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Sterols in Some Red Algae

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Abstract

The sterol composition of the red algae, *Meristotheca papulosa*, *Gracilaria textorii*, and *Porphyridium cruentum*, was investigated. In gas-liquid chromatography on SE-30, QF-1, XE-60, and NGS-XE-60, infrared absorption spectra, and mass spectrometry, the sterols isolated from *M. papulosa* and *G. textorii* were found to contain only one component corresponding to cholesterol. The sterols isolated from *P. cruentum* which was cultured on a chemically-defined medium, were identified as 22-dehydrocholesterol (60%), cholesterol (5%), desmosterol (20%), ergosterol (5%), and C_{29}-sterol (10%) by gas-liquid chromatography on SE-30, QF-1, NGS-XE-60, and OV-17. 22-Dehydrocholesterol was further confirmed by mass spectrometry.

TSUDA *et al.* (1957, 1958a, 1858b) demonstrated that cholesterol peculiar to animals occurred in red algae. Their report was the first case showing the occurrence of it in the plant kingdom. After that, it has been suggested by SAITO and IDLER (1966), GIBBONS *et al.* (1967), IDLER *et al.* (1968), ALCAIDE *et al.* (1968), and IDLER and WISEMAN (1970) that the principal sterol of red algae is cholesterol. Moreover, it has also been found that green algae (IKEKAWA *et al.* (1968), ORCUTT and RICHARDSON (1970)), brown algae (IKEKAWA *et al.* (1968), PATTERSON (1968), KNIGHTS (1970)), and Chrysophyta (COLLINS and KALNINS (1969)) contain cholesterol. Recently, the authors have demonstrated the occurrence of cholest-4-en-3-one in the red algae, *Meristotheca papulosa* (KANAZAWA and YOSHIOKA (1971)) and *Gracilaria textorii* (KANAZAWA and YOSHIOKA (1972)), and the formation of cholest-4-en-3-one from cholesterol in these red algae. Therefore, the presence of cholesterol in the red algae, *M. papulosa* and *G. textorii*, is suggested. The present paper deals with the sterol composition of these red algae.

AARONSON and BAKER (1961) have reported that the unicellular red alga, *Porphyridium cruentum* lacks sterols. In the present study, the composition of sterols found in *P. cruentum* is discussed.

Materials and Methods

Red algae. *Meristotheca papulosa*, which is used as edible red algae in Japan, was obtained from the commercial source. *Gracilaria textorii* was collected in May 1969 at Hanase-Point, Kagoshima.

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Kagoshima University. *P. cruentum* was grown on the ASP-6 medium (PROVASOLI *et al.* (1957)) with the following solutes per liter: NaCl, 12.0 g; KCl, 0.4 g; $MgSO_4 \cdot 7H_2O$, 4.0 g; $CaCl_2 \cdot 2H_20$, 0.2 g; $NaNO_3$, 0.5g; $NaHPO_4 \cdot 12H_2O$, 0.05 g; Tris, 0.5 g; Fe (Cl^-), 2.0 mg; Mn(Cl^-), 1.0 mg; Zn(Cl^-), 0.5 mg; Cu(Cl^-), 20 μg; Co(Cl^-), 10 μg; HBO_3, 20 mg; Mo (Na salt), 0.5 mg; EDTA, 30 mg; thiamine, 0.2 mg; nicotinic acid, 0.1 mg; pyridoxine, 0.04 mg; pyridoxamine, 0.02 mg; Ca-pantothenate, 0.1 mg; choline, 0.5 mg; inositol, 1.0 mg; *p*-aminobenzoic acid, 0.01 mg; biotine, 0.5 μg; folic acid, 0.25 μg; riboflavin, 5.0 μg; vitamin B_{12}, 0.5 μg; pH 7.6. The cultivation was carried out in 1 liter Erlenmeyer flask containing 700 ml of the culture medium with aeration (300 ml/min.) at 20°C under white-fluorescent lamps. After 2 weeks, the cells were harvested by centrifugation (3000 rpm, 10 min.) and washed twice with distilled water. The average yield of the cells was about 4.0 g fresh weight per flask.

Isolation of sterols. From *M. papulosa* and *G. textorii*, the lipids were extracted with 7 volumes of dichloromethane, and then chromatographed on a column of silica gel (Kieselgel G, Merck) with hexane-acetone as an eluent (KANAZAWA and YOSHIOKA (1971)). The steroid fraction gained was rechromatographed on a column of alumina (grade II, Merck) with hexane-benzene as an eluent (KANAZAWA and YOSHIOKA (1971)). After recrystallization from methanol, the purified sterols were obtained.

The lipids from *P. cruentum* were extracted with chloroform-methanol according to the method of BLIGH and DYER (1959), and then saponified with ethanolic potassium hydroxide. The sterols were isolated from the unsaponifiable matters by the digitonin-precipitation method (IDLER and BAUMANN (1952)) and recrystallized from methanol. After acetylation by addition of acetic anhydride-dry pyridine (1: 1), the steryl acetate mixture was chromatographed on a silver nitrate-impregnated silicic acid with hexane-benzene (VROMAN and COHEN (1967)).

Gas-liquid chromatography (GLC). GLC was conducted with a Shimadzu GC-3AF unit by using 1.5 % SE-30, 1.0 % QF- 1, 1.0 % XE-60 %, 1.0 % NGS-1.0 % XE-60 (1: 1), and 1.5 % OV-17 as a column. The identification of peaks was performed by comparing retention times to those of authentic sterols. Conditions of GLC were essentially the same as described previously for the identification of crustacean sterols (TESHIMA and KANAZAWA (1971)).

Mass and infrared spectral analyses. Mass spectrum was measured with a Hitachi RMU-6D instrument (chamber voltage, 70 eV). Infrared absorption spectrum was obtained with a Nippon Bunko DS-301 spectrometer in chloroform.

Results

Sterol content. The sterol contents of *M. papulosa*, *G. textorii*, and *P. cruentum* are given in Table 1. The red algae examined contained 0.011-0.065 % of sterols. The both sterols of *M. papulosa* and *G. textorii* purified by crystallizations from methanol gave the melting points of 149-150°C.

Table 1, Sterol contents of red algae, *M. papulosa*, *G. textorii*, and *P. cruentum*.

Species	Weight (g)	Lipids		Unsap. matters		Sterols	
		g	%	mg	%	mg	%
M. papulosa	810*	6.0	0.74	—	—	450	0.055
G. textorii	600*	5.8	0.97	—	—	390	0.065
P. cruentum	57**	0.26	0.46	56	0.10	6	0.011

* Dry weight ** Fresh weight

Sterol composition. The results of GLC analyses of sterols isolated from *M. papulosa* and *G. textorii* are shown in Table 2. In GLC on SE-30, QF-1, XE-60, and NGS-XE-60, the sterols from *M. papulosa* and *G. textorii* were found to contain only one component corresponding to authentic cholesterol. To confirm the identity of cholesterol, the sterols were further analyzed by infrared absorption and mass spectrometry. The mass spectra of the algal sterols gave one molecular ion peak (M^+) at m/e 386 and other prominent peaks at m/e 371 (M^+-CH_3), 368 (M^+-HOH), 353[$M^+-(CH_3+HOH)$], 273(M^+-R, R=alkyl side chain of cholesterol), 255 [$M^+-(R+HOH)$], 231 [$M^+-(R+42)$], and 213 [$M^+-(R+42+HOH)$]. These data strongly supported that the sterol from *M. papulosa* and *G. teytorii* is cholesterol. The infrared absorption spectra showed also the identity of both the algal sterols with cholesterol.

Table 2. GLC analyses of the sterols isolated from *M. papulosa* and *G. textorii*.

Sterol	Relative retention time*			
	Column			
	SE-30	QF-1	XE-60	NGS-XE-60**
M. papulosa sterol	1.83	2.82	4.30	0.97
G. textorii sterol	1.83	2.82	4.29	0.97
Authentic cholesterol	1.83	2.82	4.30	0.97

* Relative to cholestane

** The trimethylsilyl derivatives of sterols were subjected to GLC

The GLC of sterols from *P. cruentum* revealed the presence of seven components, as shown in Table 3. The peaks 2, 3, 4, 5, and 6 were identical with 22-dehydrocholesterol (60 %), cholesterol (5 %), desmosterol (20 %), ergosterol (5 %), and C_{29}-sterol (10 %) in the retention times, respectively. Three mg of 22-dehydrocholesterol was isolated as acetate by column chromatography on a silver nitrate-impregnated silicic acid. After saponification, the free sterol obtained was subjected to mass spectral analysis. The mass spectrum of this sterol gave the molecular ion peak at m/e 384 (M^+) and other prominent peaks at m/e 369 (M^+-CH_3), 366(M^+-HOH), 299[$M^+-(CH_3+C_{23}-C_{27}+1H)$], 273($M^+-R$, R=alkyl side chain of

Table 3. GLC analyses of the sterols isolated from *P. cruentum*.

Peak	Relative retention time (Column)				Composition (%)	Identification
	SE-30*	QF-1*	NGS-XE-60**	OV-17**		
1	1.31	—	0.41	0.71	<1	Unknown
2	1.66	2.55	0.92	0.94	60	22-Dehydrocholesterol
3	1.82	2.82	1.00	1.00	5	Cholesterol
4	2.15	3.09	1.23	1.19	20	Desmosterol
5	2.22	3.12	1.41	1.35	5	Ergosterol
6	2.96	4.60	1.65	1.58	10	C_{29}-sterol
7	3.60	—	—	1.85	<1	Unknown

* Relative to cholestane
** Relative to cholesterol

22-dehydrocholesterol), 271[$M^+-(R+2H)$], 255[$M^+-(R+HOH)$], and 213 [$M^+-(R+42+HOH)$]. These data supported that this sterol is 22-dehydrocholesterol.

Discussion

Cholesterol isolated from many red algae was found also to occur in the red algae, *M. papulosa* and *G. textorii*, in the present study. In the previous papers, the authors have reported that the labeled cholest-4-en-3-one was formed as a metabolite of cholesterol-4-^{14}C in the tissues of *M. papulosa* and *G. textorii*. A part of cholesterol in these algae may be used as a precursor of keto-steroid, as in animals.

AARONSON and BAKER (1961) examined the presence of sterols in *P. cruentum* by LIEBERMAN-BURCHARD reaction and chromatography. But, sterols were not found in it. In the present study, *P. cruentum* contained 0.011 % of sterols, and 22-dehydrocholesterol and desmosterol were identified as a major component. In red algae, 22-dehydrocholesterol has been isolated from *Hypnea japonica* (TSUDA *et al.* (1960)), *Dilsea carnosa* and *Polyides caprinus* (GIBBONS *et al.* (1667)), and *Rhodymenia palmata* (IDLER *et al.* (1968)). Desmosterol has also been detected in a number of red algae (GIBBONS *et al.* (1967), IDLER *et al.* (1968), ALCAIDE *et al.* (1968), and IDLER and WISEMAN (1970)). The sterols found in *P. cruentum* may be synthesized by this red alga. However, percentage of content and composition of sterols in *P. cruentum* differs according to conditions of culture. This question requires further investigation.

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