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LIPID COMPOSITION OF THE MARINE DINOFLAGELLATE *SCRIPPSIELLA TROCHOIDEA*

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Key Word Index—*Scrippsiella trochoidea*; Dinophyceae; dinoflagellates; lipids; sterols; fatty acids; 3-ketosteroids; hydrocarbons; taxonomy.

Abstract—Cellular lipid concentrations of fatty acids, sterols, alcohols, hydrocarbons and 3-keto steroids were determined in laboratory cultures of the marine dinoflagellate *Scrippsiella trochoidea*. Fatty acids comprise 90% (180 pg/cell) of the total lipids, with C_{14} to C_{26} chain lengths present. Polyunsaturated fatty acids account for a substantial portion of the total fatty acids (30%) and include C_{18}, C_{20} and C_{22} acids. A complex distribution of sterols were present with a total of 25 4-methyl and 4-desmethyl sterols in greater than 0.2% relative abundance. The 15 4-methyl sterols encountered account for 83.7% of total sterols present with saturated species as well as nuclear unsaturation at Δ^5, Δ^7 and $\Delta^{8(14)}$ positions represented. Side chain alkylations among 4α-methyl sterols include C-23 methyl, and C-23, C-24 dimethyl substituents in addition to unsaturation sites at the Δ^{22} and $\Delta^{17(20)}$ positions. Dinostanol (4α, 23*R*, 24*R*-trimethyl-5α-cholestan-3β-ol) was the most abundant sterol and accounted for 27.7% of the total sterols. Twenty-one 3-keto steroids were tentatively identified based on mass spectral data, the most abundant being the 23*S* and 23*R* isomers of dinostanone (4α,23*S*,24*R*-trimethylcholestan-3-one and 4α,23*R*,24*R*-trimethylcholestan-3-one) which together account for almost 50% of the total ketones present. The distribution of 3-keto steroids was highly correlated with that of the sterols. The biosynthetic and biogeochemical implications of the lipid distribution are discussed.

INTRODUCTION

Dinoflagellates represent a major component of the marine phytoplankton and their production forms the basis of the food web in many marine environments. The classification of dinoflagellates is often based on cell morphology, pigment composition, storage products and cell division studies. For chemotaxonomic studies lipids are of particular interest and a number of detailed analyses have demonstrated their utility as a classification guide [for review see 1]. For example, the Dinophyceae differ from other marine and lacustrine algae in that their sterols are dominated by 4α-methyl sterols, including dinosterol (4α,23,24-trimethylcholest-22-en-3β-ol) which is present in almost all the species examined [1–7], and thus allows a rapid indication of taxonomic position. As a unique 'biological marker', dinosterol has also been of considerable interest for organic geochemical studies as evidence for the contribution of dinoflagellates to sedimentary organic matter in several areas [8, 9].

Scrippsiella trochoidea (Peridinales, Peridiniaceae) is a widely distributed dinoflagellate of temperate marine waters surrounding the British Isles [10] and elsewhere [11]. This organism was originally described as *Glenodinium trochoidea* by Stein in 1883, but was later transferred to the genus *Peridinium* by Lemmerman [12]. Using chromosomal numbers, cell division studies and scanning electron microscopy, the genus was reassigned as *S. trochoidea* (Stein) by Loeblich in 1976 [12]. Additional chemotaxonomic information based on lipid distributions would aid in the classification of dinoflagellates as well as other algae.

For *S. trochoidea*, previous lipid analyses have been restricted to the fatty acids [13, 14], which appear to be of limited use as a taxonomic guide for the Dinophyceae. More recently, however, fatty alcohols and sterols as well as fatty acids have been described in a single culture of this organism used in a zooplankton feeding study [15]. Herein we report the detailed analysis of fatty acids, alcohols, hydrocarbons, sterols and steroidal ketones in four replicate cultures of *S. trochoidea*. The identification of steroidal ketones in this alga is novel and, in addition to its use in chemotaxonomy it is important on both biosynthetic and biogeochemical grounds. Thus, the relationship between steroidal ketones and total sterol distribution can provide insight into the biosynthesis of sterols in dinoflagellates and other alga. Moreover, as steroidal ketones have also been recognized as possible intermediates during the diagenesis of sterols in both sedimenting particulate matter [16] and marine sediments [17], their origin and distribution in phytoplankton is of importance for geochemical studies.

Concentration of lipid classes

Total lipids were extracted ultrasonically in CH_2Cl_2–MeOH from four replicate cultures of washed cells harvested at late log phase. Cellular concentrations and relative abundance of total (free and esterified) lipids

Table 1. Lipid class distribution in *Scrippsiella trochoidea*

Lipid class	Cellular concentration* pg/cell	Relative abundance (%)
Total lipids†	199.3	—
Fatty acids	180.0	90.3
Sterols	16.5	8.3
Alcohols	1.8	0.9
Steroidal ketones	0.6	0.3
Hydrocarbon	0.5	0.2

*Quantification of lipids is based on FID response by GC.
†Sum of individual lipid classes.

were then determined for each class and are shown in Table 1. The lipid concentration for all cells averaged 199 pg/cell, with fatty acids accounting for over 90% of the total solvent extractable lipids. The fatty acid:sterol ratio (10.9) is similar to that reported for marine dinoflagellates [6, 18, 19] and is substantially lower than that found in other marine alga such as diatoms (54:1) [20]. As noted in ref. [6], such low ratios appear to be a consequence of the higher sterol concentrations in dinoflagellates compared with those of other algal groups [21], and lend support to the hypothesis that they are quantitatively important contributors of sterols to marine sediments even in areas where they may not be the most numerically abundant phytoplankton. Alcohols, hydrocarbons and 3-keto steroids account for a smaller proportion of the total lipids and are discussed below.

Fatty acids

S. trochoidea contains a typical distribution of C_{14} to C_{26} fatty acids, with 16:0 as the dominant species (25.7%) (Table 2). Lesser amounts of 14:0 (10.3%) and $18{:}1\Delta^9$ (15.6%) are also present. Polyunsaturated fatty acids (PUFA) account for a substantial portion of the total fatty acids (30.6%) and include C_{18}, C_{20} and C_{22} chain lengths. Both Δ^9 and Δ^{11} isomers of 16:1, 18:1 and 20:1 occur with concentrations of the Δ^9 isomer equal to or greater than the Δ^{11} isomer in all cases. Small amounts of several odd chain acids also appear and include both the *iso* and *anteiso* branched C_{15} and C_{17} acids. In several studies utilizing sediment fatty acid profiles as indicators of the resident biota, the appearance of branched chain acids and the monoenoic $18{:}1\Delta^{11}$ have been suggested as bacterial in origin, [22, 23]. There are, however, several reports of such acids in higher organisms including other algae [24] and dinoflagellates [6]. While the culture conditions employed in this study cannot be considered axenic, all cultures were grown in 0.2 μm filtered and autoclaved seawater; bacterial numbers were low as monitored by epifluoresence microscopy [25] and contributed <0.5% to biomass by volume. Moreover, analysis of several replicate cultures showed good agreement between short branched chain and $18{:}1\Delta^{11}$ acid concentrations and the cellular abundance of other lipid classes. While bacterial sources cannot be excluded, the major input appears to originate from the alga.

Table 2. Distribution of total fatty acids and alcohols in *Scrippsiella trochoidea*

Fatty acid methyl ester	Relative percentage	(s.d.)
14:1	0.2	(0.1)
14:0	10.3	(2.2)
15:1	Tr*	—
15:0 *iso*	0.2	(0.0)
15:0 *anteiso*	0.2	(0.1)
15:0	0.2	(0.1)
$16{:}1\Delta^9$	3.1	(0.4)
$16{:}1\Delta^{11}$	0.7	(0.1)
16:0	25.7	(3.0)
17:0 *iso*	Tr	—
17:0 *anteiso*	0.2	(0.0)
17:0	0.1	(0.0)
$18{:}6^?+{:}5\Delta^3$	3.5	(0.9)
$18{:}4\Delta^5$	8.1	(2.1)
$18{:}3\Delta^9$	2.7	(0.0)
$18{:}2\Delta^9$	2.6	(0.1)
$18{:}1\Delta^9$	15.6	(2.9)
$18{:}1\Delta^{11}$	6.4	(1.4)
18:0	5.3	(1.0)
$20{:}5\Delta^5$	1.7	(0.6)
$20{:}1\Delta^9$	0.1	(0.0)
$20{:}1\Delta^{11}$	0.1	(0.0)
20:0	0.3	(0.1)
$22{:}6\Delta^3$	12.5	(1.2)
24:0	0.3	(0.1)
Total	100.0	(16.4)
Total PUFA†		30.6
Total monoenoic acids		18.3
Total saturated acids		30.6
Total alcohols		
16:0	0.6	(0.3)
18:0 *iso*	0.8	(0.1)
18:0 *anteiso*	0.6	(0.2)
18:0	0.7	(0.1)
Phytol	94.8	(9.2)
20:0 *br*	0.8	(0.3)
20:0	0.6	(0.2)
21:0	0.4	(0.1)
Total	100.0	(10.5)

*Tr, trace = <0.1%.
†PUFA, Polyunsaturated fatty acid with ≥2 double bonds. Standard deviation (s.d.) is based on the analysis of four cultures.
?Double bond position could not be determined due to coelution with the 18:5. Estimated ratio of 18:5 to 18:6 = 7:3.

Alcohols and hydrocarbons

The isoprenoid 3,7,11,15-tetramethylhexadec-2-enol (phytol) is the predominant alcohol observed in saponified extracts and represents 94.8% of the total alcohols

(Table 2). Small amounts of several branched chain species constitute the remaining alcohols and include both *iso* and *anteiso* forms of the C_{18} alcohol. Although the presence of alcohols suggested the possibility of wax esters, none could be detected by TLC or GC of unhydrolysed extracts. Only a single hydrocarbon, the polyunsaturated 21:6 (heneicosahexaene), was found at concentrations equivalent to 0.46 pg/cell (Table 1). Although this lipid appears in substantial amounts in many photosynthetic dinoflagellates [26] it is also common to a number of other planktonic and benthic algae [27] and thus appears to have little diagnostic specificity for dinoflagellates as a group.

Sterols and steroidal ketones

The sterol distribution in *S. trochoidea* is complex and contains a total of 25 components in greater than 0.1% relative abundance (Table 3). 4-Methyl sterols account for the greatest number of biosynthetically related sterols (a total of 15) and comprise 83.7% of the total sterols. Included are saturated species as well as nuclear unsaturation at Δ^5, Δ^7 and $\Delta^{8(14)}$ positions. Side chain alkylations among 4α-methyl sterols include the 23 methyl and the 23, 24 dimethyl substituents in addition to unsaturation sites at either Δ^{22} or $\Delta^{17(20)}$. The most abundant sterol is 4α,23*R*,24*R*-trimethyl-5α-cholestan-3β-ol (dinostanol) accounting for 27.7% of the total sterols. This stanol was also found to be the most abundant component in *Gonyaulax polygramma* [7]. The presence of significant amounts of its likely precursor, dinosterol (4α,23,24-trimethylcholest-22-en-3β-ol) (7.5%), is in agreement with its occurrence in other species of dinoflagellates [2, 4, 6, 21, 28,] and corroborates the inclusion of *S. trochoidea* in the group Dinophyceae. Other abundant 4-methyl sterols include 4α,24-dimethylcholestan-3β-ol (8.4%), 4α-methylcholesta-8(14),22-dien-3β-ol (9.6%), 4α,24-dimethylcholesta-8(14),22-dien-3β-ol (7.2%) and 4α,23*S*,24*R*-trimethyl-5α-cholestan-3β-ol (5.2%).

Among the 4-methyl sterols is peridinosterol (4α,23,24-trimethylcholest-17(20)-en-3β-ol) which was first isolated from the dinoflagellate *Peridinium* (i.e. *Glenodinium*; [11]) *foliaceum* by Withers *et al.* [29]. More recently, peridinosterol has been observed in lacustrine sediments in which organic inputs are presumed to be from the dinoflagellate *Peridinum cinctum* fa. westii (Lemm.) [30]. Two possible origins for the formation of the unusual $\Delta^{17(20)}$ double bond have been suggested [31], either (i) via double bond

Table 3. Total free and esterified 3-β sterols of *Scrippsiella trochoidea*

Peak no.	RR_t	Identification	Abundance (%)	(s.d.)	[M]†* *m/z*
1	1.00	Cholest-5-en-3β-ol	3.2	(1.1)	458
2	1.03	5α-Cholestan-3β-ol	0.6	(0.3)	460
3	1.06	27-Nor-24 methyl-5α-cholestan-3β-ol	0.4	(0.0)	460
4	1.14	4α-Methylcholesta-8(14),22-dien-3β-ol	9.6	(0.8)	470
5	1.16	4α-Methylcholest-22-en-3β-ol	0.6	(0.1)	472
6	1.19	Cholest-7-en-3β-ol†			458
7	—	24-Methyl-5α-cholest-22-en-3β-ol‡	1.5	(0.1)	472
8	1.26	4α-Methylcholest-8(14)-en-3β-ol	0.3	(0.0)	472
9	1.32	4α-Methylcholestan-3β-ol	0.6	(0.2)	474
10	1.34	24-Methylcholest-8(14)-en-3β-ol	1.6	(0.6)	472
11	1.39	24-Methyl-5α-cholestan-3β-ol	3.4	(0.6)	474
12	1.47	4α,24-Dimethylcholesta-8(14),22-dien-3β-ol	7.2	(0.1)	484
13	1.51	4α,24-Dimethyl-5α-cholest-22-en-3β-ol	3.4	(0.0)	486
14	1.55	24-Methylcholest-7-en-3β-ol	3.6	(0.0)	472
15	1.59	C 29 Stenol	0.9	(0.0)	486
16	1.62	C 29 Stenol (dien)	1.1	(0.2)	484
17	1.66	4α,24-Dimethylcholest-8(14)-en-3β-ol	4.7	(0.6)	486
18	1.71	4α,24-Dimethyl-5α-cholestan-3β-ol	8.4	(0.4)	488
19	1.75	4α,23,24-Trimethylcholesta-5,22-dien-3β-ol	0.8	(0.2)	498
20	1.79	4α,23,24-Trimethylcholest-22-en-3β-ol	7.5	(0.1)	500
21	1.82	4α,24-Dimethylcholest-7-en-3β-ol	0.4	(0.1)	486
22	1.90	4α,23,24-Trimethylcholest-17(20)-en-3β-ol	4.1	(0.1)	500
23	1.93	4α,23,24-Trimethycholest-8(14)-en-3β-ol	3.2	(0.3)	500
24	1.98	4α,23*S*,24*R*-Trimethyl-5α(H)-cholestan-3β-ol	5.2	(0.1)	502
25	2.04	4α,23*R*,24*R*-Trimethyl-5α(H)-cholestan-3β-ol	27.7	(2.0)	502
Total			100.0	(8.2)	
Total 4-methyl sterols			83.7%		
Total 4-desmethyl sterols			16.3%		

†RR_t, relative retention time as the TMSi ether; cholesterol = 1.00, dinosterol = 1.79.

*$[M]^+$ shown for the TMSi ether.

‡Coeluting peaks—relative percent equals sum of both components.

Standard deviation (s.d.) is based on the analysis of four cultures.

Peaks in order of elution on a 25 m methyl silicone capillary column (OV-1), 0.31 mm id, 0.17 μm film thickness.

migration from a Δ^{22} precursor or (ii) through a cyclopropyl precursor such as demethylgorgosterol (22,23-methylene-4α,24-dimethylcholest-5-en-3β-ol) which is initially isomerized to the $\Delta^{20(22)}$ 23,24-dimethyl intermediate with subsequent double bond migration to the $\Delta^{17(20)}$. Our inability to detect the $\Delta^{20(22)}$ 23,24 dimethyl intermediate or any sterols having the 22,23 methylene structure, together with the prevalence of possible Δ^{22} precursors (e.g. dinosterol), suggest that double bond migration is more likely in *S. trochoidea*.

Although sterols in which the methyl group at C-4 is absent (4-desmethyl) comprise only 16.3% of the total sterols in *S. trochoidea*, the 10 4-desmethyl sterols have a diversity of molecular species and a range of nuclear and side chain modifications comparable with that of the 4-methyl sterols. 24-methylcholest-7-en-3β-ol is the most abundant 4-desmethyl sterol (3.6% of total sterols, Table 3) with lesser amounts of the 24-methylcholestan-3β-ol (3.4%), cholest-5-en-3β-ol (3.2%) and 24-methylcholesta-8(14),22-en-3β-ol (1.6%). Small amounts of 5α-cholestan-3β-ol, 27-nor-24-methyl-5α-cholestan-3β-ol, cholest-7-en-3β-ol and 24-methyl-5α-cholest-22-en-3β-ol were also present. The most unusual of these is the 27-nor sterol which is uncommon in marine algae. Although several nor-sterols have been previously observed in *Gymnodinium simplex* [28], to our knowledge this is the first report of the saturated species in a dinoflagellate. While the biosynthetic origin of this sterol is unknown, both *de novo* synthesis or demethylation have been considered as possible pathways [28]. In *S. trochoidea* the most likely precursor for demethylation, the 24-methyl-5α-cholestan-3β-ol, is present in significant amounts. Two minor C-29 desmethyl sterols could not be fully identified.

Sterols possessing highly alkylated side chains, and in particular the 23,24-dimethyl substitution are prevalent in *S. trochoidea* as in many dinoflagellates [e.g. 1]. Using evidence from labelling studies, Withers *et al.* [29] have suggested that formation of the 23,24 dimethyl side chain found in sterols 19, 20, 22–25 proceeds via a 24-methylene → 24-methyl → Δ^{22}, 24-methyl → Δ^{22},23,24-dimethyl sequence. An alternative view has been suggested by Djerassi [32] after sterols were isolated which contained the Δ^{22},23-monomethyl side chain. In this mechanism, bioalkylation at C-23 could occur prior to methylation at C-24 without requiring the C-24 methylene intermediate. In our cultures, however, no sterols containing the Δ^{22},23-methyl side chain or other methylated intermediates were observed to substantiate this sequence. From these observations, it appears that sterol biosynthesis in *S. trochoidea* shows a general agreement with the sequence of side chain alkylation proposed by Withers [29]. Although a key intermediate in the scheme, 24-methylene cholesterol, was not observed, it may not accumulate in the alga at sufficient quantities for detection. The presence of a number of nonalkylated Δ^{22} bonds among the *S. trochoidea* sterols, however, does suggest that Δ^{22} bonds may be easily introduced without requiring methyl additions as has been postulated elsewhere [32]. Such a duality of side chain synthesis and modification would account for the abundance of nonalkylated Δ^{22} side chains (sterols 4,7) in *S. trochoidea*, and the presence of 24- and 23,24-dimethyl substitutions. Alternatively, biomethylation could proceed directly via *S*-adenosylmethionine [32]. It should be stressed, however, that sterol side chain biosynthesis in *S. trochoidea* remains speculative. Detailed tracer experiments are required.

One striking characteristic of the sterols in *S. trochoidea* is the number and abundance of sterols with the $\Delta^{8(14)}$ double bond. The five sterols present with this unsaturation account for 26.6% of the total sterols, with four of the group also containing the 4-methyl substitution (Table 3). The presence of sterols with the 8(14) double bond is seldom encountered in algae [33], although such sterols have been isolated from dinoflagellates of the genus *Amphidinium* [1] and one species of the genus *Glenodinium* [4]. In eucaryotic systems, sterols possessing the 8(14) double bond generally function as intermediates in cholesterol biosynthesis by allowing demethylation to occur at C-14 [34]. While *S. trochoidea* does contain significant amounts of cholesterol, the predominance of $\Delta^{8(14)}$ sterols implies that double bond migration after elimination of the methyl group at C-14 is a minor process. The low concentrations of another intermediate in cholesterol biosynthesis, the Δ^{7} sterols, supports this view. Rather, *S. trochoidea* may be similar to the genus *Amphidinium* which is postulated to have the biosynthesis of 'normal' sterols impeded [1], resulting in the predominance of either the $\Delta^{8(14)}$ bond or saturated nuclei.

The distribution and abundance of steroidal ketones in *S. trochoidea* is shown in Table 4. The most abundant 3-keto steroids are the 23*S* and 23*R* isomers of dinostanone (4α,23*S*,24*R*-trimethylcholestan-3-one and 4α,23*R*,24*R*-trimethylcholestan-3-one) which together account for almost 50% of the total ketones present. Several of the steroidal ketones have been previously identified in dinoflagellates, in particular 4α,23,24-trimethylcholest-22-en-3-one (dinosterone) [18,35,36] which is the third most abundant component. Less abundant 3-keto steroids include the 4α-cholesta-8(14),22-dien-3-one (8%), 4α,24-dimethylcholestan-3-one (9.7%) and 4α,23,24-trimethylcholest-8(14)-en-3-one (7.1%). Among the 3-keto steroids a number of side chain modifications occur, with the Δ^{22} bond the most prevalent.

It is interesting to note that while the 3-keto steroids are present in small concentrations (3.7% of the total sterols), the distribution is very similar to that of their corresponding sterols. Almost all nuclear and side chain varieties were present, including both 4-methyl and 4-desmethyl components. While little information on the sequence in which sterols are synthesized in dinoflagellates is available, the presence of 3-keto steroids having the final side modification in place suggests that final reduction to the corresponding sterol occurs after such modifications are completed. The absence of a ketone corresponding to the 27-nor sterol also implies that demethylation at C-28 occurs after final reduction at C-3 to the alcohol.

Biogeochemical implications

In marine sediments, sterols having saturated nuclei (stanols) are often considered the result of microbial reduction of the unsaturated stenol precursor either during sedimentation or after deposition. More recently, evidence for the direct contribution to sediments of both stenols and stanols from dinoflagellates has been reported [9]. In *S. trochoidea*, stanols alone account for 59.3% of the total sterols, allowing for a substantial input of saturated components directly to the underlying sedi-

Table 4. Distribution of 3-keto steroids in *Scrippsiella trochoidea*

Peak no.	RR_t*	Identification	Abundance† (%)	M†
1	0.81	4α-Cholesta-8(14),22-dien-3-one†	10.0‡	396
2	0.81	24-Methylcholesta-5,22-dien-3-one	—	412
3	0.85	4α-Methylcholest-22-en-3-one	0.2	398
4	0.88	24-Methylcholest-22-en-3-one†	0.9‡	398
5	0.88	Cholest-7-en-3-one	—	384
6	0.99	4α-Methylcholest-8(14)-dien-3-one	1.3	384
7	1.01	4α-Methyl-cholestan-3-one	0.5	400
8	1.17	4α,24-Dimethylcholesta-8(14),22-dien-3-one	1.9	410
9	1.19	4α,24-Dimethylcholest-22-en-3-one	0.5	412
10	1.23	24-Methylcholest-7-en-3-one	1.4	398
11	1.29	$[M]^+$ 486 En-3-one	0.5	412
12	1.32	$[M]^+$ 484 Dien-3-one	0.9	398
13	1.36	4α,24-Dimethylcholest-8(14)-en-3-one	1.6	412
14	1.44	4α,24-Dimethylcholestan-3-one	9.7	410
15	1.48	4α,23,24-Trimethylcholesta-5,22-dien-3-one	0.5	412
16	1.53	4α,23,24-Trimethylcholest-22-en-3-one	10.8	426
17	1.59	4α,24-Dimethylcholest-7-en-3-one ?	0.3	412
18	1.64	4α,23,24-Trimethylcholest-17(20)-en-3-one	1.9	426
19	1.68	4α,23,24-Trimethylcholest-8(14)-en-3-one	7.1	426
20	1.74	4α,23*S*,24*R*-Trimethylcholestan-3-one	13.5	428
21	1.79	4α,23*R*,24*R*-Trimethylcholestan-3-one	36.4	428

*RR_t; Relative retention time, cholesterol = 1.00, dinosterol = 1.79.

†The small quantities involved precluded the analysis of separate cultures. Analytical error for replicate analyses = ±6%.

‡Coeluting peaks. Percent composition equals the sum of both components. Peaks in order of elution on a 25 m methyl silicone capillary column (OV-1), 0.31 mm id, 0.17 μm film thickness.

ments. This does not exclude the microbial reduction of other sterols, and in *S. trochoidea* a number of unsaturated moieties are present (Table 3). A similar consideration must be made for 27-nor-24-methyl-5α-cholestan-3β-ol, whose occurrence in a number of marine sediments has been proposed as a product of microbial demethylation at C-28 [37, 38]. Its occurrence in a *S. trochoidea*, albeit in small quantities, necessitates that a direct phytoplankton input must also be considered.

The appearance of 3-keto steroids in sedimenting particulate material [17, 39], have also been considered intermediates in the degradation of sterols. Moreover, laboratory studies have demonstrated the appearance of both 3- and 4-keto steroids in the microbial degradation of cholest-5-en-3β-ol [40]. The widespread distribution of 3-keto steroids in *S. trochoidea*, however, indicates that their presence in sinking particulate matter and sediments may also originate from a direct dinoflagellate input. Thus, the presence of 3-keto steroids observed in sediment traps [16, 39] and surface sediments [41, 42] could arise from a direct phytoplankton input as well as by their formation by microbial transformation of sterols.

EXPERIMENTAL

Four replicate cultures of *S. trochoidea* (Stein) (Plymouth Culture Collection No. 104) were grown at 15 ° in f/2 medium [43] made up to 32% salinity in 0.2 μm filtered and autoclaved seawater. Illumination was provided by banks of 'white' fluorescent tubes producing 160–215 $\mu E/m^2$. A 12 hr light: 12 hr dark light regime was maintained throughout the growth period with cells harvested in the late log phase. Large aggregates were first removed by filtering cultures through a 60 μm nylon mesh, and the remaining cells were then sepd from the culture medium by sieving on to a 10 μm nylon mesh. Retained cells were rinsed and resuspended in filtered seawater, a known portion removed for cell counts and the remainder collected onto a precleaned GF/C filter pad (pre-combusted at 500° for 24 hr) and stored in redist CH_2Cl_2–MeOH (1:1) at −20° until analysis.

Lipid extraction and analysis. Each filter pad was ultrasonically extracted ×3 in CH_2Cl_2–MeOH (1:1). Extracts for lipid analysis were concd by rotary evapn and saponified in 7 ml 0.1 N KOH–MeOH plus 1 ml dist. H_2O under reflux for 2 hr. Additional H_2O was then added and the saponified neutral fraction partitioned ×3 into hexane–Et_2O (9:1). The remaining ext was acidified with conc HCl to pH 1 and the polar fraction partitioned in a similar manner. Extraction and partitioning of control filter pads spiked with a known amount of 16:0 and 5α-cholestane demonstrated recovery efficiencies greater than 96% for both compounds. A known aliquot of the saponified neutral fraction was treated with BSTFA to convert sterols to their TMSi derivatives while the remainder was further sepd into major neutral classes by TLC on silica gel G using hexane–EtOAc (17:3). The entire polar fraction was treated with BF_3–MeOH and the fatty acid Me esters partitioned into hexane–Et_2O. Lipid classes on TLC were identified by comparison with authentic standards. The spots were then scraped off and eluted with a series of solvents of increasing polarity and stored in CH_2Cl_2 at −20°C until analysis. Using this procedure, total saponified neutral and polar fractions for quantitative

measurements as well as individual lipid classes for structural identification could be obtained. This was particularly important for the identification of the 3-keto steroids which are present in low concns and have overlapping GC R_ss with the 3-β-sterols.

Lipid identification and quantitation was obtained by capillary GC and computerized GC/MS. GC was carried out on a capillary FID instrument fitted with a 25 m × 0.3 mm id cross linked Me silicone (OV1) column operated in the splitless mode. Fatty acid Me esters were analysed using H_2 as carrier (0.5 kg/cm^2) and temp. prog. 80–280° at 4°/min. Sterols, steroidal ketones and alcohols were similarly analysed with teep. prog. from 100 to 300° at 2°/min. All data was processed using a laboratory data system with quantification of all compounds based on individual peak area compared to an int std (5α-cholestane) added before inj.

GC-MS was performed on a capillary chromatograph interfaced to a quadrupole MS instrument. EIMS (40 eV, 1 scan/sec) were obtained using the same conditions as for GC analysis with the exception that He was used as carrier (0.3 kg/cm^2). All MS were acquired and processed using a dedicated data system. The position and geometry of the double bond in monounsaturated fatty acids was determined by reaction of a subsample of TLC purified fatty acid Me esters with 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene to form Diels–Alder adducts [44]. Interpretation of EIMS of adducts utilized the loss of Δ and ω fragments from the $[M]^+$ for identification. Molecular identification for all compounds were made on the basis of coinjection with authentic stds, comparison with ref and/or lit spectra [45, 46] and MS interpretation.

Diagnostic ions in the MS of TMSi sterol derivatives in Table 3 were as follows. GC-MS (40 eV) sterol 3; 460$[M]^+$ (10), 445 (15), 305 (15), 215 (70), 75 (100), sterol 4: 470$[M]^+$ (5), 269(20), 243 (20), 227 (5), 69(100), sterol 5: 472$[M]^+$ (40), 271 (100), 229 (20), sterol 6: 458$[M]^+$ (10), 353 (10), 255 (25), 229 (10), 213 (15), sterol 7: 472$[M^+]$ (10), 371 (15), 345 (25), 257 (60), 69 (100), sterol 8: 472$[M]^+$ (100), 269 (20), 243 (30), 227 (20), sterol 9: 474$[M]^+$ (10), 459 (15), 417 (5), 384 (10), 369 (15), 215 (65), sterol 10: 472 $[M]^+$ (40), 3677(15), 255 (40), 229 (50), 213 (45), sterol 11: 474 $[M]^+$ (10), 384 (10), 369 (15), 215 (70), 75 (100), sterol 12: 484 $[M]^+$ (15), 469 (10), 357 (20), 269 (15), 243 (15), 243 (15), 69 (100), sterol 13: 486$[M]^+$ (30), 388 (35), 359 (40), 343 (25), 271 (30), 69 (100), sterol 14: 472$[M]^+$ (40), 367 (10), 255 (100), 229 (40), 213 (55), 75 (75), sterol 17: 486$[M]^+$ (30), 471 (5), 269 (15), 243 (20), 227 (30), 98 (100), sterol 18: 488$[M]^+$ (10), 473(20), 398(15), 359 (25), 261 (30), 229 (30), 75 (100), sterol 19: 498$[M]^+$ (30), 408 (10), 363 (5), 269 (10), 69 (100), sterol 20: 500$[M]^+$ (10), 388 (20), 359 (40), 271 (15), 69 (100), sterol 21: 486$[M]^+$ (50), 269 (100), 227 (50), sterol 22: 500$[M]^+$ (5), 402 (25), 387 (30), 297 (35), 283 (20), 95 (100), sterol 23: 500$[M]^+$ (55), 485 (10), 410(10), 395 (15), 269 (25), 227 (50), sterol 24: 502$[M]^+$ (10), 412 (40), 373 (10), 261 (20), 229 (35), 75 (85), sterol 25: 502 $[M]^+$ (5) 412 (20), 373 (30), 261 (30), 229 (25), 75 (80).

3-Keto steroids showed a very similar MS fragmentation pattern as their sterol counterparts. The principal difference is the presence of the $[M]^+$ at 74 mass units less in the ketone than the TMSi ether of the corresponding sterol.

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