COMMUNICATION

Sterols of *Chaetoceros* and *Skeletonema*[1]

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Dietary sterol is required by the oyster for growth, and sterol is believed to be obtained primarily from dietary phytoplankton. Seven isolates of *Chaetoceros* and one of *Skeletonema*, which are of potential use as oyster food, were analyzed for sterol composition using gas chromatography, high-performance liquid chromatography and gas chromatography/mass spectrometry. *Skeletonema* and five isolates of *Chaetoceros* contained cholesterol as their major sterol. Two other isolates of *Chaetoceros* also contained cholesterol, but 24-methylenecholesterol was the principal sterol. Cholesterol has rarely been reported as the major sterol from phytoplankton. In view of the widespread occurrence of *Skeletonema* and *Chaetoceros* in the marine environment, these algae could be an important source of the oyster's cholesterol.
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The American oyster *Crassostrea virginica* has a complex sterol composition of some forty compounds (1–3). This complexity has been attributed to dietary sterols that are accumulated due to the oyster's inability to synthesize sterols from simple precursors (4–7). Oysters must therefore obtain sterols from their diet of phytoplankton. In carefully controlled studies, the growth rates of oysters have been correlated with several dietary factors, one of which is sterol composition (8). The principal sterol of the oyster is cholesterol (1,9); however, cholesterol is rarely found in phytoplankton species, which are used in the laboratory or in commercial hatcheries to feed oysters or clams (10). This leads to the conclusion that other as yet unexamined phytoplankton must provide the oyster's cholesterol. In studies designed to evaluate phytoplankton as food for juvenile oysters, Enright *et al.* (11,12) listed three species of *Chaetoceros* and *Skeletonema costatum* as the most effective phytoplankton for promoting growth of the juvenile oyster, *Ostrea edulis*. *S. costatum* was reported by Ballantine *et al.* (13) to contain 24-methylenecholesterol as the principal sterol, and it also contained cholesterol, 24-methylcholesterol and 24-ethylcholesterol in amounts greater than 10% of total sterol. In *Chaetoceros simplex calcitrans* the principal sterols were cholesterol and 24-methylenecholesterol (14), but in an unidentified *Chaetoceros* sp., 24-methylcholesterol and 24-ethylcholesterol were the principal sterols (15). In view of the importance of sterols to the oyster, and the reports of cholesterol in some isolates of the cosmopolitan marine genera *Chaetoceros* and *Skeletonema*, the present study was undertaken to quantitatively determine the sterol composition of all *Chaetoceros* and *Skeletonema* isolates available to us.

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Abbreviations: GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry; HPLC, high-performance liquid chromatography.

MATERIALS AND METHODS

Phytoplankton strains were obtained from the Milford Culture Collection (Strain number is listed in parenthesis in Table 1.). Five of the eight strains were bacterized, and three were axenic. Of the seven *Chaetoceros* isolates analyzed, only B-13 forms short chains (*ca.* 4 cells); the others grow as essentially solitary unicells with sizes easily consumed by post-set oysters (<10 μm). The *Skeletonema* strain produced only short chains (*ca.* 3–4 cells). Algae were cultured in enriched natural seawater medium "E" formulation (16) under aseptic conditions (17). Algae from the stationary phase were centrifuged, lyophilized and stored in a freezer before analysis. Cells from the centrifuged concentrate were diluted and counted in an Improved Neubauer Hemocytometer (American Optical Co., Buffalo, NY) with a microscope. Dry weights were determined by weighing a known number of cells collected on a glass fiber filter (Whatman, GF/F, Whatman Labsales, Hillsboro, OR), washed with ammonium formate isotonic to the growth medium and dried in an oven at 80°C. Means of two dry weight determinations were calculated for each species, ranges never exceeded 5% of the mean.

Lyophilized samples were extracted overnight in a Soxhlet apparatus with $CHCl_3$/methanol (2:1, vol/vol) and sterol ester, sterol glycoside and free sterol fractions were isolated by Biosil A column chromatography and analyzed by capillary gas chromatography (GC), as described previously (17). Sterols were identified by capillary GC and gas chromatography/mass spectrometry (GC/MS) (17). High-performance liquid chromatography (HPLC) was employed to assign C-24 stereoconfiguration.

RESULTS AND DISCUSSION

Cholesterol, desmosterol, 24-methylenecholesterol, 24-methylcholesterol, 24-ethylcholesterol, fucosterol and isofucosterol were significant components in the sterol fractions of the isolates studied. They were identified on the basis of their relative retention times in capillary GC as compared to authentic compounds and by analysis with capillary GC/MS (21).

Table 1 shows the free sterol composition of the phytoplankton examined. Ester sterol and glycoside sterol each made up less than 2% of the free sterol and were not examined further.

The sterol composition of *C. gracile* (isolates Chaet B and ARC-11), *C. muelleri* and *C. sp.* (NRAC-Chaet) were essentially identical, with cholesterol being slightly more

TABLE 1

Sterols of *Chaetoceros* and *Skeletonema*

Isolate	Dry wt μg/g	fg/cell	Sterol composition[a] (% of total sterol)						
			CHOL	DES	24MEC	24MC	24EC	FUCO	ISOFUCO
Chaetoceros gracile (Chaet B)	9.9	41	47		9			39	1
C. gracile (ARC-11)	10.1	40	49		6			33	3
C. muelleri (CHGRA)	14.4	69	48	9				41	1
C. sp. (NRAC-Chaet)	27.7	84	47		7	3		37	3
C. simplex (Chaet-G)	3.8	104	40	1	24	1		31	1
C. sp. (B-13)	3.7	51	1		35	20	23		10
C. calcitrans (Chaet-cal)	0.3	3	39	3	56				
Skeletonema costatum (NRAC-Skel)	2.0	16	71		8	3	12		1

[a]Abbreviations: CHOL, cholesterol; DES, desmosterol; 24MEC, 24-methylenecholesterol; 24MC, 24-methylcholesterol; 24EC, 24-ethylcholesterol; FUCO, fucosterol; ISOFUCO, isofucosterol.

abundant than fucosterol. Small amounts of 24-methylenecholesterol and isofucosterol were also present. The amount of sterol in three of these isolates on a per cell basis (40–84 fg/cell) was also similar, although NRAC Chaet contained appreciably more sterol on a dry weight basis (27.7 μg/g) than the other strains in this group. Cholesterol made up 40% of the sterols of *C. simplex*, but substantial quantities of both 24-methylenecholesterol and fucosterol were also present. The amount of sterol per cell in *C. simplex* (104 fg/cell) was slightly greater than in the other isolates examined (Table 1); however, cells of this strain were much larger than the others, resulting in % dry weight values less than those of the *C. gracile/muelleri* group. *Chaetoceros* sp. (B-13) is the only isolate examined that had large amounts of 24-ethylcholesterol and 24-methylcholesterol occurring. The latter sterol was examined by HPLC (18), which separates most 24α from 24β sterols. It revealed that the 24-methylcholesterol of *Chaetoceros* sp. (B-13) was all 24β-methylcholesterol (5-ergostenol). This system does not resolve 24-ethylcholesterol isomers. In each previous case where a member of the diatom order centrales was examined for stereochemistry at C-24, the β isomer was also reported (10). The sterols of *C. calcitrans* (Chaet-cal) differed from those in the other isolates in two respects. The total sterol was at least an order of magnitude less (3 fg/cell or 0.31 μg/g dry wt) in this isolate than in any other *Chaetoceros* isolate examined. Chaet-cal was the only isolate examined that did not contain C_{29} sterols. This isolate contained a small amount (3% total sterol) of desmosterol and the remaining sterol consisted only of cholesterol and 24-methylenecholesterol, with the latter sterol being most abundant. Cholesterol was prevalent in *S. costatum*, with the overall sterol content (16 fg/cell or 2.0 μg/g dry wt) being less than all *Chaetoceros* isolates except *C. calcitrans*. However, the second most abundant sterol in *Skeletonema* was the 24-ethylcholesterol and not fucosterol, as was the case in most *Chaetoceros* isolates (Table 1).

Previous indications (10) were that most algae in the families Chaetoceraceae and Thalassiosiraceae, to which *Chaetoceros* and *Skeletonema* belong, contain 24-methylenecholesterol as a principal sterol. The presence of 24-methylenecholesterol was demonstrated in each isolate examined in this study. The seven isolates of *Chaetoceros* examined showed four distinct compositional patterns. With the exception of *Chaetoceros* sp. (B-13), cholesterol was a substantial component, and was the prevalent component of six of these sterol mixtures. Cholesterol was also most abundant in *Skeletonema*. With the exception of the red algae (10), the prevalence of cholesterol in sterol mixtures is rare in most plant groups (22). Until now few phytoplankton were known to contain cholesterol, and it appeared that cholesterol in natural populations of oysters must come from dealkylation of dietary phytoplankton sterols. Rapid growth of oysters on cultured phytoplankton species that contain no cholesterol (8) suggests that dealkylation of certain phytosterols to cholesterol is likely to occur. Nevertheless, the discovery of the prevalence of cholesterol in these widely occurring marine phytoplankton isolates could point the way to locating direct phytoplankton sources of cholesterol for the oyster and other marine invertebrates in nature.

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