The Distribution of Sterols in Algae[1,2]

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ABSTRACT

Available analytical techniques are now sufficient for the separation and identification of sterols from complex mixtures in plants. Gas and thin layer chromatography and mass spectroscopy in particular, have been used to resolve some of the confusion concerning the sterol composition of algae. Red algae (Rhodophyta) contain primarily cholesterol, although several species contain large amounts of desmosterol, and one species contains primarily 22-dehydrocholesterol. Only a few Rhodophyta contain traces of C-28 and C-29 sterols. Fucosterol is the dominant sterol of brown algae (Phaeophyta), apparently the major sterol of every species examined. Most Phaeophyta also contain traces of cholesterol and biosynthetic precursors of fucosterol. The sterols of green algae (Chlorophyta) are much more varied and complex than those of other groups of algae. Whereas the Phaeophyta and Rhodophyta contain one primary sterol, many of the Chlorophyta contain a complex mixture of sterols such as occurs in higher plants. The Chlorophyta contain such sterols as chondrillasterol, poriferasterol, 28-isofucosterol, ergosterol, cholesterol and others. Sterol composition may be of value in the systematics of plants such as the Chlorophyta. Recently (for the first time) complex mixtures of sterols have been isolated in very small amounts in the blue-green algae (Cyanophyta). Available data on the sterols of other groups of algae are insufficient for making useful comparisons.

INTRODUCTION

Since their recent isolation from bacteria (1,2) and blue-green algae (3,4), sterols have

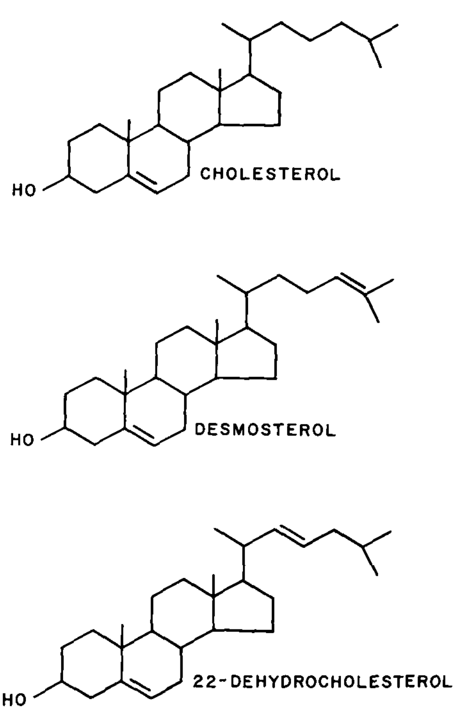

FIG. 1. Sterols of red algae.

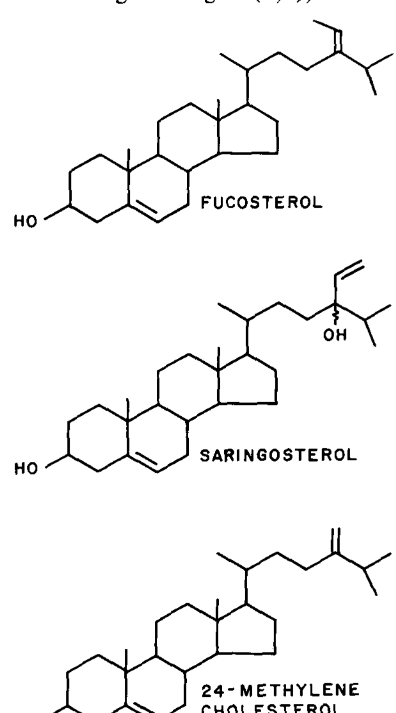

FIG. 2. Sterols of brown algae.

[1]One of 12 papers to be published from the "Sterol Symposium" presented at the AOCS Meeting, New Orleans, April 1970.

[2]Scientific Article No. A2606, Contribution No. 4331 of the Maryland Agricultural Experiment Station.

TABLE I

Sterols of Red Algae (Rhodophyta)

Order	Species	Major sterols[a]	References
Porphyridiales	*Porphridium cruentum*	none	12
Bangiales	*Porphyra purpurea*	desmo	13
Gelidiales	*Acanthopeltis japonica*	chol	32
	Gelidium amansii	chol	32
	G. japonicum	chol	33
	G. subcostatum	chol	33
	Pterocladia tenuis	chol	33
Cryptonemiales	*Dilsea earnosa*	chol	13
	Corallina officinalis	chol	13
	Gloiopeltis fureata	chol	34
	Tichocarpus crinitus	chol	34
	Grateloupia elliptica	chol	34
	Cyrtymenia sparsa	chol	34
	Polyides caprinus	chol	13
	P. rotundus	chol	11
Gigartinales	*Gracilaria verrucosa*	chol	b
	Plocamium vulgare	chol	13
	Furcellaria fastigiata	chol	13
	Hypnea japonica	22-DC	14
	Ahnfeltia stellata	chol	13
	Chondrus crispus	chol	15,35
	C. giganteus	chol	34
	C. ocellatus	chol	34
	Gigartina stellata	chol	13
	Iridophycus cornucopiae	chol	34
	Rhodoglossum pulchrum	chol	33
Rhodymeniales	*Halosaccion ramentaceum*	chol, desmo	11
	Rhodymenia palmata	desmo, chol	11, 13, 15
	Coeloseira pacifica	chol	34
Ceramiales	*Ceramium rubrum*	chol	b
	Chondria dasyphylla	chol	b
	Laurencia pinnatifida	chol	13
	Polysiphonia nigrescens	chol	13
	P. lanosa (fastigata)	chol	13
	P. subtillisima	chol	b
	Rhodomelia conferoides	chol	11,15
	R. larix	chol	34
	Dasya pedicellata	chol	b
	Grinnellia americana	chol, desmo	b
	Rytiphlea tinctoria	camp or Δ^5 erg	16

[a]Desmo, desmosterol; chol, cholestenol; 22-DC, 22-dehydrocholesterol; camp, campesterol; Δ^5 erg, Δ^5-ergostenol.

[b]Doyle and Patterson, unpublished data.

been isolated from all major groups of living organisms. Cholesterol is the primary sterol of all higher animals; lower animals may contain cholesterol or a complex mixture of 27-, 28-, or 29-carbon sterols. The 28- and 29-carbon sterols found in primitive animals apparently result from their diet; none have been shown to be synthesized by animals.

In higher plants, β-sitosterol is commonly the principal sterol, although it is frequently accompanied by campesterol and stigmasterol. Sterols other than these are relatively rare in higher plants. In algae, however, the sterols are much more varied. Early work indicated that a complex mixture of sterols occurred in algae which was similar to the sterol mixtures of higher plants (5). A mixture of sitosterols was reported in several species of green algae (Chlorophyta). However, other species of Chlorophyta were reported to contain such widely-differing sterols as ergosterol (6), chondrillasterol (7), zymosterol (8), and fucosterol (5). While all publications showed that fucosterol was the major, if not the sole sterol of brown algae (Phaeophyta), they were in complete disagreement on the sterol content of red algae (Rhodophyta) (9). Most Japanese species of Rhodophyta contain cholesterol, while Rhodophyta from British waters contain sitosterol (9). These and many other questions concerning the occurrence and identity of sterols in algae have been resolved in recent years by a reexamination of the species in question using recently available methods of analysis.

TABLE II

Sterols of Brown Algae (Phaeophyta)

Order	Species	Major sterols[a]	References
Ectocarpales	*Pylaiella littoralis*	fuco	5
	Spongonema tomentosum (Ectocarpus tomentosus)	fuco	5
Sphacelariales	*Cladostephus spongiosus*	fuco	5
	Sphacelaria pennata (cirrosa)	fuco	5
	Stypocaulon scoparium	fcou	5
Dictyotales	*Dictyopteris divaricata*	fuco	18,39
	Dictyota dichotoma	fuco	5
	Padina arborescens	fuco	36
Chordariales	*Heterochordaria abietina*	fuco	36
Dictysiphonales	*Myelophycus caespitosus*	fuco	34
Laminariales	*Alaria crassifolia*	fuco	34,39
	Chorda filum	fuco	5
	Costaria costata	fuco, 24 MC	17, 41
	Eisenta bicyclis	fuco	17
	Laminaria angustata	fuco	36
	L. digitata	fuco, 24 MC	5,19
	L. faeroensis	fuco, 24 MC	19
	L. Hyperborea (cloustonii)	fuco	37
	L. japonica	fuco	36
	L. saccharina	fuco	37
Fucales	*Ascophyllum nodosum*	fuco	5,19,37
	Cystophyllum hakodatense	fuco	34
	Fucus gardneri	fuco	40
	F. evanescens	fuco, 24 MC	17,34,39
	F. diviarcarpus	fuco, 24 MC	41
	F. ceranoides	fuco	5
	F. serratus	fuco	37
	F. spiralis	fuco	37
	F. vesiculosis	fuco	37
	Halidrys siliquosa	fuco	5
	Pelvetia wrightii	fuco	17
	P. canalicalata	fuco	37
	Sargassum muticum	fuco	40
	S. confusum	fuco	39
	S. thunbergii	fuco, 24 MC	39
	S. ringgoldianum	fuco, 24 MC	39

[a]Fuco, fucosterol; 24 MC, 24-methylene cholesterol.

STEROLS OF RED ALGAE (RHODOPHYTA)

There are two probable reasons for the confusion concerning the sterol composition of Rhodophyta. First, when the earlier work with Rhodophyta was done (10), analytical techniques were not capable of separating and identifying closely-related sterols that were known to occur in other plants. Secondly, recent work has shown (11) that the sterol composition of a single species of Rhodophyta can be markedly different from sample to sample. It is not yet clear whether these differences are seasonal variations or due to factors related to the specific nutritional environments from which the samples were collected. In spite of the fact that we know the sterol composition of nearly 40 species of Rhodophyta, specific experiments have not been conducted to determine whether these sterols are synthesized by the algae or absorbed from their environment. It may be significant that *Porphridium cruentum*, a species of Rhodophyta which is cultured on a chemically-defined medium lacks sterols (12). The species of red algae examined for sterols are listed in Table I. The earliest sterol identifications have been omitted unless they have been substantiated by modern techniques of analysis. Cholesterol is the major sterol in most Rhodophyta; in many species, it is the only sterol detected. The major sterol of *Porphyra purpura* is desmosterol (13), but in *Porphyra sp.* (11), cholesterol predominates. The sterols of *Halosaccion ramentaceum* and *Rhodymenia palmata* are primarily desmosterol and cholesterol. In a given sample either may be found to predominate (11). *Hypnea japonica* contains 22-dehydrocholesterol as its major sterol (14), and several other species contain traces of this unusual sterol (11).

The following facts about the sterols of red algae are now apparent: (a) all Rhodophyta examined contain sterols except *Porphridium*

TABLE III

Sterols of Green Algae (Chlorophyta)

Order	Species	Major sterols[a]	References
Chlorococcales	*Scenedesmus obliquus*	chond, Δ^7-erg	7, 24, 42
	Chlorella vulgaris	chond, Δ^7-erg	24, 25
	C. fusca	chond, Δ^7-erg	b
	C. emersonii	chond, Δ^7-erg	b
	C. glucotropha	chond	b
	C. miniata	chond	b
	C. vannielii	erg	6, 26
	C. simplex	erg	26
	C. nocturna	erg	26
	C. sorokiniana	erg	26
	C. candida	erg	26
	C. ellipsoidea	porif, Δ^5-erg	25
	C. saccharophila	porif, Δ^5-erg	25
	Hydrodictyon reticulatum	spin	38
Ulvales	*Ulva lactuca*	28-iso	22
	U. pertusa	chol	39
	Enteromorpha linza	28-iso	23
	E. intestinalis	28-iso	22
	Monostroma nitidum	hali	23
Cladophorales	*Chaetomorpha crassa*	chol, 24-MC	39

[a]Chond, chondrillasterol; Δ^7-erg, Δ^7-ergostenol; erg, ergosterol; porif, poriferasterol; Δ^5-erg, Δ^5-ergostenol; spin, spinasterol; 28-iso, 28-isofucosterol; chol, cholesterol; hali, haliclonasterol; 24-MC, 24-methylene cholesterol.

[b]Patterson, unpublished data.

cruentum; (b) the predominant sterol of the great majority of Rhodophyta is cholesterol; and (c) with only one exception, the major sterols of the red algae have been C-27 sterols (Fig. 1). In some studies, traces of sterols tentatively identified as 24-methylene cholesterol, brassicasterol, stigmasterol, sitosterol or fucosterol have been reported (15,11). Gibbons, et al. (13) found no trace of C-28 and C-29 sterols in the Rhodophyta they examined and suggested that these algae could be incapable of alkylation at C-24 by S-adenosyl methionine. Some Rhodophyta apparently do contain C-28 and C-29 sterols; however, only in *Rytiphlea tinctoria* are they major sterols (16). It would be of interest to determine whether these sterols are synthesized by Rhodophyta or are absorbed from the environment.

STEROLS OF BROWN ALGAE (PHAEOPHYTA)

Since the earliest work of Carter, et al. (10), it has been recognized that fucosterol is the predominant sterol of Phaeophyta. Apparently, there is no exception to this rule (Table II). In one study (17), sargasterol, the C-20 isomer of fucosterol, was identified as the major sterol in *Sargassum ringgoldianum*, but this study did not make use of chromatographic methods now available to determine if the sterol is homogenous. More recent studies on this alga have not detected sargasterol (18,39). *S. ringgoldianum* apparently contains fucosterol as its primary sterol with small amounts of cholesterol, 24-methylene cholesterol and saringosterol (18). Recent work using gas chromatography has shown, without exception, the presence of small amounts of sterols other than fucosterol in Phaeophyta. The presence in brown algae of 24-methylene cholesterol and of desmosterol, which was tentatively identified in *Laminaria* (19), can be explained, since they could be biosynthetic precursors of fucosterol. Saringosterol (hydroxy-24-vinylcholesterol) (Fig. 2) has been identified in many recently-examined Phaeophyta (18,19,39). Knights has recently presented evidence that saringosterol arises from fucosterol by air oxidation (20).

Fucosterol is the major sterol in all brown algae examined. It is even more dominant in Phaeophyta than cholesterol is in Rhodophyta. Members of Phaeophyta are not closely related to any other algae (21). It may be phylogenetically significant, then, that fucosterol has not been frequently identified in other algal species.

STEROLS OF GREEN ALGAE (CHLOROPHYTA)

The sterols of Chlorophyta are much more complex than those of Phaeophyta and Rhodophyta. Early work indicated that the

TABLE IV

Sterols of *Chlorella*, Per Cent of Total Algal Sterol

Species	$\Delta^{5,7}$ Sterols		Δ^{7} Sterols			Δ^{5}-Sterols		
	Ergosterol	Unidentified $\Delta^{5,7}$ Sterols	Δ^{7}-Ergostenol	Chondrillasterol	Δ^{7}-Chondrillastenol	Δ^{5}-Ergostenol	Poriferasterol	Clionasterol
C. vannielii	76	24						
C. sorokiniana	75	25						
C. nocturna	66	34						
C. simplex	70	30						
C. candida	76	24						
C. protothecoides var. mannophila	35	65						
C. protothecoides var. communis	16	84						
C. ellipsoidea						28	56	16
C. saccharophila						30	60	7
C. vulgaris			25	65	10			
C. glucotropha			15	75	10			
C. fusca			27	59	12			
C. emersonii			28	62	9			
C. miniata			16	59	9			

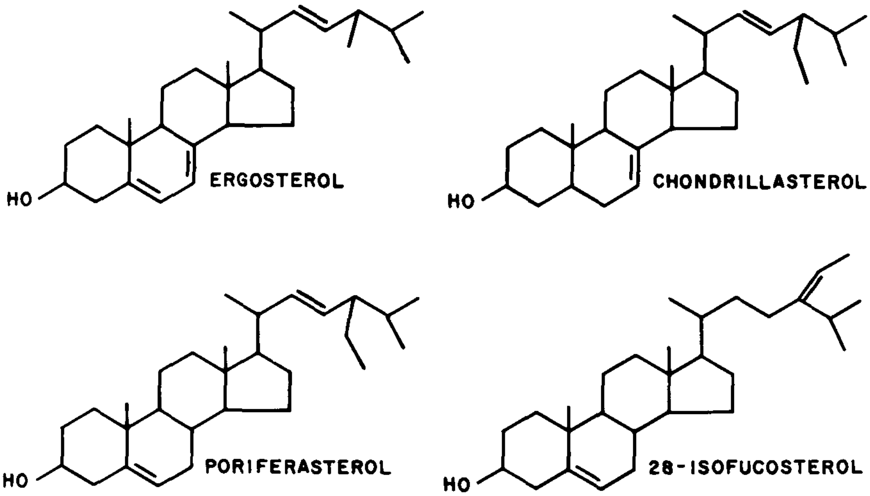

FIG. 3. Sterols of green algae.

sterols of Chlorophyta were similar to those of higher plants, that is, a complex mixture of sitosterols (5). However, a recent reexamination showed that *Ulva lactuca* and *Enteromorpha intestinalis* contain 28-isofucosterol (22), and not sitosterol as earlier work indicated (5). Other species which were shown to contain sitosterol in early work will probably be found to be in error also, since sitosterol has not been firmly identified in any green alga. These early examinations will not be considered here unless they have been substantiated by a recent reexamination. Due to the complexity of the sterol mixture in most Chlorophyta, many incorrect identifications have been made and are still being made. These mistakes may be largely avoided by use of modern techniques such as gas chromatography and thin layer chromatography and mass spectroscopy. Even with these techniques, identifications of these sterols are difficult because of the common occurrence, in Chlorophyta, of sterols with an asymmetric carbon atom at C-24. Just as a C-20 isomer of fucosterol (sargasterol) was once thought to occur in *Sargassum*, a C-20 isomer of campesterol has been tentatively identified in *Monostroma nitidum* (23). Thus the occurrence of haliclonasterol in *Monostroma* and spinasterol in *Hydrodictyon reticulatum* (38) must be regarded as tentative, since not enough data are available to determine the orientation at C-20 and C-24 (Table III). Cholesterol occurs in significant amounts in some species of Chlorophyta and may even be the primary sterol (39).

Considerable data are now available on the sterols of *Chlorella*, a unicellular green alga (Table IV). There is much variability in sterol composition within the members of this genus. Several species contain chondrillasterol and Δ^7-ergostenol with a smaller amount of Δ^7-chondrillastenol (24 and G.W. Patterson, unpublished data). Two species contain poriferasterol, Δ^5-ergostenol and clionasterol, and other species contain primarily ergosterol (26). It has been definitely established that all of these sterols contain alkyl groups at C-24

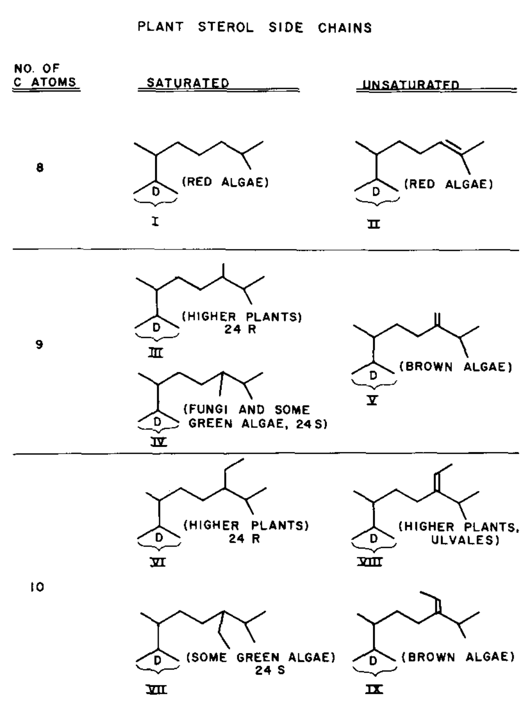

FIG. 4. Plant sterol side chains.

with the 24S configuration. The 24S configuration may be characteristic of green algal sterols in contrast to the 24R configuration of higher plant sterols (25) (Fig. 4). This certainly appears to be the case with *Chlorella*. Two strains of *Scenedesmus obliquus*, which were originally thought to contain different sterols (8), have now been found to contain chondrillasterol, Δ^7-ergostenol, and Δ^7-chondrillastenol in proportions similar to those *Chlorella* species containing these sterols (G.W. Patterson, unpublished data).

Some Chlorophyta contain cholesterol like Rhodophyta, some contain ergosterol like fungi, some contain 28-isofucosterol which occurs in higher plants and is similar to the fucosterol of Phaeophyta, and some contain sterols identical to those of higher plants (except for the configuration at C-24). Although most plant sterols have a nucleus identical to cholesterol, the structure of the side chain is quite variable. Thus the sterols of algae, particularly of the Chlorophyta, appear to have value for the systematist as a guide in taxonomy and phylogeny (Fig. 4).

STEROLS OF OTHER ALGAE

Only a few species of *Charophyta*, *Xanthophyta*, *Chrysophyta*, *Bacillariophyta* and *Euglenophyta* have been examined for sterols and most of this work was accomplished before identifications of sterols could be made with any confidence. Of the recent work, ergosterol has been identified in *Euglena gracillis* (27,28) and *Synura petersenii* contains cholesterol and sitosterol (29). Several species of *Ochromonas* have been examined for sterols. Although there was much early confusion concerning the sterols of *Ochromonas* species, it has been apparently established now that poriferasterol is the major sterol of *O. malhamensis* and *O. danica* (30). Although the sterol of *O. sociabilis* has been identified as stigmasterol (28), this sterol also seems to be poriferasterol. Recently, sterols have been isolated in very small amounts from the blue-green algae, *Phormidium luridum* (24-ethyl-Δ^7-cholestenol and 24-ethyl-$\Delta^{7,22}$-cholestadienol) (4), *Anacystis nidulans*, and *Fremyella diplosiphon* (cholesterol and 24-ethyl cholesterol) (3). The configuration at C-24 in the sterols of these algae is not yet known.

The biosynthesis of sterols in algae is just beginning to attract some interest. Cycloartenol has been shown to replace lanosterol as a biosynthetic precursor in *Fucus spiralis* (31), *O. danica* and *O. malhamensis* (30); algae may be quite similar to higher plants in the manner by which they synthesize sterols. When more data on sterol composition of algae are available, more progress may be made in the taxonomy and evolution of algae and higher plants. This information may also provide clues to the specific roles of sterols in plants.

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