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Marine Sterols. XI.[1] Polyhydroxysterols of the Soft Coral *Sarcophyton glaucum*: Isolation and Synthesis of 5α-Cholestane-1β,3β,5,6β-tetrol

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(24*S*)-24-Methylcholest-5-ene-3β,25-diol (ergost-5-ene-3β,25-diol, 3), (24*S*)-24-methyl-5α-cholestane-1β,3β,5,6β-tetrol (5α-ergostane-1β,3β,5,6β-tetrol, **2a**), and a new sterol, 5α-cholestane-1β,3β,5,6β-tetrol (**1**), were isolated from the soft coral *Sarcophyton gluacum*. The structure of **1** was confirmed by the spectroscopic data and by synthesis starting from ruscogenin. The configurations at C-24 of the known C_{28}-polyhydroxysterols (**2**—**5**) were shown to be (*S*) by proton nuclear magnetic resonance (^{1}H-NMR) analysis or by correlation to 22,23-dihydrobrassicasterol (**16a**).

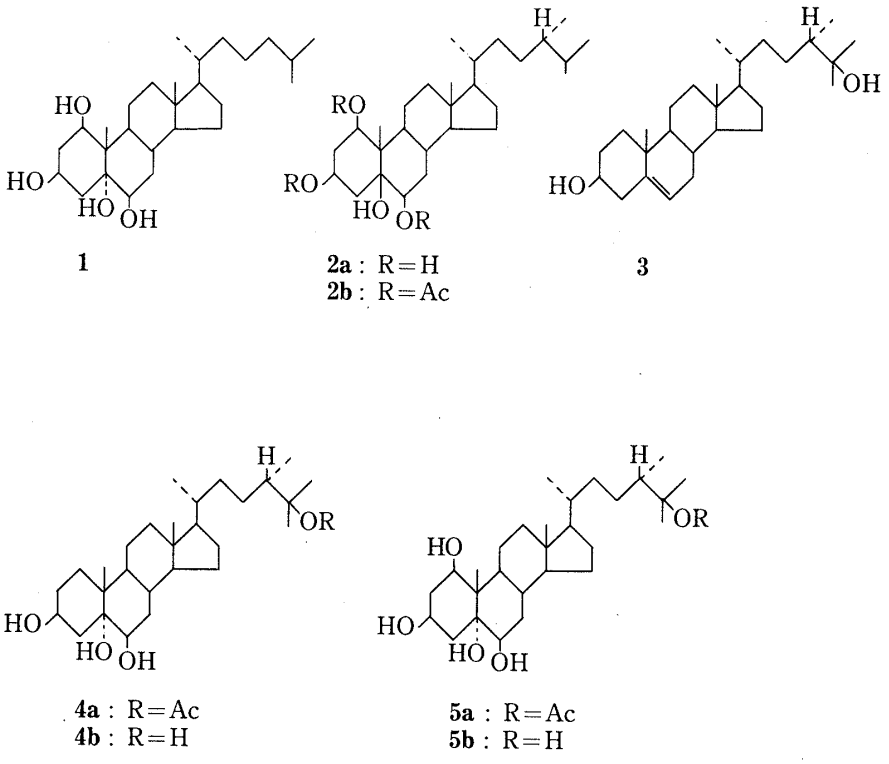
Keywords——Coelenterata; soft coral; *Sarcophyton glacum*; 5α-cholestane-1β,3β,5,6β-tetrol; (24*S*)-24-methyl-5α-cholestane-1β,3β,5,6β-tetrol; (24*S*)-24-methylcholest-5-ene-3β,25-diol

The soft coral *Sarcophyton glaucum* is a common species in Indian and Pacific coastal waters. From the lipid extract of *S. glaucum* collected at Ishigaki Island, a variety of cembrane-type diterpenes,[2] monohydroxysterols,[3] and polyhdroxysterols (**4a**, **4b**, **5a**)[4] have been characterized. Further work on the polyhydroxysterol fraction led to the isolation of 24-methylcholest-5-ene-3β,25-diol (**3**), 24-methyl-5α-cholestane-1β,3β,5,6β-tetrol (**2a**), and a new compound, 5α-cholestane-1β,3β,5,6β-tetrol (**1**). 24ξ-Methylcholest-5-ene-3β,25-diol had been isolated from a soft coral by Engelbrecht *et al.*[5] The tetrahydroxysterols **1** and **2a** were obtained as a 1 : 3 mixture in a previous study[4] and the latter was later found in the soft coral *Lobophytum pauciflorum*.[6] The characteristic 1β,3β,5α,6β-tetrahydroxy structure in compound **5a** in the previous study was deduced by comparison of the ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) chemical shifts with those of (25*R*)-5α-spirostane-1β,3β,5,6β-tetrol, which was synthesized from the sapogenin ruscogenin.[4] However, the C-24 stereochemistry of the 24-methylsterols (**2**—**5**)[4-7] remained unsettled. In the present paper, we wish to report the structure and synthesis of the new compound (**1**), and the determination of the (24*S*) stereochemistry in the known compounds **2** to **5**.

Isolation and Synthesis of 5α-Cholestane-1β,3β,5,6β-tetrol (1)

Compound **1** was obtained as a mixture with **2a** which was resistant to separation by silica gel chromatography or by reversed-phase partition chromatography.[7] The separation was accomplished by utilizing the slight difference of their mobility on normal phase partition chromatography over a Lipidex 5000 column with a mixture of hexane–acetone–methanol. Compound **1**, mp 260—263.5 °C, $[\alpha]_D$ −5.4°, showed a molecular ion (M^+) at *m/z* 436 and dehydration ions at *m/z* 418 and 400 in field desorption mass spectrometry (FD-MS). In the ^{1}H-NMR spectrum (pyridine-d_5), it showed signals of 18-Me (δ 0.80), 21-Me (δ 0.95, d, J=6.34 Hz), terminal dimethyl (δ 0.88, 6H, d, J=6.83 Hz), and 19-Me at δ 1.93. Three hydroxymethine signals were observed at δ 4.21 (1H, br s, 6α-H), 4.90 (1H, dd, J=5, 11 Hz, 1α-H), and 4.8—5.0 (1H, m, 3α-H). Similar chemical shifts, except for the side chain, were also observed in the 1β,3β,5α,6β-tetrahydroxy sterol **5a**.[4] The common feature of **1** and **5a** in the ^{1}H-NMR spectrum is the strongly deshielded nature of the 19-methyl group and of the protons at 1α, 3α, and 4β by 1,3-diaxial interaction with hydroxyl group. The 4β-axial proton appeared at δ

3.08 as a triplet (J=12 Hz). It was observed at δ 2.95 in the major polyhydroxysterol **4a** and at 3.05 in **5a**.[4] The ^{13}C-NMR chemical shifts of the carbons in the steroid ring coincided with those of **5a**, while the chemical shifts of C-20 to C-27 were the same as those of cholesterol.[8] Thus, the new C_{27} polyhydroxysterol from *S. glaucum* was assigned the structure 5α-cholestane-1β,3β,5,6β-tetrol (**1**).



1 **2a**: R=H, **2b**: R=Ac **3**

4a: R=Ac, **4b**: R=H **5a**: R=Ac, **5b**: R=H

Chart 1

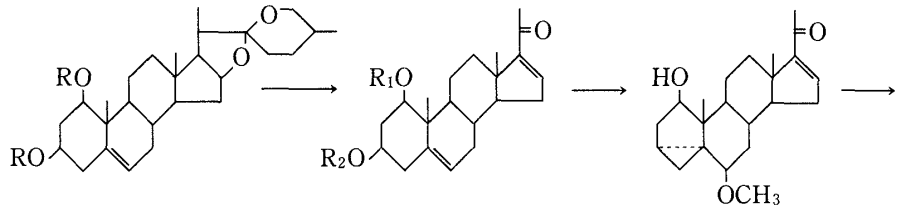
TABLE I. ^{13}C-NMR Chemical Shifts of Compounds **1**, **3** and **5a** (ppm, in pyridine-d_5)

Carbons	**1**	**3**	**5a**	Carbons	**1**	**3**	**5a**
1	73.5	37.2	73.7	15	24.6	24.3	25.1
2	42.8[a]	31.9	44.0[b]	16	28.3	28.1	28.5
3	65.2	71.7	65.4	17	56.5	56.6	56.8
4	42.0[a]	42.2	43.1[b]	18	12.4	11.9	12.6
5	76.8	140.4	77.0	19	10.5	19.4[c]	10.8
6	76.3	121.4	76.9	20	36.0	34.8	36.7
7	35.2	31.9	35.8	21	18.7	19.0[c]	19.2
8	31.6	31.6	32.0	22	36.3	36.2	35.2
9	46.7	50.0	47.1	23	23.9	27.8	28.1
10	44.5	36.4	44.9	24	39.6	45.1	42.4
11	24.8	21.0	24.9	25	28.1	73.5	85.6
12	41.1	39.7	41.4	26	22.6	26.6	23.6
13	42.4	42.2	42.8	27	22.9	27.2	23.2
14	56.3	55.7	56.7	28		14.8	14.8

a—c) These assignments may be interchanged.

The synthesis of **1** was carried out starting from the sapogenin ruscogenin (**6a**)[9] *via* the stereospecific rearrangement of the allyl ester (**11**).[10] Acid-catalyzed opening of the spiroketal ring of ruscogenin diacetate (**6b**) followed by chromic acid oxidation[11] gave pregnadienolone

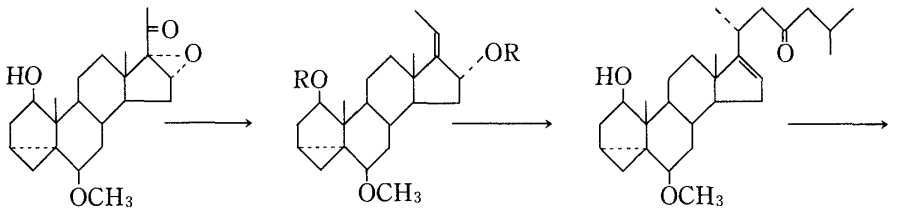
(**7a**). Partial hydrolysis of the diacetate **7a** gave the 1β-monoacetate (**7b**) which was converted to the 6β-methoxy-3α,5α-cyclo derivative (**8**, 53% from **6b**). The α,β-unsaturated ketone (**8**) was oxidized to the α,β-epoxy ketone (**9**) and converted by means of the Wharton reaction[12] with hydrazine in dimethylaminoethanol to the allyl alcohol. The 17(20)*E* geometry of the major product (**10**, 75% from **8**) was determined by the 18-Me chemical shift (δ 0.94) which agreed with the value obtained by Benn *et al.*[13] The key intermediate β-keto ester (**11**) was obtained by acylation of **10** with isovaleryl Meldrum's acid according to the method of Oikawa and Yonemitsu.[14] Carrol reaction of the β-keto ester (**11**) was carried out by heating in xylene with an excess of sodium hydride.[10] The resultant cholestane-type steroid (**12**) has the natural sterol type C-20 stereochemistry owing to the stereospecific recombination of the transient intermediate.[10] Catalytic hydrogenation of **12** gave **13** in 52% yield from **10**. Reduction of the 23-keto group followed by acid hydrolysis gave cholest-5-ene-1β,3β-diol (**15**, 46% from **13**). The 5α,6β-glycolation of **15** with hydrogen peroxide and formic acid in tetrahydrofuran[15] gave the 1β,3β,5α,6β-tetrol which was identical with compound **1** isolated from *S. glaucum*.



6a: R=H
6b: R=Ac

7a: $R_1=R_2=Ac$
7b: $R_1=Ac$, $R_2=H$
7c: $R_1=R_2=H$

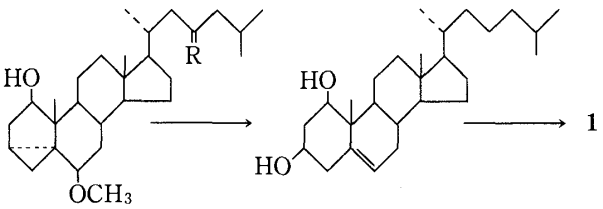
8



9

10: R=H
11: $R=COCH_2COCH_2CH(CH_3)_2$

12



13: R=O
14: $R=H_2$

15

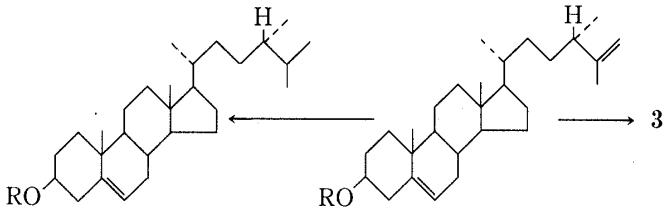
1

Chart 2

Isolation and Synthesis of (24*S*)-24-Methylcholest-5-ene-3β,25-diol (3)

Compound **3**, mp 185.5—187.5 °C, $[\alpha]_D$ −50.3°, was obtained in small amounts from the less polar fraction of the lipid of *S. glaucum*. The monounsaturated dihydroxy C_{28} sterol structure was indicated by the mass spectrum (MS), which showed ions at *m/z* 416 (M^+), 398 (M^+-H_2O), 380 (M^+-2H_2O), and at 273 (M^+—side chain). The ^{1}H-NMR (in $CDCl_3$) signals at

δ 0.68 (18-Me), 1.01 (19-Me), 3.3—3.7 (1H, m, 3α-H) and at 5.35 (1H, m, 6-H) show the presence of a conventional 3β-hydroxy-Δ^5-steroid ring.[1] The ions at *m/z* 271 (M^+—side chain, -2H), and 314 (cleavage at C-22 and C-23 by McLafferty-type fission) are those generally found from Δ^{24} or $\Delta^{24(28)}$-sterols,[16] and reflect the initial loss of H_2O from C-24 or C-25. The signals of two secondary methyl doublets at δ 0.95 (J=6 Hz, 21-Me) and 0.89 (J=7 Hz, 28-Me), and the deshielded terminal dimethyl at δ 1.16 (6H, s) indicate the presence of a 24-methylcholestane-type side chain with a hydroxyl group at C-25 in **3**. Thus the ^{1}H-NMR and mass spectral data, and the mp of **1** were almost the same as those of 24ξ-methylcholest-5-ene-3β,25-diol (mp 189.5—190.5 °C, isolated previously from an unidentified soft coral (probably *Nephthea* sp.).[5] The major monohydroxysterol in *S. glaucum* is 22,23-dihydrobrassicasterol (**16a**) having (24*S*) configuration.[3] Codisterol (**17a**) was also present (3%) in the monohydroxysterol fraction from *S. glaucum* and the (24*S*) stereochemistry was determined by correlation to **16a**.[1] Hydration at C-25 of **17a** by the oxymercuration method[17] gave (24*S*)-24-methylcholest-5-ene-3β,25-diol (70%) which was identical with the dihydroxysterol **3** from *S. glaucum*.



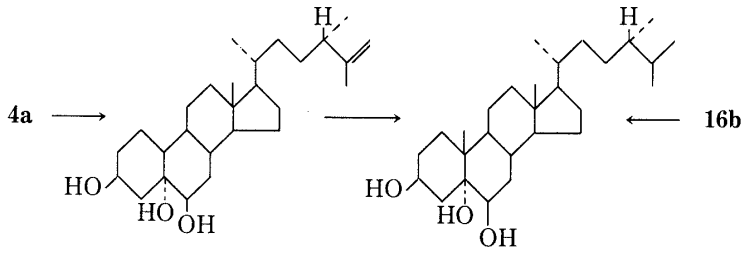
16a : R=H
16b : R=Ac

17a : R=H
17b : R=Ac

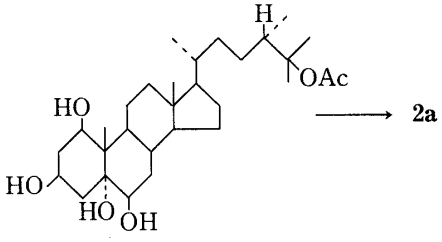
Chart 3

Stereochemistry at C-24 of 24-Methylpolyhydroxysterols (2a, 3, 4a, 4b, 5a)

Five 24-methylpolyhydroxysterols (**2a, 3, 4a, 4b, 5a**) have been isolated from *S. glaucum*. The major compound 24ξ-methyl-5α-cholestane-3β,5,6β,25-tetrol 25-monoacetate (**4a**) was first isolated from the soft coral *S. elegans* by Moldwan *et al.*[7] The C-24 stereochemistry of the compounds **2a**, **4**, and **5a** remained unsettled. On biogenetic grounds, it was expected to have (24*S*) (24β) configuration, since almost all the 24-methylsterols isolated from soft corals or their symbiont dinoflagellates have 24β-methyl stereochemistry.[18,19]



18 **19**



5a

Chart 4

Pyrolysis of **4a** gave 24-methyl-5α-cholest-25-ene-3β,5,6β-triol (**18**). Hydrogenation of **18** gave a triol which was identical with the triol (**19**) obtained from 22,23-dihydrobrassicasterol acetate (**16b**) indicating the (24*S*) stereochemistry in **4a**. Similarly, pyrolysis of **5a** followed by hydrogenation gave the tetrol, which was identical with **2a**. Compound **2a** was also indicated to have the (24*S*) stereochemistry by ^{1}H-NMR (in $CDCl_3$) analysis. In the (24*R*/*S*) pair of 24-methylcholesterols, one of the doublets of the terminal dimethyl signals occurs at significantly different positions.[20] The 26,27-dimethyl signals occur at δ 0.85 and 0.80 in the (24*R*) isomer (campesterol) and at δ 0.85 and 0.78 in the (24*S*) isomer (22,23-dihydrobrassicasterol, **16a**), and are clearly discernible by 200 MHz ^{1}H-NMR spectroscopy. The hydroxyl groups in the A and B rings have little effect on the side chain methyl signals.[21] The triacetate (**2b**) showed the terminal dimethyl signals at δ 0.85 and 0.78, as in the triol (**19**) (δ 0.85 and 0.78), and the signals resembled those of the 24*S* isomer **16a**. Consequently, the stereochemistry at C-24 of all the 24-methylpolyhydroxysterols (**2** to **5**) isolated from *S. glaucum* is considered to be (*S*).

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were determined on a JASCO DIP-4 digital polarimeter. ^{1}H-and ^{13}C-NMR spectra were determined on JEOL-FX 100 and 200 spectrometers at 100 and 200 MHz (^{1}H-NMR) and 25.00 MHz (^{13}C-NMR). Mass spectra were determined on JEOL JMS D-300 (EI-MS) and JEOL 01SG-2 (FD-MS) spectrometers. Infrared (IR) spectra were taken on a JASCO A-102 spectrometer. Gas chromatography (GC) was carried out on a Shimadzu GC4BPF gas chromatograph using a glass column (2m×3 mm. i.d.) packed with 1.5% OV-17 on 80—100 mesh Shimalite W at 265°C, with N_2 carrier gas at a flow rate of 60 ml/min.

(24*S*)-24-Methylcholest-5-ene-3β,25-diol (**3**)——The crude lipid extract (400 g) of *S. glaucum* was chromatographed over a column of silica gel in previous work.[4] The mixture (30 mg), which contained **3**, was eluted after the cembrane diol sarcophytol-B with $CHCl_3$.[2] Crystallization from EtOAc gave 18.7 mg of **3**, mp 185.5—187.5°C, $[\alpha]_D$ −50.3° (*c*=0.95, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3300. EI-MS *m/z*: 416 (M^+), 401 (M^+-CH_3), 398 (M^+-H_2O), 383 (M^+-H_2O, CH_3), 380 (M^+-2H_2O), 273 (M^+—side chain), 271 (M^+—side chain, 2H). ^{1}H-NMR 100 MHz, $CDCl_3$) and ^{13}C-NMR ($CDCl_3$), see text.

Synthesis of 3 from Codisterol (**17a**)——A solution of **17a** (100 mg) in 0.4 ml of tetrahydrofuran (THF) was added dropwise to a mixture of $Hg(OAc)_2$ (100 mg) in H_2O (0.2 ml) and THF (0.2 ml) at 0°C, and the mixture was stirred at 0°C for 2.5 h then at room temperature for 1 h. The mixture was added to 3 N NaOH soln. (0.1 ml) and mixed with $NaBH_4$ (420 mg) in 3 N NaOH (2 ml) and stirred for 10 min. The excess $NaBH_4$ was decomposed by adding 2 N HCl slowly, and the mixture was extracted with $CHCl_3$. The extract was worked up as usual and the residue was subjected to flash chromatography over a column of silica gel (50 g) with 18% EtOAc in $CHCl_3$. The fractions which contained dihydroxysterol were concentrated and recrystallized from $CHCl_3$–MeOH to give 70 mg of **3**; mp and mixed mp with the diol (**3**) from *S. glaucum*, 185—187°C; $[\alpha]_D$ −54° (*c*=0.49, $CHCl_3$). The ^{1}H-NMR and MS were the same as those of **3** from *S. glaucum*.

5α-Cholestane-1β,3β,5,6β-tetrol (**1**) **and (24*S*)-Methyl-5α-cholestane-1β,3β,5,6β-tetrol** (**2a**)——The mixture (207.7 mg) obtained from fraction III in a previous study[4] was subjected to chromatography. A half of the mixture was dissolved in 30 ml of a mixture of cyclohexane–acetone–MeOH (90: 7: 3). The solution was subjected (in fifteen portions) to chromatography over a column of Lipidex 5000 (Packard, 2.2×45 cm) and eluted with the same solvent at a flow rate of 0.5 ml/min. Aliquots of the fractions (10 ml each) were checked as the trimethylsilyl ethers by GC and the fractions were combined accordingly. Compound **1** was eluted immediately after **2**. Compound **1** (16.4 mg), mp 260—263.5°C from MeOH–acetone. $[\alpha]_D$ −54° (*c*=1.0, MeOH). For ^{1}H-and ^{13}C-NMR and MS, see text. Compound **2a** (25.5 mg), mp 273—275.4°C from MeOH–acetone. $[\alpha]_D$−13.2° (*c*=1.14, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3480. FD-MS *m/z*: 450 (M^+), 432 (M^+-H_2O). EI-MS *m/z*: 432 (M^+-H_2O), 414 (M^+-2H_2O), 396 (M^+-3H_2O), 287 (M^+—side chain, $2H_2O$). ^{1}H-NMR (200 MHz, pyridine-d_5) δ: 0.800 (18-Me), 0.82 (6H, d, *J*=6.35,26,27-Me), 0.88 (3H, d, *J*=6.84 Hz, 28-Me), 0.9$\mathcal{L}$ (3H, d, *J*=6.5 Hz, 21-Me,)1.93 (19-Me), 4.23 (1H, br s, 6α-H), 4.8—5.1 (1H, m, 3α-H), 4.92 (1H 'dd, *J*= 5, 11 Hz, 1α-H). ^{1}H-NMR (200 MHz, $CDCl_3$), see text.

1β,3β-Dihydroxy-pregna-5,16-dien-20-one 1-Monoacetate (**7b**)——A mixture of ruscogenin diacetate (**6b**, 17.4 g) and pyridine hydrochloride (5.5 g) in acetic anhydride (70 ml) was refluxed for 4 h. The mixture was concentrated to 40 ml and poured into H_2O. Extraction with $CHCl_3$ and the usual work-up gave the crude pseudoruscogenin triacetate as a solid. It was dissolved in AcOH (100 ml) containing 4.5 g NaOAc. A solution of CrO_3 (6.55 g) in 80% AcOH (25 ml) was added dropwise to the pseudoruscogenin solution at 11—13°C over a period of 25 min, then the mixture was stirred at 22—23°C for 1 h. It was poured into H_2O and extracted with Et_2O. The extract was worked up as usual and the solvent was evaporated off. The residue

was dissolved in *tert*-BuOH (350 ml). This solution was mixed with a solution of KOH (10 g) in H_2O (12 ml) and the mixture was stirred at 30—35°C for 75 min. Most of the solvent was evaporated off *in vacuo* at low temperature. The mixture was added to 1 l of H_2O, neutralized with AcOH and then extracted with Et_2O. Usual work-up and evaporation of Et_2O gave crude **7b** (13 g) as an oil, $[\alpha]_D$ −17° (c=1.16, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3400, 1725, 1660, 1580. ^{1}H-NMR (100 MHz, CD_3OD) δ: 0.91 (18-Me), 1.18 (19-Me), 2.03 (OAc), 2.25 (21-Me), 3.2—3.8 (1H, m, 3α-H), 4.60 (1H, dd, J=5, 11 Hz, 1α-H), 5.6 (1H, m, 6-H), 6.72 (1H, br s, 16-H). A portion of **7b** was hydrolyzed by refluxing the mixture in 5% KOH in MeOH to give the diol (**7c**), mp 235—238°C from MeOH, $[\alpha]_D$ −25° (c=0.48, $CHCl_3$). *Anal.* Calcd for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 75.99; H, 8.89.

1β-Hydroxy-6β-methoxy-3α,5α-cyclopregn-16-en-20-one (8)——The crude unsaturated ketone (**7b**, 13 g) was mixed with TsCl (13 g) in freshly distilled pyridine (120 ml) and the mixture was left at room temperature overnight. The mixture was poured into ice-water, and extracted with Et_2O, and the extract was subjected to usual work-up. The residue was dissolved in a solution of KOAc (5.7 g) in MeOH (400 ml) and refluxed for 30 min. It was concentrated to 150 ml, diluted with H_2O, and extracted with Et_2O. The extract was worked up as usual and the solvent was evaporated off. The residue was dissolved in *tert*-BuOH (350 ml), mixed with a solution of KOH (12.5 g) in H_2O (15 ml) and stirred at 80°C for 45 min. The mixture was concentrated, diluted with H_2O, and extracted with Et_2O. The extract was worked up as usual and the residue was chromatographed over a column of silica gel (500 g). Elution with a gradient of MeOH (2—5%) in $CHCl_3$ gave **8** (5.63 g, 47% from **6a**), mp 150—154°C from MeOH, $[\alpha]_D$ +68° (c=0.48, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3440, 1645, 1585. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 0.97 (18-Me), 1.15 (19-Me), 2.25 (21-Me), 2.77 (6α-H), 3.38 (CH_3O–), 3.97 (1H, br d, J=6 Hz, 1α-H), 6.72 (1H, br s, 16-H). EI-MS m/z: 344 (M^+), 326 (M^+-H_2O), 312 (M^+—MeOH), 294 (M^+-H_2O, MeOH). *Anal.* Calcd for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.65; H, 9.34.

6β-Methoxy-3α,5α-cyclopregn-17(20)*E*-ene-1β,16α-diol (10)——The α,β-unsaturated ketone **8** (5.6 g) in MeOH (320 ml) was mixed with 4 N NaOH solution (12.5 ml) and then with 30% H_2O_2 (24 ml) at room temperature. After 15 min, the mixture was poured into H_2O (800 ml) and extracted with Et_2O (350 ml). The extract was worked up as usual and the solvent was evaporated off. The residue was dissolved in *N*,*N*-dimethylethanolamine (50 ml) and mixed with KOH (5.2 g) and hydrazine hydrate (100%, 5.2 ml). The mixture was refluxed under N_2 for 1 h, poured into H_2O, and extracted with Et_2O. The extract was worked up as usual and the solvent was evaporated off. Thin-layer chromatography (TLC) of the residue (4.7 g, 75% from **8**) with 5% MeOH in $CHCl_3$ on a 15% silver nitrate-impregnated silica gel plate showed that it was composed of **10** and a trace of a less polar compound. A portion of the product was recrystallized from benzene–hexane to give pure **10**, mp 130—135°C, $[\alpha]_D$ +17° (c=0.6, $CHCl_3$). ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 0.94 (18-Me), 1.11 (19-Me), 1.80 (3H, d, J=7 Hz, 21-Me), 2.75 (1H, m, 6α-H), 3.35 (MeO–), 3.94 (1H, br d, J=6 Hz, 1α-H), 4.45 (1H, m, 16β-H), 5.61 (1H, br q, J=7 Hz, 20-H). EI-MS m/z: 346 (M^+), 328 (M^+-H_2O), 314 (M^+—MeOH), 296 (M^+-H_2O, MeOH). *Anal.* Calcd for $C_{22}H_{34}O_3$: C, 76.36; H, 9.89. Found: C, 76.05; H, 9.72.

1β-Hydroxy-6β-methoxy-3α,5α-cyclocholestan-23-one (13)——Meldrum's acid (950 mg) was acylated with isovaleryl chloride according to the published procedure.[14] The isovaleryl Meldrum's acid thus obtained was stored in dry CH_2Cl_2 (0.2 mmol/ml). Twenty-two ml of this isovaleryl Meldrum's acid solution was concentrated at 15—20°C and mixed with a solution of the allyl alcohol **10** (674 mg) in benzene (12 ml). The mixture was refluxed for 1 h. The benzene solution was washed with 5% $NaHCO_3$, H_2O, and saturated NaCl solution and the solvent was evaporated off. The residue was chromatographed over a column of silica gel (15 g) and eluted with a mixture of hexane–$CHCl_3$ (1 : 1) to give 700 mg of β-ketoester (**11**). It was dissolved in dry xylene (20 ml), then NaH (140 mg) was added and the mixture was refluxed for 2 h. Excess NaH was decomposed with a small amount of MeOH and the mixture was diluted with H_2O and extracted with Et_2O. After the usual work-up, the solvent was evaporated off. The residue was refluxed in 5% KOH in MeOH (20 ml) for 6 h, diluted with H_2O, and extracted with Et_2O. The extract was worked up as usual and the solvent was evaporated off. The residue was dissolved in MeOH (20 ml) and hydrogenated over 150 mg of PtO_2 catalyst at 65°C for 2 h. Filtration and evaporation of the solvent gave a residue, which was purified over a column of silica gel (20 g). Elution with 1% MeOH in $CHCl_3$ gave **13** (440 mg, 52% from **10**) as an oil, $[\alpha]_D$ +34° (c=0.91, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3440, 1705. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 0.73 (18-Me), 1.10 (19-Me), 0.86 (6H, d, J=6 Hz, 26,27-Me), 0.89 (3H, d, J=6 Hz, 21-Me), 2.72 (1H, m, 6α-H), 3.33 (MeO–), 3.9 (1H, br d, J=7 Hz, 1α-H). EI-MS m/z: 430 (M^+), 412 (M^+-H_2O), 398 (M^+—MeOH).

Cholest-5-ene-1β-3β-diol (15)——Compound **13** (1.96 g) was dissolved in diethyleneglycol (80 ml), and hydrazine hydrate (100%, 11 ml) was added. The mixture was heated gradually from 20 to 125°C during 30 min and kept at 125—130°C for 10 min. Pellets of KOH (11 g) were added to the mixture and the temperature was raised gradually to 210°C during 30 min and kept at 210—215°C for another 3.5 h. After cooling, the mixture was poured into H_2O and the precipitate was extracted with Et_2O. The extract was worked up as usual and the solvent was evaporated off. The residue was dissolved in a 4 : 1 mixture of dioxane and H_2O (110 ml) containing 100 mg of conc. H_2SO_4, and the mixture was refluxed for 20 min. It was diluted with H_2O until the solution became slightly cloudy and was then concentrated *in vacuo* to *ca.* 70 ml. The precipitate was collected by suction and the filter cake was washed with H_2O. It was purified by chromato-

graphy over a column of silica gel (50 g). Elution with 2% MeOH in $CHCl_3$ gave **15** (0.81 g, 46% from **13**), mp 181—182.5°C, $[\alpha]_D$ −39° (c=0.78, $CHCl_3$). EI-MS m/z: 402 (M^+), 384 (M^+-H_2O), 366 (M^+-2H_2O), 351 (M^+-2H_2O, Me). *Anal.* Calcd for $C_{27}H_{46}O_2\cdot 1/4H_2O$: C, 79.65; H, 11.51. Found: C, 79.91; H, 11.29.

5α-Cholestane-1β,3β,5,6β-tetrol (**1**)——A solution of **15** (140 mg) in THF (4.5 ml) was cooled in an ice bath. A mixture of 88% formic acid (2.3 ml) and 30% H_2O_2 (0.22 ml) was added dropwise at 0°C with stirring. The whole was further stirred for 18 h at room temperature, the excess H_2O_2 was decomposed with Na_2SO_3 solution. The mixture was diluted with H_2O, extracted with Et_2O and worked up as usual. The residue was hydrolyzed by refluxing it in 3% KOH in MeOH for 2 h. The mixture was concentrated at 20—30°C and diluted with H_2O. The precipitate was collected and purified over a column of silica gel (6 g) with a gradient (5—10%) of MeOH in $CHCl_3$ to give 90 mg (60%) of **1**; mp and mixed mp with the tetrol **1** from *S. glaucum*, 261—264°C (from acetone–hexane), $[\alpha]_D$ −6° (c=1.15, MeOH). MS and ^{1}H-NMR (200 MHz, pyridine-d_5) spectra were identical with those of **1** from *S. glaucum*. *Anal.* Calcd for $C_{27}H_{48}O_4\cdot 1/4H_2O$: C, 73.50; H, 11.07. Found: C, 73.38; H, 10.75.

(24*S*)-24-Methyl-5α-cholestane-3β,5,6β-triol(5α-Ergostane-3β,5,6β-triol, 19)——a: The 5α, 6β-glycolation of **16b** (150 mg, mp 148—149°C, $[\alpha]_D$ −49°)[3] was carried out according to the same procedure as described for compound **1** and gave 130 mg of **19**, mp 241.5—244°C from acetone, $[\alpha]_D$ −4° (c=0.54, MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$), see text. ^{1}H-NMR (200 MHz, pyridine-d_5) δ: 0.75 (18-Me), 1.67 (19-Me), 0.82 (6H, d, J=6.83 Hz, 26,27-Me), 0.88 (3H, d, J=6.84 Hz, 28-Me), 0.99 (3H, d, J=6.35, 21-Me), 2.97 (1H, t, J=12 Hz, 4β-H), 4.20 (1H, br s, 6α-H), 4.8—5.0 (1H, m, 3α-H). EI-MS m/z: 434 (M^+), 416 (M^+-Me), 398 (M^+-2H_2O), 383 (M^+-2H_2O, Me), 380 (M^+-3H_2O).

b: Compound **4a** (184 mg) in a small flask was heated in an oil bath at 280°C for 5 min. Chromatography of the crude product over a column of silica gel (20 g) with 5% MeOH in $CHCl_3$ gave 115 mg of **18** mp 214—215°C, $[\alpha]_D$ −3.4° (c=0.64, MeOH). EI-MS m/z: 432 (M^+), 414 (M^+-H_2O), 396 (M^+-2H_2O). ^{1}H-NMR (200 MHz, pyridine-d_5) δ: 1.67 (6H, s, 19- and 27-Me), 4.82 (2H, br s, 26-H). A solution of **18** (110 mg) in EtOH (30 ml) and AcOH (1 ml) was hydrogenated over 10% Pd–C catalyst (110 mg). The catalyst and the solvent were removed and the residue was recrystallized from acetone–MeOH to give **19** (100%), mp 241.5—244°C. Mixed mp with the triol (**19**) in a), 241.5—244°C, $[\alpha]_D$ −2.4° (c=0.5, MeOH). The MS and ^{1}H-NMR (200 MHz, $CDCl_3$ and pyridine-d_5) spectra were identical with those of the product obtained in a). *Anal.* Calcd for $C_{28}H_{50}O_3\cdot 1/4H_2O$: C, 76.56; H, 11.59. Found: C, 76.37; H, 11.33.

Conversion of 5a to 2a——Compound **5a** (1 mg) in a small flask was heated gently over an open flame until the crystals melted and evolved gas. After cooling, the residue was hydrogenated over 10% Pd-C catalyst as above. The product was found to be virtually pure on TLC, and the ^{1}H-NMR spectrum (200 MHz, pyridine-d_5) was identical with that of **2a**. A portion of the product was recrystallized from EtOAc to give pure **2a**, mp and mixed mp 270—274°C.

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