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IDENTIFICATION OF (24*S*)-24-METHYLCHOLESTA-5,22-DIEN-3β-OL AS THE MAJOR STEROL OF A MARINE CRYPTOPHYTE AND A MARINE PRYMNESIOPHYTE

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Key Word Index—*Cryptomonas* sp.; *Isochrysis galbana*; Cryptophyceae; Prymnesiophyceae; marine algae; sterols; (24*S*)-24-methylcholesta-5,22-dien-3β-ol.

Abstract—The major sterol in the two unicellular marine algae *Cryptomonas* sp. and *Isochrysis galbana* has been identified as (24*S*)-24-methylcholesta-5,22-dien-3β-ol (epibrassicasterol). The production of a sterol with the 24α-configuration by cryptophycean and prymnesiophycean algae and by diatoms contrasts with the situation in many other algae which produce sterols with the 24β-configuration.

A large array of sterols, many having unprecedented structures, have been identified in marine invertebrates [1–3]. Although marine invertebrates of several phyla are recognized to possess a limited capacity for sterol synthesis it seems probable that in most cases this is restricted to the production of C_{27}-sterols [2, 4], although some molluscs may perhaps have the ability to alkylate the sterol side chain [4, 5]. Since many of the sterols encountered in marine organisms have additional side chain alkyl groups, which is typical of phytosterols, it is now considered that these may be predominantly of algal origin [2–4]. The algae may be either members of the phytoplankton population or the symbiotic zooxanthellae which are found in species of several marine phyla. The sterols produced by such algae are then assimilated into various other animals via the marine food chain. With this view in mind marine algae have attracted renewed attention in order to identify the primary producers of some of the more unusual marine sterols [3, 6–8].

We now report the identity of sterols found in the cryptophycean *Cryptomonas* sp. and in the prymnesiophycean *Isochrysis galbana*. Both algae contained one major sterol (*ca* 95%) which had a GC retention time and mass spectrum (*m/z* 398 $[M]^+$) indicating it to be 24-methylcholesta-5,22-dien-3β-ol. This sterol has previously been reported to be a constituent of *I. galbana* [8, 9] but the configuration at C-24 was not determined to ascertain whether the sterol was the 24β-epimer (brassicasterol) or the 24α-epimer (epibrassicasterol, crinosterol). The sterols from both *Cryptomonas* sp. and *I. galbana* were acetylated, purified and the ^1H NMR spectra recorded in order to resolve this point. The two steryl acetates had identical spectra (Experimental) and showed the expected methyl group proton resonances for 24-methylcholesta-5,22-dien-3β-yl acetate. However, the doublet for the C-21 methyl group proton permitted the C-24 configuration to be unambiguously assigned [10–12] as 24*S* (i.e. 24α-). In the two algal steryl acetates this doublet was at δ 1.003, compared to the H-21 doublet for a sample of brassicasteryl acetate (the 24*R*-epimer, i.e. 24β-) which resonated at 1.009. (24*S*)-24-Methylcholesta-5,22-dien-3β-ol is a recognized constituent of invertebrate sterol mixtures where it co-occurs with the (24*R*)-epimer [1, 2, 11].

This is the first report of the occurrence of epibrassicasterol in the Cryptophyceae. It has previously been isolated from a diatom, *Phaeodactylum tricornutum* [13] and from *Emiliania huxleyi* [14]. The latter is in the class Prymnesiophyceae (= Haptophyceae) [15], order Isochrysidales (family Gephyrocapsaceae) as is *Isochrysis galbana* (family Isochrysidaceae). Other members of that order, in the family Hymenomonadaceae, *Hymenomonas carterae* [14] and *Hymenomonas pringsheimii* [Goad, L. J., Holz, G. G., Jr. and Beach, D. H., unpublished observation], and the order Coccosphaerales (family Zygosphaeraceae) *Crystallolithus hyalinus* [14], also contain 24-methylcholesta-5,22-dien-3β-ol but the configuration at C-24 has not been determined. In contrast, members of the order Pavlovales (family Pavlovaceae), *Pavlova lutheri* [16] and *Pavlova gyrans* [Goad, L. J., Holz, G. G., Jr. and Beach, D. H., unpublished observation] do not contain 24-methylcholesta-5,22-dien-3β-ol. Finally, 24-methylcholesta-5,22-dien-3β-ol has been found in another cryptophycean, *Chroomonas salina* [Goad, L. J., Holz, G. G., Jr. and Beach, D. H., unpublished observation].

It is now apparent that the production of epibrassicasterol may be more widespread among algal classes than had been recognized.

EXPERIMENTAL

Culture of algae. Isochrysis galbana Plymouth 1 strain and *Cryptomonas* sp. WH 1 strain were grown in DV medium [17, 18] at 15° with continuous illumination (2500 lx).

Extraction of sterols. The harvested cells were extracted with $CHCl_3$–MeOH (2:1) for 3 hr under N_2, filtered and the cell residue was re-extracted for 2 hr. The combined filtrates were reduced in vol. and resuspended in $CHCl_3$–MeOH (19:1) satd with H_2O. The non-lipid contaminants were removed by passage of the lipid extract through a Sephadex G-25 column employing the same solvent as eluent. The eluate was taken to dryness under

red. pres. and the resulting lipid resuspended in $CHCl_3$ and separated into neutral and polar fractions by CC on Si gel (10 g). Neutral lipids were eluted with $CHCl_3$. The neutral lipids were rechromatographed on a column of alumina (Brockmann, grade III) and eluted with petrol–Et_2O mixtures to yield the sterol-containing fraction, which was subjected to prep. TLC (Si gel; $CHCl_3$–EtOH, 98 : 2) to yield the sterols. After GC/MS analysis, the sterols were acetylated (pyridine–Ac_2O), and the steryl acetates purified on a column of alumina by elution with petrol–Et_2O mixtures prior to ^1H NMR analysis.

Cryptomonas *sp. sterol.* GC/MS on 3% OV-17 (270°): MS *m/z* (rel. int.): 398[M]$^+$ (50), 380 (7), 355 (5), 337 (10), 300 (28), 271 (29), 255 (50), 213 (15), 69 (100). ^1H NMR spectrum ($CDCl_3$, 220 MHz) of the purified (24*S*)-24-methylcholesta-5,22-dien-3β-yl acetate: δ 0.690 (*s*, H-18), 0.815 (*d*, J = 6.8 Hz, H-26), 0.832 (*d*, J = 6.8 Hz, H-27), 0.906 (*d*, J = 6.8 Hz, H-28), 1.003 (*d*, J = 6.5 Hz, H-21), 1.016 (*s*, H-19), 2.031 (*s*, acetate), 4.5–4.7 (*m*, H-3α), 5.16–5.19 (*m*, H-22 and H-23), 5.38 (*m*, H-6).

Isochrysis galbana *sterol.* MS *m/z* (rel. int.): 398[M]$^+$ (24), 383 (4), 300 (5), 365 (4), 337 (7), 300 (16), 271 (19), 255 (37), 229 (6), 213 (12), 69 (100). Purified (24*S*)-24-methylcholesta-5,22-dien-3β-yl acetate: ^1H NMR spectrum identical to that of steryl acetate isolated from *Cryptomonas* sp.

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