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Marine Sterols. XV.[1] Isolation of 24-Vinyloxycholesta-5,23-dien-3β-ol from the Brown Alga *Sargassum thumbergii*

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A new minor sterol component, 24-vinyloxycholesta-5,23-dien-3β-ol, was isolated from the brown alga *Sargassum thumbergii* (MERTENS) O. KUNTZE. Its structure was deduced from spectroscopic data and confirmed by chemical conversion to 24-ketocholesterol.

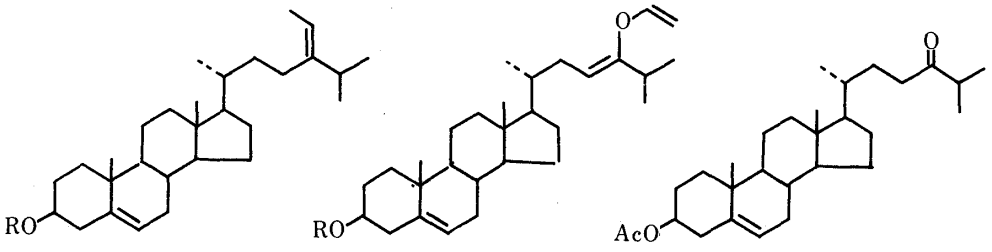
Keywords——brown algae; *Sargassum thumbergii*; 24-vinyloxycholesta-5,23-dien-3β-ol; 24-ketocholesterol

The sterols of brown algae are known to be composed mainly of fucosterol (**1a**) with smaller amounts of such conventional sterols as 24-methylenecholesterol and cholesterol.[2] In view of the recent discovery of biogenetically unusual sterols in marine organisms, including micro algae,[3] it has become desirable to reinvestigate the minor sterol components of marine algae. Isolation of such chemically modified sterol components present generally in very small amounts was not possible in earlier studies on the sterols of marine organisms.

The sterol mixture of the typical brown alga *Sargassum thumbergii* (MERTENS) O. KUNTZE was separated by the usual method. Examination of the mixture by gas chromatography (GC) showed the general pattern of the sterol profile of *Sargassum* species, having **1a** as the predominant component (95%), associated with 24-methylenecholesterol (2%), and cholesterol (2%). The mixture was acetylated and recrystallized several times to remove the bulk of the major sterol components. The sterol mixture obtained from the mother liquor was subjected to chromatography on a silver nitrate-impregnated silica gel column. Repeated separation of the mixture gave a small amount of a non-crystalline unknown component (**2b**) whose relative retention time in GC was very close to that of fucosterol acetate (**1b**), but whose mobility on silver nitrate-impregnated thin-layer chromatography (TLC) was slower. Alkaline hydrolysis of **2b** gave the free sterol **2a**.

Compound **2a** was found to be a triunsaturated C_{29} sterol having the molecular formula $C_{29}H_{46}O_2$ and a common 3β-hydroxy-Δ^5-steroid skeleton. Since the TLC on a silica gel plate did not differentiate **1a** and **2a**, the extra oxygen atom was suggested to form an ether linkage. This was confirmed by the proton nuclear magnetic resonance (^{1}H-NMR) spectra, which showed a typical pattern of a vinyl ether group[4] at δ 4.05 (1H, dd, J=1.5, 6.4 Hz), 4.35 (1H, dd, J=1.5, 13.7 Hz), and 6.30 (1H, dd, J=6.4, 13.7 Hz). The chemical shifts of the C-18, C-19, and C-21 protons were observed in the same positions as those of cholesterol, but the signals of the C-26 and C-27 protons were observed at δ 1.05, which is significantly deshielded from that of cholesterol (δ 0.86) or **1a** (δ 0.98).[5] The mass spectrum (MS) of **2a** showed ions at m/z 426 (M^+) and 383 ($M^+ - C_2H_3O$). It also showed the ion derived by the cleavage at C-20 and C-22 with loss of H_2O at m/z 283. The ion due to a $C_8H_{13}O$ fragment was observed at m/z 125 as the base peak. Since the signal of the olefinic proton of the remaining trisubstituted double bond was observed at δ 4.83 as a triplet (J=6.8 Hz), the vinyloxy group was supposed to be linked at C-24. This was confirmed by the fact that hydrolysis of **2b** with *p*-toluenesulfonic acid in acetone gave 24-ketocholesterol acetate (**3**). The structure of **2a** was thus established as

(23*E*)-24-vinyloxycholesta-5,23-dien-3β-ol or its C-23 geometric isomer, a new type of sterol in marine organisms. The occurrence of **2a** was confirmed in fresh *S. thumbergii* collected later in a nearby area. Artifactual formation of **2a** from acetaldehyde and 24-ketocholesterol by dehydration is unlikely since severe conditions were not employed in the separation process. A biogenetically possible process would be the one that involve the removal of some leaving group at C-23 of fucosterol 24,28-oxide, with simultaneous cleavage of the C-24,28 bond.



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

Fig. 1

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. ^{1}H-NMR spectra were determined on a JEOL-FX 200 spectrometer at 200 MHz in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. MS were determined on a JEOL JMS D-300 spectrometer.

Isolation of 24-Vinyloxycholesta-5,23-dien-3β-ol (2a)——Dried and pulverized *S. thumbergii* (5 kg), collected at Muroran, May 1980, was extracted with a 1:1 mixture of methanol and chloroform. The extract (310 g) was partitioned by Folch's method[6] and the lipid (150 g) obtained was subjected to chromatography on a column of silica gel (1.8 kg). Elution with a 1:1 mixture of chloroform and hexane gave16.4 g of crude sterol mixture. This was acetylated by a usual method (pyridine–acetic anhydride) and most of the sterol acetate was removed by successive crystallizations of the mixture from methanol. The mother liquor (2.2 g) contained a large amount of non-steroidal impurities which was removed by chromatography as described above. The sterol mixture (0.98 g) thus obtained was recrystallized again. The mother liquor (240 mg) was purified again by chromatography as above. The sterol components (62 mg) were subjected to chromatography on a 12.5% silver nitrate-impregnated silica gel column (50 g) and eluted with a gradient mixture of benzene and hexane. Elution with benzene–hexane (3:7) gave **2b** as a non-crystaline solid (3 mg). Hydrolysis of 2.5 mg of **2b** with 2.5% KOH–methanol gave **2a**, ^{1}H-NMR δ: 0.68 (3H, s, 18-H), 1.01 (3H, s, 19-H), 0.91 (3H, d, J=6.35 Hz, 21-H), 1.05 (6H, d, J=6.84 Hz, 26, 27-H), 3.53 (1H, m, 3α-H), 5.35 (1H, m, 6-H), 4.05 (1H, dd, J=1.5, 6.4 Hz, 29-H), 4.35 (1H, dd, J=1.5, 13.7 Hz, 29-H), 4.83 (1H, t, J=6.8, 23-H), 6.30 (1H, dd, J=6.4, 13.7 Hz, 28-H). MS *m/z*: 426.3483 (M^+, Calcd for $C_{29}H_{46}O_2$, 426.3496), 408 ($M^+ - H_2O$), 383.3268 (Calcd, 383.3313, $M^+ - C_2H_3O$), 283.2423 (Calcd, 283.2425, allylic cleavage at C-20 and C-22, with loss of H_2O), 271.2053 (Calcd, 271.2060, M^+ – side chain with 2H transfer), 125.0973 (Calcd, 125.0967, $C_8H_{13}O$, base peak).

Hydrolysis of 2b——A trace of *p*-toluenesulfonic acid was added to a solution of compound **2b** (0.5 mg) in 2 ml of acetone and the mixture was left at room temperature overnight. The mixture was evaporated at 20 °C and the residue was charged on a column (0.5 × 4 cm) of silica gel. Elution with benzene gave 24-ketocholesterol acetate, mp 120—122 °C (lit.,[7] mp 127—129 °C). MS *m/z*: 382 (M^+ – AcOH), 367 (M^+ – Me, –AcOH), 296 (M^+ – C-23 to C-27, –1H, –AcOH). ^{1}H-NMR δ: 0.67 (3H, s, 18-H), 1.01 (3H, s, 19-H), 1.08 (6H, d, J=6.8 Hz, 26, 27-H), 5.38 (1H, m, 6-H).

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