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STEROLS OF *NITELLA FLEXILIS* AND *CHARA VULGARIS**

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Key Word Index—*Nitella flexilis*; *Chara vulgaris*; Charophyta; clionasterol; 28-isofucosterol.

Abstract—The principal sterols of *Nitella flexilis* were identified as clionasterol and 28-isofucosterol—58 and 36%, respectively, of the total sterol. Clionasterol and 28-isofucosterol were also the principal sterols of *Chara vulgaris*, making up 39 and 54% of the total sterol, respectively. Small quantities of cholesterol and 24-methylenecholesterol were also detected in both algae.

INTRODUCTION

BEFORE the advent of GLC, TLC and MS, there was much confusion about the sterol composition of plants, especially the algae.[1] In the last few years, however, much additional work with the red algae has been accomplished.[2] It now appears that, with few exceptions, cholesterol is the primary sterol of red algae and that fucosterol is the primary sterol of brown algae. The situation with green algae is still very confused. The early literature[3] indicated that larger green algae contain sitosterol as do higher plants. However, more recently these algae have been discovered to contain as the primary sterol, 28-isofucosterol,[4,5] or in one case, cholesterol.[6] Sitosterol has not been conclusively demonstrated in any green algae although its 24β isomer, clionasterol, has been isolated from *Chlorella ellipsoidea*.[7]

Sitosterol and fucosterol were reported in *Nitella opaca*[3]—an alga placed in the Chlorophyta by some phycologists and in a separate division, the Charophyta, by others.[8] In view of the difficulties in classifying these algae, and the interest in algal sterols with regard to dietary requirements of aquatic animals, *Nitella flexilis* and *Chara vulgaris* were collected and their sterols analyzed.

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RESULTS AND DISCUSSION

Sterols made up 0·09 and 0·06% of the dry weight of *Nitella flexilis* and *Chara vulgaris*, respectively. Two principal sterols were isolated from both species. M.ps of these sterols and their acetates, as well as the optical rotations of the sterols and acetates, are presented in Table 1. These data indicate that the two major sterols of both algae are clionasterol (24β-ethylcholesterol) and 28-isofucosterol (24-*Z*-ethylidenecholesterol). Both sterols give a slow Liebermann–Burchard reaction and have optical rotations typical of Δ^5-sterols. The m.ps of the isolated 28-isofucosterol are significantly higher than those of fucosterol. Clionasterol can also be distinguished from sitosterol by the higher m.p. of its acetate compared to that of sitosterol.

TABLE 1. A COMPARISON OF SOME PHYSICAL CHARACTERISTICS OF *Chara* AND *Nitella* STEROLS WITH KNOWN STEROLS

Sterol	m.p. (Sterol)	m.p. (Sterol acetate)	α_D^{23} (Sterol)	α_D^{23} (Sterol acetate)
(*Chara vulgaris* sterols)				
Clionasterol	137–140	137–139	−37	−45
28-Isofucosterol	133–136	131–132·5	−41	−43
(*Nitella flexilis* sterols)				
Clionasterol	143–144	137–139·5	−43	−46
28-Isofucosterol	135–137	132–134	−46	−47
(Known sterols)				
Sitosterol[10]	137·5–138	131–122	−33	−37
Clionasterol[7]	143–144	139–141	—	—
28-Isofucosterol[11]	137	134	−38	−40
Fucosterol[12]	124	118	−40	−45

GLC of the fractions collected from column chromatography in four systems[9] confirmed the presence of clionasterol and 28-isofucosterol. Although clionasterol is indistinguishable from sitosterol on GLC, 28-isofucosterol is easily distinguished from fucosterol. The sterols of *Chara* and *Nitella* are similar except that the most abundant sterol of *Chara* is 28-isofucosterol and that of *Nitella* is clionasterol. Small amounts of cholesterol and 24-methylenecholesterol were identified in both species by GLC. Traces of sterols with retention times corresponding to 24-methylcholesterol (presumably Δ^5-ergostenol) and brassicasterol were also observed from *Chara*. *Nitella* contained traces of a sterol with retention times roughly corresponding to those of $\Delta^{5,25}$-stigmastadienol.

The primary sterols of the Charophyta appear to be clionasterol and 28-isofucosterol rather than sitosterol and fucosterol as reported for *Nitella opaca*.[3] Clionasterol and 28-isofucosterol have been identified in members of the Chlorophyta, but they have not been previously identified together in the same organisms. Sitosterol and 28-isofucosterol were both found in the pine, and 28-isofucosterol was reported to be a biosynthetic pre-

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cursor of sitosterol.[13] It appears that the enzyme systems of algae, which always seem to produce sterols with the 24β configuration, convert 28-isofucosterol to clionasterol, rather than sitosterol, as occurs in higher plants.[13]

EXPERIMENTAL

Nitella flexilis (L.) Ag. was collected from a fresh-water stream near Roanoke, Va. in April 1971 and *Chara vulgaris* L. was collected in September 1971 in a fresh-water stream near Frederick, MD. The algal material was washed free of contaminating matter, freeze dried, and extracted with $CHCl_3$–MeOH (2:1). Sterols were isolated by saponification of the lipid, recovery of the non-saponifiable fraction, and precipitation of the sterols as described by Doyle *et al.*[14] GLC of the free sterols on alumina[15] failed to show the presence of any sterols containing 4,4-dimethyl or 4-methyl groups. After acetylation, the sterol acetate mixture was chromatographed on a silica gel–$AgNO_3$ column,[15] which was monitored by GLC. Purified compounds were recrystallized from MeOH and m.ps were obtained on a calibrated Fisher–Johns apparatus. Optical rotations were taken with $CHCl_3$ as solvent at 23°. Quantitative sterol analyses were made by measurement of peak areas on GLC. The GLC systems used have recently been described.[9]

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