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Isolation of 23-Methylcholesta-5,22-dien-3β-ol from the Soft Coral *Sarcophyton glaucum*

A new marine C_{28} sterol was isolated from the soft coral, *Sarcophyton glaucum*, and its structure was proposed as (22*E*)-23-methylcholesta-5,22-dien-3β-ol on spectral evidences, especially by the comparison of its ^{13}C-NMR chemical shifts with those of 23,24-dimethylcholesta-5,22-dien-3β-ol, which is present in the same soft coral.

Keywords——23-methylcholesta-5,22-dien-3β-ol; soft coral; *Sarcophyton glaucum*; dinoflagellate; marine sterol; ^{13}C-NMR

Previously we reported the occurrence of a minor unidentified sterol (I) which shows the slightly longer retention time than cholesterol on gas chromatography (GC), along with the biogenetically interesting 23,24-dimethylcholesta-5,22-dien-3β-ol (II) and 23,24-dimethylcholesta-5,17(20)-dien-3β-ol (III), in the sterol mixture of the soft coral *Sarcophyton glaucum*, collected at Ishigaki Island.[1] The major components were (24*S*)-24-methylcholesterol and gorgosterol, and compound I represented less than 0.5% of total sterols. Repeated column chromatography of the sterol mixture (30 g) over silver nitrate-impregnated silica gel (benzene–hexane) and partition chromatography using Lipidex 5000 (hexane–methanol)[2] afforded 11 mg of a gas chromatographically pure specimen as needles from methanol.

Compound I showed mp 140.5—142.5°, $[\alpha]_D$ −39.2° (c=0.98, $CHCl_3$). The mass spectrum of I showed a molecular ion (M^+) at *m/e* 398 and other ions at 380 (M^+-H_2O), 383 (M^+-CH_3), 365 (M^+-CH_3 and H_2O), and 355 ($M^+-C_3H_7$) indicating that it is a diunsaturated C_{28} sterol. A series of fragments, *m/e* 271 (M^+—side chain and 2H), 255 (M^+—side chain and H_2O) and 213 (M^+—side chain, H_2O, and ring D cleavage) are those normally observed with sterols having an unsaturated nucleus and unsaturated side chain.[3] Furthermore, an intense peak at *m/e* 125 (C_9H_{17}) seemed to match the entire side chain, excluding the possibility of an extra methyl group's presence on the nucleus. The proton magnetic resonance (PMR) signals for 18-Me (δ 0.71), 19-Me (1.01), hydroxymethine (3.3—3.76, 1H, m) and olefinic proton (5.36, 1H, m) suggested that I belongs to the conventional 3β-hydroxy-Δ^5 sterols[4] as was the case with all of the other ten sterol components in the same organism.[1] Assuming that I bears a methylated cholestane-type side chain, three doublets at δ 0.93 (3H, J=6.5 Hz), 0.83 (3H, J=6 Hz), and at 0.81 (3H, J=6 Hz) are assignable to 21-, 26- and 27-methyl signals respectively. Also the olefinic signals at δ 1.56 (3H, d, J=0.5 Hz) and 4.88 (1H, broad d, J=10 Hz) indicate the presence of a trisubstituted double bond bearing one methyl group in the side chain. The appearance of the doublet at δ 4.88 confined the location of the double bond to a position adjacent to a tertiary carbon (*i.e.* Δ^{22} or Δ^{23}), and thus the location of the olefinic methyl group is set at C-23. An intense ion at *m/e* 300 (cleavage at C-20 and C-22 with 1H transfer) in the mass spectrum is characteristic of Δ^{22}-sterols with a nuclear double bond while that of Δ^{23}-sterols is *m/e* 301.[3b] From these results the most plausible structure of I would be 23-methylcholesta-5,22-dien-3β-ol. The previously determined homologous sterol (II) showed a similar PMR spectrum and mass spectral fragmentation pattern.[1]

1) M. Kobayashi, A. Tomioka, and H. Mitsuhashi, Steroids, in press. The GC was carried out using 1.5% OV-17 on 80—100 mesh Shimalite W at 260°. The relative retention time (Rt_R) of I to cholesterol was 1.06.
2) P.M. Hyde and W.H. Elliott, *J. Chromatogr.*, **67**, 170 (1972).
3) *a*) B.A. Knights, *J. Gas Chromatogr.*, **5**, 273 (1967); *b*) S.G. Wyllie and C. Djerassi, *J. Org. Chem.*, **33**, 305 (1968).
4) R.F. Zürcher, *Helv. Chim. Acta*, **40**, 2054 (1963).

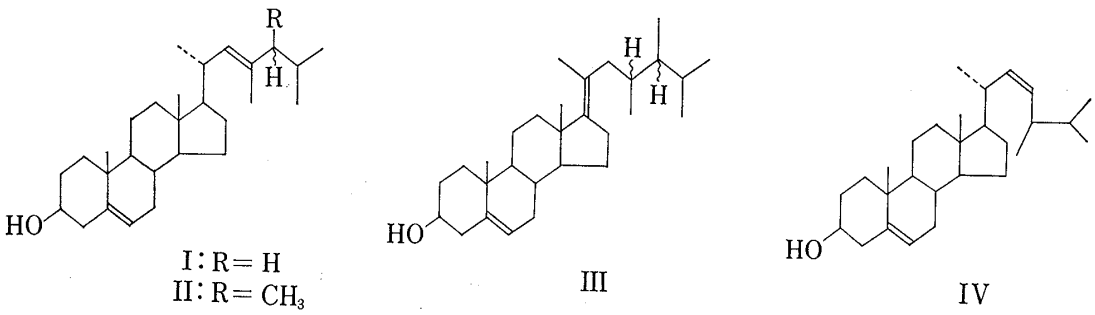

Fig. 1

The ^{13}C-NMR spectrum of I and its comparison with that of II (Table I) provided strong evidence to support the structure of I. The both compounds showed almost the same chemical shifts for carbons 1 to 22. The difference is that compound I showed the signal of C-25 higher by 5.9 ppm and those of C-26 and C-27 slightly lower than the corresponding signals in II. These discrepancies can be well explained by the β- and γ-effects of the 24-methyl group.[5] The geometry of Δ^{22} double bond of I and II was deduced as E from the chemical shifts of C-24 and olefinic methyl. The chemical shifts of C-24 (I, 49.6 ppm; II, 50.1 ppm) are reasonably close to the calculated value when the β-methyl substituent effect (+8 ppm)[6] was taken into account on those of (22E)-cholesta-5,22-dien-3β-ol (42.0 ppm) and (22E)-24-methylcholesta-5,22-dien-3β-ol (43.1 ppm).[7] The chemical shifts of olefinic methyls (I, 16.2 ppm; II, 16.9 ppm) show the influence of the shielding effect by vicinal Z carbon (C-20) which is similar to that of (E)-polyisoprene (16.0 ppm) in contrast to (Z)-polyisoprene (23.6 ppm).[8] The geometry of the similar 23,24-dimethyl system in dinosterol, a 4α-methyl-5α,6-dihydro analog of II, from the toxic dinoflagellate *Gonyaulax tamarensis* by Shimizu *et al.* was also shown to have the E geometry by X-ray crystallography.[9]

The proposed structure, (22E)-23-methylcholesta-5,22-dien-3β-ol, represents the second member of a class of 23-methylcholestane-type sterols found in marine organisms. The first example, 4α,23-dimethyl-5α-cholest-22-en-3β-ol, had been found in the cultured dinoflagellate, *Gonyaulax diagenesis* by Alam, *et al.*[10] Compound I may also be derived from the symbiotic dinoflagellate of *S. glaucum*.

Interestingly, I shows the spectroscopic and chromatographic data very similar to those reported for a C_{28} sterol from a clam *Tapes philippinarum* by Teshima *et al.*[11] Although we were unable to do the direct comparison, the published PMR and mass spectra are almost identical with those of I except for a minor difference. The sterol from the clam was assigned a structure of (22Z)-24-methylcholesta-5,22-dien-3β-ol (IV) on the basis of PMR (δ 4.79—4.88 and 4.15—4.22) and very weak infrared absorption (680 cm^{-1}). One of the signals (δ 4.15—4.22) attributed to the olefinic proton, however, is evidently too high for such an isolated Z-Δ^{22} disubstituted double bond which usually occurs at δ 4.9—5.1.[12]

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Table I. ^{13}C-NMR Chemical Shifts in Compounds I and II (in ppm)

Carbons	I	II	Carbons	I	II
1	37.2	37.7	15	24.3	24.3
2	31.9	31.9	16	28.2	27.8
3	71.7	71.7	17[a]	56.7	56.7
4	42.2	42.2	18	12.1	12.1
5	140.4	140.4	19	19.4	19.4
6	121.4	121.4	20	34.9	34.5
7	31.9	31.6	21	20.7	20.6
8	31.6	30.7	22	132.9	131.4
9	50.1	50.1	23	130.1	134.8
10	36.5	36.4	24	49.6	50.1
11	21.1	21.0	25	26.0	31.9
12	39.7	39.7	26[b]	22.3	20.0
13	42.1	42.1	27[b]	22.6	21.7
14[a]	56.6	56.7	28	—	13.2
			29[c]	16.2	16.9

a, *b*) Chemical shifts may be interchanged.
c) Methyl at C-23.

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