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OCCURRENCE OF (24*S*)-24-METHYLCHOLESTA-5, 22*E*-DIEN-3β-OL IN THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

IAN RUBINSTEIN* and L. JOHN GOAD

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

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Key Word Index—*Phaeodactylum tricornutum*; Bacillariophyceae; Chrysophyta; diatom; sterol.

Abstract—The principal sterol of the marine diatom *Phaeodactylum tricornutum* was identified as (24*S*)-24-methylcholesta-5,22*E*-dien-3β-ol. Two deuterium atoms were incorporated into this sterol when the diatom was cultured in the presence of [CD_3]methionine indicating a 24-methylene intermediate.

INTRODUCTION

THE STEROLS of many marine algae of the Divisions Rhodophyta, Phaeophyta and Chlorophyta had been identified,[1] but by contrast the diatoms have received less attention. An early study[2] indicated that *Nitzschia closterium* contained fucosterol (24*E*-ethylidenecholest-5-en-3β-ol) while *Navicula pelliculose* was reported[3] to possibly contain chondrillasterol [(24*R*)-24-ethyl-5α-cholesta-7,22*E*-dien-3β-ol]. More recently the diatoms *Cyclotella nana* and *Nitzschia closterium* have been investigated[4] and the sterol in both species reported as brassicasterol [(24*R*)-24-methylcholesta-5,22*E*-dien-3β-ol]. As part of an investigation of the sterols of marine invertebrates[5] and their origin through the marine food chain we have now examined the sterol content of the diatom *Phaëodactylum tricornutum* (Chrysophyta; Bacillariophyceae). This diatom was formally known[6] as *Nitzschia closterium* forma *minutissima* (Allen and Nelson).

RESULTS AND DISCUSSION

The sterol obtained from *P. tricornutum* was shown by GLC to contain one principal component with a retention time corresponding to brassicasterol. The IR, MS and NMR spectral data were consistent with the identification of the sterol as 24-methylcholesta-5,22*E*-dien-3β-ol. To determine the configuration at C-24 the 100 M Hz spectrum of the diatom steryl acetate was recorded at a sweep width of 250 Hz and compared with the NMR spectra obtained under the same conditions for samples of (24*R*)-24-methylcholesta-5,22*E*-dien-3β-yl acetate (brassicasteryl acetate) and (24*S*)-24-methylcholesta-5,22*E*-dien-3β-yl acetate. This latter compound was isolated from the sterol mixture extracted from a

* Present address: Institut de Chimié, Universite Louis Pasteur, Strasbourg, France.

[1] PATTERSON, G. W. (1971) *Lipids* **6**, 120.
[2] HEILBRON, I. M. (1942) *J. Chem. Soc.* 79.
[3] LOW, E. M. (1955) *J. Mar. Res.* **14**, 199.
[4] KANAZAWA, A., YOSHIOKA, M. and TESHIMA, S. (1971) *Bull. Jap. Soc. Sci. Fish.* **37**, 899.
[5] GOAD, L. J., RUBINSTEIN, I. and SMITH, A. G. (1972) *Proc. Roy. Soc. Lond.* **180B**, 223.
[6] LEWIN, J. C., LEWIN, R. A. and PHILPOTT, D. E. (1958) *J. Gen. Microbiol.* **18**, 418.

brittle star (*Ophicomina nigra*), and shown to have the (24*S*)-configuration by ozonolysis to give the side chain fragment which was isolated as the *p*-phenyl phenacyl ester. This ester had the opposite ORD curve to the corresponding side chain derivative prepared from the (24*R*)-compound,ergosta-5,7,22*E*-trien-3β-ol.[7] The spectra of the diatom steryl acetate and (24*S*)-24-methylcholesta-5,22*E*-dien-3β-yl acetate were very similar but by comparison, in the spectrum of brassicasteryl acetate there was a significant downfield shift of the doublet for the C-21 methyl protons and also to a lesser extent of the C-28 methyl doublet (Fig. 1). A similar comparative difference in the positions of the C-21

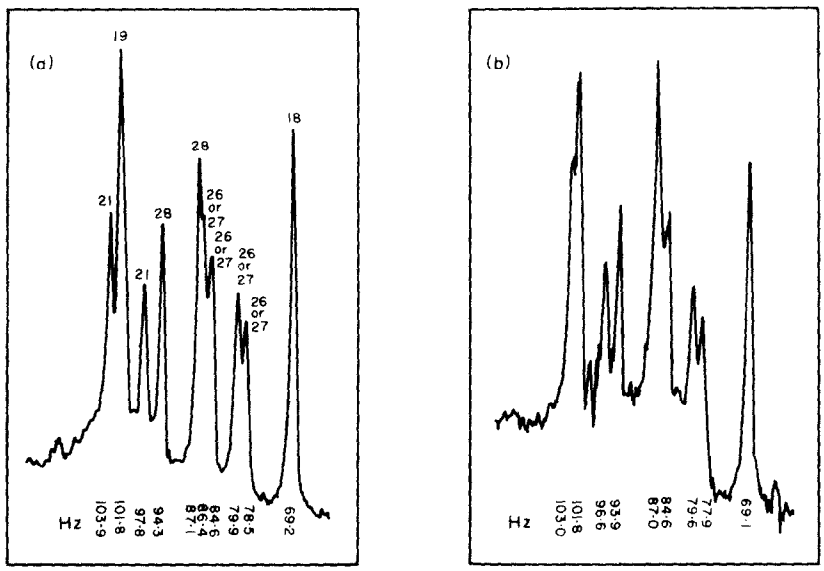

FIG. 1. NMR SPECTRA OF (a) (24*R*)-24-METHYLCHOLESTA-5,22-DIEN-3β-YL ACETATE, AND (b) THE STERYL ACETATE OBTAINED FROM *Phaeodactylum tricornutum*.

methyl protons doublets is observed[7,8] in the NMR spectra of the C-24 epimers (24*S*)-24-ethylcholesta-5,22*E*-dien-3β-ol (stigmasterol) and (24*R*)-24-ethylcholesta-5,22*E*-dien-3β-ol (poriferasterol). It is therefore concluded that the diatom sterol is most probably (24*S*)-24-methylcholesta-5,22*E*-dien-3β-ol. It is noteable that the m.p. of both the free sterol (147–148°) and the steryl acetate (157–158°) were in good agreement with the literature[9] values for brassicasterol (148°) and its acetate (158°) and were also identical to the m.p.s given for the sterol and its acetate obtained previously from *C. nana* and *N. closterium*.[4] Sterols previously isolated from a crinoid (crinosterol)[10] and a mollusc (pincsterol)[11] were suggested to be (24*S*)-24-methylcholesta-5,22*E*-dien-3β-ol, while we have identified this compound in an ophiuroid.[7] It now seems probable that these marine invertebrates derive this compound via the food chain and the present identification of this sterol in a diatom suggests that these algae are possibly the primary source.

[7] RUBINSTEIN, I. and GOAD, L. J. unpublished results.
[8] THOMPSON, M. J., DUTKY, S. R., PATTERSON, G. W. and GOODEN, E. L. (1972) *Phytochemistry* **11**, 1781.
[9] FERNHOLTZ, E. and STAVELY, H. E. (1940) *J. Am. Chem. Soc.* **62**, 1875.
[10] BOLKER, H. I. (1967) *Nature* **213**, 905.
[11] ASHIKAGA, C. (1957) *Nippon Nogei Kagaku Kaishi* **31**, 115.

To obtain information on the mode of biosynthesis of the diatom sterol, *P. tricornutum* was cultured in the presence of [CD_3]methionine. MS of the purified steryl acetate showed that the relative abundance of the ion at *m/e* 382 (M^+ + 2-acetate) increased from 5% for the control unlabelled steryl acetate to 19% for the steryl acetate obtained from the alga grown with [CD_3]methionine. The incorporation of two deuterium atoms indicates that a 24-methylene intermediate is produced in the C-24 alkylation mechanism.[12,13] It is interesting that the subsequent reduction of the 24-methylene compound in *P. tricornutum* appears to lead to a sterol with opposite C-24 configuration to that found[14,15] in the sterols of *Ochromonas* species (Chrysophyta; Chrysophyceae). An extension of this type of study to other members of the Bacillariophyceae and Chrysophyceae may provide useful phylogenetic information.[15]

EXPERIMENTAL

Methods were generally as described previously.[16] *Phaeodactylum tricornutum* (Bohlin) was grown in Erdschreiber medium[17] at ambient temp. under continual 'Gro-lux' illumination and a stream of 5% (v/v) CO_2 in air. The cells (25 g fr. wt) from 16 l. of medium were harvested after 14 days by centrifugation, broken in a Braun homogenizer and the non-saponifiable lipid extracted in the usual manner. The sterol (m.p. 147–148°) was obtained by preparative TLC [silica gel developed with 2% (v/v) EtOH:$CHCl_3$], acetylated and further purified by TLC [10% (w/w) $AgNO_3$:silica gel developed with pure $CHCl_3$] to give the steryl acetate (5·5 mg) which was homogeneous by GLC. m.p. 157–158°. IR: ν_{max} cm^{-1}, 1720, 970, 955, 795. MS: *m/e* (%), 380(100), 365(4), 313(2), 282(2), 255(19), 228(2), 213(13). *NMR*: (100 *MHz*) (δ), 5·37 (*m*, 1H, C-6), 5·15 (*m*, 2H, C-22, C-23), 4·45 (*m*, 1H, C-3α), 2·01 (*s*, 3H, $CH_3CO_2^-$), 1·02 (*s*, 3H, C-19), 1·03 and 0·97 (*d*, 3H, C-21), 0·94 and 0·87 (*d*, 3H, C-28), 0·87, 0·80 and 0·85, 0·78 (*d*'s, 6H, C-26 and C-27), 0·69 (*s*, 3H, C-18).

Incorporation of [CD_3]*methionine. P. tricornutum* was cultured in 200 ml batches of Erdschreiber medium containing 50 mg of [CD_3]methionine. The sterol was isolated and purified as the acetate as described above and then examined by MS.

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[12] JUAREGUIBERRY, G., LAW, J. A., MCCLOSKY, J. A. and LEDERER, E. (1965) *Biochemistry* **4**, 347.
[13] GOAD, L. J. and GOODWIN, T. W. (1972) *Prog. Phytochem.* **3**, 113.
[14] GERSHENGORN, M. C., SMITH, A. R. H., GOULSTON, G., GOAD, L. J., GOODWIN, T. W. and HAINES, T. H. (1968) *Biochemistry* **7**, 1698.
[15] GOAD, L. J., LENTON, J. R., KNAPP, F. F. and GOODWIN, T. W. (1974) *Lipids* in press.
[16] LENTON, J. R., GOAD, L. J. and GOODWIN, T. W. (1973) *Phytochemistry* **12**, 1135.
[17] FØYN, B. (1934) *Arch. Protist.* **83**, 154.