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STEROL COMPOSITION OF SOME MEDITERRANEAN GREEN ALGAE

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Key Word Index—Chlorophyceae; Siphonales; Siphonocladales; Ulotrichales; Cladophorales; chemotaxonomy; green algae; sterols.

Abstract—The distribution of sterols in ten Mediterranean green algae has been examined. Clionasterol is typical for the Siphonocladales and all the species of the Siphonales not belonging to the genus *Codium*, which is characterized by clerosterol. 28-Isofucosterol has been confirmed to be characteristic of the Ulotrichales.

INTRODUCTION

In 1976 Goad [1] reviewed the most significative works dealing with the sterol distribution in marine green algae. Notwithstanding the relatively few data existing at that time in the literature it became apparent that the sterol profiles of Chlorophyceae could be of taxonomic interest. Nevertheless the exiguous number of algae which had been examined at that time did not allow meaningful correlations among some species belonging to the same order. In fact, while the four species of the Ulotrichales examined were consistent in containing isofucosterol (**6**) as the main component, the only two species of the Siphonales reported showed a very contrasting sterol composition, since in *Codium fragile* the principal component (95%) is the rare clerosterol (**7**), and in *Halimeda incrassata* clionasterol (**8**) predominates. Recently [2] we reported the sterol profiles of eight Mediterranean Chlorophyceae; our results confirmed that isofucosterol is representative of the Ulotrichales while the only species of the Cladophorales examined, in agreement with the previous data, was shown to contain a high proportion of cholesterol (**1**). As for the Siphonales we found that clerosterol is the dominant sterol in *C. tomentosum*, *C. aderens* and *C. bursa*, while it is completely absent in *H. tuna*, which like *H. incrassata*, is characterized by a high content of clionasterol.

A more extensive survey of the sterols of algae belonging to the division Chlorophyta seemed warranted to enable chemotaxonomic correlations at the level of lower systematic categories to be made.

In pursuing our taxonomically oriented work on Mediterranean green algae [3–5] we examined the sterol fraction of ten Chlorophyceae and the results are reported in this paper.

RESULTS AND DISCUSSION

The unsaponifiable fraction from the chloroform extract of each alga was chromatographed on a Si gel column and the sterol fraction was acetylated and analysed by GC/MS. The sterol acetates were fractionated by chromatography on Si gel impregnated with silver nitrate. The various column fractions were monitored by GC and combined accordingly.

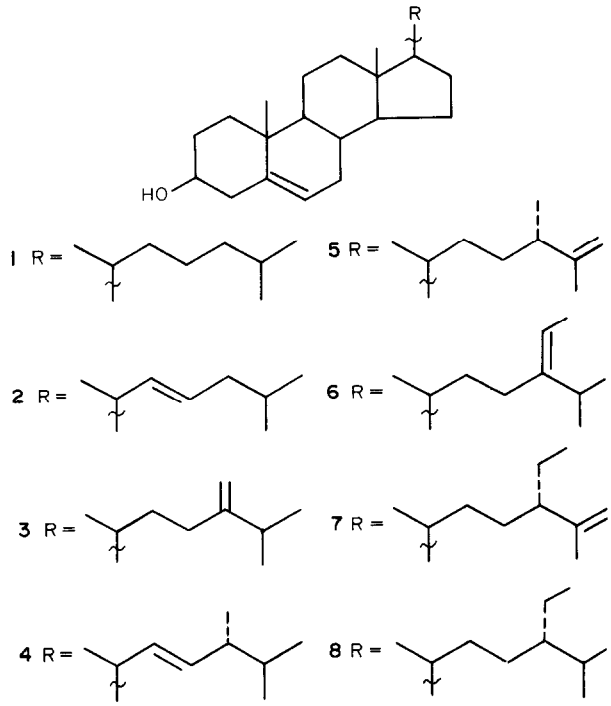

Fig. 1. Sterol composition of some Mediterranean green algae.

The structures of sterols isolated as pure acetates were established by comparison of their physicochemical properties (^{1}H NMR, IR, MS, mp and $[\alpha]_D$)

Table 1. Sterol composition of some Mediterranean green algae

Order	Species	Sterol (mg/kg dry alga)*							
		1†	2	3	4	5	6	7	8
Ulotrichales	*Ulva lactuca*	46	*t*	46	22	—	465	—	—
Cladophorales	*Cladophora laetevirens*	112	—	140	107	—	—	—	94
	Chaetomorpha aurea	179	*t*	195	61	—	—	—	161
Siphonales	*Caulerpa prolifera*	45	—	25	*t*	—	—	—	413
	Bryopsis plumosa	64	*t*	24	18	—	—	—	491
	B. muscosa	45	*t*	25	18	—	—	—	530
	Udotea petiolata	48	*t*	32	19	—	40	—	630
	Codium vermilara	t	—	*t*	—	*t*	—	419	134
Siphonocladales	*Dasycladus vermicularis*	62	*t*	23	14	—	—	—	348
	Acetabularia mediterranea	65	—	35	10	—	—	—	350

*—Not detectable; t, trace amounts.

†**1**, Cholesterol; **2**, 22-dehydrocholesterol; **3**, 24-methylenecholesterol; **4**, brassicasterol; **5**, codisterol; **6**, 28-isofucosterol; **7**, clerosterol; **8**, clionasterol.

with those of an authentic sample. The identification of the other steryl acetates was based upon their GC retention time and comparison of their GC/MS spectra with those of an authentic specimen. The configuration at C-24 of the steryl acetates identified only by GC/MS was only tentatively assigned as *S*, in view the preponderance of 24*S*-alkyl sterols in the green algae [6]. Our results, listed in Table 1, fully confirm the taxonomic significance of sterol distribution in Chlorophyceae.

With another five species of the order Siphonales examined, it is now apparent that clerosterol is representative of the genus *Codium*, the other species being characterized by the presence of clionasterol as the dominant sterol. It is to be noted that clionasterol is also characteristic of the Siphonocladales, as shown by the present data and those previously reported [2].

The examination of the sterol profiles of *Cladophora laetevirens* and *Chaetomorpha aurea* again indicates the lack of a representative sterol for the order Cladophorales. The only significant feature is a high proportion of cholesterol which can be explained by a relative inefficiency of the transmethylation reaction in sterol biosynthesis of the algae belonging to this order.

Finally, the re-examination of *Ulva lactuca* showed that 28-isofucosterol is not a unique sterol as previously reported, but certainly it is the dominant one, thus further confirming that this sterol is indeed characteristic of the Ulotrichales. The presence of isofucosterol and other 24-ethylidene sterols with the *Z*-configuration in many vascular plants is a fact of some interest in view of the possible ancestral role of the Ulotrichales in higher plant evolution [1].

EXPERIMENTAL

Plant material. Algae listed in Table 1 were collected from the littoral zone of the Tyrrenian coast of Italy from Salerno to Napoli between April and July 1981.

Isolation and identification of sterols. A freshly collected sample of each alga (usually *ca* 500 g) was freeze-dried and extracted with $CHCl_3$ (3 × 600 ml) at room temp. Combined extracts were evaporated to dryness and the residue was refluxed with 10% KOH in 80% EtOH (50 ml). After extraction with Et_2O the organic phase, taken to dryness, was chromatographed on a Si gel column using C_6H_6–Et_2O (4:1) as eluent. The sterol fraction was collected and, after acetylation with Ac_2O–C_5H_5N (1:1) for 12 hr at room temp., fractionated on a $AgNO_3$–Si gel (1:2) column (eluent: petrol–C_6H_6, 7:3).

GC/MS analysis (1.5 m × 5 mm glass column packed with 3% SE 30; flow of N_2: 30 ml/min) allowed the identification of individual steryl acetates. When a particular fraction was shown to be a single compound, identification was confirmed by comparison of its physical properties (mp; $[\alpha]_D$; IR; MS; ^1H NMR) with those of an authentic sample. Quantitation was performed by GC of steryl acetates (cholestane as int. standard) using integrated areas of peaks.

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