Notes

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Marine Sterols. XIV.[1] Isolation of (24*S*)-24-Methyl-5α-cholestane-3β,5,6β,25ξ,26-pentol from the Soft Coral *Sarcophyton glaucum*

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(24*S*)-24-Methyl-5α-cholestane-3β,5,6β,25ξ,26-pentol (**1**) was isolated from the soft coral *Sarcophyton glaucum*. The structure of **1** was confirmed by the spectroscopic data and by the synthesis of a C-25 isomeric mixture of **1** starting from codisterol acetate (**5a**), which is one of the main components in the 3β-monohydroxysterol fraction of *S. glaucum*.

Keywords——coelenterata; soft coral; *Sarcophyton glaucum*; (24*S*)-24-methyl-5α-cholestane-3β,5,6β,25ξ,26-pentol; polyoxysterol; codisterol

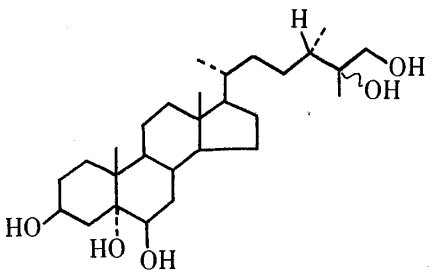
The soft coral *Sarcophyton glaucum* is commonly found in the Indo–Pacific coastal waters; for example, it appears as large colonies in the shallow waters around Okinawa. Its lipid content is high (about 1.6 kg from 17 kg of wet material),[2a] and the major lipid was found to be a cembrane diterpene sarcophytol-A (14ξ-hydroxycembrane-1,3,7,11-tetraene),[2a] which represented nearly 25% of the total lipid extract of *S. glaucum* collected at Ishigaki Island.[2b] Interestingly, *S. glaucum* collected in the Red Sea contained little sarcophytol-A.[3] Thus, the chemical components of soft corals vary according to their habitats, possibly due to the variation of their symbiont microalgae, zooxanthelae.

S. glaucum also contains novel mono- and polyoxysterols. One of the most interesting compounds is glaucasterol (24ξ,25ξ-24,26-cyclocholesta-5,22-dien-3β-ol, **4**).[4] Glaucasterol was isolated in very small amounts from the monohydroxysterol fraction and it also appears to occur in several deep sea gorgonians which belong to the same subclass, octocollaria.[5] The polyoxysterol fraction of *S. glaucum* is a complex mixture and we have hitherto identified six compounds having androstane, cholestane, and 24-methylcholestane skeletons (**2a**—**2e**, **3**).[1,6] A common functionality of these compounds was the 5α,6β-glycol group. In the present paper, we wish to report the structure of a minor new polyoxysterol (**1**) and the correlation of codisterol ((24*S*)-24-methylcholesta-5,25-dien-3β-ol, **5b**) to **1**.

Repetitive flash chromatography[7] of the polyhydroxysterol fraction from the crude lipid extract (840 g) of *S. glaucum* gave 120 mg of compound **1**, mp 262—264 °C, $[\alpha]_D$ −14°. The elemental analysis indicated the molecular formula $C_{28}H_{50}O_5$. Compound **1** did not show the molecular ion (M^+) in the mass spectrum, as was the case with six other polyoxysterols,[6] but showed several dehydration ions due to the loss of one to four molecules of H_2O at *m/z* 448, 430, 412 and 394. The proton nuclear magnetic resonance (^{1}H-NMR) signals of **1** due to the steroid ring and 21-Me were virtually the same as those of the major compound **2a**, as reported previously.[6b] The spectrum showed signals of 18-Me (δ 0.73), 19-Me (1.66), 21-Me (1.00, d, J=6.35 Hz), 3α-H (4.9, m), 6α-H (4.19, br s), and 4β-H (2.98, t, J=11.7 Hz). The significantly deshielded nature of 19-Me, 3α-H, and 4β-H is a result of the 1,3-diaxial interaction with the hydroxyl groups and it was further intensified by pyridine-induced

deshielding.[6b] The mass spectrum of **1** showed ions due to cleavage of the side chain with successive loss of three molecules of H_2O, at *m/z* 289, 271, and 253. The ^{1}H-NMR also showed the signals of a secondary methyl at δ 1.27 (d, J=6.83 Hz), a tertiary methyl which is geminal to oxygen at δ 1.47 (s), and almost coalesced doublets (J=11 Hz) due to a hydroxymethyl group at δ 3.99 and 4.00. Thus the mass and ^{1}H-NMR spectra suggested that compound **1** is 24-methylcholestane-3β,5α,6β,25,26-pentol. This was supported by the presence of several fragment ions due to the loss of H_2O and hydroxymethyl at *m/z* 417 (M^+-H_2O, CH_2OH), 399 (M^+-2H_2O, CH_2OH), and 381 (M^+-3H_2O, CH_2OH).

The structure of **1** was confirmed by synthesis from codisterol (**5b**) which occurs simultaneously (3% of total monohydroxysterols) in *S. glaucum*.[8] Codisterol was first found in a green alga *Codium fragile* by Goad *et al.*[9] Before we found **5b** in *S. glaucum*, only a Caribbean sponge, *Verongia cauliformis*, was known to contain **5b** and its C-24 isomer in small amounts.[10] The C-24 stereochemistry of **5b** from *S. glaucum* was confirmed as (*S*) by converting **5b** to 22,23-dihydrobrassicasterol. Simultaneous glycolation of the two double bonds of **5a** with *m*-chloroperbenzoic acid followed by acid hydrolysis[11] and then alkaline hydrolysis gave a C-25 isomeric mixture of (24*S*)-24-methyl-5α-cholestane-3β,5,6β,25,26-pentol which was homogeneous on chromatography and resistant to separation. Its mass spectrum was identical with that of **1**. The ^{1}H-NMR was also identical with that of **1** except for the signals due to 27-Me and 28-Me, and splitting of the hydroxylmethyl signal into a pair of signals in 2:3 intensity ratio. The major signals were due to the C-25 isomer of **1** and appeared at δ 3.92 and 3.94 (each d, J=11 Hz, C-26), 1.39 (s, C-27), and 1.10 (d, J=6.83 Hz, C-28), while the minor signals appeared at the same positions as those of **1**. Thus, the minor polyoxysterol from *S. glaucum* was identified as (24*S*)-24-methyl-5α-cholestane-3β,5,6β,25ξ,26-pentol (**1**).



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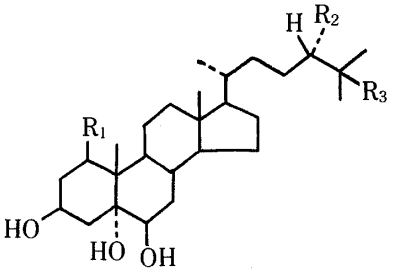
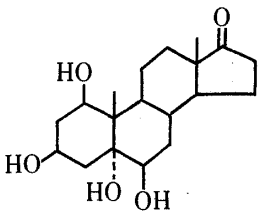


2a : R_1=H, R_2=Me, R_3=OAc
2b : R_1=H, R_2=Me, R_3=OH
2c : R_1=OH, R_2=H, R_3=H
2d : R_1=OH, R_2=Me, R_3=H
2e : R_1=OH, R_2=Me, R_3=OAc



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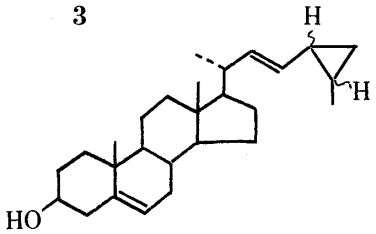

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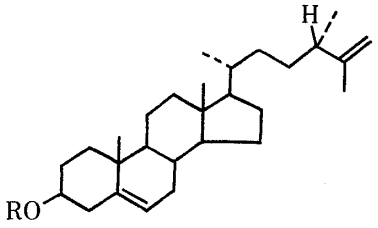

5a : R=Ac
5b : R=H

Chart 1

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 a JEOL-FX 200 spectrometer at 200 MHz (^{1}H-NMR) and 50 MHz (^{13}C-NMR) in pyridine-d_5 solutions. Mass spectra were determined on a JEOL JMS D-300 spectrometer.

Isolation of 1——The lipid extract (840 g) of *S. glaucum*, which was obtained in a previous study,[6b] was partitioned with a mixture of solvents, hexane–MeOH–H_2O (20 : 10 : 2), and separated into upper (590 g) and lower (151 g) extracts. Monohydroxysterols and other non-polar compounds were extracted in the upper layer while the lower layer contained polyhydroxysterols and other polar compounds. The polar lipid fraction was chromatographed over a column of silica gel (1.5 kg) with a mixture of benzene–$CHCl_3$ (1 : 1, 40 l), $CHCl_3$ (50 l), and a gradient of 0 to 20% MeOH in $CHCl_3$ (110 l). The fractions containing **1**—**3** were eluted with 18—20% MeOH in $CHCl_3$. Further chromatography of this mixture over a column of silica gel with 10% MeOH in $CHCl_3$ gave 11.5 g of a mixture containing **1** and **2b**—**2e** and 250 mg of a mixture which contained **1** and **3**. Both mixtures were separated in portions by flash chromatography with 10% MeOH in $CHCl_3$ and provided 95 mg of **3**, 1.14 g of **2b**, 9 g of a mixture containing **2c**—**2e**, and a mixture (0.67 g) containing **1**. The mixture containing **1** was separated by flash chromatography with 4% MeOH in ethyl acetate several times and gave 120 mg of **1**, mp 262—264 °C (acetone–hexane), $[\alpha]_D$ −14° (c=1.4, MeOH). *Anal.* Calcd for $C_{28}H_{50}O_5 \cdot 1/2H_2O$: C, 70.69; H, 10.81. Found: C, 70.65; H, 11.03. Mass spectrum, see the text. Other ions, m/z: 305 (M^+ − side chain, 2H), 262 (M^+ − side chain, H_2O, C-16, 17), 244 (M^+ − side chain, $2H_2O$, C-16,17), 247 (M^+ − side chain, H_2O, CH_3, C-16, 17), 229 (M^+ − side chain, $2H_2O$, CH_3, C-16, 17), 211 (M^+ − side chain, $3H_2O$, CH_3, C-16, 17). ^{1}H-NMR, see the text. ^{13}C-NMR, δ: 32.5 (C-1), 33.3 (C-2), 67.4 (C-3), 42.9 (C-4), 75.9 (C-5), 76.3 (C-6), 35.7 (C-7), 31.2 (C-8), 46.0 (C-9), 39.2 (C-10), 21.8 (C-11), 40.7 (C-12), 43.1 (C-13), 56.5(C-14), 24.6 (C-15), 28.6 (C-16), 56.5 (C-17), 12.4 (C-18), 17.2 (C-19), 36.8 (C-20), 19.3 (C-21), 35.4 (C-22), 28.6 (C-23), 41.4 (C-24), 74.9 (C-25), 68.8 (C-26), 21.5 (C-27), 14.5 (C-28).

Synthesis of C-25 Isomeric Mixture of 1——Codisterol acetate (**5a**, 140 mg, 0.32 mmol) in 10 ml of CH_2Cl_2 was treated with 240 mg (1.4 mmol) of *m*-chloroperbenzoic acid at 0 °C and the mixture was left at room temperature overnight. The solution was washed with saturated $NaHCO_3$ solution, H_2O, and saturated NaCl solution and the solvent was evaporated off at 30 °C. The residue was dissolved in a mixture of tetrahydrofuran (THF) (12 ml) and H_2O (2.5 ml) and treated with 0.1 ml of 78% $HClO_4$ solution overnight. The mixture was neutralized with diluted Na_2CO_3 solution and the solvent was evaporated off at 30 °C. The crude reaction mixture was dissolved in 5 ml of 5% KOH in MeOH and refluxed for 30 min, then the solvent was evaporated off at 30 °C. The residue was triturated with $CHCl_3$. Most of the non-polar by-products were extracted by $CHCl_3$. The residue was again triturated with 20% MeOH in $CHCl_3$ and the extract was directly mixed with 5 g of silica gel. The silica gel suspension containing the crude product was dried at room temperature and mounted on a column of silica gel (35 g). Elution with 15% MeOH in $CHCl_3$ gave 120 mg (84%) of pentol mixture, mp 260—261 °C (acetone–hexane). The mass spectrum was identical with that of natural **1**. ^{1}H-NMR, see the text. *Anal.* Calcd for $C_{28}H_{50}O_5$: C, 72.06; H, 10.80. Found: C, 71.91; H, 11.07.

References and Notes

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