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STEROLS OF THE SIPHONOUS MARINE ALGA *CODIUM FRAGILE*

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Abstract—The marine siphonous green alga, *Codium fragile*, was shown to contain two 25-methylene sterols. These were identified as (24*S*)-24-ethylcholesta-5,25-dien-3β-ol and the previously unknown (24*S*)-24-methylcholesta-5,25-dien-3β-ol for which the trivial name codisterol is proposed.

INTRODUCTION

THE STEROL composition of algae is receiving increasing attention[1] and the mechanisms of sterol biosynthesis operative in the various classes of algae may assume some phylogenetic significance.[2] Most members of the Rhodophyceae are reported to contain C_{27} sterols with cholesterol predominating whereas in the Phaeophyceae the C_{29} compound fucosterol is the major sterol.[1] By comparison algae of the Chlorophyceae have been less extensively studied and those so far examined have contained a diverse array of sterols.[1] Thus 28-isofucosterol was isolated[3,4] from some members of the Ulotrichales while a variety of 24-methyl and 24-ethyl sterols were found[1,5] in algae from the Chloroccales. We now report the sterol composition of the marine alga *Codium fragile* (Chlorophyceae; Siphonales).[6]

RESULTS AND DISCUSSION

GLC analysis of the sterol mixture isolated from *Codium fragile* showed it to contain two components with the sterol of longer retention time constituting about 94% of the mixture. The two compounds were separated by chromatography of their acetates on silver nitrate impregnated alumina and by preparative TLC on silver nitrate–silica gel. The major component had a retention time on GLC indicating a C_{29} steryl acetate and this was confirmed by the MS which had a base peak at *m/e* 394 (M^+-acetate) and ions at *m/e* 255 (M^+-side chain-acetate) and 213 (M^+-side chain-42-acetate) which are consistent with a C_{29} Δ^5 steryl acetate containing a side chain double bond. The nuclear double bond was located in the Δ^5 position by peaks in the NMR spectrum at δ 0·66 and 1·01 for the

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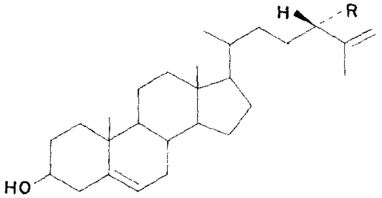
C-18 and C-19 methyl protons[7] respectively and by a multiplet at 5·36 due to the C-6 proton. The IR spectrum showed peaks at 980 and 1641 cm^{-1} for a terminal methylene group and this was confirmed by the NMR spectrum which revealed the two protons of a methylene group resonating at δ 4·64 and 4·72. A resonance at 1·56 due to three protons of a methyl group attached to an olefinic carbon was assigned to C-27 and thus located the methylene group as C-26. The presence of the 25-methylene group was substantiated by isomerization of the steryl acetate by treatment with iodine which gave 24-ethylcholesta-5,24-dien-3β-yl acetate.[8,9] A triplet at δ 0·71, 0·79, 0·86 in the NMR spectrum of the *C. fragile* steryl acetate was assigned to the three protons at C-29 of the side chain 24-ethyl group. The configuration at C-24 was determined by reduction of the 25-methylene group to give a C_{29} Δ^5 steryl acetate with a m.p. (139·5–141°) in agreement with the reported[10] m.p. (139°) of (24*S*)-24-ethylcholest-5-en-3β-yl acetate. Moreover the methyl proton region of the 100 M Hz NMR spectrum recorded at 250 Hz sweep width was similar to the spectrum[11] of (24*S*)-24-ethylcholest-5-en-3β-yl acetate but was significantly different in detail from the NMR spectrum of a sample[12] of (24*R*)-24-ethylcholest-5-en-3β-yl acetate. The major sterol of *C. fragile* was therefore identified as (24*S*)-24-ethylcholesta-5,25-dien-3β-ol (**1**). This compound, trivial name clerosterol, has previously been reported in higher vascular plants.[13–15]



(**1**) R = CH_2Me
(**2**) R = Me

The acetate of the minor sterol component isolated from *C. fragile* had a MS with ions at *m/e* 380 (M^+-acetate), 255 (M^+-side chain-acetate) and 213 (M^+-side chain-42-acetate) showing it was a diunsaturated C_{28} steryl acetate. This was again identified as a 25-methylene compound by the IR peaks at 980 and 1641 cm^{-1} and by the peaks in the NMR spectrum at δ 4·66 for the two C-26 protons and at 1·62 for the three protons of the C-27 methyl group. Peaks in the NMR spectrum at δ 0·66 and 1·02 for the C-18 and C-19 methyl protons respectively placed the nuclear double bond at the Δ^5 position. A C-24 methyl was indicated by a doublet at 0·93, 1·00 for the three C-28 protons. Reduction of the 25-methylene bond of the steryl acetate gave 24-methylcholest-5-en-3β-yl acetate which had a m.p. (145–149°) in closer agreement with the value (146–148°) reported[16] for the (24*S*)-isomer

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than the m.p. (141–142°) of the (24*R*)-epimer.[17] Also the NMR spectrum of the reduced steryl acetate was identical to the spectrum[11] of (24*S*)-24-methylcholest-5-en-3*β*-yl acetate. The minor component of the *C. fragile* sterol mixture was thus characterized as (24*S*)-24-methylcholesta-5,25-dien-3*β*-ol (**2**). We believe this to be the first report of this sterol in Nature and propose the trivial name codisterol for this compound.

The occurrence of 25-methylene sterols in a member of the Order Siphonales is interesting in view of the suggested derivation of the siphonous green algae from the Chlorococcales.[18] Studies[19,20] on the C-24 alkylation mechanisms operative in a *Trebouxia* sp. (Chlorococcales) have revealed that elaboration of the 24-methyl and 24-ethyl sterols proceeds with the production of 25-methylene sterol intermediates. The 25-methylene sterols found in *C. fragile* probably arise by a similar alkylation mechanism to that operating in the *Trebouxia* sp.[19] but they presumably accumulate due to the absence, or very low activity of, a suitable Δ^{25}-reductase.

EXPERIMENTAL

Methods were generally as described previously.[21] NMR spectra were measured at 100 M Hz in $CDCl_3$ with TMS as internal standard by the Physico Chemical Measurements Unit, Harwell, U.K.

Codium fragile (Sur) Hariot subspecies *tomentosoides* (van Goor) Silve was very kindly provided by Dr. E. M. Burrows and was collected in 1972 at Kimmeridge Bay, Dorset, U.K. The seaweed (10·23 kg wet wt) was homogenized in acetone, filtered and the residue extracted a second time with acetone. The combined acetone extracts were reduced to a small volume and partitioned with petrol. Evaporation of the light petroleum gave the total lipid (30·50 g, dark green oil) which was saponified by refluxing for 1·5 hr in 10% (w/v) KOH in 85% (v/v) aqueous EtOH. Conventional work up produced the non-saponifiable lipid (3·51 g) which was chromatographed on 200 g alumina Brockmann grade III eluting with increasing percentages of Et_2O in petrol. (b.p. 40–60°). The eluants were monitored by TLC and the sterol containing fraction (1·375 g) acetylated (pyridine–acetic anhydride). GLC on 2% OV17 showed two components comprising 6% and 94% of the mixture respectively. The steryl acetates (1·029 g) were chromatographed on a column of 50 g of 14% (w/w) $AgNO_3$ impregnated alumina which was eluted successively with 500 ml fractions of petrol. containing increasing proportions of Et_2O. GLC monitoring of the eluants showed that the major component of longest retention time, was eluted first from the column. This compound (626 mg) was identified (see below) as (24*S*)-24-ethylcholesta-5,25-dien-3*β*-yl acetate. The later fractions (104 mg) eluted from the $AgNO_3$-alumina column contained the minor C_{28} component of shorter GLC retention time accompanied by an approximately equal amount of the C_{29} steryl acetate. These compounds were separated by preparative TLC on 10% (w/w) $AgNO_3$–silica gel developed 2 × with freshly purified $CHCl_3$. Two bands were observed, the most polar was eluted (30 mg) and identified (see below) as (24*S*)-24-methylcholesta-5,25-dien-3*β*-yl acetate.

(24*S*)-24-Ethylcholesta-5-25-dien-3*β*-yl acetate: m.p. 126–126·5° (lit.[13] m.p. 124–127°). MS: *m/e* (%), 454(5), 394(100), 313(8), 255(7), 228(5), 213(6). NMR: (*δ*, ppm) 0·66 (*s*, 3H, C-18), 1·01 (*s*, 3H, C-19), 0·71, 0·79 and 0·86 (*t*, 3H, C-29). 0·86 and 0·93 (*d*, 3H, C-21), 1·56 (*s*, 3H, C-27), 2·02 (*s*, 3H, CH_3COO), 4·65 (*m*, 1H, C-3α), 4·64 and 4·72 (2H, C-26), 5·36 (*m*, 1H; C-6). IR: ν_{max} cm^{-1} 980, 1641 ($>C{=}CH_2$), 1750 (acetate). The terminal methylene group of a sample of the steryl acetate was reduced using tris-(triphenylphosphine)-rhodium chloride as catalyst[22] to give (24*S*)-24-ethylcholest-5-en-3*β*-yl acetate, m.p. 139·5–141° (lit.[10] m.p. 139°). MS: *m/e* 396 (M^+-acetate). NMR: (*δ*, ppm) 0·68 (*s*, 3H C-18), 1·02 (*s*, 3H, C-19), 0·78, 0·85 and 0·80, 0·86 (*d*'s, 6H, C-26 and C-27), 0·90 and 0·95 (*d*, 3H, C-21), 0·78, 0·85 and 0·92 (*t*, 3H, C-29) 2·02 (*s*, 3H, CH_3COO), 4·65 (*m*, 1H, C-3α), 5·35 (*m*, 1H, C-6). A sample of the (24*S*)-24-ethylcholesta-5,25-dien-3*β*-yl acetate (100 mg) was isomerized[8,9] by reflux for 6 hr. with iodine (45 mg) in benzene (50 ml). After addition of 10% (w/v) sodium thiosulphate the steryl acetates were extracted into Et_2O. GLC (2% OV17) showed two components in approximately equal proportions which were separated by column chromatography on 10 g of 14% (w/w) $AgNO_3$–alumina followed by preparative TLC on 10% (w/w) $AgNO_3$–silica gel. This gave starting material and the less polar 24-ethylcholesta-5,24-dien-3*β*-yl acetate (40 mg), m.p. 134° (lit.[8] m.p. 133–134°). MS: *m/e* 394 (M^+-acetate, 100%) 296 (M^+-acetate-98, 95%). NMR:

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(δ, ppm) 0·68 (*s*, 3H, C-18), 1·02 (*s*, 3H, C-19), 0·85, 0·93 and 1·00 (*t*, 3H, C-29), 1·62 (*s*, 6H, C-26 and C-27) 2·02 (*s*, 3H, CH_3COO), 4·63 (*m*, 1H, C-3α), 5·37 (*m*, 1H, C-6).

(24*S*)-24-Methylcholesta-5,25-dien-3β-yl acetate: m.p. 118–121°. MS: *m/e* (%) 440(3), 380(100), 313(4), 255(6), 228(13), 213(13). NMR: (δ, ppm), 0·66 (*s*, 3H, C-18), 1·02 (*s*, 3H, C-19), 0·93 and 1·00 (*d*, 3H, C-28), 0·87 and 0·95 (*d*, 3H, C-21), 1·62 (*s*, 3H, C-27), 2·02 (*s*, 3H, CH_3COO), 4·65 (*m*, 1H, C-3α), 4·66 (2H, C-26), 5·35 (*m*, 1H, C-6). IR: υ_{max} cm^{-1}, 890, 1645 ($>C{=}CH_2$), 1750 (acetate). Reduction[22] of a sample with tris-(triphenyl phosphine)-rhodium chloride catalyst gave (24*S*)-24-methylcholest-5-en-3β-yl acetate, m.p. 145–149° (lit.[16] m.p. 146–148°). MS: *m/e* 382 (M$^+$-acetate). NMR: (δ, ppm) 0·68 (*s*, 3H, C-18) 1·03 (*s*, 3H, C-19), 0·83 and 0·89 (*d*, 3H, C-26), 0·75 and 0·83 (*d*, 3H, C-27), 0·89 and 0·95 (*d*, 3H, C-21), 0·75 and 0·82 (*d*, 3H, C-28), 2·02 (*s*, 3H, 3H, CH_3COO), 4·65 (*m*, 1H, C-3α), 5·36 (*m*, 1H, C-6).

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