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Sterols of the Killer Clams, Mollusca Pelecypoda

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The sterols of the killer clams, *Tridacna squamosa, Tridacna noae, Tridacna crocea* and *Hipponps hipponps,* were investigated by using mainly gas-liquid chromatography. In these killer clams, cholesterol, brassicasterol, 22,23-dihydrobrassicasterol and 24-methylenecholesterol were present as prominent sterols. The sterol composition of killer clams was fairly different with that of other pelecypods, and pointed out to be unique in the occurrence of large amounts (34-65 % in total sterols) of 22,23-dihydrobrassicasterol.

In earlier studies, the great majority of works have shown that complex sterol mixtures occur in marine mollusks[1]. Recently, AUSTIN[2] has extensively reviewed the sterol composition of marine invertebrates and plants from the viewpoint of chemotaxonomy. Also, IDLER and WISEMAN[3] have reviewed minutely the sterols of mollusks. The most reasonable conclusion to be drawn from available data is as follows: (1) The classes Gastropoda and Cephalopoda contain mainly cholesterol; (2) the class Pelecypoda contains relatively large amounts of brassicasterol and 24-methylenecholesterol besides cholesterol; (3) the class Amphineura, the most primitive mollusk, essentially contains 7-cholestenol but no Δ^5-sterols. However, little is known about the sterols of the killer clams, belonging to the family Tridacnidae (phylum Mollusca, class Pelecypoda, order Eulamellibranchia), except the giant clam, *Tridacna gigas*[4]. Hence, the authors intended to investigate the sterols of the killer clams. As a result, the sterol composition of the killer clams was pointed out to be unique as compared with other pelecypods. This paper deals with these results and discussion.

Materials and Methods

Killer clams. The family Tridacnidae consists of two genera which involve six species of mollusks. In the present study, the four killer clams, *Tridacna squamosa, Tridacna noae, Tridacna crocea* and *Hipponps hipponps,* were subjected to the analysis of sterol composition. These mollusks were collected at the Hatoma Island in Okinawa or at the Kakeroma Island in Amami. After sampling, the mollusks were dipped into methanol and transported to this labolatory.

Isolation of sterols. From the killer clams, the unsaponifiable materials were isolated by the essentially same method as described in a previous paper[5]. The sterols were obtained by column chromatography on alumina (Merck, grade II-III) with hexane-benzene[5] and then purified by crystallizations from methanol.

Identification of sterols. The identification of sterols was performed by gas-liquid chromatography (GLC) using two columns, 1.5 % SE-30 (non-selctive phase) and

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1.5 % OV-17 (selective phase)[5]. In GLC, free sterols and trimethylsilyl derivatives were analyzed by using 1.5 % SE-30 and 1.5 % OV-17, respectively. The trimethylsilyl derivatives of sterols were prepared by the method of ENEROTH *et al.*[6] Sterols were identified by comparing retention times with authentic sterols and/or by means of the steroid number (SN) devised by VANDENHEUVEL and HORNING[7].

In the case of *T. crocea*, the sterols were further subjected to mass spectrometry at low voltage (8 eV) in order to confirm the identity of components. Under this conditions, the mass spectrum was found to gave only the molecular ion peaks corresponding to each sterol component[8]. The percentage composition of sterol components was determined by hight of molecular ion peaks.

Results and Discussion

The yield of the lipids, unsaponifiable materials, and sterols isolated from the four killer clams are given in Table 1. As shown in Fig. 1, the GLC on 1.5 % SE-30 showed that the sterols from the killer clams were composed of number of components. However, better separation of the components was achieved by GLC on 1.5 % OV-17 as shown in Fig. 2 and Table 2. The GLC on 1.5 % OV-17 indicated that the sterols of the four killer clams contained at least eleven to thirteen components. As to the sterol mixture of *T. crocea*, the identity of sterol components was further confirmed by mass spectrometry. As shown in Table 3, the sterols from *T. crocea* gave the molecular ion peaks at m/e 426, 414, 412, 400, 398, 396, 384, and 370. Considering both the percentage compositions of sterols in GLC on 1.5 % OV-17 and mass spectrometry, each sterol component was elucidated as follows: peak a-1, 22-*trans*-24-norcholesta-5,22-dien-3β-ol; peak a-2, 22-dehydrocholesterol ; peak a-3, cholesterol ; peak a-4, brassicasterol ; peak a-5, 22,23-dihydrobrassicasterol (or campesterol) ; peak a-6, 24-methylenecholesterol ; peak a-8, β-sitosterol (or clionasterol) ; peak a-11, gorgosterol. The peak a-10 was assumed to be 28-isofucosterol (moleuclar weight 412).

In every killer clam examined, cholesterol (12-29%), brassicasterol (5-17 %), 22,23-dihydrobrassicasterol (34-65 %), and 24-methylenecholesterol (4-13 %) were present as prominent sterols. Cholesterol, brassicasterol, and 24-methylenecholesterol have been also found in most pelecypods as a principal sterol[3]. 22,23-Dihydro-

Table 1. Yields of the lipid fractions from the killer clams.

Species	Total lipids	Unsaponifiable materials		Sterols	
	mg	mg	% *	mg	% *
T. crocea	2140	220	10.3	103	4.8
T. noea	3445	504	14.7	160	4.6
T. squamosa	4100	410	10.0	236	5.8
H. hipponps	722	70	9.7	27	3.7

* Per total lipids

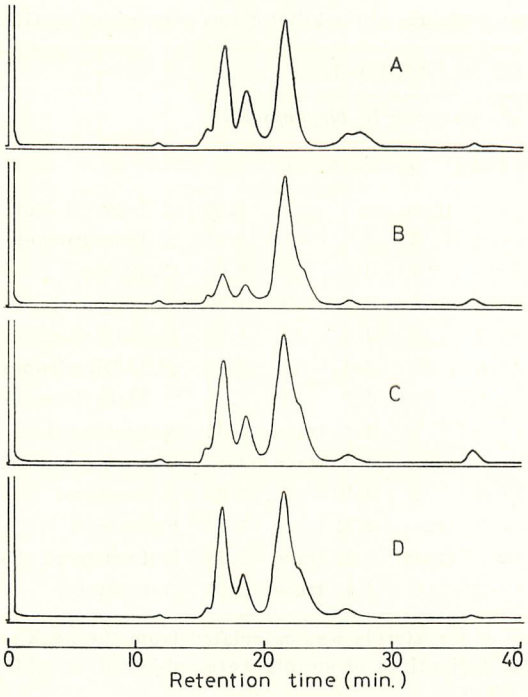

Fig. 1. GLC on 1.5 % SE-30 of the sterols isolated from the killer clams. A, *T. crocea*; B, *T. noae*; C, *T. squamosa*; D, *H. hipponps*

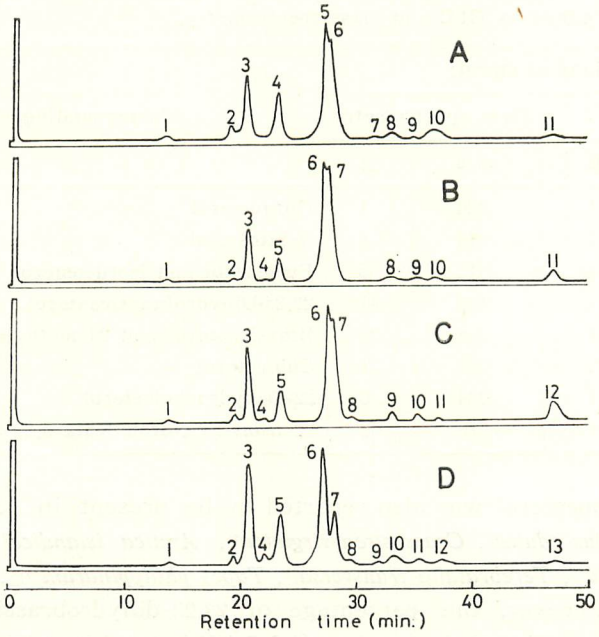

Fig. 2. GLC on 1.5% OV-17 of the sterols isolated from the killer clams. A, *T. crocea*; B, *T. noae*; C, *T. squamosa*; D, *H. hipponps*.

Table 2. Sterol compositions of the killer clams determined by GLC on 1.5% OV-17.

Sterols detected in GLC on 1.5% OV-17*								RRT**	Identified as
T. crocea		*T. noae*		*T. squamosa*		*H. hipponps*			
Peak	%	Peak	%	Peak	%	Peak	%		
a-1	1	b-1	trace	c-1	trace	d-1	trace	0.66	22-*trans*-24-Norcholesta-5,22-dien-3β-ol
a-2	4	b-2	1	c-2	3	d-2	3	0.94	22-Dehydrocholesterol
a-3	22	b-3	12	c-3	24	d-3	29	1.00	Cholesterol
	—	b-4	trace	c-4	1	d-4	3	1.07	Unknown storol
a-4	17	b-5	5	c-5	11	d-5	14	1.13	Brassicasterol
a-5	44	b-6	65	c-6	39	d-6	34	1.30	22,23-Dihydrobrassicasterol
a-6	4	b-7	13	c-7	10	d-7	13	1.35	24-Methylenecholesterol
	—		—	c-8	1	d-8	trace	1.42	Stigmasterol
a-7	trace		—		—	d-9	trace	1.53	Unknown sterol
a-8	3	b-8	trace	c-9	3	d-10	4	1.60	β-Sitosterol
a-9	trace	b-9	trace	c-10	trace	d-11	trace	1.70	Fucosterol
a-10	4	b-10	trace	c-11	trace	d-12	trace	1.78	Isofucosterol (?)
a-11	1	b-11	4	c-12	6	d-13	trace	2.26	Gorgosterol

Percentage composition of the sterols was calculated from the peak area in GLC.

* The trimethylsilyl derivatives of sterols were subjected to GLC on 1.5 % OV-17. Column temp. 235 °C

** Relative retention time to cholesterol (retention time, 20.9 min.)

Table 3. Percentage compositions of the sterol components of *T. crocea* determined by GLC and mass spectrometry.

% Compositions of sterols				Corresponding sterols
GLC		Mass spectrometry		
Peak	%	m/e	%	
a-11	1	426	1	Gorgosterol
a-8	3	414	4	β-Sitosterol
a-9 and a-10	4	412	5	Fucosterol and isofucosterol
a-5	44	400	41	22,23-Dihydrobrassicasterol
a-4 and a-6	21	398	20	Brassicasterol and 24-methylenecholesterol
a-3	22	386	21	Cholesterol
a-2	4	384	6	22-Dehydrocholesterol
a-1	1	370	2	22-*trans*-24-Norcholesta-5,22-dien-3β-ol

cholesterol or campesterol was also reported to be present in some pelecypods: *Nucula* sp.,[9] *Mytilus edulis*[9], *Crassostrea virginica*[9], *Arctica islandica*[9], *Spisula solidissima*[9], *Mya arenaria*[9], *Terebratalia transversa*[9], *Tapes philippinarum*[10,11], and *Placopecten magellanicus*[12]. However, the percentage of 22,23-dihydrobrassicasterol in the sterols of the above pelecypods was low (0.6-5.4 % in total sterols). Compared with other families of pelecypods, the killer clams appear to be unique in the respect

that they contains large amounts of 22,23-dihydrobrassicasterol in their tissues.

In plants, it has been proposed that 24-methylcholesterol such as campesterol is synthesized from 24-methylenecholesterol which is formed from C_{27}-sterols by transmethylation from S-adenosyl-methionine[13,14]. In addition, FAGERLUND and IDLER[15] have demonstrated that the clam, *Saxidomus giganteus*, belonging to the same Pelecypoda as the killer clams, is capable of forming 24-methylenecholesterol from cholesterol. Considering these facts, the occurrence of large amounts of both 22, 23-dihydrobrassicasterol and 24-methylenecholesterol in the tissues of killer clams may postulate the possibility that they probably possess the ability for the interconversion of both the sterols. On the other hand, the symbiosis with the zooxanthellae in the mollusks of the family Tridacnidae is a well-known fact[16]. Also, CIERESZKO *et al.*[8] have shown that the zooxanthellae from the giant clam, *T. gigas*, contained C_{27}-(molecular weight 386; 28 % of total sterols) and C_{28}-(molecular weight 400; 72 % of total sterols) sterols with one double bond. The authors presume that C_{28}-sterol reported by CIERESZKO *et al.*[8] was probably 22,23-dihydrobrassicasterol. Therefore, it may be assumed that a part of 22,23-dihydrobrassicasterol occurring in the killer clams originates from the symbiotic zooxanthellae.

In addition to the four sterols mentioned above, the killer clams examined contained small amounts of 22-*trans*-24-norcholesta-5, 22-dien-3β-ol (trace-1 %), 22-dehydrocholesterol (1-4 %), stigmasterol (0-1 %), β-sitosterol (trace-4 %), fucosterol (trace-4 %), gorgosterol (trace-6%), and unknown sterols. Gorgosterol, a C_{30}-sterol, has been found in a number of coelenterates and their zooxanthellae[8,17,18]. However, the zooxanthellae from the sea anemone, *Anthopleura elegantissima*, and the giant clam, *T. gigas*, were found to contain no gorgosterol[8]. In the present study, the killer clams, *T. crocea*, *T. noea* and *T. squamosa*, were clarified to contain small amounts of gorgosterol.

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