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## Studies on the Lipids of Shell-fish

### III. On the fatty acid and sterol compositions of a purple and a lischke's tegula top shell snails

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#### Abstract

This report presents studies on the fatty acid and sterol compositions of the acetone-soluble lipids obtained from the flesh and viscera of two species of snails, i.e., a purple, *Thais clavigera*, and a lischke's tegula top shell, *Chlorostoma argyrostoma lischkei*, which were collected from the same habitat during the same season.

The predominant fatty acid components were 20:1 and 20:5 acids for the purple, and 16:0 and 18:1 acids for the lischke's tegula top shell. It was inferred that significant differences in the fatty acid compositions between the two species were related to their different feeding habits, that of the purple, being carnivore, and of the lischke's tegula top shell, herbivore.

While the unsaponifiables of both species were composed of large amounts of sterols, mainly cholesterol (87-93%), small amounts of hydrocarbons and glyceryl ethers were also detected.

#### Introduction

Shell-fishes are roughly classified into three main classes from the viewpoint of their feeding habits<sup>1)</sup>, that is, herbivore, carnivore and filter feeder; and besides, the omnivore, a transitive stage of herbivore and carnivore, and the detritus feeder, a kind of filter feeder, are added to them.

As to the lipids of shell-fishes, numerous studies have been reported on their characteristics<sup>2-7)</sup>, fatty acid compositions<sup>8-13)</sup>, sterols<sup>14-18)</sup> and phosphatides<sup>19-22)</sup>, however, there were few investigations on the correlation between the lipid compositions of shell-fishes and their feeding habits.

The authors have been studying on the lipid compositions of shell-fishes as a clue to elucidate the correlation between the lipids of marine organisms and their food-chain or habitat. Previously, they have studied on the visceral lipid composition of the abalone<sup>23)</sup>, the herbivore snail, and on those of the flesh and viscera of the Japanese prickly scallop<sup>24)</sup>, a filter feeder bivalve. From the results, it was inferred that both lipids of the abalone and the J. p. scallop were slightly affected by each dietary lipid.

They have successively studied on the lipid compositions of two snails, i.e.,

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a purple, *Thais clavigera*, and a lischke's tegula top shell, *Chlorostoma argyrostoma lischkei*.

The purple is classified with the Family Muricidae of the Order Neogastropoda and the l. t. top shell with the Family Trochidae of the Order Archaeogastropoda<sup>25</sup>). Both snails widely inhabit the rocky shores on the tideland of the sea. The purple has been known as a kind of carnivore snail destructive to the cultivation of oysters, designated as oyster drill or screw borer<sup>26</sup>). One of the authors had observed that several purples clambered up the l. t. top shells in which holes were made on the shore being at the ebb at dawn. On the other hand, the feeding habits of the l. t. top shell are not known in detail; however, this snail may possibly be herbivore, when considering the fact that the diets of *Trochus* spp.<sup>27</sup>), belonging to the Family Trochidae, are algae or algal detritus, and that many kinds of herbivore snails belong to the Order Archaeogastropoda.

Then, as to the lipids of these two species, there are few reports on the characteristics and  $\Delta^5,7$ -sterol contents of the acetone-soluble fractions in the ether-extracts from the dried shucked purple<sup>4</sup>), and on the sterols in unsaponifiables of the ethanol-ether-extracts from the raw purple and the l. t. top shell<sup>6</sup>).

The present paper describes the fatty acid and sterol compositions of the acetone-soluble lipids in the fleshy and visceral lipids obtained from the purple and l. t. top shell snails.

## Materials and Methods

### *Materials*

A purple (1630 g, wt. included shells) and a l. t. top shell (2000 g), collected on the shore of Toi near Hakodate, Hokkaido, May 1970, were boiled to shuck easily for 15 min, individually. Then each flesh (230 and 310 g) and viscera (140 and 270 g) of both snails were used for the extraction of lipids.

### *Experimental methods*

Experimental methods, i.e., purification of solvents, lipids extraction, fractionation of acetone-soluble lipids, preparations of fatty acids and unsaponifiables, determination of fatty acids by gas-liquid chromatography (GLC) using the column packed 10% DEGS on 80-100 mesh Chromosorb W AW, GLC of sterols, acetylation of sterols for GLC analyses, thin-layer chromatography (TLC), ultra-violet spectroscopy (UV) and colorimetry of sterols, were done after the same methods or conditions described previously<sup>23</sup>).

### *Fractionation of sterols*

The unsaponifiables dissolved in chloroform (10% soln) were applied zonally

to the origin of the activated preparative silica gel plates (ca. 50 mg of sample/0.5 mm thick), which had been developed previously with benzene, and then with a solvent system of petroleum ether-diethyl ether (40:60, v/v). Sterol bands of a situation lower than that of hydrocarbons on the plates were scrapped off and extracted with diethyl ether.

## Results and Discussion

### *Lipid contents and characteristics of acetone-soluble lipids*

The contents of the total lipids and of the acetone-soluble lipids in the total lipids, and the characteristics of the acetone-soluble lipids of the flesh and the viscera from the purple and the l. t. top shell, are given in Table 1. The total lipid contents of the viscera were larger than those of the flesh from both species, and the results of the visceral lipids had large amounts of acetone-soluble lipids and small amounts of conjugated lipids such as phospholipids agreeing with those of the abalone<sup>23</sup>) and the J. p. scallop<sup>24</sup>). Iodine values of the flesh from both species were barely high in comparison with those of the viscera, and moreover the unsaponifiables contents in the flesh were as high as the results obtained from the J. p. scallop<sup>24</sup>).

Table 1. *Lipid contents and properties of acetone-soluble lipids.*

		Total lipid	Acetone-soluble lipid					USM (%)
		content (% wet wt.)	content (% total lipid)	$n_D^{20}$	A.V.	S.V.	I.V.	
Purple	Flesh	1.5	30.5	1.4804	14.7	168.0	155.3	36.4
	Viscera	5.9	72.5		6.7		150.6	15.4
L. t. top shell	Flesh	1.4	22.7	1.4806	25.0	190.8	157.9	43.6
	Viscera	5.6	82.4		12.8		139.8	7.7

### *Fatty acid compositions*

The fatty acid compositions of the acetone-soluble lipids fractionated from each fleshy and visceral lipids of the two species are given in Table 2. The fatty acids of both flesh and viscera of the purple contained relatively large amounts of 20:1, 20:5, 16:0, 18:1, 20:4 and 18:4 acid, and it appeared that the viscera exceeded the flesh a little in the acid amounts of 16:0, 18:1 and 18:4. The fatty acids of each flesh and viscera had 21.5 and 23.0% saturated acids, 34.8 and 37.1% mono-unsaturated acids, 43.6 and 39.9% polyunsaturated acids respectively, both compositions of the flesh and viscera being similar on the whole. On the other hand, the fatty acids of both flesh and viscera of the l. t. top shell contained relatively large acid amounts of 16:0, 18:1, 20:4 and 20:5, and it appeared that the flesh

Table 2. *Fatty acid compositions of acetone-soluble lipids.*

Number of carbon atoms and double bonds	Purple		L. t. top shell	
	Flesh	Viscera	Flesh	Viscera
	Peak area %			
10:0	0.1	0.1		
12:0	0.1	0.1	trace	0.2
:1	0.1	trace	0.2	trace
13:0	0.1	0.1	trace	0.2
:1	trace	0.1	trace	0.2
14:0	2.9	3.8	3.2	6.7
:1	trace	0.4	0.8	0.7
15:0	0.8	0.5	0.9	0.3
:1	0.6	0.2	0.5	0.2
16:0	10.9	12.8	22.4	16.7
:1	3.9	4.5	5.4	4.9
17:0	2.1	1.4	2.1	1.1
:1	1.9	0.2	2.2	0.5
18:0	4.5	4.2	6.2	2.9
:1	7.4	10.2	14.8	26.2
:2	2.0	2.4	4.2	5.1
:3	1.0	1.2	2.3	3.2
:4	6.0	7.9	2.5	6.5
19:0	trace			
:1	0.4	0.8	0.7	0.2
20:0				trace
:1	18.0	18.7	3.6	4.5
:2	2.4	1.6	0.5	0.5
:4	9.3	7.0	14.7	6.2
:5	15.5	14.5	10.4	9.5
21:1	0.8	0.8	0.2	0.9
:5	1.6	0.9	0.8	0.4
22:1	1.7	1.2	0.4	1.3
:2	0.5	trace	trace	0.3
:5	3.6	2.3	1.0	0.6
:6	1.7	2.1	trace	trace
24:1	trace	trace		trace

exceeded the viscera a little in the acid amounts of 16:0, 18:0 and 20:4, contrariwise in the acid amounts of 14:0, 18:1 and 18:4. The fatty acids of each flesh and viscera had 34.8 and 28.1% saturated acids, 28.8 and 39.6% monounsaturated acids, 36.4 and 32.3% polyunsaturated acids respectively. The amounts of saturated and polyunsaturated acids of the flesh were somewhat larger than those of the viscera, conversely those of monounsaturated acids were smaller.

Pointing out the significant differences in fatty acids between the purple and the l. t. top shell, it was noticed that the former had larger acid amounts of 20:1 and 20:5 than the latter, and contrariwise the latter had larger acid amounts of 16:0 and 18:1 than the former. Moreover polyunsaturated acids were predominant in the former and saturated acids in the latter. 18:1 acid contents of both flesh and viscera of the l. t. top shell were larger than those of the purple. In this

respect the authors have found that the visceral lipids of the abalone<sup>23</sup>, herbivore, had relatively large acid amounts of 18:1. Shimma and Taguchi<sup>8</sup>) have also pointed out that this acid content of the abalone or kid-abalone, herbivore, had about twice the quantity of the other shell-fishes of filter feeders. In addition, 18:2 and 18:3 acids, derived from the dietary plant, in the l. t. top shell were comparatively larger than those in the purple, and their contents had some resemblance to those in the abalone<sup>23</sup>).

Considering the fatty acid compositions of these two tested snails, collected from the same habitat during the same season, it was thinkable that the fatty acid metabolism of the l. t. top shell was different from that of the purple, carnivore. And the composition of the l. t. top shell was rather similar comparatively to that of the abalone<sup>23</sup>), herbivore, which had showed a characteristic pattern of fatty acids. Consequently, it was inferred that the feeding habits of the l. t. top shell would be mainly algal-feeding.

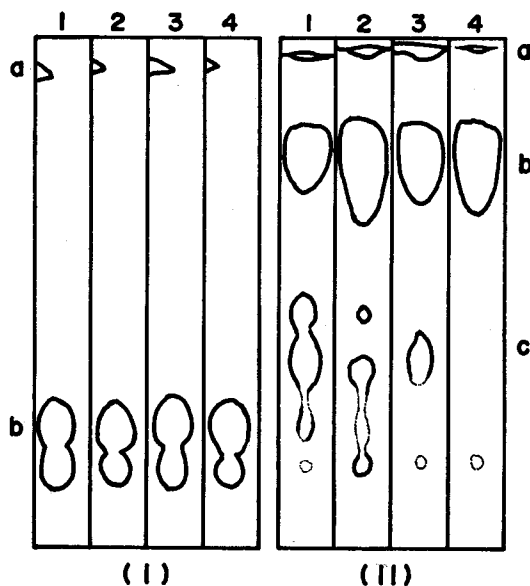


Fig. 1. Thin-layer chromatograms of unsaponifiables.  
Purple: 1. Viscera 2. Flesh, L. t. top shell: 3. Viscera 4. Flesh  
(I) Benzene (II) Petroleum ether: diethyl ether (40: 60,v/v)  
a: hydrocarbons b: sterols c: glyceryl ethers

#### *Sterol compositions*

The unsaponifiables of the acetone-soluble lipids from the flesh and the viscera of the purple and the l. t. top shell were analyzed by TLC, and the chromatogram results are shown in Fig. 1. The major unsaponifiable components of both species were sterols, hydrocarbon and glyceryl ether spots were also detected. Apparently,

the viscera had larger spots of glyceryl ethers than those of the flesh of both species. From the results of both species sterols were the most important unsaponifiable components corresponding to those of the abalone<sup>23)</sup> and the J. p. scallop<sup>24)</sup>. Moreover the sterol components were determined relatively in detail as follows. The contents of  $\Delta^{5,7}$ -sterols contained in the sterols of the flesh and viscera of the l.t. top shell, which was obtained by calculating from the equation<sup>28)</sup>, were 0.57% and 1.72%, respectively. These results agree with the data of 1.8%  $\Delta^{5,7}$ -sterols determined by Kita and Toyama<sup>6)</sup>. The changes of the Liebermann-Burchard reaction

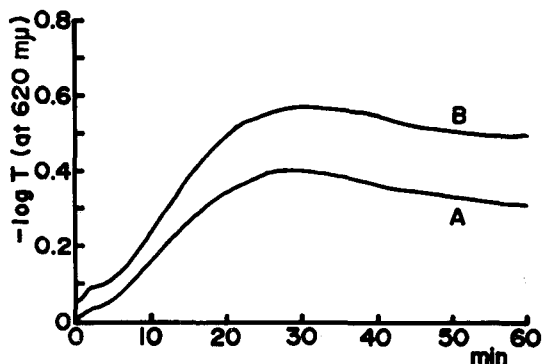


Fig. 2. Rate of Liebermann-Burchard reaction of sterols obtained from the flesh (A) and viscera (B) of a lischke's tegula top shell.

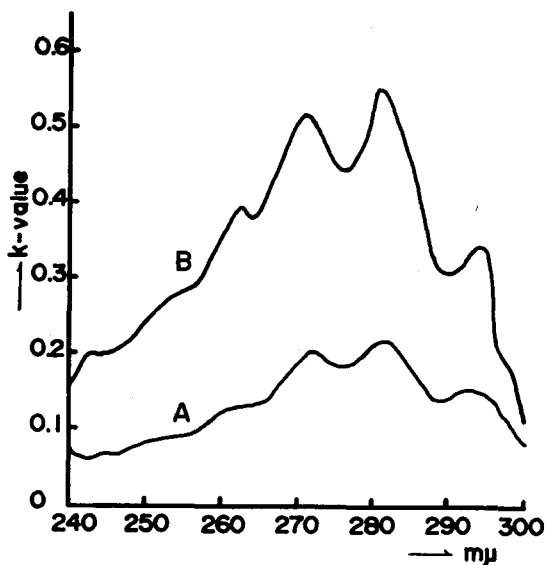


Fig. 3. UV spectra of sterols obtained from the flesh (A) and viscera (B) of a lischke's tegula top shell. (in ethanol)

Table 3. *Compositions of sterols.*

r <sup>*</sup>	Component	Purple		L. t. top shell	
		Flesh	Viscera	Flesh	Viscera
		Peak area %			
0.35	unknown	0.2	0.6		
0.43	unknown	3.8	2.8	3.3	1.7
0.64	unknown	0.7	0.7		
1.00	cholesterol	92.3	86.6	92.2	92.6
1.27	2,4-methylenecholesterol	1.6	4.7	2.8	2.9
1.63	$\beta$ -sitosterol	1.4	4.6	1.7	2.8

\* compared with cholesterol=1.00.

at 620  $m\mu$  and the UV spectra of sterols of the flesh and the viscera from the l.t. top shell are shown in Fig. 2 and Fig. 3, respectively. Kita and Toyama<sup>6)</sup> have reported that the sterols of marine invertebrates had a specific absorption at 255  $m\mu$ , nevertheless, as seen in Fig. 3, this absorption was not recognized in our samples, rather, the pattern of the l. t. top shell was similar to that of the J.p. scallop<sup>24)</sup>. On the other hand, as shown in Fig. 2, the pattern of the l. t. top shell took a tendency of a  $\Delta^5$ -sterols type, but not the pattern of the J. p. scallop<sup>24)</sup>.

The gas-chromatographic data of the flesh and viscera of the two species are given in Table 3. In both species, sterols consisted of 87–93% cholesterol, and other minor sterols were considered to be 2,4-methylenecholesterol and  $\beta$ -sitosterol, comparing with the data of the relative retention from literatures<sup>20–22)</sup>. From the results of the two species, cholesterol was the most important component of sterols being similar to that of the abalone<sup>23)</sup>, but not to that of the J. p. scallop<sup>24)</sup>. These data obtained from the two species agree with the fact that sterols of snails are mainly cholesterol, as was obvious from the past report<sup>22)</sup>. These results may be related to the fact that numerous components were found in animals of a lower order and they were made into cholesterol, thus decreasing the kind of sterols with the progress of evolution<sup>29)</sup>. A biosynthesis of sterols in Mollusca, however, has not been studied sufficiently yet.

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