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Studies on the Sterols in Some Marine Phytoplanktons

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Summary

1. The sterols from four species of diatoms, *Skeletonema costatum*, *Chaetoceros gracilis*, *Chaetoceros didymus* and *Thalassiosira decipience*, and one dinoflagellate, *Prorocentrum micans*, were investigated.

2. The sterol components were identified by combined gas chromatography and mass spectrometry.

3. Each of the four diatoms has a different major sterol. The major sterol of *S. costatum* was 24-methylenecholest-5-enol, that of *C. gracilis* was cholest-5-enol, that of *C. didymus* was 24-ethylidenecholest-5-enol and that of *T. decipience* was 24-methylcholest-5-enol.

4. *P. micans* contained cholest-5-enol as the major sterol, accompanied by 4, 23, 24-trimethylcholest-22-enol and 4, 23-dimethyl-cholest-22-enol.

5. C_{26} sterol was not detected in the phytoplanktons which were investigated in this paper.

After extended research in the sterols from marine organisms, many novel sterols have been isolated and identified (1, 2). Phytoplanktons, which are one of the primary producers in the marine food chain, are generally considered to be responsible for the distribution of the sterols isolated from marine invertebrates. The sterol composition of many marine algae have already been reported, but there is little information about phytoplanktons (1, 3). As a part of our investigation into the sterol metabolism in marine mollusks, we investigated the sterols of marine diatoms *Skeletonema costatum*, *Chaetoceros grecilis*, *Chaetocetos didymus*, *Thalassiosira decipience* and a dinoflagellate *Prorocentrum micans* cultured in the laboratory. The present report shows the results of the analysis of the sterol composition of some marine phytoplanktons.

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Materials and Methods

Diatoms were grown in Matsudaira medium (4) and the dinoflagellate was grown in Guillard F medium (5). Culture media were filtrated with a membrane filter (0.45 μm ; TM-2, TOYO ROSHI). Ehlenmeyer flasks (5 l) containing the incubation medium were exposed to a light period of 24 hours. *S. costatum* was grown in 35 l, *C. gracilis* 20 l, *C. didymus* 25 l, *T. decipience* 25 l and *P. micans* 50 l. After 7-14 days, these cells were harvested by centrifugation and washed with distilled water.

The lipids were extracted with chloroform-methanol accoeding to the procedure of Folch (6). The lipids obtained were saponified in the usual manner. The sterols were isolated from the nonsaponifiable materials by thin layer chromatography (TLC).

The sterols were acetylated with dry pyridine-acetic anhydride (1 : 1, v/v) and trimethylsilylated. The composition of the sterols was determined by gas-liquid chramatography (GLC) which was performed using a Hitachi 163 Gas Chromatograph equipped with a 2 m×3 mm i.d. column, packed with 1.5% Silicon OV-17 (80-100 mesh) on Chromosorb W (Applied Science). Steryl acetates and TMS derivatives were identified by a combined gas chromatography and mass spectrometry (GC-MS) with a 0.75% Silicon OV-17 column.

Results

The weight of phytoplanktons harvested and the contents of lipids and sterols are given in TABLE 1. The structure of sterol side chains and nuclei identified are shown in FIG 1.

Sterols of S. costatum

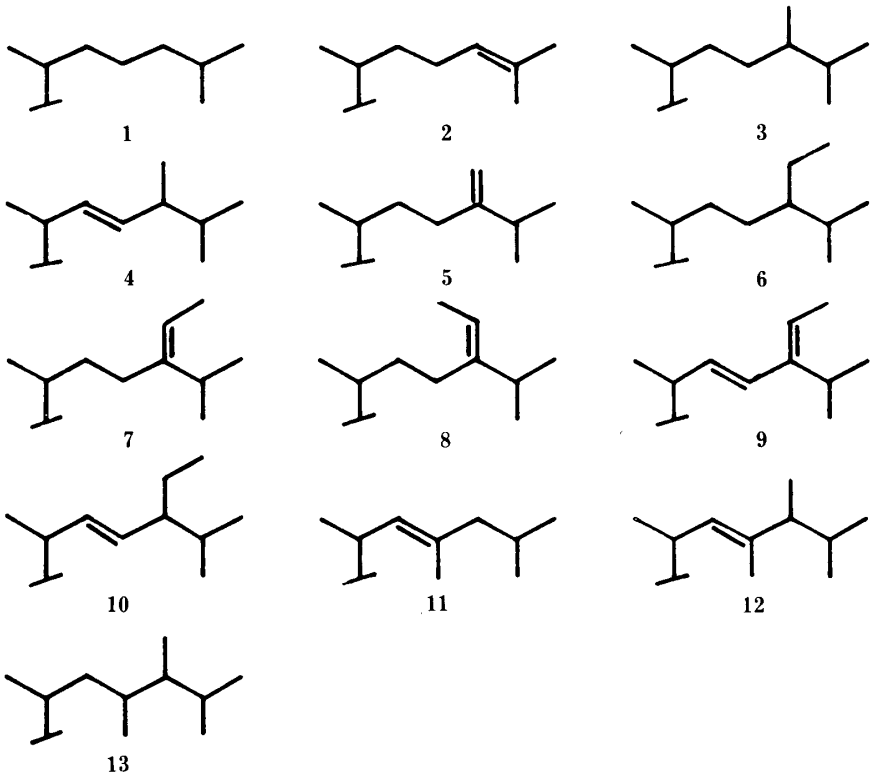
The gas chromatogram of stery acetates isolated from *S. costatum* indicated that the sterol mixture of this plankton involved at least 6 sterols : the mass

TABLE 1. *Sterol Contents of the Marine Phytoplanktons*

Planktons	Fresh weight	Lipids		Sterols		
	(g)	(mg)	(%)*	(mg)	(%)*	(%)**
Skeletonema costatum	12.5	91	0.7	33	0.3	36.3
Chaetoceros gracilis	14.2	204	1.4	41	0.3	20.1
Chaetoceros didymus	35.0	287	0.8	9	0.03	3.1
Thalassiosira decipience	33.4	391	1.2	23	0.07	5.9
Prorocentrum micans	7.1	346	4.9	15	0.2	4.3

* Percentage to fresh weight

** Percentage to lipid weight



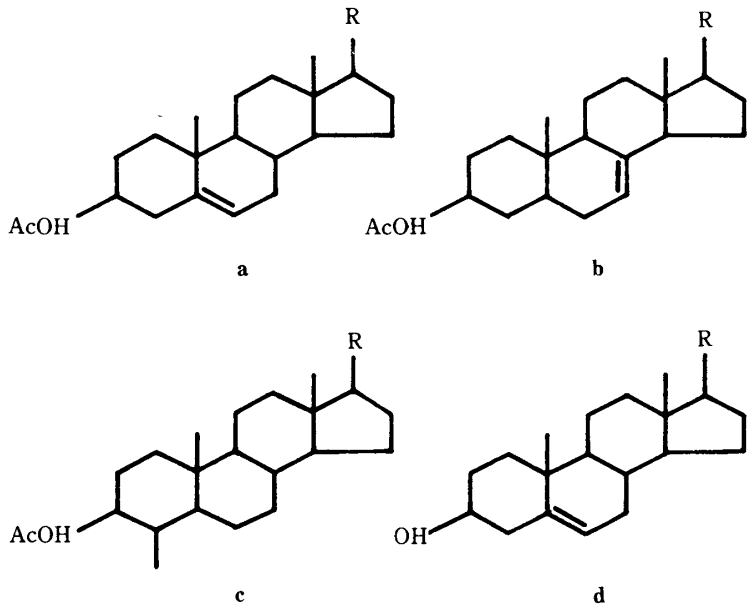


FIG. 1. Sterol side chains and nuclei.

spectrum pattern of S-1 : m/e 368 [M-AcOH (60), relative intensity 100%], 353 [M-60-CH_3 (15), 22%], 260 [M-60-108 (C_8H_{12}), 27%], 247 [M-60-121 (C_9H_{13}), 22%], 255 [M-side chain-60, 22%], 213 [M-side chain-60-42, 18%]. This pattern indicated the monoene steryl acetate and side chain saturated steryl acetate. Peak **S-1** was identified as cholest 5-enyl acetate (**1a**). **S-2** (relative retention time to cholesterol=rrt 1.19) : m/e 366 [M-60, 100%], 351 [M-60-15, 29%], 253 [M-side chain-60-2 H, 48%]. **S-2** was identified as cholest-5, 24-dienyl acetate (**2a**). Peak **P-3** (rrt 1.37) was the major component of the steryl acetate mixture isolated from *S. costatum*. Its fragment pattern showed m/e 380 [m-60, 100%], corresponding to C_{28} dien stery acetate and other ion at m/e 365 [m-60-15, 22%], 296 [M-60-(C_{22}-C_{23})-H, 50%], 281 [M-60-15-(C_{22}-C_{23})-H, 22%] 253 [35%]. The peaks at m/e 296 and 281 indicated that double bond must be in position C-24. **S-3** was identified as 24-methylenecholest-5-enyl acetate (**5a**). **S-4** (rrt 1.57) : m/e396 [M-60, 100%], 255 [27%], 213 [20%]. These indicated that S-4 was C_{29} monoene steryl acetate. And other fragment ion at m/e 288 [M-60-108, 25%] and 275 [M-60-121, 18%] indicated saturated side chain such as **S-1.** Thus **S-4** was identified as 24-ethylcholest-5-enyl acetate (**6a**). **S-5** and **S-6** (rrt 1.64 and 1.72) showed the same mass fragment patterns. The ion at m/e 394 [M-60, 29%] was indicative of C_{29} dine steryl acetate. And other ions were showed at m/e 379 [M-60-15, 7%], 296 [100%], 281 [23%], 253 [18%] and 213 [14%]. Thus **S-5** and **S-6** were identified as 24-ethylidenecholest-5-enyl acetate. Judging from these relative retention times, **S-5** were fucosterol acetate (**8a**) (24**E**-ethylidenecholest-5-enyl acetate) and **S-6** was isofucosterol acetate (**7a**) (24*Z*-ethylidenecholest-5-enyl acetate).

Sterols of C. gracilis

The gas chromatogram of the steryl acetate mixture isolated from *C. gracilis* indicated that the sterol mixture of this plankton involved at least 10 sterols. Cg-1 (880 1.00) was the major steryl acetate component of this plankton. Mass fragment patterns indicated that **Cg-1** was cholest-5-enyl acetate (**1a**). **Cg-2** (rrt 1.16) : m/e 380 [M-60, 100%] indicated that Cg-2 was C_{28} diene steryl acetate and m/e 365 [M-60-15, 15%], 255 [54%], 253 [47%] and 213 [20%] suggested that the double bond was located in both ring and side chain. Further ions at m/e 337 [M-60-43 ; 43=terminal isopropyl at C_{25} to C_{27}, 5%] and 282 [M-60-15-(C_{22}-C_{23}), 5%] indicated that the double bond must occupy position 22. Thus **Cg-2** was identified as 24-methylcholesta-5, 22-dienyl acetate (**4a**). **Cg-3** (rrt 1.37) was identified as 24-methylenecholest-5-enyl acetate (**5a**). **Cg-4** (rrt 1.57) was identified as 24-ethylcholest-5-enyl acetate (**6a**). **Cg-5** and **Cg-6** were identified as 24 *E*-ethylidenecholest-5-enyl acetate (**8a**) and 24*Z*-ethylidenecholest-5-enyl acetate (**7a**) respectively. **Cg-7** (rrt 1.83) showed the molecular ion at m/e 454 [M, 3%], corresponding to C_{29} diene steryl acetate the double bond of which was

not located at position 5. And other ions at m/e 439 [M-15, 2%], 394 [M-60, 31%], 379 [M-60-16, 9%], 313 [M-side chain-2H, 6%], 296 [100%] and 281 [26%] indicated that double bond at position 24 (28). Further ions were present at m/e 253 [37%], 228 [M-side chain-60-27, 13%] and 213 [18%]. **Cg-7** was considered as 24-ethylidenecholest-7-enyl acetate (**7b**). **Cg-8** (rrt 2.01) : m/e 452 [M, 2%]. 392[M-60, 26%] and 213 [18%], these were corresponding to C_{29} triene steryl acetate. Further ions at m/e 296 [52%] and 281 [21%] indicated that double bond at 24 (28), and ions at m/e 349 [M-60-43, 5%] and 282 [24%] indicated that double bond occupied position 22. **Cg-8** was identified as 24-ethylidenecholesta-7, 22-dienyl acetate (**9b**). **Cg-9** (rrt 2.94) : m/e 410 [M-60, 100%] indicated that **Cg-9** was C_{30} monoene steryl acetate. Further ions at m/e 302 [M-60-108, 13%] and 289 [M-60-121, 17%] indicated saturaated side chain.

Sterols of C. didymus

The gas chromatogram of the steryl acetate mixture isolated from *C. didymus* indicated that the sterol mixture of this plankton involved at least 4 sterols. The chromatogram showed a more simple steryl acetate mixture than those of the former two phytoplanktons. **Cd-1** (rrt 1.00) was identified as cholest-5-enyl acetate (**1a**), and **Cg-2** (rrt 1.57) was 24-ethylcholest-5-enyl acetate (**6a**). **Cd-3** (rrt 1.64) which was the major steryl acetate peak of this mixture isolated from *C. didymus* was identified as (24*E*)-24-ethylidenecholest-5-enyl acetate (**8a**). **Cd-4** (rrt 1.72) showed the same mass frogment pattern as **Cd-3** identified as (24*Z*)-24-ethylidenecholest-5-enyl acetate (**7a**).

Sterols of T. decipience

The gas chromatogram of the steryl acetate mixture isolated from *T. dicipience* indicated that the sterol mixture of this plankton involved at least 7 sterols. **T-1** (rrt 1.00) was identified as cholest-5-enyl acetate (**1a**). **T-2** (rrt 1.30) was the major steryl acetate of this mixture from *T. decipience*. Its fragment pattern showed at m/e 382 [M-60, 100%], 367 [M-60-15, 44%] that indicated monoene steryl acetate. Further ions at m/e 274 [M-60-108, 38%] and 261 [M-60-121, 41%] indicated that the double bond was not located in the side chain. **T-2** was identified as 24-methylcholest-5-enyl acetate (**3a**). **T-3** (rrt 1.57) was identified as 24-ethyl-cholest-5-enyl acetate (**6a**). **T-4** was (24*E*)-24-ethylidenecholest-5-enyl acetate (**8a**), and **T-5** (rrt 1.72) was (24*Z*)-24-ethylidenecholest-5-enyl acetate (**7a**).

Sterols of P. micans

The gas chromatogram of the steryl acetate mixture isolated from *P. micans* indicated that the sterol mixture of this plankton involved at least 6 sterols. **P-1** (rrt 1.00) which was the major steryl acetate of this plankton was identified as

TABLE 2. *Salient Mass Spectrometric Data of the Acetylated Sterols Isolated from the Marine Phytoplanktons*

	1a	**2a**	**3a**	**4a**	**5a**	**6a**	**7, 8a**	**7b**	**9b**	**11c**	**10a**	**12c**	**13c**
rrt*	1.00	1.19	1.30	1.16	1.37	1.57	1.64,1.72	1.83	2.01	1.26	1.40	1.51	1.75
Molecular ion(M^+)	—	—	—	—	—	—	—	454(3)	452(2)	456(89)	—	470(22)	472(36)
M^+-CH_3(15)	—	—	—	—	—	—	—	439(2)	—	—	—	—	—
M^+-AcOH(60)	368(100)	366(100)	382(100)	380(100)	380(100)	396(100)	394(29)	394(31)	392(26)	396(13)	394(100)	—	412(72)
M^+-60-15	353(22)	351(29)	367(44)	365(15)	365(22)	381(18)	379(7)	379(9)	377(7)	381(3)	379(7)	—	397(42)
M^+-60-(C_{22}-C_{23}-H)**	—	—	—	—	296(50)	—	296(100)	296(100)	296(52)	—	—	—	—
M^+-60-15-(C_{22}-C_{23}-H)	—	—	—	—	281(22)	—	281(23)	281(26)	281(21)	—	—	—	—
M^+-60-(C_{24}-C_{25})	—	—	—	337(5)	—	—	—	—	349(5)	—	351(8)	—	—
M^+-60-15-(C_{22}-C_{23})	—	—	—	328(5)	—	—	—	—	282(24)	—	282(90)	—	—
M^+-(C_{20}-C_{22})	—	—	—	—	—	—	—	—	—	358(89)	—	358(65)	—
M^+-60-108(C_8H_{12})	260(27)	—	274(38)	—	—	288(25)	—	—	—	—	—	—	—
M^+-60-121(C_9H_{13})	247(22)	—	261(41)	—	—	275(18)	—	—	—	—	—	—	—
M^+-side chain-2H	—	—	—	—	—	—	—	—	—	329(70)	—	329(100)	—
M^+-side chain-60	255(22)	255(54)	255(56)	255(54)	255(17)	255(27)	255(8)	—	—	271(100)	255(42)	271(89)	271(20)
M^+-side chain-60-2H	253(8)	253(48)	253(16)	253(47)	253(35)	253(15)	253(18)	253(37)	253(100)	—	253(60)	—	—
M^+-side chain-60-42	213(18)	213(15)	213(65)	213(20)	213(22)	213(20)	213(18)	213(18)	213(26)	229(27)	213(38)	299(15)	229(100)

* rrt : retention time to cholesterol acetate

** C_{22}-C_{23} : cleavage at C_{22}, C_{23}

1a : cholest-5-enyl acetate, **2a** : cholesta-5, 24-dienyl acetate, **3a** : 24-methylcholest-5-enyl acetate, **4a** : 24-methylcholesta-5, 24-dienyl acetate, **5a** : 24-methylenecholest-5-enyl acetate, **6a** : 24-ethylcholst-5-enyl acetate, **7a** : 24-ethylidenecholst-5-enyl acetate, **7b** : 24-ethylidenecholest-7-enyl acetate, **9b** : 24-ethylidenecholesta-7, 22-dienyl acetate, **11c** : 4, 23-dimethylcholest-22-enyl acetate, **10a** : 23, 24-dimethylcholesta-5, 22-dienyl acetate, **12c** : 4, 23, 24-trimethylcholest-22-enyl acetate, **13c** : 4, 23, 24-trimethylcholestanyl acetate.

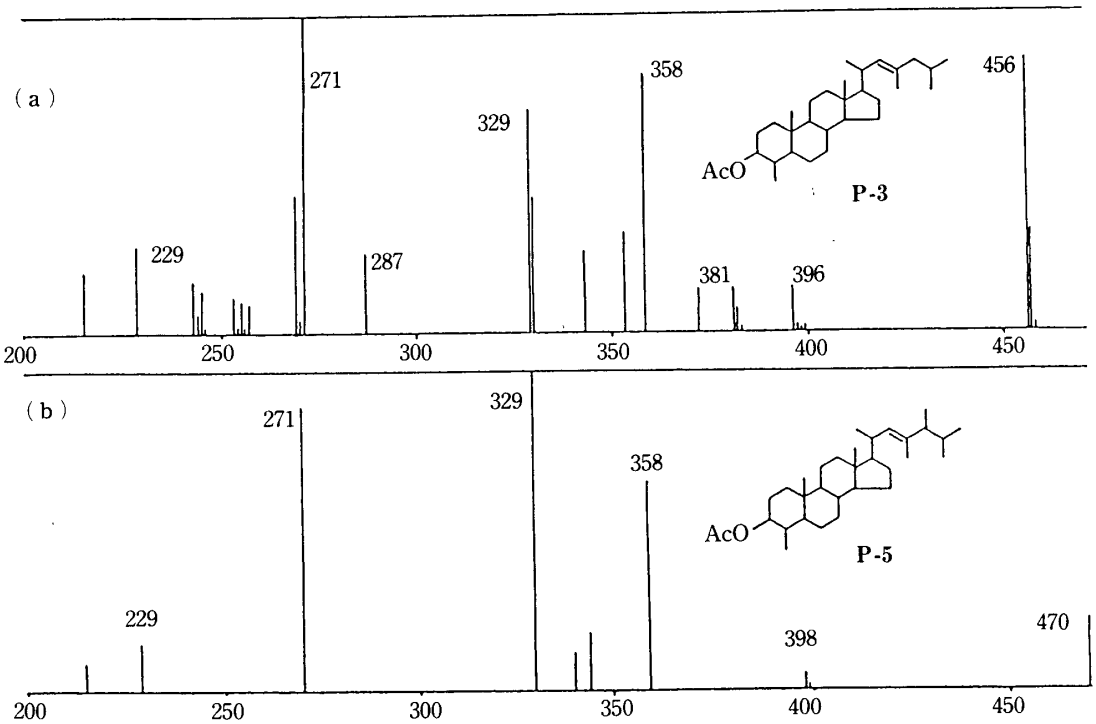


FIG. 2. Mass spectra of (a) acetylated 24-demethyldinosterol and (b) acetylated dinosterol

TABLE 3. *Sterol Composition of the Marine Phytoplanktons Determined by GLC (%)*

Sterol	rrt	*S. costatum*	*C. gracilis*	*C. didymus*	*T. decipience*	P. micans
1a	1.00	17.5%	61.9	18.8	6.1	28.8
4a	1.16	—	4.4	—	—	8.6
2a	1.19	17.1	—	—	—	—
11a	1.26	—	—	—	—	20.9
3a	1.30	—	—	—	62.3	—
5a	1.37	52.3	4.7	—	—	—
10a	1.40	—	—	—	—	14.2
12c	1.51	—	—	—	—	14.5
6a	1.57	6.7	4.5	19.0	13.9	—
7,8a	1.64,1.72	6.4	11.3	62.2	14.6	—
13c	17.5	—	—	—	—	10.9
7b	1.83	—	4.2	—	—	—
9b	2.01	—	3.6	—	—	—
*	2.94	—	2.8	—	—	—
unknown		—	2.6	—	3.1	—

* C_{30} monoene steryl acetate

cholest-5-enyl acetate (**1a**). **P-2** (rrt 1.16) was identified as 24-methylcholesta-5, 22-dienyl acetate (**4a**). **P-3** (rrt 1.26) : m/e 456 [M, 89%], 396 [M-60, 13%], 381 [M-60-15, 8%], 358 [M-cleavage at C_{20} and C_{22}, 89%], 329 [M-side chain-2H, 70%], 271 [M-side chain-60, 100%], 229 [M-side chain-60-42, 27%]. This fragment ion pattern has been already reported by Alam *et al.* (7) from dinoflagellate *Gonyaulax diagenesis* and identified as 4, 23-dimethylcholest-22-enyl acetate (24-demethyldinosterol acetate) (**11c**). **P-4** (rrt 1.40) : m/e 394 [M-60, 100%], 379 [M-60-15, 22%], 351 [M-60-42, 8%], 282 [90%]. **P-4** was identified as 24-ethylcholesta-5, 22-dienyl acetate (**10a**). **P-5** (rrt 1.51) : m/e 470 [M, 22%], 358 [65%], 329 [100%], 271 [89%], 229 [15%]. This fragment ion pattern and relative intensity indicated that **P-5** was 4, 23, 24-trimethylcholest-22-enyl acetate (**12c**) which was already known as dinosterol acetate. And this sterol was widely distributed in dinoflagellates. **P-6** (rrt 1.75) : its fragment pattern and relative intensity were shown in Fig. 2. The structure of this steryl acetate was considered to indicate that side chain saturation occurred in dinosterol acetate (**13c**).

The sterol composition of these planktons is given in TABLE 3.

Discussion

From the results of this investigation we determined that there are some characteristic nature in sterols of diatoms. First, diatoms do not alwasys contain a simple sterol composition. *S. costatum* has at least 6 sterols, *C. gracilis* has at least 10 sterols, *T. decipience has at least 5 sterols, and C. didymus* contains at least 4 sterols. From previous reports, we may conclude that diatoms may be divided into two groups, one containing a simple sterol composition, and the other cotaining a relatively complex sterol mixture (8-13). In this analysis, sterols isolated from diatoms vary in carbon number from 27 to 30. Especially *S. costatum* and *C. gracilis* contain unidentified sterols which have relative retention times that are longer than C_{30} sterols, so these sterols may be consider to be composed of more than 30 carbons. While diatoms contain a complex sterol mixture, every diatom that we investigated in this analysis contained cholest-5-enol (**1d**), 24-methylcholest-5-enol (**3d**) and 24-ethylidenecholest-5-enol (**7** and **8d**). But C_{26} sterols which are widely distributed in marine invertebrates could not be detected in the diatoms investigated. From previous reports, we can conclude that diatoms were not responsible for distribution of C_{26} sterols in marine invertebrates.

Each diatoms contains a major sterol which occupies the larger percentage of the total sterol mixture. The major sterol of each diatom that was investigated in this analysis was different from each other. The major sterol isolated from *S. costatum* is 24-methylenecholest-5-enol (45.1%, **5d**), that from *T. decipience* is 24-methylcholest-5-enol (60.2%, **3d**) and that from *C. gracilis* is cholest-5-enol (61.

9%, **1d**). But the major sterol from the same genus *C. didymus* is (24*E*)-24-ethylidenecholest-5-enol (**8d**).

24-Methylcholesta-5,22-dienol (Brassicasterol or epibrassicasterol, **4d**) which was said to be the only sterol in diatoms was detected in *C. gracilis* as a minor component (4.4%).

Dinoflagellates were the sterol source for the marine invertebrates as well as diatoms. *P. micans* contained C_{27} sterol to C_{30} sterol. And the major sterol was cholest-5-enol (28.8%, **1d**), but the characteristic sterols were 4, 23, 24-trimethylcholest-22-enol and this analogs which were not detected in any other organisms. And almost all the dinoglagellates which were investigated contained 4-methylsterols (14-16). Dinosterol was considered a precursor of gorgosterol which has a biogenetically fascinating cyclopropane group in the side chain isolated from Anthozoa associated with a dinoflagellate symbiont (17-19). 23, 24-Dimethyl-cholesta-5, 22-dienol has been isolated from Order Alcyonaria *Sarcophyta elegans* (20). C_{26} sterol was not detected in the dinoflagellates so far examined. Dinoflagellates were also not responsible for the production of C_{26} sterols

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