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

MASARU KOBAYASHI and HIROSHI MITSUHASHI*

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

(Received March 28, 1983)

(24S)-24-Methyl-5α-cholestan-3β,5,6β,25ξ,26-pentol (I) was isolated from the soft coral Sarcophyton glaucum. The structure of I was confirmed by the spectroscopic data and by the synthesis of a C-25 isomeric mixture of I 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; (24S)-24-methyl-5α-cholestan-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), and the major lipid was found to be a membrane diterpene saponofytol-A (14ξ-hydroxycembrane-1,3,7,11-tetraene), which represented nearly 25% of the total lipid extract of S. glaucum collected at Ishigaki Island. Interestingly, S. glaucum collected in the Red Sea contained little saponofytol-A. 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 glaucosterol (24ξ,25ξ-24,26-cyclocholesta-5,22-dien-3β-ol, 4). Glaucosterol 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. The polyoxysterol fraction of S. glaucum is a complex mixture and we have hitherto identified six compounds having androstane, choleslate, and 24-methylcholestan skeletons (2a—2e, 3). 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 (I) and the correlation of codisterol (24S')-24-methylcholesta-5,25-dien-3β-ol, 5b) to 1.

Repetitive flash chromatography of the polyoxysterol fraction from the crude lipid extract (840 g) of S. glaucum gave 120 mg of compound 1, mp 262—264 °C, [α]D -14 °C. 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, 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 (¹H-NMR) signals of I due to the steroid ring and 21-Me were virtually the same as those of the major compound 2a, as reported previously. 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
The mass spectrum of 1 showed ions due to cleavage of the side chain with successive loss of three molecules of H$_2$O, at $m/z$ 289, 271, and 253. The $^1$H-NMR also showed the signals of a secondary methyl at $\delta$ 1.27 (d, $J$ = 6.83 Hz), a tertiary methyl which is geminal to oxygen at $\delta$ 1.47 (s), and almost coalesced doublets ($J$ = 11 Hz) due to a hydroxymethyl group at $\delta$ 3.99 and 4.00. Thus the mass and $^1$H-NMR spectra suggested that compound 1 is 24-methylcholest-3β,5α,6β,25,26-pentol. This was supported by the presence of several fragment ions due to the loss of H$_2$O and hydroxymethyl at $m/z$ 417 (M$^+$ - H$_2$O, CH$_2$OH), 399 (M$^+$ - 2H$_2$O, CH$_2$OH), and 381 (M$^+$ - 3H$_2$O, CH$_2$OH).

The structure of 1 was confirmed by synthesis from codisterol (5b) which occurs simultaneously (3% of total monohydroxysterols) in S. glauum. Codisterol was first found in a green alga Codium fragile by Goad et al. Before we found 5b in S. glauum, only a Caribbean sponge, Verongia cauliformis, was known to contain 5b and its C-24 isomer in small amounts. The C-24 stereochemistry of 5b from S. glauum 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 and then alkaline hydrolysis gave a C-25 isomeric mixture of (24S)-24-methyl-5α-cholestan-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 hydroxymethyl 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 $\delta$ 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. glauum was identified as (24S)-24-methyl-5α-cholestan-3β,5,6β,25,26-pentol (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. 1H- and 13C-NMR spectra were determined on a JEOL-FX 200 spectrometer at 200 MHz (1H-NMR) and 50 MHz (13C-NMR) in pyridine-d5 solutions. Mass spectra were determined on a JEOL JMS D-300 spectrometer.

Isolation of 1—The lipid extract (840 g) of S. glauces, which was obtained in a previous study, was partitioned with a mixture of solvents, hexane-MeOH-H2O (20:10:2), and separated into upper (590 g) and lower (151 g) extracts. Monohydrosylosters and other non-polar compounds were extracted in the upper layer while the lower layer contained polyhydrosylosters and other polar compounds. The polar lipid fraction was chromatographed over a column of silica gel (1.5 kg) with a mixture of benzene-CHCl3 (1:1, 40 l), CHCl3 (50 l), and a gradient of 0 to 20% MeOH in CHCl3 (110 l). The fractions containing 1—3 were eluted with 18—20% MeOH in CHCl3. Further chromotography of this mixture over a column of silica gel with 10% MeOH in CHCl3 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 CHCl3 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), [α]D —14 ° (c = 1.4, MeOH). Anal. Caled for C25H48O5; 1H2O: 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, H2O, C-16, 17), 244 (M+ — side chain, 2H2O, C-16, 17), 247 (M+ — side chain, H2O, CH3-16, 17), 229 (M+ — side chain, 2H2O, CH3-16, 17), 211 (M+ — side chain, 3H2O, CH3-16, 17). 1H-NMR, see the text. 13C-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 CHCl3 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 NaHCO3 solution, H2O, 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 H2O (2.5 ml) and treated with 0.1 ml of 78% HClO4 solution overnight. The mixture was neutralized with dilute Na2CO3 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 CHCl3. Most of the non-polar by-products were extracted by CHCl3. The residue was again triturated with 20% MeOH in CHCl3 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 CHCl3 gave 120 mg (84%) of pentol mixture, mp 260—261 °C (acetone-hexane). The mass spectrum was identical with that of natural 1. 1H-NMR, see the text. Anal. Caled for C25H49O5; C, 72.06; H, 10.80. Found: C, 71.91; H, 11.07.

References and Notes

4) M. Kobayashi and H. Mitsuhashi, Steroids, 40, (1982), “in press.” The structure and the trivial name glaucosterol were presented at the 11th International Symposium on Marine Natural Products, Tenerife, Spain, July 1982, and referred to by P. J. Scheuer in his lecture at the subsequent XIIIth International Symposium on the Chemistry of Natural Products, Pretoria, South Africa, August 1982. Nevertheless, they later coined a new trivial name “papakusteron” for apparently the same compound from gorgonians.