
Sterols in Microalgae

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1 Introduction

Sterols are tetracyclic triterpenoids biosynthesized by all eukaryotic organisms (Desmond and Gribaldo 2009). A generalized structure with carbon atoms numbered is shown in Fig. 1. Sterol distributions in microalgae display a wide range of structures reflecting subtle differences in the sterol biosynthetic pathway used by different organisms (e.g. Goad et al. 1974; Volkman 1986, 2003, 2005; Volkman et al. 1998; Nes 2011). In some cases the presence of specific sterols follows taxonomic classifications quite closely. In other classes, a wide range of structures can be found (Table 1) either because the taxonomic grouping is actually polyphyletic or the alga accumulates sterols produced at different stages in the biosynthetic pathway perhaps due to a defect in later biosynthetic steps.

Sterol biosynthesis occurred very early in the history of life on Earth (Kodner et al. 2008), and the presumed last common ancestor of the eukaryotes likely had a large panel of enzymes for sterol biosynthesis (Desmond and Gribaldo 2009). These biosynthetic pathways have continued to evolve over geological time and thus one might expect to see certain “unusual” or rare sterols in particular algal classes due to specific changes in one or more of the many genes needed for sterol biosynthesis.

This review provides characteristic features of the sterol distributions in each of the major microalgal classes and builds on previous reviews by this author (Volkman 1986, 2003, 2005; Volkman et al. 1998). However, a difficulty immediately arises as to which taxonomic scheme to use. Ideally this should reflect the evolution of the different algal classes, but a consensus is not yet available. There are 15 traditionally recognized phyla in microalgae and most of the

papers cited in this review have assigned species based on this classification. These 15 phyla are: Cyanophyta (cyanobacteria, also referred to as blue-green algae in the older literature), Rhodophyta (red algae), Euglenophyta (euglenoids), Cryptophyta (cryptomonads), Pyrrophyta (dinoflagellates), Raphidophyta (raphidophytes), Chrysophyta (chrysophytes, golden-brown algae), Xanthophyta (=Tribophyta, yellow-green algae), Chlorophyta (green algae), Eustigmatophyta (eustigmatophytes), Phaeophyta (brown algae), Prasinophyta (prasinophytes), Bacillariophyta (diatoms), Glaucophyta (glaucophytes).

Undoubtedly these classification schemes will continue to be refined as new gene sequence data become available. While it would have been more satisfying to structure the paper around an agreed taxonomic scheme that reflects the evolution of microalgal classes, this has not been possible. The taxonomy of many microalgal groups is in a state of flux and it is not uncommon to see some species transferred to completely different classes and species names being changed several times. Accordingly the data are structured according to the major phyla and where possible assigned to different algal classes within each phylum. Revised taxonomic assignments have been used based on those presented in *AlgaeBase* (Guiry and Guiry 2015), with a reference back to the original classification used in the paper cited where there has been a revision. The chapter “*Systematics, Taxonomy and Species Names: Do They Matter?*” of this book (Borowitzka 2016) also provides the names and current taxonomically accepted affiliation of all taxa mentioned in the present chapter.

2 Sterol Biosynthesis

Sterols are an end-product of the cyclization of the C_{30} isoprenoid 2,3-oxidosqualene.

Cyclisation either produces lanosterol as in animals, fungi, and some algae and bacteria or cycloartenol as in higher plants and most algae (Fig. 2). Many plants also

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Fig. 1 A generalized structure of a sterol showing numbering of carbon atoms and positions of alkyl substituents and double bonds

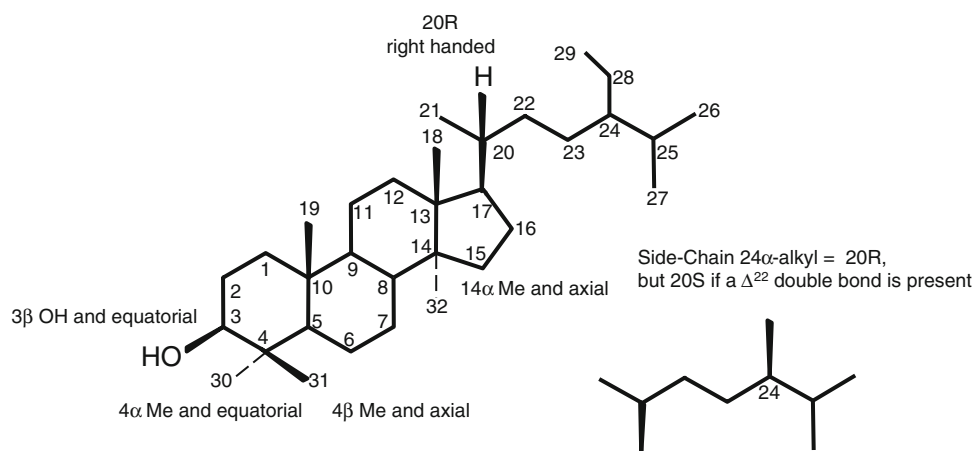
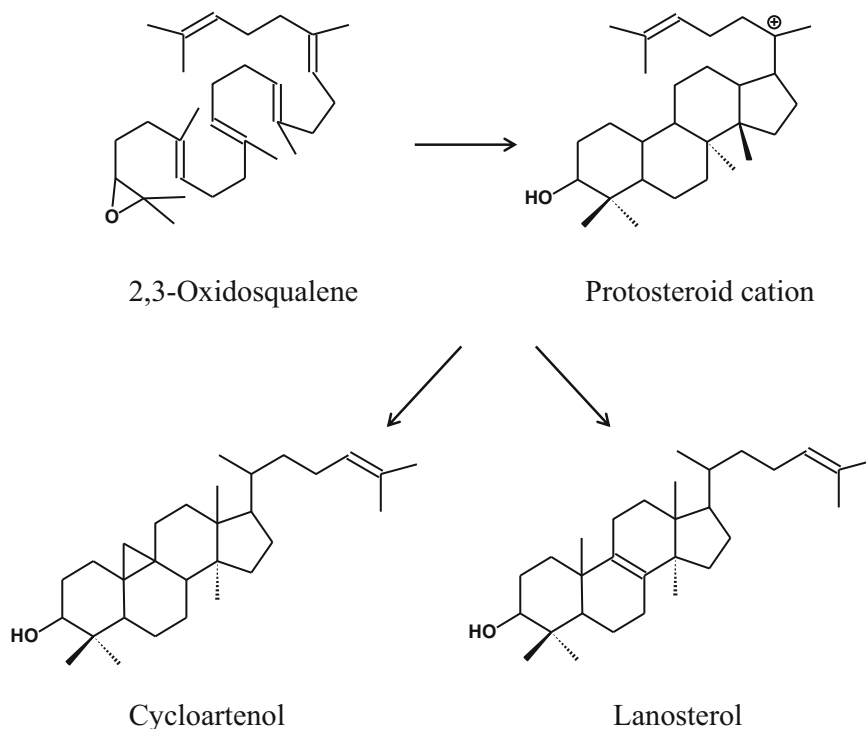


Table 1 Systematic and trivial names for sterols found in microalgae

Sterol C. no.	Structure	Systematic name	Trivial name
27:0	IIb	5 α -cholestan-3 β -ol	5 α -cholestanol
27:1	Ib	Cholest-5-en-3 β -ol	Cholesterol
27:1	Ia	27-Nor-(24S)-cholesta-5,22E-dien-3 β -ol	Ocellasterol
27:2	Ic	Cholesta-5,22E-dien-3 β -ol	22-dehydrocholesterol
27:2	Id	Cholesta-5,24-dien-3 β -ol	Desmosterol
28:0	IIe	24 α -methyl-5 α -cholestan-3 β -ol	Campestanol
28:1	Ie	24 α -methylcholest-5-en-3 β -ol	Campesterol
28:1	Ie	24 β -methylcholest-5-en-3 β -ol	5-ergosterol
28:1	IIIe	24 β -methyl-5 α -cholest-7-en-3 β -ol	7-ergosterol
28:2	If	24 α -methylcholesta-5,22E-dien-3 β -ol	<i>epi</i> Brassicasterol or diatomsterol
28:2	If	24 β -methylcholesta-5,22E-dien-3 β -ol	Brassicasterol
28:2	Ih	23-methylcholesta-5,22E-dien-3 β -ol	None
28:2	Ig	24-methylcholesta-5,24(28)-dien-3 β -ol	24-methylenecholesterol
28:2	IVe	24 β -methyl-cholesta-5,7-dien-3 β -ol	5,7-ergostadienol
28:2	IIIIf	24 β -methyl-5 α -cholesta-7,22E-dien-3 β -ol	7,22-ergostadienol
28:3	IVf	24 β -methyl-cholesta-5,7,22-trien-3 β -ol	Ergosterol
29:0	IIIi	24 α -ethyl-5 α -cholestan-3 β -ol	Sitostanol
29:1	Ii	24 α -ethylcholest-5-en-3 β -ol	Sitosterol
29:1	Ii	24 β -ethylcholest-5-en-3 β -ol	Clionasterol
29:2	Ij	24 α -ethylcholesta-5,22E-dien-3 β -ol	Stigmasterol
29:2	Ij	24 β -ethylcholesta-5,22E-dien-3 β -ol	Poriferasterol
29:2	Ik	24 α -ethylcholesta-5,24(28)E-dien-3 β -ol	Fucosterol
29:2	Il	24 α -ethylcholesta-5,24(28)Z-dien-3 β -ol	Isofucosterol
29:2	IIIj	24 β -ethyl-5 α -cholesta-7,22E-dien-3 β -ol	Chondrillasterol
29:3	IVj	24 β -ethylcholesta-5,7,22-trien-3 β -ol	7-dehydroporiferol
30:1	Im	22,23-methylene-23,24-dimethylcholest-5-en-3 β -ol	Gorgosterol
30:0	Vn	4 α ,23,24-trimethyl-5 α -cholestan-3 β -ol	Dinostanol
30:1	Vo	4 α ,23,24-trimethylcholest-22E-en-3 β -ol	Dinosterol
30:1	Vj	4 α -methyl,24-ethyl-5 α -cholest-22E-en-3 β -ol	–
31:1	Fig. 2	4,4,14 α -trimethyl-9,19-cyclo-5 α -cholest-24-en-3 β -ol	Cycloartenol
30:2	Fig. 2	4,4,14 α -trimethyl-5 α -cholesta-8,24-dien-3 β -ol	Lanosterol

Fig. 2 The first products in sterol biosynthesis are either cycloartenol as found in higher plants and many microalgae or lanosterol as produced mostly by animals, fungi and some bacteria



convert 2,3-oxidosqualene to pentacyclic triterpenes such as α - and β -amyrin while bacteria generally synthesize cyclic triterpenes such as hopenes by cyclization of squalene rather than oxidosqualene (Buntel and Griffin 1994; Volkman 2005). For a comprehensive review of the biosynthesis of cholesterol and other sterols, primarily in higher plants and fungi, and the enzymes involved the reader is referred to the review by Nes (2011).

In microalgae, sterol biosynthesis can give rise to sterols having C_{26} to C_{31} carbon atoms and structural variations including 4,4-dimethyl substituents, a 4-methyl substituent or no methyl group at C-4 (so-called 4-desmethylsterols that comprise the majority of sterols in microalgae) (reviewed by Volkman 2003, 2005). Addition of one to four methyl substituents to the side-chain provides a wide variety of structures including 24-methyl, 24-methylene, 24-ethyl, 24-ethylidene, 24-*n*-propyl, 24-isopropyl, 23,24-dimethyl, 22,23-methylene (cyclopropyl) and 23-methyl sterols (Fig. 3).

Further variety is provided by the different locations for the double bonds including Δ^5 , Δ^7 , $\Delta^{8(9)}$, $\Delta^{8(14)}$, Δ^{22} , $\Delta^{5,7}$, $\Delta^{5,22}$, $\Delta^{5,24}$, $\Delta^{7,22}$, $\Delta^{5,24(28)}$, $\Delta^{8(14),24(28)}$ and $\Delta^{5,7,9(11)}$ amongst others. Structures of the main variants are shown in Fig. 3. Double bonds are generated by hydrogen transfers to produce different isomers. Early in the sterol biosynthesis pathway the double bond position is at Δ^8 , which is subsequently isomerized to Δ^7 . Introduction of a second double bond at C-5 leads to $\Delta^{5,7}$ sterols and reduction of the Δ^7 double bond gives rise to the Δ^5 sterols (Dempsey 1965; Doyle et al. 1972) (Fig. 4), which dominate the sterol distributions in

most microalgae. Side-chain alkylation can occur at any of these stages giving rise to C-24 alkylated sterols with unusual double bond positions in the ring system.

The presence of particular sterols in an alga can often be understood by considering them as products of the many different steps involved in sterol biosynthesis. The biosynthesis of sterols will not be covered in detail here and the reader is referred to other papers outlining the key steps (e.g. Goad et al. 1974; Chappell 2002; Nes 2011; Miller et al. 2012; Xue et al. 2012). While sterol synthesis is undoubtedly very ancient, some steps may have evolved more recently.

2.1 Side-Chain Biosynthesis

The sterol distributions of most microalgae are characterized by a high proportion of sterols that are alkylated in the side-chain with either a methyl group, ethyl group (two methyl transfers), or rarely a propyl group at C-24 (e.g. Goad et al. 1974; Raederstorff and Rohmer 1984; Patterson 1994). Other variants include the 23,24-dimethyl side-chains found in the 4-methylsterols of dinoflagellates (e.g. Withers 1987) and in the 4-desmethylsterols of some diatoms and haptophytes (e.g. Volkman et al. 1993; Rampen et al. 2009b). These alkylations are brought about by S-adenosyl-L-methionine (AdoMet=SAM) sterol methyltransferases (SMTs: e.g. Nes 2003; Nes et al. 2003). SMTs do not occur in animal systems (Nes 2000). Goad et al. (1974) describe

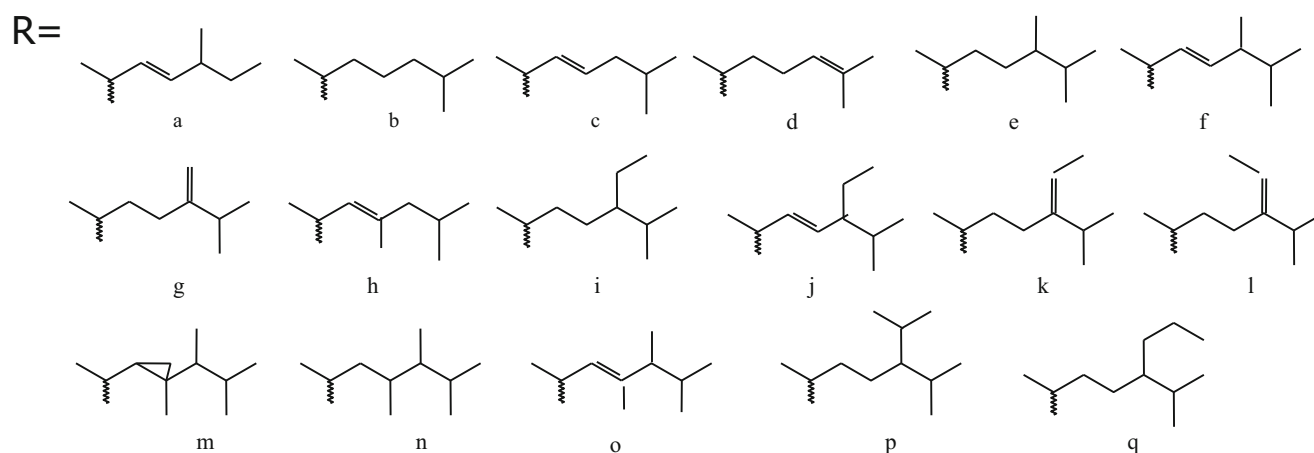
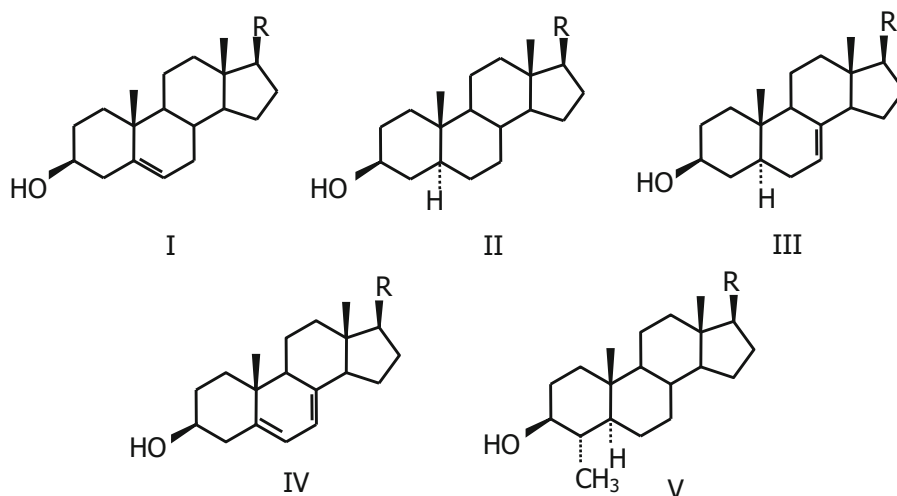
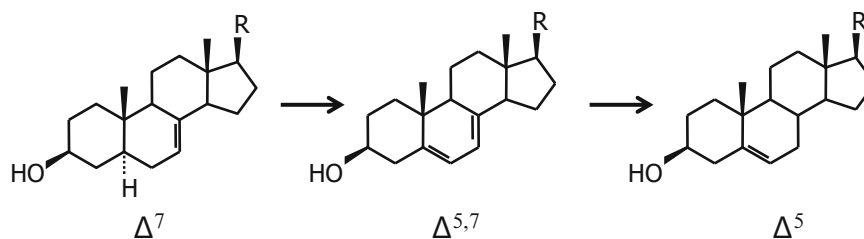


Fig. 3 Structural variation found in the main sterols of microalgae illustrating the main ring structures, double bond positions and alkylation (highly unusual sterols are not shown). R = sterol side-chain

Fig. 4 Stages in the formation of Δ^5 sterols from Δ^7 sterols in microalgae



six different mechanisms by which alkyl groups might arise in the side-chains of sterols. Nes and co-workers have proposed an active-site model, which they term the “steric-electric plug” model to describe C-24 alkylation (reviewed by Nes 2003; Nes et al. 2003). These pathways are complex and show phylogenetic groupings.

Work by Dennis and Nes (2002) showed that the SMT from *Glycine max* expressed in *Escherichia coli* cells catalyses the step-wise conversion of cycloartenol to 24(28)-meth-

ylencycloartenol and then to a mixture of stereochemically related $\Delta^{24(28)Z}$ -ethylidene-, $\Delta^{24(28)E}$ -ethylidene- and $\Delta^{25(27)}$ -24 β -ethylcyclosterols. Campesterol (24 α -methylcholesterol) and dihydrobrassicasterol (24 β -methylcholesterol), typical C₂₈ sterols in higher plants, are biosynthesized from desmosterol via 24-methylenecholesterol and 24-methyl-desmosterol. The typical plant C₂₉-sterol, sitosterol (24 α -ethylcholesterol), is produced from 24-methylenecholesterol via isofucosterol and 24-ethyl-desmosterol.

2.2 C-24 Stereochemistry

The stereochemistry of the alkyl substituents in the side-chain can be α - or β - oriented depending on the biosynthetic pathways by which the side-chain is alkylated. Different algal classes usually produce sterols with either 24α or 24β stereochemistry, but only rarely do both occur (e.g. in some diatoms; Gladu et al. 1991b). For example, the C_{28} and C_{29} Δ^5 unsaturated sterols of vascular plants generally have the 24α configuration as do the C_{28} sterols of most diatoms (Maxwell et al. 1980; Nes and Nes 1980; Gladu et al. 1991b). In contrast, green algae and dinoflagellates biosynthesize sterols with the 24β configuration (Goad et al. 1974; Bohlin et al. 1981; Goad and Withers 1982; Miller et al. 2012).

Note that due to the IUPAC-IUB JCBN sequence rules, 24α corresponds to $24R$ when the side-chain is saturated, but it becomes $24S$ when a Δ^{22} double bond is present (Fig. 1). The side-chain α - and β - nomenclature is not related to that which is used to describe the orientation of methyl substituents on the ring system (see Goad and Akihisa 1997 for further explanation). 24β -Sterols (which are common in green algae) arise from $\Delta^{25(27)}$ sterols. Reduction of $\Delta^{24(28)}$ sterols can give rise to either 24α or 24β sterols (Goad et al. 1974). Fungi apparently use this pathway exclusively for the production of 24β epimers.

The majority of published data on algal sterols has been obtained by GC-MS analysis with reliance on relative retention data (e.g. Itoh et al. 1982) and reference mass spectra to identify specific isomers (e.g. de Leeuw et al. 1983; Jones et al. 1994; Gerst et al. 1997; Volkman et al. 1997). While GC-MS is a sensitive technique and very useful for identifying small amounts of sterols in complex mixtures it does not provide information on the configuration at C-24 (Gerst et al. 1997; Giner et al. 2008). The epimers can be separated on very long, polar glass capillary columns (Maxwell et al. 1980; Thompson et al. 1981; Ikekawa et al. 1989), but analysis times are very long and to date the method has not been widely exploited. HPLC techniques are also now available (Chitwood and Patterson 1991), but are also rarely utilized. Unambiguous assignment of C-24 stereochemistry can be obtained by NMR (e.g. Wright et al. 1978; Chui and Patterson 1981; Goad and Akihisa 1997; Miller et al. 2012), but this requires isolation and purification of each sterol within the complex mixtures found in most microalgae.

Identification of the stereochemistry at C-24 can indicate which of the many sterol pathways operates in that alga. Such data combined with information from incubation with $[CD_3]$ -methionine and labeled mevalonate can be powerful tools for studying sterol biosynthesis in microalgae (Goad et al. 1974). Several algal species, including a diatom, two prymnesiophytes and two cryptophytes, have been shown to produce 24α -methylcholesta-5,22E-dien-3 β -ol (*epibrassicasterol*; Rubinstein and Goad 1974; Maxwell et al. 1980; Goad et al.

1983). Green algae on the other hand mainly contain 24β sterols. In some cases both epimers can be found (Raederstorff and Rohmer 1984) as in an unidentified alga believed to be a chrysophyte (Kokke et al. 1984); 24α -ethylcholest-5-en-3 β -ol comprised 10.2 % of 4-desmethyl sterols compared with 3.0 % for the 24β epimer.

C-24 stereochemistry is not always consistent within a single taxonomic grouping. For example, Gladu et al. (1990) examined nine strains of phytoplankton which contain 24-methylcholesta-5,22E-dien-3 β -ol as their principal sterol. The two strains provisionally identified as *Isochrysis* (a haptophyte) contained brassicasterol (24 β -methylcholesta-5,22E-dien-3 β -ol); whereas all other species examined (two haptophytes, two rhodophytes and two diatoms) contained primarily *epibrassicasterol* 24 α -methylcholesta-5,22E-dien-3 β -ol).

2.3 Biochemical Forms of Sterols

The methods used in most studies of sterols in microalgae do not provide information about the chemical form in which the sterol exists since the extracts are usually saponified and any steryl esters are converted to the free sterols. In most microalgae, the sterols mainly exist in a non-esterified (i.e. free) form but this is not always the case. For example, Véron et al. (1996) studied the sterols of seven unicellular algae including representatives from the Prasinophyceae, Haptophyceae, Eustigmatophyceae, and Bacillariophyceae (as then defined). All synthesized free sterols and esterified forms (steryl esters, acyl steryl glycosides, and steryl glycosides), but free sterols predominated in five of the species. In contrast, the eustigmatophyte *Nannochloropsis oculata* and the diatom *Thalassiosira pseudonana*, contained mostly esterified sterols whereas in the diatom *Chaetoceros calcitrans* glycosylated forms represented over 60 % of total sterols. The pennate diatom *Haslea ostrearia* synthesized large amounts of steryl glycosides consisting mainly of the uncommon sterol 23,24-dimethylcholest-5-en-3 β -ol.

2.4 Effects of Environmental Condition on Sterol Compositions

Minor differences in the relative proportions of sterols with culture age and growth state have been observed (Ballantine et al. 1979), but it is comparatively rare for sterol distributions to change dramatically with changes in environmental conditions in contrast to fatty acid compositions. Sterol compositions thus are usually good chemotaxonomic tools for studying microalgal relationships and biochemistry. Sterol concentrations per cell or dry weight, however, can be affected. For example, Mercer et al. (1974) found no change

in sterol composition with culture age in three species of xanthophytes, but noted that aeration increased the cholesterol content. A detailed review of this topic is not possible here, but some leading references are provided to guide the reader.

Piepho et al. (2010) studied the sterol content in freshwater green algae *Desmodesmus communis* (as *S. quadricauda*) and *Chlamydomonas*, the cryptophyte *Cryptomonas ovata* and the diatom *Cyclotella meneghiniana*, and found that sterol contents increased significantly with increasing light in three out of four species and that sterol content decreased with increasing light at low phosphorus supply. Piepho et al. (2012) observed that sterol concentrations were higher at 25 °C than at 10 °C in *D. quadricauda* and *C. meneghiniana*, but were not affected by temperature in *C. ovata*. Temperature and phosphorus supply interacted to affect sterol concentrations in *C. meneghiniana* presumably due to the bioconversion of 24-methylene-cholesterol to 24-methylcholesta-5,22E-dien-3 β -ol. Jo et al. (2004) found the same major sterols in autotrophically grown and heterotrophically grown *Tetraselmis suecica*, but the proportions of these and total amounts varied with culture age.

3 Sterols in Cyanobacteria

The suggestion that cyanobacteria (blue-green algae) contain sterols has been much debated. Some early studies suggested that their presence was due to contamination since on repeated purification the amount of sterols declined (Ourisson et al. 1987) and others have obtained similar data (Volkman, unpublished data; Summons et al. 2001). However, there are more than a dozen reports of sterols being found in cyanobacteria (summarized by Volkman 1986, 2003). These include simple mixtures in which cholesterol and 24-ethylcholest-5-en-3 β -ol predominate, but Δ^7 and $\Delta^{5,7}$ -sterols including ergosterol have also been reported (De Souza and Nes 1968). In almost all cases the amounts of sterols isolated are quite small (Hai et al. 1996). Summons et al. (2001) suggested that the sterols present in cyanobacterial cultures are derived from contaminating yeasts and fungi. The balance of evidence seems to indicate that cyanobacteria do not synthesize sterols although a few eubacteria can synthesize sterols that are not alkylated in the side-chain (reviewed by Volkman 2005).

4 Sterols in the Phylum Rhodophyta

The division or subphylum Rhodophyta contains both microalgae and macroalgae, generally termed red algae. Most members are marine macroalgae, but unicellular marine coccids are known including *Porphyridium*, *Rhodorus* and

Rhodella. Unicellular red algae that form mucilaginous colonies are considered primitive. The taxonomy has been through a number of changes. The Bangiophyceae, as defined traditionally, is paraphyletic and taxonomic identification of species has been difficult because of a lack of distinct morphological features, and the presumed morphological plasticity of the species. It has since been merged with the Floridophyceae to form the Rhodophyceae.

Most macrophyte red algae contain primarily C₂₇ sterols with cholesterol predominant (e.g. Palmerno et al. 1984), although several species contain large amounts of cholesta-5,24-dien-3 β -ol (desmosterol) (Gibbons et al. 1967). Only a few red macroalgae contain traces of C₂₈ (e.g. 24-methylcholesta-5,22E-dien-3 β -ol) and very rarely C₂₉ sterols. This is in sharp contrast to the sterol distributions found in microscopic red algae which, while often dominated by cholesterol, can also show high contents of C₂₈ 4-desmethyl sterols and occasionally biosynthetically "primitive" 4-methyl sterols.

4.1 Class Bangiophyceae

The sterols of the macroalgae *Pyropia/Porphyra* are dominated by cholesterol (60 %) whereas *Stylonema alsidii* (as *Goniotrichum elegans*) contains both 24-methylcholesta-5,22E-dien-3 β -ol and cholesterol (Brothers and Dickson 1980). Sterol analyses of microalgae in this class appear not to have been reported.

4.2 Class Porphyridiophyceae

The microalga *Porphyridium cruentum* contains 24-methylcholesta-5,7,22-trien-3 β -ol (ergosterol) and cholesta-5,22-dien-3 β -ol (Beastall et al. 1971). In contrast, other species of *Porphyridinium* contain unusual 4-methyl Δ^8 -unsaturated sterols including 4 α -methyl-5 α -cholesta-8,22-dien-3 β -ol, 4 α ,24-dimethyl-5 α -cholesta-8,22-dien-3 β -ol, 4-methylcholest-8-en-3 β -ol and 4,24-dimethylcholest-8-en-3 β -ol (Beastall et al. 1974), perhaps due to the lack of a Δ^8 to Δ^7 isomerase. Duperon et al. (1983) showed that *Porphyridinium* sp. contains free sterols, steryl glycosides and acylated steryl glycosides.

4.3 Class Florideophyceae

Desmosterol was the major sterol found in three samples of the macroalga *Palmaria (Rhodymenia) palmata* (82 %, 97.2 % and 60.4 %) while cholesterol (92.3 %) predominated in a fourth (Idler et al. 1968). There was also significant variation in the principal sterol of two samples of the macroalga

Devaleraea ramentacea (as *Halosaccion ramentaceum*). One contained predominantly cholesterol (85.4 %), the other desmosterol (81.8 %).

4.4 Class Stylonematophyceae

The major sterol in the microalgae *Rhodorus* sp. (CS-210) and *Rhodorus lens* (= *R. salina*?) is the C₂₈ sterol 24-methylcholesta-5,22E-dien-3 β -ol (Gladu et al. 1990; Dunstan et al. 2005) as found in some cryptomonads, diatoms and haptophytes. *Rhodorus* sp. (CS-210) contains in addition small amounts of cholesterol (2 %) and various Δ^7 -sterols including the rare 4-methyl-5 α -cholest-7-en-3 β -ol (5.8 %) and 4-methyl-5 α -cholesta-7,22-dien-3 β -ol (12.3 %).

5 Sterols in the Phylum Chlorophyta

The Viridiplantae comprises the green algae and their descendants the land plants. One clade, the Chlorophyta, comprises the early diverging prasinophytes, which gave rise to the core chlorophytes. The other clade, the Streptophyta, includes the charophyte green algae from which land plants evolved. Many uncertainties about the evolution of this phylum persist, including the branching orders of the prasinophyte lineages, the relationships among core chlorophyte clades (Chlorodendrophyceae, Ulvophyceae, Trebouxiophyceae and Chlorophyceae), and the relationships among the streptophytes (Leliaert et al. 2012). As a consequence, it is difficult to classify some of the reported sterol distributions of various species due to changes in their taxonomic assignments.

The Chlorophyta contain chlorophylls *a* and *b* and store starch inside the chloroplast. There are thought to be between 9,000 and 12,000 species. Green algae became ecologically important about 600–800 Ma ago (Knoll et al. 2007; Kodner et al. 2008), possibly in response to increases in Fe content in the ocean (Canfield et al. 2008). Morphological analogues of the Ulvophyceae have been found in Spitsbergen sediments 700–750 Ma (Butterfield et al. 1994). Green algae are thought to be responsible for the high abundance of C₂₉ steranes seen in some Cambrian sediments and crude oils (e.g. Kodner et al. 2008). However, when one examines the sterols of modern groups of green algae the situation is much more complex. Many chlorophytes contain mixtures of Δ^7 , $\Delta^{5,7}$ and $\Delta^{7,22}$ sterols (Holden and Patterson 1982), but a few contain mainly Δ^5 -unsaturated sterols. In the latter group, 24-methylcholest-5-en-3 β -ol and 24-ethyl-cholesta-5,22E-dien-3 β -ol usually predominate with moderate amounts of 24-ethylcholest-5-en-3 β -ol.

5.1 Class Chlorophyceae

This class is primarily freshwater and includes genera such as *Chlamydomonas*, *Pyramimonas*, *Scenedesmus* and *Oedogonium*. *Chlamydomonas* contains more than 600 species and has been shown to be polyphyletic (Proschold et al. 2001) which makes assessment of the early sterol literature difficult. It has been proposed that species in clades other than that containing *C. reinhardtii* must be transferred to other genera (Proschold et al. 2001).

Miller et al. (2012) have shown that the green alga *Chlamydomonas reinhardtii* synthesizes cycloartenol and converts it to ergosterol and 24-ethyl-5 α -cholesta-7,22-dien-3 β -ol (7-dehydroporiferasterol) (both having a C₂₄ β -alkyl group) through a highly conserved sterol C₂₄-methylation-C₂₅-reduction pathway that is distinct from the acetate-mevalonate pathway that produces fungal lanosterol and thence to ergosterol by the $\Delta^{24(28)}$ -olefin pathway.

Volkman et al. (1994) identified the major sterols in *Pyramimonas cordata* as 24-ethyl-cholesta-5,24(28)Z-dien-3 β -ol (88.5 %) with a small amount of the 24(28)E isomer (0.8 %) and an unusual dihydroxylated C₂₉ sterol 24-ethylcholesta-5,28(29)-dien-3 β ,24-diol (saringosterol) probably formed by oxidation of the major sterol.

Dunaliella minuta is unusual in that under stationary phase conditions it produces mainly C₂₇ sterols (Ballantine et al. 1979). *Dunaliella salina* has been reported to produce C₂₇ sterols at high salinities (4 M) (Kelly 2009) although C₂₉ sterols are more commonly associated with this species (Peeler et al. 1989).

The freshwater species *Desmodesmus communis* (as *Scenedesmus quadricauda*) contains only Δ^7 sterols 24-methyl-5 α -cholest-7-en-3 β -ol, 24-ethyl-5 α -cholesta-7,22-dien-3 β -ol and 24-ethyl-5 α -cholest-7-en-3 β -ol (Cranwell et al. 1990). In contrast, freshwater *Eudorina unicocca* contains 5 sterols: 24-methylcholesterol (32 %), cholesterol (27 %), 24-ethylcholesta-5,22E-dien-3 β -ol (22 %), 24-ethyl-5 α -cholest-7-en-3 β -ol and 24-methylcholesterol (4 %) (Cranwell et al. 1990). This species is unusual in that it contains both Δ^5 and Δ^7 sterols.

5.2 Class Ulvophyceae

Very few data are available for microalgal members of this class of green algae. The freshwater species *Ulothrix zonata* contains a complex mix of sterols with 24-methylenecholesterol (41 %), 24-methylcholesterol (23 %), 24-ethylcholesta-5,24(28)Z-dien-3 β -ol (16 %) and 24-ethylcholesterol (12 %) as major sterols (Cranwell et al. 1990).

5.3 Class Mamiellophyceae

This class is primarily marine and includes the smallest eukaryotic algal genus *Micromonas* which contains a single species, *M. pusilla*, which is the dominant photosynthetic picoeukaryote in some marine ecosystems (e.g. Throndsen 1976). Unlike many marine algae, it is distributed widely in both warm and cold waters. Under the light microscope it is easily mistaken for a rapidly swimming bacterium. Based on pigment analysis, *Micromonas* shows affinities with the Mamiellales group of the Prasinophyceae, but it lacks the characteristic scales of this group. Note that some still classify this genus in the Prasinophyceae. The sterols of *Micromonas pusilla* (strain CS-98) and a tropical strain *Micromonas* aff. *pusilla* (CS-170) were reported by Volkman et al. (1994) who found the same suite of 4 major sterols, but in very different proportions. The major sterol in CS-98 was isofucosterol (71.7 %), but in CS-170 this was only 19.4 % of total sterols. The second most abundant sterol in CS-98 was 24-methylenecholesterol (15.9 %), but this was the major sterol in CS-170 (54.4 %). This difference seems to reflect a greater extent of alkylation of the 24(28) double bond in CS-98. Both strains contained an unusual dihydroxylated C₂₉ sterol 24-ethylcholesta-5,28(29)-dien-3 β ,24-diol (saringosterol; 3.1 % of total sterols in CS-98 and 14.2 % in CS-170). It seems likely that saringosterol is formed from enzymatic oxidation of 28-isofucosterol.

5.4 Class Chlorodendrophyceae

This class includes freshwater and marine species and now includes the marine flagellate *Tetraselmis* (previously assigned to the Prasinophyceae). Patterson et al. (1993b) examined 11 isolates of *Tetraselmis* and found only 3 sterols. The principal sterol in eight isolates was either 24-methylenecholesterol or 24-methylcholesterol, both C₂₈ sterols, with the latter identified as the 24 α -isomer campesterol in each case. In the other three isolates, cholesterol was the principal sterol, which is highly unusual for a green alga, together with smaller amounts of 24-methylenecholesterol and campesterol.

Volkman (1986) and Volkman et al. (1994) reported the sterol composition for *Tetraselmis chui* and found a single major sterol (>96 %) identified as 24-methylcholesterol. In contrast, Ballantine et al. (1979) found a much greater variety of sterols in *Tetraselmis tetrathele* which included 24-methylcholesterol (34 %) and 24-methylcholesta-5,22-dien-3 β -ol (57.7 %). Similar data were reported by Lin et al. (1982) for another species *Tetraselmis suecica* where the proportions of these two sterols were 48.1 % and 50.9 % respectively.

Jo et al. (2004) examined the sterol dynamics of a strain of *Tetraselmis suecica* grown both autotrophically and heterotrophically. Six major sterols were found in the photoautotrophically grown cells: cholesta-5,22-dien-3 β -ol, 24-methylcholesterol, cholesterol, 24-methylcholesta-5,22-dien-3 β -ol, 24-methylcholesta-5,24-dien-3 β -ol, and 24-ethylcholesta-5,24-dien-3 β -ol in decreasing order of abundance. The total amounts of sterols after 1 week of culture were quite similar as was the composition, but total amounts declined in weeks 2 and 3 and 24-methylcholesterol became the major sterol. Similar changes were found when the cells were grown heterotrophically.

The effect of culture renewal rate (RR) on sterol amounts in *T. suecica* was studied by Fabr egas et al. (1997). The major sterol 24-methylcholesterol ranged from 137 fg cell⁻¹ with a 10 % RR to 40 fg cell⁻¹ with a 40 % RR and 24-methylenecholesterol ranged from 403 fg cell⁻¹ with a 10 % RR to 80 fg cell⁻¹ with a renewal rate of 50 %.

5.5 Class Trebouxiophyceae

The Trebouxiophyceae contains the three orders: Chlorellales, Prasiolales and Trebouxiales. *Botryococcus braunii* is perhaps the best known member of this class of microalgae. *Chlorella* was formerly assigned to the Chlorophyceae but most species have now been assigned to the Trebouxiophyceae, although Huss et al. (1999) have proposed that only four species should be kept in the genus *Chlorella* within the Trebouxiophyceae, i.e. *C. vulgaris*, *C. lobophora*, *C. sorokiniana* and *C. kessleri* (= *Parachlorella kessleri*). Since the description of the type species *Chlorella vulgaris*, more than a hundred ‘‘Chlorella’’ species have been established in the literature (Goers et al. 2010), but most have been assigned to other genera.

Extensive data on the sterols in *Chlorella* species have been published by Patterson and coworkers (Patterson 1967, 1969, 1974; Dickson et al. 1972; Dickson and Patterson 1973; Patterson et al. 1974, 1992). These data were collated and expanded by Holden and Patterson (1982) who analysed the sterols in 35 species. From these data they were able to group the algae into six categories according to their sterol distribution. Group Ia contained Δ^5 sterols including cholesterol, 24 β -ethylcholesta-5,22-dien-3 β -ol (poriferasterol), 24 β -methylcholesterol (5-ergosterol) and 24 β -ethylcholesterol (clionasterol), all with 24 β stereochemistry. The two isolates in Group 1B contained a related composition, but the major product was 24 β -methylcholesterol (69–73 % of total sterol) and significant amounts of the C₂₉ homolog of ergosterol, 24 β -ethylcholesta-5,7,22-trien-3 β -ol (7-dehydroporiferasterol), was also found. Group II contained Δ^7 sterols

presumably due to the lack of genes needed for the introduction of the Δ^5 double bond. The major sterol was the C_{29} sterol, 24 β -ethylcholesta-7,22-dien-3 β -ol (chondrillasterol). Group IIIa contained double bonds at both Δ^5 and Δ^7 presumably due to the lack of a Δ^7 reductase in some species. These isolates lack the ability to introduce a second alkyl group at C-24 resulting in the production, exclusively, of the C_{28} sterols ergosterol with lesser amounts of its mono- and diunsaturated derivatives. Group IIIb was similar, but these species are able to introduce a second alkyl group at C-24, producing the C_{29} homologue of ergosterol, 24 β -ethylcholesta-5,7,22-trien-3 β -ol, plus 24 β -ethyl-5 α -cholesta-7,22-dien-3 β -ol and 24 β -ethyl-5 α -cholest-7-en-3 β -ol (7-chondrillasterol). The presence of C_{29} sterols in ergosterol-synthesizing organisms is a rare occurrence. The last type of sterol biosynthetic pattern, Group IIIc, was encountered in only one strain of *Chlorella*. This contained unique Δ^8 and $\Delta^{8,22}$ sterols along with ergosterol, 24 β -methylcholesta-5,7-dien-3 β -ol (5,7-ergostadienol), and 24 β -methyl-5 α -cholest-7-en-3 β -ol (7-ergostenol) as originally published by Patterson et al. (1974). These groupings align well with the various stages in the sterol biosynthetic pathway and this would seem to be a useful way to categorize sterol patterns. However, the taxonomic status of some of these strains is uncertain and it remains to be seen whether they match closely with modern views on *Chlorella* taxonomy.

Chlorella autotrophica (now *Chlorella vulgaris* var. *autotrophica*) contains a complex mixture of C_{28} and C_{29} sterols with Δ^7 , $\Delta^{5,7}$ and $\Delta^{5,7,9(11)}$ nuclear double bond systems (Patterson et al. 1992). This alga also contained the rare tetraunsaturated sterols, 24-methylcholesta-5,7,9(11),22-tetraen-3 β -ol and 24-ethylcholesta-5,7,9(11),22-tetraen-3 β -ol.

Akihisa et al. (1992) identified ergosterol (51 %) and 7-dehydroporiferasterol (30 %), as the principal sterols of *Chlorella vulgaris*. They also identified the unusual 24 β -methyl- $\Delta^{9(11)}$ -sterols, 24 β -methyl-5 α -cholest-9(11)-en-3 β -ol and 14 α ,24 β -dimethyl-5 α -cholest-9(11)-en-3 β -ol, and the same two 24 β -alkyl- $\Delta^{5,7,9(11),22}$ -sterols, 24 β -methylcholesta-5,7,9(11),22E-tetraen-3 β -ol (9(11)-dehydroergosterol) and 24 β -ethylcholesta-5,7,9(11),22E-tetraen-3 β -ol found in *C. autotrophica*.

More recent work by Goers et al. (2010) has confirmed that sterol composition is a reliable chemotaxonomic marker within several groups of *Chlorella* and found high contents of ergosterol in nine species, all from the Chlorellaceae. More distant relatives within the Trebouxiophyceae or representatives of the Chlorophyceae did not contain ergosterol. The sterols in *Mucidosphaerium* (*Dictyosphaerium*) *pulchellum* were reported by Cranwell et al. (1990). The major sterol was cholesterol (90 %) with a small quantity of 24-ethylcholesterol (8 %).

5.6 Class Prasinophyceae

Prasinophytes can be important constituents of the phytoplankton in oceanic waters. Volkman et al. (1994) reported sterol compositions for *Pyramimonas cordata*, and *Pycnococcus provasolii* and found relatively simple distributions of Δ^5 -sterols. The major sterols were 24-methylenecholesterol (which also occurs in diatoms), 24-methylcholesterol and 24-ethylcholesta-5,24(28)Z-dien-3 β -ol (28-isofucosterol). They proposed that 24-methylcholesterol may be a useful marker for these microalgae. Minor amounts of C_{30} *n*-propylcholestane were produced after hydrogenation of the sterol mixture indicating the presence of C_{30} sterols that were not characterized.

Patterson et al. (1992) found that an un-named strain of *Pyramimonas* contained only 24-methylenecholesterol as a major sterol component (99 %) together with a trace of cholesterol. In marked contrast, *Pyramimonas grossii* contained a complex mixture of C_{28} and C_{29} sterols with Δ^7 , $\Delta^{5,7}$ and $\Delta^{5,7,9(11)}$ nuclear double bond systems. Sterols were found both with and without the C-22 side chain double bond; ergosterol and 24 β -ethylcholesta-5,7,22-trien-3 β -ol (7-dehydroporiferasterol) were the principal sterols. Rare tetraene sterols, 24-methylcholesta-5,7,9(11),22-tetraen-3 β -ol and 24-ethylcholesta-5,7,9(11),22-tetraen-3 β -ol were found in *P. grossii* as in *Chlorella vulgaris* var. *autotrophica* and *Dunaliella tertiolecta* (Patterson et al. 1992).

6 Sterols in the Phylum Charophyta

6.1 Class Conjugatophyceae

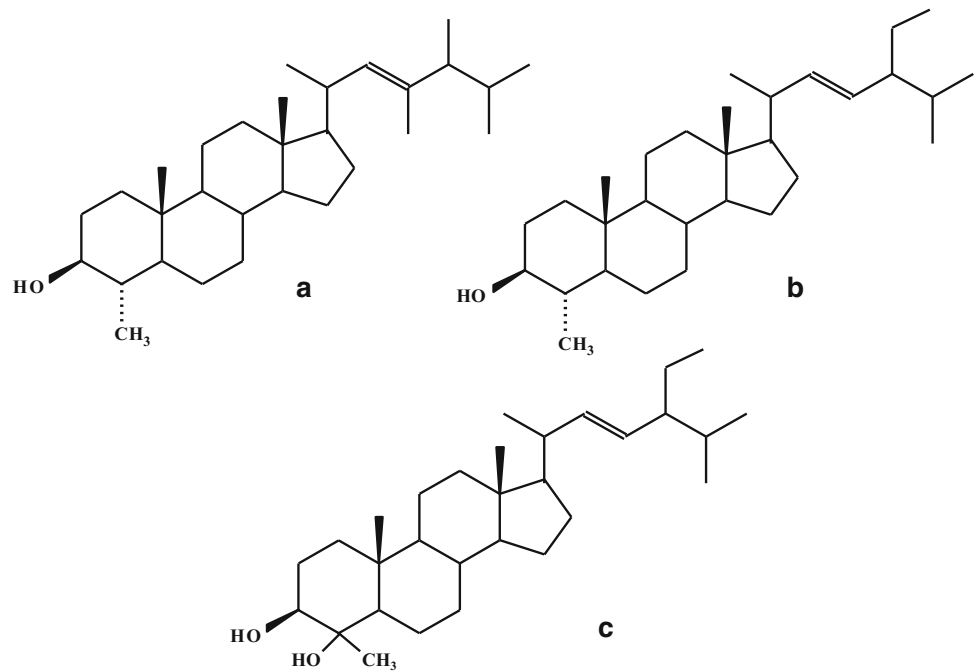
Few data are available for this class. Cranwell et al. (1990) analysed the sterols in the two desmids *Cosmarium bioculatum* and *Xanthidium subhastiferum*. Both contained three main sterols: 24-methylcholesterol, 24-ethylcholesterol and 24-ethylcholesta-5,22E-dien-3 β -ol, which are the same three sterols found in higher plants.

7 Sterols in the Phylum Dinophyta

7.1 Class Dinophyceae

Dinoflagellates (Dinophyceae) are generally considered to be the major source of 4-methyl sterols in aquatic environments (e.g. de Leeuw et al. 1983; Volkman 2003 and refs therein), but they are also potential contributors of 4-desmethyl sterols. In most species, 4-methyl sterols predominate (e.g. Leblond and Chapman 2002), but exceptions are known (e.g. Teshima et al. 1980). Complex mixtures of sterols are usually found (Withers et al. 1979; Wengrovitz

Fig. 5 Comparison of structures of two C_{30} sterols: (a) dinosterol ($4\alpha,23,24$ -trimethyl- 5α -cholest- $22E$ -en- 3β -ol) as found in many dinoflagellates and structurally isomeric 4α -methyl, 24 -ethyl- 5α -cholest- $22E$ -en- 3β -ol (b) as found in haptophytes from the *Pavlovo*phyceae. Also shown is the structure of a dihydroxylated 4 -methyl sterol termed a pavlovol (c) that appear to be unique to the *Pavlovo*phyceae



et al. 1981; Piretti et al. 1997; Amo et al. 2010), and many species contain unusual sterols having Δ^7 or Δ^8 double bonds (e.g. Hallegraeff et al. 1991) and unusual patterns of side-chain alkylation such as 23,24-dimethyl substitution (Leblond and Lasiter 2012 and refs therein).

The major sterol in many dinoflagellates is the 4-methyl sterol dinosterol ($4\alpha,23,24$ -trimethyl- 5α -cholest- $22E$ -en- 3β -ol; Fig. 5) which has no double bond in the ring system and an unusual 23,24-dimethyl side-chain alkylation (Shimizu et al. 1976; Boon et al. 1979). Because of its specificity as a dinoflagellate marker it has been widely used for paleoclimate studies (e.g. Boon et al. 1979; Makou et al. 2010; Castaneda et al. 2011). Methods to isolate purified dinosterol and other sterols from dinoflagellates for structural or isotopic analysis are now available using reverse phase and normal phase HPLC (Atwood and Sachs 2012). Although dinosterol appears to be a reliable biomarker for dinoflagellates, it should be noted that some dinoflagellates do not contain this sterol at all (Teshima et al. 1980; Kokke et al. 1981; Goad and Withers 1982).

Recently Leblond et al. (2010) published a review of the sterols and steroid ketones in dinoflagellates and related these to taxonomic assignments based on 18 rDNA phylogeny. These authors compiled a database from 102 published analyses of dinoflagellates which contained 58 distinct identified sterols and steroid ketones. Data on both sterols and 18S rDNA were available for 82 of the 102 strains. These data were clustered into six groups:

1. Mainly *Karenia* and *Karlodinium* species containing 4-desmethyl sterols with $\Delta^{8(14),22}$ diunsaturation such as $4\alpha,24$ -dimethyl- 5α -cholesta- $8(14),22$ -dien- 3β -ol and 27 -nor- $4\alpha,24$ -dimethyl- 5α -cholesta- $8(14),22$ -dien- 3β -ol;
2. Mainly *Amphidium* species containing 4α -methyl- 5α -cholest- $8(14)$ -en- 3β -ol and $4\alpha,24$ -dimethylcholesta- $8(14),24(28)$ -dien- 3β -ol. Note that both groups 1 and 2 are distinguished by sterols having a $\Delta^{8(14)}$ double bond which is uncommon in other dinoflagellates;
3. This grouping contained *Polarella glacialis*, *Protoceratium reticulatum*, *Lingulodinium polyedra* and *Gymnodinium simplex*. Major sterols were cholesta- $5,22Z$ -dien- 3β -ol, 24 -methylcholesta- $5,22E$ -dien- 3β -ol, and $4\alpha,24$ -dimethyl- 5α -cholestan- 3β -ol;
4. This contained *Akashiwo sanguinea* and prominent sterols 24 -methylcholest- 5α -cholest- $22E$ -en- 3β -ol, $23,24$ -dimethyl- 5α -cholest- $22E$ -en- 3β -ol and $4\alpha,24$ -dimethyl- 5α -cholestan- 3β -ol;
5. This contained genera such as *Alexandrium*, *Prorocentrum*, and *Symbiodinium*. Major sterols were cholesterol and $4\alpha,23,24$ -trimethyl- 5α -cholest- $22E$ -en- 3β -ol (dinosterol);
6. This contained *Alexandrium*, *Gymnodinium*, *Heterocapsa*, *Pfiesteria*, *Pyrocystis* and *Thoracosphaera*. Prominent sterols were $4\alpha,24$ -dimethyl- 5α -cholestan- 3β -ol, $4\alpha,23,24$ -trimethyl- 5α -cholestan- 3β -ol (dinostanol) and dinosterol.

Several of these groupings matched well with DNA-based phylogeny, but note that some genera occur in two groups as do some sterols. Volkman et al. (1999b) examined the sterols of four species of the dinoflagellate genus *Prorocentrum* and found over 20 sterols which varied considerably in abundance between the species. LeBlond et al. (2010) found that all *Prorocentrum* species were closely related by 18S rDNA analysis, but based on their sterols they were distributed in both clusters 5 and 6. All contained 23,24-dimethylcholesta-5,22E-dien-3 β -ol, dinosterol and dinostanol. Those in cluster 5 also produced cholesterol not found in cluster 6 while those in cluster 6 produced 24-methylenecholesterol not found in cluster 5. Similarly, *Pyrocystis lunula* and *Pyrocystis noctiluca* (= *Pyrocystis pseudonoctiluca*), although closely related by 18 S rDNA analysis had quite different sterol distributions with dinosterol abundant in *P. noctiluca* but absent from *P. lunula* (Dahmen and Leblond 2011).

Sterols containing a cyclopropyl ring in the side-chain such as gorgosterol are known from various marine animals, particularly coelenterates and a few dinoflagellates (Withers 1987). Some species of *Gonyaulax* contain large amounts of cholesterol but there does not appear to be a single distribution of 4-desmethyl sterols which characterizes these algae.

Karenia brevis has been shown (Leblond and Chapman 2002) to possess two major sterols, (24S)-4 α ,24-dimethyl-5 α -cholesta-8(14),22-dien-3 β -ol (abbreviated ED in their work) and its 27-nor derivative (NED). These novel structures are also found in *Karenia mikimotoi* and *Karlodinium micrum* (= *Karlodinium veneficum*), two dinoflagellates closely related to *K. brevis* (Leblond and Chapman 2002). They are also found as minor components of the more complex sterol profiles of other members of the *Gymnodinium/Pteridinium/Prorocentrum* (GPP) taxonomic group (Leblond and Chapman 2002).

A predominance of the 4-methyl and 4-desmethyl $\Delta^{8(14)}$ sterols and a lack of dinosterol was reported by Mooney et al. (2007) for species in the Karenaceae. Unusual sterols included 23-methyl-27-nor-24-methylcholesta-8(14),22-dien-3 β -ol (*Karenia papilionacea*, 59–66 %); 27-nor-(24R)-4 α ,24-dimethyl-5 α -cholesta-8(14),22-dien-3 β -ol (brevesterol; *Takayama tasmanica* 84 %, *Takayama helix* 71 %, *Karenia brevis* 45 %, *Karlodinium* sp.? 40 %, *Karenia mikimotoi* 38 %); and 4 α ,24-dimethyl-5 α -cholesta-8(14),22-dien-3 β -ol (gymnodinosterol; *K. mikimotoi* 48 %, *Karenia umbella* 59 %, *Karlodinium veneficum* 71–83 %). In *Takayama* species, five steroid ketones were identified, including for the first time the 3-keto form of brevesterol and gymnodinosterol. Steroid ketones have also been reported in *Scripsiella trochoidea* (Harvey et al. 1988), *Prorocentrum* spp. (Volkman et al. 1999b) and *Cryptothecodinium cohnii* (Withers et al. 1978).

8 Sterols in the Phylum Haptophyta

Haptophytes usually contain from one to five major sterols, and commonly cholesterol or 24-methylcholesta-5,22E-dien-3 β -ol predominates (Volkman et al. 1981). Moderate amounts of the C₂₉ sterols 24-ethylcholesterol and 24-ethylcholesta-5,22E-dien-3 β -ol are found in several species (Conte et al. 1994).

8.1 Class Coccolithophyceae

Well known examples of this class include the coccolithophorids *Emiliania huxleyi* and *Gephyrocapsa oceanica*. Both are major sources of organic matter in marine ecosystems and can form large blooms easily visible to satellites due to shedding of their coccolith scales. The sterol distributions of both species are very simple and are dominated (>90 %) by 24-methylcholesta-5,22E-dien-3 β -ol (Volkman et al. 1980b, 1995). The principal sterol in *Pleurochrysis carterae* and an unidentified haptophyte strain CCMP1215 was also shown to be 24-methylcholesta-5,22E-dien-3 β -ol by Ghosh et al. (1998). In *E. huxleyi*, the stereochemistry at C-24 has been shown to be 24 α (Maxwell et al. 1980) as found also in the sterols of *Pleurochrysis carterae* which contains stigmasterol (24 α -ethylcholesta-5,22E-dien-3 β -ol) and *epibrassicasterol* (Gladu et al. 1990). *Chrysolita lamellosa* (= *Ruttenra lamellosa*) also contains 24 α -methylcholesta-5,22E-dien-3 β -ol (*epibrassicasterol*) as well as significant amounts of Δ^5 - and $\Delta^{5,22}$ -C₂₉ sterols (Rontani et al. 2004). Goad et al. (1983) identified 24 α -methylcholesta-5,22E-dien-3 β -ol as the major sterol of the marine haptophyte *Isochrysis galbana*.

Phaeocystis pouchetii is a major phytoplankton species in polar oceans and a major food source for krill (Hamm et al. 2001). Its dominant sterol (93–100 %) is 24-methylcholesta-5,22E-dien-3 β -ol irrespective of culture age or life stage (Nichols et al. 1991). Small amounts of cholesterol (8 %) were found in one strain (A1-3) and strain DE10 contained 24-methylenecholesterol (Nichols et al. 1991). In marked contrast to the above results, Ghosh et al. (1998) showed that *Prymnesium parvum* contained only small amounts of sterols consisting solely of cholesterol.

Hymenosulphate, a novel sterol sulphate with Ca-releasing activity has been isolated from the cultured marine haptophyte *Hymenomonas* sp. by Kobayashi (1989).

8.2 Class Pavlovophyceae

A number of papers have reported the unusual sterol compositions of species in the genus *Pavlova*. Volkman et al. (1990) reported the presence of 4-methylsterols, 5 α (H)-stanols,

4-desmethyl sterols and unusual dihydroxylated sterols called pavlovols (Fig. 5). The major 4-desmethyl sterol in each of the species analysed by these authors was 24-ethylcholesta-5,22E-dien-3 β -ol which occurred with smaller amounts of 24-ethylcholesterol and in some species cholesterol. Two species also contained significant amounts of the 5 α (H)-stanol 24-ethyl-5 α -cholest-22E-en-3 β -ol. The major 4-methyl sterol was a C₃₀ sterol identified as 4 α -methyl-24-ethyl-5 α -cholest-22E-en-3 β -ol. This sterol has a similar structure to dinosterol, which occurs in dinoflagellates, except that the side-chain contains a 24-ethyl group rather than 23,24-dimethyl substitution (Fig. 5). Minor 4-methylsterols included 4,24-dimethyl-5 α -cholest-22E-en-3 β -ol and the fully saturated stanol 4,24-dimethylcholestanol.

Pavlovols have a second hydroxyl group at C-4 and a C-4 methyl group (Fig. 5) in the sterol ring system (e.g. Volkman et al. 1990; Gladu et al. 1991a; Patterson et al. 1992; Véron et al. 1996; Rauter et al. 2005) and have been proposed as a chemotaxonomic marker for this subgroup of haptophytes (Volkman et al. 1997). Mass spectra of the TMSi-ether derivatives of 4 α ,24-dimethyl-5 α -cholestan-3 β ,4 β -diol and 4 α -methyl,24-ethyl-5 α -cholestan-3 β ,4 β -diol found in *Pavlova pinguis* and 4 α ,24-dimethyl-5 α -cholest-22E-en-3 β ,4 β -diol found in *Diacronema vikianum* can be found in Volkman et al. (1997).

9 Sterols in the Phylum Euglenophyta

9.1 Class Euglenophyceae

This genus was established by Ehrenberg to accommodate those euglenoid organisms that have eyespots. *Euglena* (*Astasia*) *longa*, a natural mutant of *Euglena* that has lost all potential for photosynthesis contains cycloartenol metabolites indicative of biosynthesis by a plant-type mechanism (Anding et al. 1971; Rohmer and Brandt 1973) since lanosterol was not detected (Anding et al. 1971). Anding and Ourisson (1973) reported the presence of ergosterol in both light-grown and dark-grown *Euglena gracilis* and an unusual 4-methylsterol 4 α ,24-dimethyl-5 α -cholest-8(9)-en-3 β -ol has also been reported (Anding et al. 1971).

Brandt et al. (1970) found that free sterols predominate over bound sterols in light-grown green-coloured *E. gracilis* whereas in dark-grown white cells the reverse is true. The free sterols of green cells consist almost exclusively of Δ^7 -sterols (98 %) while in white cells Δ^5 -sterols make up 31 % of the sterols.

Zielinski et al. (1982) reported that the freshwater *Eutreptia viridis* contained 18 different sterols including a novel sterol with the rare Δ^{23} -unsaturation 24-ethylcholesta-5,7,23Z-trien-3 β -ol. The free sterols were dominated by $\Delta^{5,7}$ -diunsaturated sterols (ca. 80 %).

10 Sterols in the Phylum Cryptophyta

10.1 Class Cryptophyceae

The Cryptophyceae is a class of algae within the Pyrrhophyta in some systems of classification. Cryptomonads are aquatic unicellular eukaryotes that inhabit both marine and freshwater environments. Most cryptomonads are photosynthetic (and are thus also referred to as cryptophytes) and possess plastids that are very diverse in pigmentation. Cryptomonads are common in freshwater systems but can also be found in marine and brackish habitats. Each cell is around 10–50 μ m in size and flattened in shape, with typically two slightly unequal flagella.

Dunstan et al. (2005) examined the sterols of seven cryptophytes. The major sterol in *Rhodomonas* spp. (CS-215, CS-694), *Rhodomonas salina* (CS-174, CS-24), and *Proteomonas sulcata* (CS-412) was 24-methylcholesta-5,22E-dien-3 β -ol (91–99 % of total sterols) together with small amounts of cholesterol (1–2.7 %). *Rhodomonas maculata* (CS-85) had the same two sterols, but cholesterol was more abundant (17.7 %). *Chroomonas placoidea* (CS-200) contained in addition the C₂₉ sterol 24-ethylcholesta-5,22E-dien-3 β -ol (35.5 %).

11 Sterols in the Phylum Glaucophyta

11.1 Class Glaucophyceae

Glaucophytes (or Glaucocystophytes) are freshwater algae that have an almost intact cyanobacterium, referred to as a cyanelle, as the photosynthetic organelle. Heimann et al. (1997) reported the presence of “sitosterol” and an identified sterol in *Cyanophora paradoxa*. A more recent analysis by Leblond et al. (2011) found that *C. paradoxa* and *Glaucocystis nostochinearum* contained very simple sterol distributions consisting of sterols more typically found in higher plants: 24-methylcholesterol, 24-ethylcholesta-5,22E-dien-3 β -ol, and 24-ethylcholesterol.

12 Sterols in the Phylum Picozoa

Picoeukaryotes (defined as cells <3 μ m) are now known to be ubiquitous in surface waters of all oceans and are likely to be the most abundant eukaryotes in the sea. Most are phototrophic, but some are heterotrophic, especially in oligotrophic coastal sites. In 2007, a novel and widespread picoeukaryotic lineage with affinities to cryptophytes and katablepharids, the “picobiliphytes” was reported from 18S environmental clone library sequences (Not et al. 2007). Until the work of Seenivasan et al. (2013) these heterotrophs

(which may be related to glaucocystophytes) had remained uncultured. These authors described *Picomonas judraskeda* gen. et sp. nov., from marine coastal surface waters, and established a new phylum, Picozoa. No sterol data are available as yet from cultures of these ecologically important organisms.

13 Sterols in the Phylum Cercozoa

13.1 Class Chlorarachniophyceae

Chlorarachniophytes are marine unicellular algae that possess secondary plastids of green algal origin. Although chlorarachniophytes are a small group (the phylum of Chlorarachniophyta contains 14 species in 8 genera), they have variable and complex life cycles that include amoeboid, coccoid, and/or flagellate cells (Hirakawa et al. 2011). They are typically mixotrophic and photosynthetic and have the form of small amoebae, with branching cytoplasmic extensions that capture prey and connect the cells together. The amoeboid morphology may be the result of secondary endosymbiosis of a green alga by a nonphotosynthetic amoeba or amoeboflagellate. The only sterols present in genera *Bigelowiella*, *Gymnochlorella*, and *Lotharella* were identified as 24 α -methylcholesta-5,22E-dien-3 β -ol) and one of the epimeric pair poriferasterol/stigmasterol (24-ethylcholesta-5,22E-dien-3 β -ol) (Leblond et al. 2005).

14 Sterols in the Phylum Heterokontophyta (Ochrophyta)

The heterokonts (or stramenopiles) are chromists with chloroplasts surrounded by four membranes. There are more than 100,000 known species. Given the diversity of species present and wide variety of evolutionary paths involved (Leipe et al. 1994) it is not surprising that a great diversity of sterol patterns exist for this Phylum.

14.1 Diatom Classes

Diatoms are photosynthetic secondary endosymbionts found throughout marine and freshwater environments, and are believed to be responsible for around one-fifth of the primary productivity on Earth (Bowler et al. 2008 and refs therein). In spite of the fact that the pennate and centric lineages have only been diverging for 90 million years, their genome structures are dramatically different and a substantial fraction of genes (ca. 40 %) are not shared.

The sterols of diatoms have been the best studied of all the algal classes with data available for more than 100 species (e.g. Orcutt and Patterson 1975; Volkman 1986; Barrett et al. 1995; Rampen et al. 2009a, b, c, 2010). Rampen et al. (2010) analysed the sterols of over 100 diatom strains and detected 44 different sterols of which 11 were considered to be major sterols (i.e. >10 % of total sterols). Two-thirds of the species contained 24-methylenecholesterol, but 24 α -methylcholesta-5,22E-dien-3 β -ol (*epibrassicasterol*) which is often used as a diatom marker was only the fifth most common sterol. Cholesterol was abundant in a few species, but in *Amphora* species 24-ethylcholesta-5,22E-dien-3 β -ol predominates (Gladu et al. 1991b).

Some diatoms have been reported to contain large amounts of Δ^7 -unsaturated sterols (e.g. *Thassiosira pseudonana*, *Odontella (Biddulphia) aurita* and *Fragilaria* sp.; Orcutt and Patterson 1975), but this seems to be rare. Giner and Wikfors (2011) have recently reported Δ^7 sterols in *Ditylum brightwellii*.

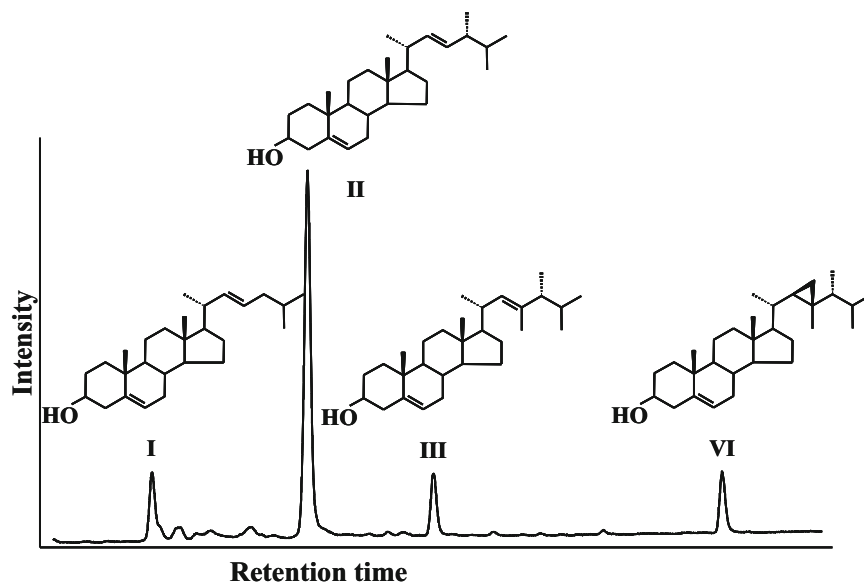
A few diatoms lack appreciable amounts of C-24 alkylated sterols. One example is *Biddulphia sinensis* (now *Odontella sinensis*) which contains mostly cholesta-5,22E-dien-3 β -ol (Volkman et al. 1980a). In contrast *Biddulphia aurita* (now *Odontella aurita*) analysed by Orcutt and Patterson (1975) contained none of this sterol and among the six identified sterols were the uncommon sterols 24-methylcholest-8(9)-en-3 β -ol (22.8 %) and 24-methyl-5 α -cholesta-7,22E-dien-3 β -ol (18 %).

One interesting report is the presence of the C₃₀ sterol gorgosterol in species from the genus *Delphineis* (Rampen et al. 2009c). Figure 6 shows a chromatogram of the sterols found in this alga. This sterol contains a cyclopropyl group in the side-chain which is more commonly associated with the sterols of jellyfish and highlights the diversity of sterol biosynthesis pathways developed in these microalgae. Giner and Wikfors (2011) have confirmed using NMR that the pennate diatom *Delphineis* sp. (CCMP 1095) contains gorgosterol, as well as the 27-nor C₂₇ sterol ocellasterol.

Volkman et al. (1993) showed that a strain of *Navicula* sp. (CS-146) contained small amounts of the dinoflagellate marker sterol dinosterol plus other 4-methyl sterols including 4 α ,24-dimethyl-5 α -cholest-22E-en-3 β -ol (9.7–12.6 %), 4 α ,24-dimethyl-5 α -cholestan-3 β -ol (2.0–3.4 %) and 4 α ,23,24-trimethyl-5 α -cholestan-3 β -ol (dinostanol or its C-23/C-24 epimer; 0.3–0.6 %). The 4-desmethyl sterol fraction included the common diatom sterol 24-methylcholesta-5,22E-dien-3 β -ol (20.2–30.5 %), but the major sterol was 24-ethylcholesterol (31.0–38.6 %) more usually associated with higher plants.

Sterols containing 23-methyl group rather than the more usual 24-methyl group are also found in diatoms. 23-Methylcholesta-5,22E-dien-3 β -ol was found in 14 out of 106 diatom cultures studied by Rampen et al. (2009a) thus

Fig. 6 Chromatogram showing the main sterols present in *Delphineis* sp. CCMP 1095. (I) Cholesta-5,22E-dien-3 β -ol; (II) 24-Methylcholesta-5,22E-dien-3 β -ol; (III) 23,24-Dimethylcholesta-5,22E-dien-3 β -ol; (IV) 22,23-Methylene-23,24-dimethylcholesta-5-en-3 β -ol (gorgosterol) with structures of the major sterols (From Rampen et al., 2009c)



confirming earlier reports of such sterols in diatoms (Barrett et al. 1995). These data confirm that this unusual pattern of side-chain alkylation is not restricted to the dinoflagellates. Rampen et al. (2009b) further showed that diatoms are a likely source of steroidal hydrocarbons containing 23,24-dimethyl alkylation in sediments and petroleum since their precursor 4-desmethyl-23,24-dimethyl sterols were present in 22 of the cultures they studied.

Giner and Wikfors (2011) re-examined the occurrence of sterols with 23,24-dimethyl side-chains in diatoms. The centric diatom *Triceratium dubium* (= *Biddulphia* sp. CCMP 147) contained a high proportion of 23-methylated sterols of which 23,24-dimethylcholesta-5,22E-dien-3 β -ol was 37.2 % of total sterols. They also showed that the sterol composition of *Ditylum brightwellii* (CCMP 358) is very complex and includes 5 α (H)-stanols and Δ^7 -sterols, in addition to the predominant Δ^5 -sterols. A pair of previously unknown sterols, 24-ethylcholesta-5,24,28-trien-3 β -ol and 24-ethylcholesta-24,28-dien-3 β -ol, were also detected and their structures determined by NMR and by synthesis of the former sterol derived from saringosterol. Also detected in *D. brightwellii* was the previously unknown 23-methyl-5 α -cholesta-7,22-dien-3 β -ol.

14.2 Class Eustigmatophyceae

Eustigmatophytes are a small group of coccoid microalgae. Most are freshwater or live in soils, with the main marine species represented by the genus *Nannochloropsis*. Their colour is distinctive due to the presence of the accessory pigments violaxanthin and β -carotene. Eustigmatophytes contain unusual long chain C₂₈–C₃₂ *n*-alkyl-1,15-diols, and the

corresponding hydroxy ketones (Volkman et al. 1992, 1999a; Méjanelle et al. 2003).

Sterol data for this algal class are rather limited. Volkman et al. (1999a) analysed three freshwater species *Eustigmatos vischeri*, *Vischeria helvetica* and *Vischeria punctata* and demonstrated that the sterol distributions consisted predominantly of 24-ethylcholesterol with small amounts of cholesterol, 24-methylcholesterol, 24-ethylcholesta-5,22E-dien-3 β -ol and isofucosterol.

The high proportion of C₂₉ sterols in freshwater species is in marked contrast to marine species from the genus *Nannochloropsis* which contain a dominance of cholesterol (Volkman et al. 1992; Patterson et al. 1994; Méjanelle et al. 2003). Volkman et al. (1992) studied the lipids of *N. oculata*, *N. salina* and an un-named species and found simple distributions of sterols dominated by cholesterol (>75 %) together with small amounts of the C₂₉ sterols 24-ethylcholesta-5,24(28)E-dien-3 β -ol (fucosterol) and 24-ethylcholesta-5,24(28)Z-dien-3 β -ol (isofucosterol). *N. salina* also contained 24-ethylcholesterol. The sterol composition of *N. gaditana* is similar with a predominance of cholesterol and lesser amounts of 24-ethylcholesterol (Méjanelle et al. 2003). Véron et al. (1996) showed that the sterols in *N. oculata* were mostly esterified rather than being present as free sterols.

The value of sterols as a chemotaxonomic tool was demonstrated by Gladu et al. (1995) who studied strain UTEX 2341 which had previously been identified as *Chlorella minutissima*. This strain contained cholesterol as the principal sterol along with 24-methylenecholesterol, fucosterol, and isofucosterol which was inconsistent with any of 35 *Chlorella* strains analyzed at that time. This finding and other data showed that the strain was actually a eustigmatophyte.

14.3 Class Synurophyceae

The Synurophyceae are keterokonts closely related to the Chrysophyceae. Both have a long “flimmer” flagellum and a short “whiplash” flagellum. Fucoxanthin is the main pigment responsible for their “golden brown” colouration. Synurophytes have bristles and scales which are taxon-specific. Silicified resting stages called stomatocysts are unique to the Chrysophyceae and Synurophyceae and may be preserved in sediments facilitating their use as palaeoenvironmental indicators. The oldest silicified scales and bristles are found in Middle Eocene freshwater sediments from northwest Canada, but the oldest stomatocysts have been found in Early Cretaceous marine sediments from the Southern Ocean.

An early study by Collins and Kalnins (1969) of the sterols in *Synura petersenii* (then assigned to the Chrysophyta) identified only two sterols identified as cholesterol and sitosterol.

14.4 Class Chrysophyceae

Chrysophytes have been subject to considerable revision with many species moved to other classes such as the closely related Synurophyceae and Pelagophyceae (Jordan and Iwataki 2012). One of the first lipid studies of *Ochromonas danica* was by Halevy et al. (1966) who found four sterols and identified the C₂₈ sterol ergosterol and C₂₉ sterol 24-ethylcholesta-5,22E-dien-3 β -ol (reported as the 24 α isomer stigmaterol). Further work on *Ochromonas danica* and *Poteriochromonas (Ochromonas) malhamensis* was carried out by Gershengorn et al. (1968) who found cycloartenol and 24-methylenecycloartanol but no lanosterol thus providing evidence of the plant-type biosynthetic pathway used by these algae (Fig. 2). The C₂₉ sterol 24 β -ethylcholesta-5,22E-dien-3 β -ol (poriferasterol) was identified as the major sterol. Melting points were used to define the stereochemistry as 24 β which is opposite to the 24 α stereochemistry implied by the identification of stigmaterol by Halevy et al. (1966). The C₂₈ sterol 24 β -methylcholesta-5,22E-dien-3 β -ol (brassicasterol) has been identified in an unidentified species assigned to the order Sarcinochrysidales (Chrysophyceae) (Rohmer et al. 1980; Kokke et al. 1984).

The sterols of *Ochromonas danica* have been identified as ergosterol, brassicasterol, 22-dihydrobrassicasterol, clionasterol, poriferasterol, and probably 7-dehydroporiferasterol. By contrast *Poteriochromonas malhamensis* contains only poriferasterol as the major sterol component.

Poteriochromonas (Ochromonas) malhamensis was one of the first species used to probe the biosynthetic pathways occurring in microalgae. For example, Knapp et al. (1971) were able to show that the three 24-ethylidene sterols fucos-

terol, iso-fucosterol and 24-ethyl-5 α -cholesta-7,24(28)-dien-3 β -ol could all be transformed to poriferasterol, by *P. malhamensis*, but with varying degrees of efficiency,

Billard et al. (1990) showed that the C₂₉ sterols 24-ethylcholesta-5,22E-dien-3 β -ol, 24-ethylcholesterol and fucosterol predominated in the genera *Chrydoderma*, *Chrysowaernella*, *Chrysomeris* and *Giraudyopsis*. The status of the first genus is uncertain, but the latter three are now classified in the class Chrysomerophyceae. This is in marked contrast to the sterols found in *Sarcinochrysis* and *Nematochryopsis* which contain the C₃₀ sterol 24-n-propylidenecholesterol (previously assigned to the Chrysophyceae, but now considered to be part of the Pelagophyceae).

A paleoenvironmental application by Soma et al. (2007) linked the profile of 24-ethylcholesta-5,22E-dien-3 β -ol in steryl chlorin esters (SCEs) in sediments of Lake Baikal over the past 28,000 years with the reported distribution of chrysophyte cysts during the Holocene.

14.5 Class Chrysomerophyceae

Chrysomeris ramosa contains 24-ethylcholesterol as the major sterol together with 24-ethylcholesta-5,22E-dien-3 β -ol, 24-methylcholesterol and cholesterol (Billard et al. 1990), but percentage data were not provided. *Giraudyopsis stellifer* also contained 24-ethylcholesterol as the major sterol, but other sterols included fucosterol, 24-ethylcholesta-5,22E-dien-3 β -ol, 24-methylenecholesterol, 24-methylcholesta-5,22E-dien-3 β -ol and cholesterol (Billard et al. 1990).

14.6 Class Pelagophyceae

These small unicellular algae were formerly grouped with Chrysophyceae and are classified into two orders: Pelagomonadales and Sarcinochrysidales (Giner et al. 2009). These are the only algae known to date that synthesize the C₃₀ sterol 24-propylidene cholesterol. In a comprehensive study, Giner et al. (2009) analysed the sterol compositions of 42 strains of pelagophyte algae including 17 strains of *Aureococcus anophagefferens* using a combination of GC and HPLC techniques. ¹H-NMR data were obtained for 17 strains. All strains analyzed contained 24-propylidenecholesterol. All strains from the order Sarcinochrysidales contained the (24E)-isomer, while all strains in the order Pelagomonadales contained the (24Z)-isomer, either alone or together with the (24E)-isomer. The occurrence of Δ^{22} and 24 α -sterols was limited to the Sarcinochrysidales. The first occurrence of 24-n-propylcholesta-5,22E-dien-3 β -ol in an alga, CCMP

1410, was reported. Traces of the rare sterol 26,26-dimethyl-24-methylenecholesterol were detected in *Aureococcus anophagefferens*, and the (25R)-configuration was proposed, based on biosynthetic considerations. Traces of a novel sterol, 24-propylidenecholesta-5,25-dien-3 β -ol, were detected in several species. Raederstorff and Rohmer (1984) analysed the sterols of *Nematochryopsis roscoffensis* (= *N. marina*) and also found the C₃₀ sterols 24(E)-24-*n*-propylidenecholesterol and 24-*n*-propylcholesterol.

14.7 Class Xanthophyceae

There are about 600 species and 100 families of Xanthophyceae, but most are very rare. Common genera include *Botrydium*, *Tribonema* and *Vaucheria*. These yellow-green microalgae are mostly found in fresh water or wet soil, but a few are marine. They can be confused with green algae because of their pigmentation, but they are actually secondary endosymbionts that evolved from protists that engulfed a microalga and assimilated its chloroplasts. Most xanthophytes are coccoid or filamentous, but some are siphonous. 18S ribosomal RNA gene analysis indicates that the Xanthophyceae is most closely related to the Phaeophyceae and that the class may be paraphyletic (Potter et al. 1997).

Mercer et al. (1974) studied the sterols of *Botrydium granulatum*, *Tribonema aequale* and *Monodus subterraneus* (= *Monodus subterranea*). In each case, the major sterols were cholesterol and 24 β -ethylcholesterol (clionasterol), and their proportions did not vary with age of the cultures. Small amounts of cycloartenol and 24-methylenecycloartanol were also found in all three algae. The biosynthesis of clionasterol in *M. subterranea* was studied by Mercer and Harries (1975). Clionasterol grown in the presence of labelled methionine showed the participation of a 24-ethylidene sterol intermediate in its biosynthesis. Data from cells incubated with labelled mevalonic acid showed that the 24-ethylidene sterol intermediate is reduced directly to clionasterol and not isomerized to a Δ^{24} -sterol which is then reduced.

14.8 Class Dictyochophyceae

Dictyocha is the generic name for all extant silicoflagellates, or for species in which the basket-shaped skeleton consists of a single ring with several connecting bars (Kristiansen and Preisig 2001). Few data are available for these microalgae. Patterson and Van Valkenburg (1990) analysed the sterols from cultured *Dictyocha fibula* and found only 4-methyl-cholesta-5,22E-dien-3 β -ol and 24-methylenecholesterol.

14.9 Class Raphidophyceae

Marine and freshwater raphidophytes form a monophyletic group and 18S ribosomal RNA gene sequences suggest that the Raphidophyceae is a sister taxon to the Phaeophyceae-Xanthophyceae clades, but the bootstrap value was only 40 % (Potter et al. 1997). These microalgae are of interest for their propensity to form harmful algal blooms in both marine (brown pigmented species) and freshwater (green pigmented species) environments. The lipids of Raphidophyceae microalgae still remain understudied and the taxonomy is not clearly established. Fatty acids and pigments have been used as chemotaxonomic markers for these algae (Mostaert et al. 1998) as well as sterols (Nichols et al. 1987; Patterson and Van Valkenburg 1990; Marshall et al. 2002; Giner et al. 2008; Leblond et al. 2013).

Nichols et al. (1987) reported data on the sterols and fatty acids of the red tide flagellates *Heterosigma akashiwo* and *Chattonella antiqua* (= *Chattonella marina* var. *antiqua*) and this work was expanded by Marshall et al. (2002) who reported the sterols and fatty acids in six marine raphidophyte algae. The dominant sterol in *Chattonella* spp., *H. akashiwo* and *Fibrocapsa japonica* was identified as 24-ethylcholesterol, but the configuration at C-24 was not specified at that time (Nichols et al. 1987; Marshall et al. 2002); a reinvestigation of the sterols of *C. marina* using ¹H NMR spectrometry indicated the presence of the 24 α -configuration (sitosterol) (Giner et al. 2008). The *Chattonella* species also contained small amounts of the uncommon sterol cholest-8(9)-en-3 β -ol (1.8–5 %). *F. japonica* contained the highest proportion of 24-ethylcholesterol (84–92 %) with small amounts of cholesterol (5–11 %) and isofucosterol (24-ethylcholesta-5,24(28)Z-dien-3 β -ol; ca. 2 %; Marshall et al. 2002).

Giner et al. (2008) also analysed the sterols and fatty acid compositions of three harmful algal species previously classified in the genus *Chattonella* (Raphidophyceae). These were “*Chloromorom toxicum*” (ex a North American strain identified originally as *Chattonella* cf. *verruculosa*, and probably a member of the Raphidophyceae; Edvardsen et al. 2007), *Verrucophora farcimen* (= *Pseudochattonella farcimen*; Dictyochophyceae), and *Verrucophora verruculosa* (= *Pseudochattonella verruculosa*; Dictyochophyceae). *C. toxicum* contained the 24 β -ethyl sterols, poriferasterol (24 β -ethylcholesta-5,22E-dien-3 β -ol) and clionasterol (24 β -ethylcholesterol), as its major sterols. In contrast, the stereochemistry of the 24-ethyl sterols of *Chattonella marina* and *Heterosigma akashiwo*, was determined to be 24 α and 24 β , respectively (Giner et al. 2008). Both *Pseudochattonella* (*Verrucophora*) strains contained the 27-nor C₂₇ sterol ocellasterol as the only detected sterol.

Patterson and Van Valkenburg (1990) reported that *Olisthodiscus luteus* contained 24-ethylcholesterol,

24-ethylcholestanol, 24-methylcholesterol and cholesterol. The presence of a fully saturated stanol in microalgae is unusual. The only sterol analysis of a freshwater species is by Leblond et al. (2013) who reported sterol compositions for 21 isolates of the green-pigmented, raphidophyte *Gonyostomum semen* from Scandinavian lakes. All contained the C₂₉ sterols 24-ethylcholesta-5,22E-dien-3 β -ol and 24-ethylcholesterol as major components together with smaller amounts of 24-methylcholesterol. The same three sterols occur in higher plants.

15 Conclusions

The published literature on the sterol compositions of microalgae continues to increase but it is still far from comprehensive. Extensive data are now available for green algae, diatoms and dinoflagellates from which it has been possible to assemble groupings of species containing common sterol distributions. In some cases these align with taxonomic groupings but in others species that are only distantly related can have similar compositions. Unfortunately, sterol data for some algal classes are still restricted to just a handful of species and some supposed characteristics may be challenged as additional compositional data are obtained. It is apparent that some earlier sterol identifications are now known to be in error (particularly with regard to C-24 stereochemistry) and many sterols are more widely distributed than had been thought. This should not be a surprise since sterol biosynthesis is an ancient microalgal trait and variations in biosynthesis are to be expected over such geologically long time periods. As sequence data become more readily available it will be useful to test these sterol groupings against taxonomies defined from genetic data and to discover what changes in genetic makeup are responsible for variations in sterol compositions.

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