Comparative Study on the Sterol Composition of Marine Mollusks

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From the viewpoint of comparative biochemistry, the sterol compositions of mollusks belonging to the class Gastropoda (three orders, nine families, sixteen genera, seventeen species), Pelecypoda (two orders, three families, three genera, three species), and Polyplacophora (one species) were investigated by using gas-liquid chromatography on 1.5% OV-17.

The gastropods were found to contain cholesterol as a main sterol. In the case of *Murex asianus*, *Cymatiidae* sp., *Charonia sauliae*, and *Conomurex luhuanus*, however, other C₂₇- and C₂₈-sterols were detected in addition to cholesterol (42-64%). Compared to the gastropods, the pelecypods appeared to contain relatively small amount of cholesterol (26-35%) and revealed the very complex composition of sterols. On the other hand, the chiton, *Liolophura japonica*, belonging to the most primitive mollusks, was found to contain 7-cholestenol (Δ^7 -sterol) contrasting that of the other mollusks.

It is a well-known fact that a variety of C₂₇-, C₂₈- and C₂₉-sterols occur in marine mollusks¹⁾. During the works of the past decade, the several sterols which had not been found in natural sources or animals have been isolated from the marine mollusks such as pelecypods. For example, 24-methylenecholesterol²⁾, 22-*trans*-24-norcholesta-5,22-dien-3 β -ol^{3,4)}, and 22-*cis*-22-dehydrocholesterol⁵⁾ have been isolated from the scallop, *Placopecten magellanicus*, and/or the clam, *Tapes philippinarum*, as a new sterol. By using usual column and thin-layer chromatographic techniques, however, it is generally difficult to separate completely the individual components of sterol mixtures occurring in marine mollusks. Recently, a few workers have attempted to elucidate the sterol composition of marine mollusks by means of gas-liquid chromatography (GLC)⁶⁻¹²⁾ and the combined GLC-mass spectrometry^{3,5)}. However, the knowledge on the sterol composition of marine mollusks is still little.

In the previous study¹³⁾, the authors have extensively clarified the sterol components of the short-necked clam, *T. philippinarum*, by using both GLC and spectral analyses. The present investigation was undertaken to obtain further knowledge on the sterol composition of marine mollusks in the viewpoint of comparative biochemistry. This paper describes the sterol composition of the marine mollusks (twenty-one species) determined by GLC.

Materials and Methods

Mollusks. The mollusks examined in the present study belong to the class Poly-

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placophora (one species), Gastropoda (seventeen species), and Pelecypoda (three species) as shown in Table 1. These mollusks were caught at the Yoron Island and the Cape of Sata in Kagoshima during August, 1970, and during April, 1971, respectively. The mollusks were dipped into methanol and transported to this laboratory.

Table 1. The mollusks examined in the present study.

Class	Order	Family	Species	Japanese name	
Poly-placophora	Ischnochitonida	Chitonidae	<i>Liolophura japonica</i>	Hizaragai	
		Haliotidae	<i>Haliotis gurneri</i>	Tokobushi	
		Acmaeidae	<i>Cellana nigrisquamata</i>	Kasagai	
	<i>Cellana nigrolineata</i>		Matsubagai		
	<i>Patelloida saccharina</i>		Unoashi		
	Archaeogastropoda	Trochidae	<i>Monodonta labio</i>	Ishidatami	
			<i>Teculatus maximus</i>	Gintakahama	
			<i>Trochus maculatus</i>	Nishikiuzu	
		Turbinidae	<i>Astraliu haematragerum</i>	Urauzugai	
			<i>Turbo cornutus</i>	Sazae	
Gastropoda			Strombidae	<i>Conomurex luhuanus</i>	Magakigai
	Mesogastropoda	Cypraeidae		<i>Peribolus arabica</i>	Yakujimadakara
		Cypraeidae		<i>Ravitronea capterpentis</i>	Hanamaruyuki
		Cymatiidae		<i>Charonia sauliae</i>	Boshubora
<i>Cymatiidae sp.</i>			—		
Neogastropoda	Muricidae	<i>Murex asianus</i>	Onisazae		
		<i>Purpura bronni</i>	Reishi		
	Eascoliariidae	<i>Pleuroplaca trapezium</i>	Himeitomakibora		
		Pelecypoda	Pteriomorphia	Pinnidae	<i>Atrina pectinata</i>
Mytilidae	<i>Mytilus edulis</i>			Murasakiigai	
Heterodonta	Veneidae		<i>Tapes philippinarum</i>	Asari	

Isolation of sterols. From the mollusks, the lipids were extracted with chloroform-methanol according to the method of BLIGH and DYER¹⁴⁾, and then saponified with ethanolic potassium hydroxide. The sterols were isolated from the unsaponifiable matters by using column chromatography on alumina with hexane-benzene¹⁵⁾. The details of the procedure for isolation of sterols have been described in the previous papers¹³⁾. After recrystallization from methanol, the purified sterols were subjected to GLC.

GLC. GLC was conducted with a Shimadzu GC-3AF unit by using 3 m long, 4 mm I.D. silanized glass column, packed with 1.5% OV-17 on 80-100 mesh Shimalite W. The column was operated at 243°C with nitrogen carrier gas (2.0 kg/cm²). The identification of peaks was performed by comparing retention times to those of both authentic sterols and the sterol mixture of the short-necked clam, *T. philippinarum*.

Table 2. Sterol content of the marine mollusks.

Species	Fresh weight (g)	Lipids		Unsap. matters		Sterols	
		(g)	(%)*	(mg)	(%)*	(mg)	(%)*
<i>L. japonica</i>	75	0.65	0.85	84	0.11	52	0.070
<i>H. gurneri</i>	100	1.27	1.27	870	0.87	91	0.091
<i>C. nigrisquamata</i>	100	4.50	4.50	185	0.19	116	0.116
<i>C. nigrolineata</i>	11	0.21	1.9	23	0.21	11	0.10
<i>P. saccharina</i>	23	0.69	3.0	46	0.20	30	0.13
<i>M. labio</i>	90	1.83	2.0	425	0.48	123	0.13
<i>T. maximus</i>	50	1.20	2.4	55	0.11	35	0.07
<i>T. maculatus</i>	10	0.23	2.3	38	0.38	20	0.20
<i>A. haematrugum</i>	12	0.30	2.5	26	0.22	17	0.16
<i>T. cornutus</i>	100	4.50	4.5	185	0.19	116	0.12
<i>C. luhuanus</i>	42	0.90	2.1	98	0.23	92	0.22
<i>P. arabica</i>	122	3.31	2.5	980	0.80	112	0.09
<i>R. captserpentis</i>	9.0	0.079	0.81	48	0.53	3.9	0.04
<i>C. sauliae</i>	125	3.77	3.0	712	0.57	110	0.09
<i>Cymatiidae sp.</i>	24	0.49	2.1	59	0.28	36	0.15
<i>M. asianus</i>	25	0.44	1.7	218	0.87	50	0.20
<i>P. bronni</i>	30	0.57	1.9	54	0.18	30	0.10
<i>P. trapezium</i>	24	0.30	1.3	51	0.21	36	0.15
<i>A. pectinata</i>	30	0.80	2.7	51	0.17	30	0.10
<i>M. edulis</i>	80	1.51	1.9	380	0.47	171	0.21
<i>T. philippinarum</i>	20	0.31	1.5	101	0.50	12	0.06

* Per fresh matter

Results

The content of sterols isolated from the twenty-one species of mollusks is given in Table 2. The mollusks examined contained 0.06% to 0.21% of sterols in their tissues. The sterol composition of mollusks determined by GLC on 1.5% OV-17 is shown in Table 3.

It was found that most gastropods contain large amount of cholesterol and other minor sterols. In the case of *Conomurex luhuanus*, *Charonia sauliae*, *Cymatiidae sp.*, and *Murex asianus*, however, the percentages of cholesterol were low (42%–64%) as compared with the other gastropods. In addition, these mollusks contained a substantial amount of 22-dehydrocholesterol (5%–6%), brassicasterol (4%–22%), desmosterol (trace–33%), campesterol (0–16%), 24-methylenecholesterol (0–5%) and β -sitosterol (4%–10%).

On the other hand, the sterol composition of the pelecypods was very complex in contrast to the gastropods. The three species of pelecypods examined contained a number of sterol components (7–10 components) besides cholesterol. Also, in these pelecypods the percentages of cholesterol were low (26%–35%) as compared with those of the gastropods. Furthermore, these pelecypods were found to contain relatively high amount of other C_{27} -, C_{28} -, and C_{29} -sterols. For example, the sterols isolated from

Table 3. Sterol composition of the marine mollusks determined by GLC.

Species	Composition (%) of sterols											
	A	B	C	D	E	F	G	H	I	J	K	L
<i>L. japonica</i>	—	—	—	4	—	—	93	3	—	—	—	—
<i>H. gurneri</i>	1	—	6	93	—	—	—	—	t*	—	—	—
<i>C. nigrisquamata</i>	—	—	—	86	3	10	—	2	—	—	—	—
<i>C. nigrolineata</i>	—	—	—	98	—	2	—	—	—	—	—	—
<i>P. saccharina</i>	—	—	—	100	—	—	—	—	—	—	—	—
<i>M. labio</i>	—	—	t	99	t	t	—	—	—	—	—	—
<i>T. maximus</i>	—	—	4	79	3	t	—	6	t	1	8	t
<i>T. maculatus</i>	—	—	2	82	5	—	—	7	—	—	5	—
<i>A. haematrangum</i>	—	—	—	78	3	—	—	7	—	—	8	4
<i>T. cornutus</i>	—	—	t	99	—	t	—	—	t	—	t	t
<i>C. luhuanus</i>	—	—	5	64	9	—	—	—	2	t	10	—
<i>P. arabica</i>	t	t	4	80	4	2	—	t	4	2	5	t
<i>R. captserpentis</i>	—	—	—	100	—	—	—	—	—	—	—	—
<i>C. sauliae</i>	t	—	1	42	4	35	—	—	5	t	4	—
<i>Cymatiidae sp.</i>	—	t	6	52	22	—	—	16	—	—	4	—
<i>M. asianus</i>	t	t	5	56	17	t	—	12	3	t	5	1
<i>P. bronni</i>	—	—	11	86	4	—	—	—	—	—	—	—
<i>P. trapezium</i>	—	—	1	83	9	—	—	3	—	—	4	—
<i>A. pectinata</i>	1	1	16	26	23	—	—	t	21	2	6	1
<i>M. edulis</i>	2	4	25	30	23	4	—	—	12	—	2	—
<i>T. philippinarum</i>	4	1	6	35	14	9	—	t	17	4	8	3

* Trace

Relative retention times (RRT) to cholesterol (27.8 min.) were as follows: A (22-*trans*-24-norcholesta-5,22-dien-3 β -ol, 0.66), B (unknown, 0.88), C (22-dehydro-cholesterol, 0.92), D (cholesterol, 1.00), E (brassicasterol, 1.12), F (Desmosterol, 1.18), G (7-cholestenol, 1.19), H (campesterol, 1.28), I (24-methylene cholesterol, 1.32), J (stigmaterol, 1.39), K (β -sitosterol, 1.57) and L (fucosterol, 1.73).

In the case of the following mollusks, other unknown components were detected: *M. asianus* (RRT 1.61, 1%; RRT 1.82, trace), *A. pectinata* (RRT 1.48, 1%; RRT 1.82, trace), and *C. sauliae* (RRT 1.48, trace; RRT 1.61, 1%; RRT 1.82, 2%).

Atrina pectinata were composed of 22-*trans*-24-norcholesta-5,22-dien-3 β -ol (1%), unknown sterol (1%), 22-dehydrocholesterol (16%), cholesterol (26%), brassicasterol (23%), campesterol (trace), 24-methylenecholesterol (21%), stigmaterol (2%), β -sitosterol (6%), and fucosterol (1%).

In the case of *Liolophura japonica*, belonging to the most primitive mollusks, the sterols were mainly composed of 7-cholesterol (Δ^7 -sterol) in contrast to the other higher mollusks which contained Δ^5 -sterol.

Discussion

The several workers have investigated on the sterol composition of marine mollusks by using GLC, and demonstrated that in some pelecypods^{6,7)} a variety of types of C₂₇-, C₂₈- and C₂₉-sterols besides cholesterol occurred. However, relatively little is known about the details of sterol composition of gastropods. Recently, YASUDA¹²⁾ has studied on the

sterols of eight species of marine or fresh water gastropods, and showed that the sterol composition of the gastropods was simple as compared with that of the pelecypods. In the present study, it was also clarified that the most gastropods examined contained large portion of cholesterol and minor or trace amount of other sterols, although a few gastropods such as *Charonia sauliae* revealed the complex sterol composition as also occurred in the pelecypods. However, no notable relationship between the sterol composition and taxonomy was perceived among the gastropods examined.

The most reasonable conclusion to be drawn from available data appears to be as follows: 1) The class Polyplacophora, the most primitive mollusks, contains Δ^7 -cholestenol and a small amount of Δ^5 -sterols (C_{27} and C_{28}); 2) The class Pelecypoda contains 22-dehydrocholesterol, cholesterol, brassicasterol, and 24-methylencholesterol as a principal sterol. In addition, small amounts of C_{26} ($\Delta^{5,22}$), C_{27} ($\Delta^{5,24}$), C_{28} (Δ^6), and C_{29} (Δ^5 , $\Delta^{5,22}$, $\Delta^{5,24,28}$)-sterols are occasionally detected; 3) The class Gastropoda, the higher mollusks than Pelecypoda, contains large amount of cholesterol and a small amount of Δ^5 -sterols which are often found in the Pelecypoda.

Recently, a few workers have investigated on sterol-synthesizing ability in marine mollusks^{9,16-19}). However, the origin of sterols other than cholesterol occurring in marine mollusks is still obscure, except for 24-methylencholesterol^{20,21}).

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