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Glenn W. Patterson, Kyle C. McKenna, William R. Lusby, and Mary A. Bisson

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STEROLS OF THE CHAROPHYCEAE

GLENN W. PATTERSON,* KYLE C. MCKENNA,

Department of Botany, University of Maryland, College Park, Maryland 20742

WILLIAM R. LUSBY,

Insect Hormone Laboratory, USDA, Beltsville, Maryland 20705

and MARY A. BISSON

Department of Biological Sciences, State University of New York, Buffalo, New York 14260

ABSTRACT.—Sterols were identified from two species of *Chara* and from one species each from the genera *Nitella, Nitellopsis*, and *Lamprothamnium*. The principal sterol in each species examined was either 24-ethylcholesterol or 28-isofucosterol. Cholesterol was present in small quantities in each alga. Four of the six genera of the Characeae have now been examined for sterol composition. 24-Ethylcholesterol or a mixture of 24-ethyl-cholesterol and 28-isofucosterol constitutes the majority of the sterol in each species examined to date.

The algal class Charophyceae has similarities with several orders of green algae as well as with the bryophytes and higher plants. Taxonomic treatments of the class vary. Parker (1) combines the classes Chlorophyceae, Prasinophyceae, and Charophyceae to form the Division Chlorophycota. Margulis and Schwartz (2) consider the Charales as one of eight orders in the Chlorophyta. The Charophyta have numerous structural and developmental characteristics in common with other green algae. They also have numerous biochemical characteristics in common, such as the presence of chlorophylls a and b, starch as a food reserve, and cell walls of cellulose or xylose or mannose polymers (1). Sterol composition has also been shown to be related to taxonomic affinities, especially in the algae (3). This is best demonstrated in the green algae where the generally more primitive orders such as Volvocales and Chlorococcales contain a wide array of sterols, generally with 28 and 29 carbon atoms and frequently with a Δ^{22} double bond (3), while in the structurally more complex orders such as Ulvales, Caulerpales, and Cladophorales, sterols such as clionasterol and those with a C-24 (28) double bond are dominant. Only three species in the Charophyceae have been examined for sterol composition. In two very early reports, Heilbron (4,5) reported that fucosterol and sitosterol were the principal sterols of *Nitella opaca*. More recently, clionasterol and 28-isofucosterol were found to be the principal sterols of *Nitella flexilis* and *Chara vulgaris*, respectively (6). In contrast, bryophytes (7), pteridophytes (8), and in higher plants, 24α-methyl- and 24α-ethylsterols such as campesterol and sitosterol are the principal components, while 28-isofucosterol is present only in small amounts (9).

Further data on the sterols of Charophyceae would be helpful in attempts to relate the biochemistry and taxonomy of green algae and land plants. This paper reports on the sterol composition of four genera and five species in the Charophyceae.

EXPERIMENTAL

Chara australis R. Br. was originally collected from a fresh-water river near Sydney, New South Wales, Australia, by Vernon Proctor, and has been maintained in culture for many years. *Chara buckellii* G.O.A. was collected from an $MgSO_4$ lake near Saskatchewan, Canada. *Lamprothamnium papulosum* (Wallr.) J. Gr. was collected from brackish water lakes in northern New South Wales, Australia. *Nitella translucens* (Pers.) Ag. was cultured in topsoil in freshwater media supplemented with micronutrients (10). *L. papulosum* was cultured in topsoil in ⅓ strength artificial seawater ("Instant Ocean") supplemented

with micronutrients. *Nitellopsis obtusa* (Desvaux) J. Groves was provided by Dr. Maki Katsuhara of Tokyo University (11). Lipid was extracted in a Soxhlet apparatus overnight with $CHCl_3$-MeOH (2:1). The solvent was evaporated under N_2, the lipid saponified, and the non-saponifiable fraction extracted with Et_2O, followed by alumina chromatography (Grade II). The sample was dissolved in hexane and eluted with 3 column volumes of hexane, hexane-C_6H_6 (1:1), C_6H_6, Et_2O, and MeOH. The Et_2O fraction containing the sterols was analyzed on capillary gc using a 30 m × 0.25 mm i.d. SPB-1 (Supelco) fused silica column in a Varian Model 3700 gas chromatograph. Gc-ms was performed on a Finnigan-MAT Model 4512 gas chromatograph-mass spectrometer equipped with a 30 m × 0.32 mm i.d. fused silica capillary column with a 0.25 μm film of DB-1 (J&W Scientific). NH_3 (0.6 Torr) and CH_4 (0.3 Torr) at 60° were used for chemical ionization.

RESULTS AND DISCUSSION

Samples available for extraction were small, ranging from 2 to 15 g of fresh tissue. Sterols were analyzed by capillary gc of the sterol fraction eluted from the alumina column. The samples analyzed showed evidence of the presence of several fatty alcohols which eluted with the sterols from alumina. To establish the structure of the sterols and to confirm the non-steroidal nature of other peaks seen on capillary gc, gc-ms was employed. A major compound from a *Cephaleuros* sp. (unpublished) and most of these algae had an RRT (relative to cholesterol) of 1.04 on gc. Eims indicated that it was a long chain, *n*-fatty alcohol. Cims using NH_3 and CH_4 demonstrated the mol wt to be 410. The compound thus is *n*-octacosanol. From *Ch. australis*, *Na. translucens*, and *L. papulosum*, the principal sterol was 24-ethylcholesterol with a gc-RRT (relative to cholesterol) identical with that of authentic 24-ethylcholesterol (1.53) and a mass spectrum identical to that of authentic 24-ethylcholesterol. In plants there are two isomers of 24-ethylcholesterol at C-24; clionasterol which is 24β-ethyl and sitosterol which is 24α-ethyl. In the algae there is only one report (in *Hydrodictyon reticulatum*) where 24-ethylcholesterol was shown to be sitosterol (12). In higher plants, 24-ethylcholesterol is nearly always sitosterol (9).

In previous work (6) with *Ch. vulgaris* and *Na. flexilis* the 24-ethylcholesterol isolated was of sufficient quantity that it was purified and melting points and optical rotations were determined. These determinations showed that 24-ethylcholesterol was clionasterol. Later work with nmr of the *Nitella* sample of 24-ethylcholesterol (13) showed it to be 85% clionasterol and 15% sitosterol. All the data suggest that 24-ethylcholesterol of these samples is clionasterol, but definitive evidence could not be obtained with our samples. From *Ch. buck-*

TABLE 1. Sterols of Charophyceae.

Species	Sterol[a]					
	CHOL	24MEC	24EC5,22	24EC8,24	24EC	ISOFUCO
Chara buckelii	2	1	1	3	33	6
Chara australis	4	2	1	8	84	t[c]
Chara vulgaris[b]	t	t	—	—	39	54
Nitella translucens	17	—	—	—	83	—
Nitella flexilis[b]	t	5	—	t	58	36
Nitellopsis obtusa	25	—	—	—	35	40
Lamprothamnium papulosum	12	—	10	17	60	—

[a]As % of total sterols; CHOL = cholesterol; 24MEC = 24-methylenecholesterol; 24EC5,22 = 24-ethylcholesta-5,22-dienol; 24EC = 24-ethylcholesterol; ISOFUCO = 28-isofucosterol; 24EC8,24 = 24-ethylcholesta-8(14),24(28)*Z*-dienol. All sterols have a 3β hydroxyl.

[b]Data for this species are from Patterson (6).

[c]t = Trace.

elii and *Ns. obtusa*, 28-isofucosterol was determined to be the principal sterol by comparing its gc characteristics (RRT 1.57) with those of authentic compounds (RRT fucosterol, 1.53; 28-isofucosterol, 1.57). Gc-ms gave a spectrum identical to that of authentic 28-isofucosterol with a characteristic molecular ion at *m/z* 412 and a base peak at *m/z* 314. In *Ch. buckelii* and *Ns. obtusa*, 24-ethylcholesterol was an accompanying sterol in substantial amounts, although 28-isofucosterol was not an important component of species where 24-ethylcholesterol was the principal sterol. In previous work from this laboratory, clionasterol and 28-isofucosterol were both found as major components in each species studied (Table 1). Small amounts of cholesterol, 24-methylenecholesterol, 24-ethylcholesta-5,22-dienol, and a sterol tentatively identified as 24-ethylcholesta-8(14),24(28)*Z*-dienol were found in some species. The dominant sterols of each Charophyceae species examined have been either 28-isofucosterol or 24-ethylcholesterol. From the literature it appears that 28-isofucosterol occurs in substantial amounts only in the Chlorophyte orders Ulotrichales and Ulvales (14–21). In these orders none of the species examined are reported to have more than traces of clionasterol. Higher plants contain traces of 28-isofucosterol (13), but their most common sterol is 24-ethylcholesterol (sitosterol).

The Charophyceae is not a large class, consisting of a single order, Charales, which consists of a single family, Characeae. The Characeae consists of six genera: *Nitella* and *Tolypella* in the tribe Nitelleae and *Nitellopsis, Lamprothamnium, Lychnothamnus*, and *Chara* in the tribe Chareae (1). In this work, we report the occurrence of 28-isofucosterol or 24-ethylcholesterol or both in each species examined. This composition appears to be a unique characteristic of the Charophyceae.

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