The closely related ^{1}H- and ^{13}C-NMR chemical shifts due to the α-fucose moiety in the glycosides **2a**, **3a**, and **4** indicated that each fucose bears the same absolute configuration and has stereochemically the same spatial arrangement with respect to the aglycone. Acid hydrolysis of the major glycoside **2a** with 2 N HCl in MeOH caused the rearrangement of the side chain double bond and gave 24-methylcholesta-5,23-diene-3β,16β-diol (**5**, 13%, ^{1}H-NMR, δ 5.23, 1H, br t, J=7.5 Hz; 1.58, 3H, s) and 24-methylcholesta-5,24-diene-3β,16β-diol (**6**, 18%, δ 1.66,



m/z 312 (R=H)
m/z 328 (R=OH)

Chart 2

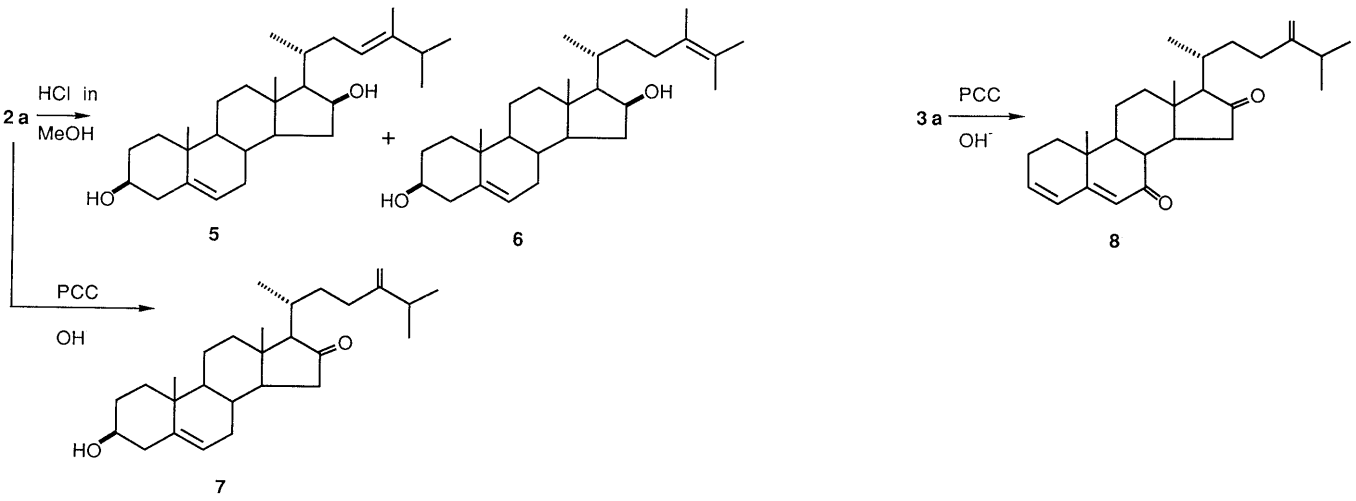


Chart 3

6H, s; 1.64, 3H, s) (Chart 3. H-3α,[15] H-6 and H-19,[13] see Experimental). Compound **5** was shown to be the 23*E*-isomer by the absence of the deshielded H-25 signal around δ 2.8, a characteristic of 23*Z*-24-methylcholest-23-ene.[15] The aqueous extract gave a methyl fucoside mixture ($[\alpha]_D$ −111°) which showed the same thin-layer chromatographic (TLC) pattern as those prepared from authentic D- and L-fucose. The specific rotation of the sugar mixture agreed with that ($[\alpha]_D$ −108°) prepared from L-fucose and was different from that ($[\alpha]_D$ +125°) from D-fucose, and indicated that the absolute configuration of the fucose units in **2a**, **3a**, and **4** is L. Treatment of **2a** with pyridinium chlorochromate (PCC) caused the decomposition of the sugar unit and after alkaline hydrolysis, it gave small amounts of a ketone **7** (Chart 3), whose infrared (IR) spectrum showed a band due to the five-membered ring ketone at 1750 cm^{-1}. Its MS showed a side chain fragment ion at *m/z* 124 and ion of an aglycone unit with loss of a methyl group (*m/z* 273), which can be generated by McLafferty-type cleavage of 16-ketosteroids.[16] The same treatment of **3a** gave the dienone **8** (MS, *m/z* 408 (M^+), ^{1}H-NMR, δ 5.63, 1H, s, H-6; 6.12, 1H, d, *J*=10.0 Hz, H-4); 6.22, 1H, dt, *J*=10.0, 4.0 Hz, H-3; IR, 1745, 1665 cm^{-1}, ultraviolet (UV) spectrum, 274 nm), which was derived by elimination of the C-3 substituent during the alkaline hydrolysis.[17,18]

In summary, three steroidal glycosides were isolated from an *Alcyonium* sp. soft coral collected off the coasts of the Andaman and Nicobar Islands. It is noteworthy, from the chemotaxonomic viewpoint, that this is the second example of *Alcyonium* sp. containing steroidal glycosides, which are rare in soft corals.

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. IR spectra were determined on a JASCO A102 spectrometer. UV spectra were determined on a Shimadzu UV-220 spectrometer. Chromatography was done by flash column chromatography[19] 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 polyoxysterols and their general isolation process were reported in the preceding paper.[1] The soft coral material, code name MF-CBR-30 (2.5 kg after extraction) gave the polyhydroxysterol derivatives MF-CBR-30-01 (compound **2a**, 97 mg), MF-CBR-30-02 (compound **3a**, 154 mg), MF-CBR-30-03 (58 mg), and MF-CBR-30-04 (compound **1**, 12 mg). MR-CBR-30-03 was found to be a mixture of compounds **3a** and **4**. This mixture (30 mg) was subjected to column chromatography with 10% MeOH in $CHCl_3$, giving the less polar compound **4** (9.0 mg).

24-Methylenecholest-5-ene-3β,7β-diol (1) Needles from MeOH, mp 160—162 °C, $[\alpha]_D^{21}$ +20.5° (*c*=0.85, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.70 (3H, s, H-18), 0.96 (3H, d, *J*=6.5 Hz, H-21), 1.02, 1.03 (each 3H, d, *J*=7.0 Hz, H-26, 27), 1.05 (3H, s, H-19), 3.55 (1H, m, H-3α), 3.85 (1H, br d, *J*=7.5 Hz, H-7), 4.66, 4.72 (each 1H, br s, H-28), 5.29 (1H, br s, H-6). (In pyridine-d_5) δ: 0.75 (3H, s, H-18), 1.03 (3H, d, *J*=6.5 Hz, H-21), 1.05 (3H, s, H-19), 1.07, 1.08 (each 3H, d, *J*=7.0 Hz, H-26, 27), 3.90 (1H, m, H-3α), 4.11 (1H, br d, *J*=8.0 Hz, H-7), 4.87 (2H, br s, H-28), 5.73 (1H, br s, H-6). ^{13}C-NMR ($CDCl_3$) δ: C-1 (37.1), C-2, 23 (31.2, 31.7), C-3 (71.5), C-4 (41.9), C-5 (143.5), C-6 (125.5), C-7 (73.4), C-8 (41.0), C-9 (48.4), C-10 (36.6), C-11 (21.2), C-12 (39.7), C-13 (43.1), C-14 (55.4), C-15 (26.4), C-16 (28.6), C-17 (56.1), C-18 (11.9), C-19 (19.2), C-20 (35.8), C-21 (18.9), C-22 (34.8), C-24 (156.6), C-25 (33.9), C-26, 27 (22.0), C-28 (106.1). MS. *m/z*: 414 (M^+), 396, 330, 312, 297, 287, 279, 269. High-resolution MS [Found (Calcd)] *m/z*: $C_{28}H_{46}O_2$ (M^+), 414.3484 (414.3497).

24-Methylenecholest-5-ene-3β,16β-diol-3-*O*-α-L-fucopyranoside (2a) Needles from MeOH, mp 245—247 °C, $[\alpha]_D^{21}$ −120° (*c*=0.33, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.89 (3H, s, H-18), 1.02 (3H, d, *J*=6.5 Hz, H-21), 1.02 (3H, s, H-19), 1.03 (6H, d, *J*=7.0 Hz, H-26, 27), 1.28 (3H, d, *J*=6.5 Hz, H-6′), 3.49 (1H, m, H-3α), 3.70—3.77 (2H, m, H-2′, 3′), 3.79 (1H, br s, H-4′), 4.03 (1H, br q, *J*=6.5 Hz, H-5′), 4.37 (1H, m, H-16α), 4.75, 4.70 (each 1H, br s, H-28), 5.00 (1H, d, *J*=3.0 Hz, H-1′), 5.35 (1H, m, H-6). (In pyridine-d_5) δ: 1.00 (3H, s, H-18), 1.05, 1.06 (each 3H, d, *J*=7.0 Hz, H-26, 27), 1.14 (3H, d, *J*=7.0 Hz, H-21), 1.15 (3H, s, H-19), 1.60 (3H, d, *J*=6.5 Hz, H-6′), 3.77 (1H, m, H-3α), 4.22 (1H, br d, *J*=2.5 Hz, H-4′), 4.43 (1H, br q, *J*=7.0 Hz, H-5′), 4.51 (1H, dd, *J*=10.0, 3.0 Hz, H-3′), 4.56 (1H, overlapped by a signal at 4.61, H-16α), 4.61 (1H, dd, *J*=10.0, 3.5 Hz, H-2′), 4.86, 4.92 (each 1H, br s, H-28), 5.32 (1H, m, H-6), 5.48 (1H, d, *J*=3.5 Hz, H-1′). ^{13}C-NMR (in pyridine-d_5) δ: C-1 (37.5), C-2 (30.1), C-3 (77.4), C-4 (39.1), C-5 (141.0), C-6 (121.7), C-7 (32.2), C-8 (31.9), C-9 (50.4), C-10 (37.0), C-11 (21.1), C-12 (40.3), C-13 (42.5), C-14 (54.8), C-15 (38.0), C-16 (71.7), C-17 (61.8), C-18 (13.4), C-19 (19.5), C-20 (30.5), C-21 (18.6), C-22 (35.0), C-23 (31.9), C-24 (157.0), C-25 (34.0), C-26, 27 (22.0), C-28 (106.3), C-1′ (99.0), C-2′ (70.1), C-3′ (71.0), C-4′ (73.3), C-5′ (67.2), C-6′ (17.2). MS *m/z*: 560 (M^+), 545, 527, 515, 485, 461, 396, 381, 379, 312, 297. High-resolution MS [Found (Calcd)] *m/z*: $C_{34}H_{56}O_6$ (M^+), 560.4076 (560.4077). Acetylation of **2a** (5 mg) in a usual way (Ac_2O–pyridine) gave the tetraacetate **2b** (4 mg) as an oil, $[\alpha]_D^{21}$ −73.6° (*c*=1.09, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.88 (3H, s, H-18), 1.01 (3H, s, H-19), 1.01 (6H, d, *J*=6.5 Hz, H-26, 27), 0.99 (3H, d, overlapped with other signals), 1.12 (3H, d, *J*=6.3 Hz, H-6′), 1.99, 2.01, 2.07, 2.16 (each 3H, s, OAc), 3.42 (1H, m, H-3α), 4.25 (1H, br q, *J*=7.0 Hz, H-5′), 4.62, 4.69 (each 1H, br s, H-28), 5.06 (1H, dd, *J*=10.5, 3.5 Hz, H-2′), 5.19 (1H, d, *J*=3.5 Hz, H-1′), 5.15—5.24 (1H, m, H-16α), 5.29 (1H, dd, *J*=3.3, 1.0 Hz, H-4′), 5.28—5.35 (1H, H-6, overlapped with other signals), 5.36 (1H, dd, *J*=10.5, 3.3 Hz, H-3′).

24-Methylenecholest-5-ene-3β,7β,16β-diol-3-*O*-α-L-fucopyranoside (3a) Needles from MeOH, mp 255—257 °C, $[\alpha]_D^{20}$ −47° (*c*=0.47, EtOH). ^{1}H-NMR (in $CDCl_3$): 0.91 (3H, s, H-18), 1.03 (6H, d, *J*=7.0 Hz, H-26, 27), 1.06 (3H, s, H-19), 1.28 (3H, d, *J*=6.0 Hz, H-6′), 3.50 (1H, m, H-3α), 3.7—3.9 (4H, m, H-7, 2′, 3′, 4′), 4.02 (1H, br q, *J*=6.5 Hz, H-5′), 4.40 (1H, m, H-16α), 4.70, 4.75 (each 1H, br s, H-28), 5.00 (1H, br s, H-1′). (in pyridine-d_5) δ: 0.96 (3H, s, H-18), 1.05, 1.06 (each 3H, d, *J*=7.0 Hz, H-26, 27), 1.16 (3H, d, *J*=6.5 Hz, H-21), 1.20 (3H, s, H-19), 1.60 (3H, d, *J*=6.5 Hz, H-6′), 3.80 (1H, m, H-3α), 4.10 (1H, br d, *J*=6.2 Hz, H-7), 4.22 (1H, br d, *J*=2.5 Hz, H-4′), 4.42 (1H, br q, *J*=6.5 Hz, H-5′), 4.51 (1H, dd, *J*=10.0, 3.0 Hz, H-3′), 4.61 (1H, dd, *J*=10.0, 3.5 Hz, H-2′), 4.6—4.7 (1H, H-16α, overlapped by a signal at 4.61), 5.48 (1H, d, *J*=3.5 Hz, H-1′), 5.66 (1H, s, H-6). ^{13}C-NMR (in pyridine-d_5) δ: C-1 (37.3), C-2 (30.1), C-3 (77.3), C-4 (40.6), C-5 (141.5), C-6 (128.5), C-7 (72.6), C-8 (40.6), C-9 (48.9), C-10 (36.8), C-11 (21.2), C-12 (40.3), C-13 (43.1), C-14 (54.7), C-15 (38.6), C-16 (71.7), C-17 (61.3), C-18 (13.4), C-19 (18.9), C-20 (30.4), C-21 (18.6), C-22 (35.1), C-23 (31.9), C-24 (157.0), C-25 (34.0), C-26, 27 (22.0), C-28 (106.3), C-1′ (99.0), C-2′ (70.0), C-3′ (71.4), C-4′ (73.3), C-5′ (67.1), C-6′ (17.1). MS *m/z*: 576 (M^+), 561, 543, 477, 429, 412, 397, 379, 328, 313, 310, 298, 287, 269, 251, 124, 75. High-resolution MS [Found (Calcd)] *m/z*: $C_{34}H_{56}O_7$ (M^+), 576.4044 (576.4026). Acetylation of **3a** (6 mg) in a usual way (Ac_2O–pyridine) gave the pentaacetate **3b** (4 mg) as an oil, $[\alpha]_D^{21}$ −8.1° (*c*=3.14, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.90 (3H, s, H-18), 1.00 (3H, d, *J*=6.5 Hz, H-21), 1.01 (6H, d, *J*=7.0 Hz, H-26, 27), 1.08 (3H, s, H-19), 1.11 (3H, d, *J*=6.5 Hz, H-6′), 1.99, 2.01, 2.02, 2.06, 2.16 (each 3H, s, OAc), 3.43 (1H, m, H-3α), 4.23 (1H, br q, *J*=6.5 Hz, H-5′), 4.62, 4.70 (each 1H, br s, H-28), 4.96 (1H, br d, *J*=8.5 Hz, H-7), 5.05 (1H, dd, *J*=10.5, 3.7 Hz, H-2′), 5.16 (1H, d, *J*=3.7 Hz, H-1′), 5.11—5.21 (1H, m, H-16α, overlapped with other signals), 5.22 (1H, br s, H-6), 5.28 (1H, br dd, *J*=3.3, 1.0 Hz, H-4′), 5.34 (1H, dd, *J*=10.5, 3.3 Hz, H-3′).

24-Methylenecholest-5-ene-3β,7α,16β-triol-3-*O*-α-L-fucopyranoside (4) Needles from MeOH, mp 241—243 °C, $[\alpha]_D^{21}$ −114° (*c*=0.33, EtOH). ^{1}H-NMR (in pyridine-d_5) δ: 1.00 (3H, s, H-18), 1.04, 1.05 (each 3H, d, *J*=7.0 Hz, H-26, 27), 1.23 (3H, s, H-19), 1.59 (3H, d, *J*=6.5 Hz, H-6′), 3.63 (1H, m, H-3α), 4.09 (1H, br s, H-7), 4.22 (1H, br s, H-4′), 4.37 (1H, br q, *J*=6.5 Hz, H-5′), 4.50 (1H, dd, *J*=10.0, 3.0 Hz, H-3′), 4.61 (1H, dd, *J*=10.0, 3.5 Hz, H-2′), 4.6—4.7 (1H, m, H-16α), 4.84, 4.90 (each 1H, H-28), 5.41 (1H, d, *J*=3.5 Hz, H-1′), 5.81 (1H, d, *J*=4.5 Hz, H-6). ^{13}C-NMR (in pyridine-d_5) δ: C-1 (37.4), C-2 (30.0), C-3 (77.1), C-4 (39.1), C-5 (144.1), C-6 (126.0), C-7 (64.7), C-8 (38.1), C-9 (42.8), C-10 (37.9), C-11 (21.0), C-12 (40.2), C-13 (42.4), C-14 (48.2), C-15 (38.3), C-16 (71.8), C-17 (61.9), C-18 (13.4), C-19 (18.7), C-20 (30.6), C-21 (18.4), C-22 (35.1), C-23 (31.9), C-24 (157.2), C-25 (34.1), C-26, 27 (22.1, 22.2), C-28 (106.4), C-1′ (99.1),

C-2′ (70.2), C-3′ (71.4), C-4′ (73.4), C-5′ (67.3), C-6′ (17.2). MS *m/z*: 576 (M^+), 561, 543, 477, 429, 412, 397, 379, 328, 313, 310, 269, 251, 124, 75. High-resolution MS [Found (Calcd)] *m/z*: $C_{34}H_{56}O_7$ (M^+), 576.4024 (576.4026).

Acid Hydrolysis of 2a A solution of **2a** (51.2 mg) in 2 N HCl in MeOH (3 ml) was refluxed for 1 h, then diluted with Et_2O, and the Et_2O layer was washed with H_2O. The Et_2O extract was subjected to chromatography over a column of silica gel, eluting with 3% Et_2O in $CHCl_3$. The eluate (17.5 mg) was further purified by chromatography over a column of 7.5% silver nitrate-impregnated silica gel, giving **5** (4.9 mg) and **6** (6.9 mg). The aqueous layer was passed through a column of Amberlite IRA 411 and the eluate was evaporated to dryness. Chromatography of the residue over a column of silica gel with 10% MeOH in $CHCl_3$ gave the crude methyl fucoside fraction as an oil, $[\alpha]_D^{21}$ −111° (c=0.49, MeOH). The same treatment of D-fucose and L-fucose gave the crude methyl fucoside, with $[\alpha]_D^{21}$ +125° (c=0.37, MeOH) and −108° (c=0.35, MeOH), respectively. The ^{1}H-NMR spectra (in pyridine-d_5) of these three samples were identical.

24-Methylcholesta-5,23-diene-3β,16β-diol (5) Oil, $[\alpha]_D^{21}$ −37° (c=0.82, $CDCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.89 (3H, s, H-18), 0.99 (6H, d, J=7.0 Hz, H-26, 27), 1.02 (3H, s, H-19), 1.58 (3H, s, H-28), 3.53 (1H, m, H-3α), 4.36 (1H, m, H-16α), 5.23 (1H, br t, J=7.5 Hz, H-23), 5.35 (1H, br d, J=5.0 Hz, H-6), MS *m/z*: 414 (M^+), 399, 396, 315, 272, 124. High-resolution MS [Found (Calcd)] *m/z*: $C_{28}H_{46}O_2$ (M^+), 414.3513 (414.3497).

24-Methylcholesta-5,24-diene-3β,16β-diol (6) Oil, $[\alpha]_D^{21}$ −33° (c=1.12, $CDCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.89 (3H, s, H-18), 1.02 (3H, s, H-19), 1.03 (3H, d, J=7.0 Hz, H-21), 1.66 (6H, s), 1.64 (3H, s), 3.53 (1H, m, H-3α), 4.37 (1H, m, H-16α), 5.35 (1H, br d, J=5.0 Hz, H-6). MS *m/z*: 414 (M^+), 399, 315, 271, 253, 213, 96. High-resolution MS [Found (Calcd)] *m/z*: $C_{28}H_{46}O_2$ (M^+), 414.3511 (414.3498).

PCC Treatment of 2a A solution of **2a** (12.4 mg) in CH_2Cl_2 (0.7 ml) was stirred with PCC (10 mg) at room temperature for 4 h, then the mixture was diluted with H_2O and extracted with Et_2O. The Et_2O extract was passed through a column of silica gel, eluting with Et_2O–$CHCl_3$ (3 : 1). The eluate was dissolved in 5% KOH in MeOH (0.7 ml) and the mixture was refluxed for 2 h, then diluted with Et_2O, washed with H_2O and saturated NaCl solution, and evaporated to dryness. Chromatography of the residue over a column of silica gel with $CHCl_3$ gave **7** (1.0 mg) as an oil, $[\alpha]_D^{21}$ −87° (c=0.14, $CHCl_3$). ^{1}H-NMR (in $CDCl_3$) δ: 0.84 (3H, s, H-18), 1.04 (3H, s, H-19), 1.02, 1.04 (each 3H, d, J=7.0 Hz, H-26, 27), 3.45—3.60 (1H, m, H-3α), 4.69, 4.72 (each less than 1H, br s, H-28), 5.35 (1H, br d, J=5.5 Hz, H-6). IR ν_{max}^{Neat} cm^{-1}: 3300, 1750, 1650, 895. MS *m/z*: 412 (M^+), 397, 379, 315, 273, 213, 124 (base peak). High-resolution MS [Found (Calcd)] *m/z*: $C_{28}H_{44}O_2$ (M^+), 412.2364 (412.2341).

PCC Treatment of 3a A solution of **3a** (34.2 mg) in CH_2Cl_2 (1.8 ml) was treated with PCC (51.2 mg) in the manner described above. Hydrolysis of the Et_2O extract with 5% KOH in MeOH (1.5 ml) followed by column chromatography with 5% MeOH in $CHCl_3$ gave **8** (6 mg) as an oil, $[\alpha]_D^{21}$ −336° (c=1.00, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, s, H-18), 0.98 (6H, d, J=7.0 Hz, H-26, 27), 1.16 (3H, s, H-19), 4.72, 4.69 (each less than 1H, br s, H-28), 5.63 (1H, s, H-6), 6.12 (1H, br d, J=10.0 Hz, H-4), 6.22 (1H, dt, J=10.0, 4.0 Hz, H-3). IR ν_{max}^{Neat} cm^{-1}: 1745, 1665, 1630, 1600, 890. UV λ_{max}^{EtOH} nm (ε): 274 (17500). MS *m/z*: 408 (M^+), 393, 390, 311, 285, 269, 124 (base peak). High-resolution MS [Found (Calcd)] *m/z*: $C_{28}H_{40}O_2$ (M^+), 408.3010 (408.3029).

Acknowledgement The authors thank the Zoological Survey of India, Calcutta, for identification of the organisms, and the Council of Scientific and Industrial Research, New Delhi, and the Department of Science and Technology, New Delhi, for financial support to CBR. Thanks are also due to Messrs. Chunduri V. L. Rao and Golakoti Trimurtulu of the School of Chemistry, Andhra University, for their technical assistance.

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