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$\Delta^{5,7}$-Sterol Constituents of Some Bivalves

Shin-ichi TESHIMA,* Akio KANAZAWA,*
and Ryuji SHIMAMOTO*

Abstract

The composition of $\Delta^{5,7}$-sterols and other sterols of six bivalves collected in Okinawa, Japan, was investigated. Sterols were identified by gas-liquid chromatography (GLC) on 1.5% OV-17 and GLC-mass spectrometry. The bivalves examined contained seven $\Delta^{5,7}$-sterols and a few Δ^{7}-sterols besides Δ^{5}-sterols commonly occurring in marine molluscs. *Saxostrea mordax* and *Tridacna crocea* contained cholesta-5,7-dienol as the major sterols (about 50% of total $\Delta^{5,7}$-sterols), whereas *Protostrea hyotis* and *Pinctada margaritifera* possessed 24-methylcholesta-5,7,22-trienol at the levels of 65% and 53%, respectively. *Atrina vexillum* contained cholesta-5,7-dienol (40%), 24-methylcholesta-5,7,22-trienol (32%), and cholesta-5,7,22-trienol as the prominent sterols. *Hippopus hippopus* involved cholesta-5,7-dienol (14%), 24-methylcholesta-5,7-dienol (12%), 24-methylcholesta-5,7,22-trienol (37%), and 24-ethylcholesta-5,7-dienol (37%). A possitive correlation was observed between the compositions of some Δ^{5}-sterol (% of total Δ^{5}-sterols) and corresponding $\Delta^{5,7}$-sterol (% of total $\Delta^{5,7}$-sterols).

Molluscan sterols have been studied in the viewpoint of comparative biochemistry and in the interest of finding new sterols due to the complexity of some species, especially pelecypods.[1-3] However, less attention has been paid to elucidate the $\Delta^{5,7}$-sterol constituents nevertheless earlier studies[4] pointed out the occurrence of abundance of $\Delta^{5,7}$-sterols in some molluscs. In the previous studies, we showed that the oyster *Crassostrea virginica*[5] and Japanese gastropods and pelecypods[6] contained a mixture of C_{26}, C_{27}, C_{28}, and C_{29} $\Delta^{5,7}$-sterols. Other recent studies have also demonstrated the occurrence of various $\Delta^{5,7}$-sterols in the gastropods, *Purpura mastoma*[7] and *Murex trunculus*,[7] the oyster *Crassostrea gigas*,[8] and seven British bivalves.[9] The present investigation is planned to obtain further information on the $\Delta^{5,7}$-sterols constituents in the viewpoint of comparative biochemistry. This paper deals with the $\Delta^{5,7}$-sterols and other sterols of six pelecypods collected in Okinawa, Japan.

Materials and Methods

Specimens of the bivalve molluscs were collected in Okinawa during July. Lipids were extracted from the alive bivalves (Table 1) by the method of BLIGH and DYER[10] and

* Faculty of Fisheries, Kagoshima University, 50-20 Shimoarata-4, Kagoshima 890, Japan.

Table 1. The pelecypods examined and their taxonomy

Class	Order	Species	Japanese name
Pelecypoda	Dysodonta	*Pretostrea hyotis*	Shakogaki
		Saxostrea mordax	Ohagurogaki
		Pinctada margaritifera	Kurochougai
		Atrina vexillum	Kurotairagi
	Heterodonta	*Hippopus hippopus*	Shagougai
		Tridacna crocea	Himejako

saponified with 10% ethanolic potassium hydroxide at 80℃ for 2 hours to isolate unsaponifiable matters in the usual manner. Sterols were isolated by alumina column chromatography with hexane-ether[11] and then acetylated with pyridine-acetic anhydride. Sterol constituents were identified by gas-liquid chromatography (GLC) on 1.5% OV−17 (2m×3 mm i.d., column temperature 260℃), argentic thin-layer chromatography ($AgNO_3$−TLC), and GLC-mass spectrometry (GLC-Mass) of the sub-fractions obtained by $AgNO_3$−TLC as described previously.[6,11,12] GLC-Mass was conducted with JEOL JGL−20K gas-chromatograph (3.0% OV−1; 2m×2 mm i.d., column temperature 285℃) and JEOL JMS−D300 mass spectrometer. As possible as we could, experiments were performed under the interception of light to prevent the decomposition of $\Delta^{5,7}$-sterols.

Results and Discussion

The sterols of six pelecypods from Okinawa were characterized by GLC and GLC-Mass. Generally, gastropods contain cholesterol as the exclusively major sterol, whereas pelecypods possesses lesser amounts of cholesterol and a variety of types of other Δ^5-sterols.[1,13] The pelecypods examined also contained a mixture of sterols commonly occurring in other marine molluscs (Table 2). Interestingly, *Pretostrea hyotis* and *Hippopus hippopus* contained larger amounts of C_{28}-sterols such as 24-methylcholesta-5, 22-dienol and 24-methylcholest-5-enol than cholesterol. Previously, we pointed out that the killer clams, *Tridacna squamosa*, *Tridacna noae*, *Tridacna crocea*, and *H. hippopus*, which were collected from Okinawa and Amami (the southern part of Japan), contained large amounts of 24-methylcholest-5-enol (34−65% of total sterols)[14] unlike other pelecypods.[1] These results suggest that the uncommon sterol compositions of *P. hyotis* and the killer clams are the relfection of their unique feeding habits.

In addition to the Δ^5-sterols, the present study showed the presence of seven $\Delta^{5,7}$-sterols in the bivalves (Table 3). The $\Delta^{5,7}$-sterols detected were grouped into 2 types. One was the sterol with a saturated side chain such as cholest-5, 7-dienol, 24-methylcholesta-5, 7-dienol, and 24-ethylcholesta-5, 7-dienol, and the other was the sterol with an unsaturated side chain such as cholesta-5, 7, 22-trienol, 24-methylcholesta-5, 7, 22-trienol, 24-methylenecholesta-5, 7-dienol, and 24-ethylcholesta-5, 7, 22-trienol. The bivalves, *Saxostres mor-*

Table 2. Composition (% of total sterols except $\Delta^{5,7}$-sterols) of Δ^{5}- and Δ^{7}-sterols of the pelecypods

Sterol*1	*P. hyotis*	*S. mordax*	*P. margaritifera*	*A. vexillum*	*H. hippopus*	*T. corcea*
24-Norcholesta-5, 22-dienol	*t**2	—	—	—	—	—
Occelasterol	—	—	—	—	—	1.3
22-Dehydrocholesterol	4.6	9.1	6.6	8.7	5.0	9.6
Cholesterol	25.8	46.7	47.4	44.5	29.2	41.0
Cholest-7-enol	1.5	—	—	1.0	—	—
24-Methylcholesta-5, 22-dienol	15.4	24.6	16.7	23.4	15.4	24.7
24-Methylcholest-5-enol	22.6	9.2	8.3	7.8	37.6	12.1
24-Methylcholesta-7, 22-dienol	3.9	—	—	—	—	—
24-Methylenecholesterol	9.1	1.3	3.0	1.5	3.1	3.0
24-Ethylcholesta-5, 22-dienol	7.2	2.5	6.3	4.3	2.5	2.5
24-Ethylcholest-5-enol	4.2	0.1	9.6	6.7	4.6	5.0
24E-24-Ethylidenecholest-5-enol	0.4	—	1.0	t	—	—
24Z-24-Ethylidenecholest-5-enol	0.4	6.1	1.1	1.2	0.6	0.2
24E-24-Ethylidenecholest-7-enol	3.9	—	—	—	1.3	—

*1 In addition to these sterols, some bivalves contained small amounns of unknown sterols (< 1%).
*2 Less than 0.1%.

Table 3. Composition (% of total $\Delta^{5,7}$-sterols) of $\Delta^{5,7}$-sterols of the pelecypods

$\Delta^{5,7}$-Sterol	*P. hypotis*	*S. mordax*	*P. margaritjfera*	*A. vexillum*	*H. hippopus*	*T. corcea*
Cholesta–5, 7-dienol	10	50	31	40	14	48
24-Methylcholesta-5, 7-dienol	8	10	3	4	12	13
24-Ethylcholesta-5, 7-dienol	2	22	11	3	37	9
Cholesta-5, 7, 22-trienol	10	3	1	15	—	7
24-Methylcholesta-5, 7, 22-trienol	65	13	53	32	37	20
24-Ethylcholesta-5, 7, 22-trienol	3	2	2	1	—	3
24-Methylenecholesta-5, 7-dienol	2	—	—	5	—	—

dax and *T. crocea*, contained cholesta-5, 7-dienol at the levels of 50% (% of total $\Delta^{5,7}$-sterols) and 48%, respectively, as the major $\Delta^{5,7}$-sterols, whereas *P. hyotis* and *Pinctada margaritifera* possessed 24-methylcholesta-5, 7, 22-trienol at the levels of 65% and 53%, respectively. *Atrina vexillum* contained cholesta-5, 7-dienol (40%), 24-methylcholesta-5, 7, 22-trienol (32%), and cholesta-5, 7, 22-trienol (15%) as the prominent $\Delta^{5,7}$-sterols. *H. hippopus* involved almost equal proportions of C_{27}(26%), C_{28}(37%), and C_{29}(37%) $\Delta^{5,7}$-sterols. The above mentioned $\Delta^{5,7}$-sterols have occurred in many other bivalve molluscs, *C. virginica*,[5] *Cerastoderma edula*,[9] *Chalamys opercularis*,[9] *Ensis soliqua*,[9] *Modiolus modiolus*,[9] *Mya arenaria*,[9] *Mytilus edulis*,[9] *Pecten maximus*,[9] *Scapharca broughtonii*,[6] *Glycymeris vestita*,[6] *Cyclina sinensis*,[6] *Metretrix petechialis*,[6] *Mactra chinensis*,[6] and *Sinonovacula constricta*.[6]

Although molluscs have long been known to be the good source of provitamin D due to a relatively large amount of $\Delta^{5,7}$-sterols in the whole body, little has been clarified about the reason why some molluscs contain $\Delta^{5,7}$-sterols. The information available suggests that molluscs, especially pelecypods, have a limited capacity for sterol biosynthesis from lower molecules such as acetate and mevalonate.[3,15-17] Also, several reports have shown that some bivalves are capable of *de novo* synthesis of C-24 alkylated sterols[16,18,19] and some others such as the oyster *Ostrea gryphea*[20] dealkylate C_{29}-sterol, fucosterol, to C_{27}-sterols, desmosterol and cholesterol. Thus, the knowledge of origin of mulluscan sterols is still scanty and sometimes contradictory. Recently, KHAN and GOAD[9] have mentioned three possible sources of $\Delta^{5,7}$-sterols in molluscs ; (1) *de novo* synthesis by the usual Δ^{5}-sterol biosynthetic route, (2) the accumulation of dietary sterols, and (3) the interconversion of dietary Δ^{5}-sterols to $\Delta^{5,7}$-sterols in the body. Some algae have been known to contain $\Delta^{5,7}$-sterols. In addition, we have demonstrated that marine occurring yeasts involve 24-methylcholesta-5, 7, 22-trienol as the major sterol.[21,22] These data suggest the possibility of accumulation of dietary $\Delta^{5,7}$-sterol in the mulluscan bodies.

However, it also seems possible that some $\Delta^{5,7}$-sterols are formed from dietary sources of corresponding Δ^{5}-sterol in molluscs. Table 4 shows the relationship between the compositions of Δ^{5}-sterol (% of total Δ^{5}-sterol) and corresponding $\Delta^{5,7}$-sterol (% of total $\Delta^{5,7}$-sterols) in the molluscs examined in our previous[6] and present studies. A possitive correlation was observed on the following three pairs : cholesterol/cholesta-5, 7-dienol (correlation coefficient $r=0.71$) ; 24-methylcholest-5-enol/24-methylcholesta-5, 7-dienol ($r=0.57$) ; cholesta-5, 22-dienol/cholesta-5, 7, 22-trienol ($r=0.60$). This suggests that cholesta-5, 7-dienol, 24-methyl-cholesta-5, 7-dienol, and cholesta-5, 7, 22-trienol may be formed from the corresponding Δ^{5}-sterols with the same side chains. Whereas, a negative or only low possitive correlation has been detected on four pairs of 24-ethylcholest-5-enol/24-ethylcholesta-5, 7-dienol ($r=-0.19$), 24-methyl-cholesta-5, 22-dienol/24-methyl-

Table 4. Relationship between the compsitions (%) of Δ^{5}-sterol and corresponding $\Delta^{5,7}$-sterol in the molluscs *1

Δ^{5}-Sterol/$\Delta^{5,7}$-Sterol	Regression line*2	Correlation coefficient (r)
Cholesterol/Cholesta-5, 7-dienol	Y = −18.3 + 1.15X	0.71
24-Methylcholest-5-enol/24-Methylcholesta-5, 7-dienol	Y = 4.73 + 0.20X	0.57
24-Ethylcholest-5-enol/24-Ethylcholesta-5, 7-dienol	Y = 13.1 − 0.78X	−0.19
Cholesta-5, 22-dienol/Cholesta-5, 7, 22-trienol	Y = 0.16 + 1.17X	0.60
24-Methylcholesta-5, 22-dienol/24-Methylcholesta-5, 7, 22-trienol	Y = 53.2 − 0.81X	−0.14
24-Methylenecholesterol/24-Methylenecholesta-5, 7-dienol	Y = 0.82 + 0.77X	0.46
24-Ethylcholesta-5, 22-dienol/24-Ethylcholesta-5, 7, 22-trienol	Y = 5.99 − 0.43X	−0.16

*1 The data obtaind in the previous and present studies were used for the calculation of regression line and correlation coefficient.

*2 X, each Δ^{5}-sterol (% of total Δ^{5}-sterols) ; Y, each $\Delta^{5,7}$-aterol (% of total $\Delta^{5,7}$-sterols).

cholesta-5, 7, 22-trienol ($r=-0.19$), 24-methylenecholesterol/24-methylenecholesta-5, 7-dienol ($r=0.46$), and 24-ethylcholesta-5, 22-dienol/24-ethylcholesta-5, 7, 22-trienol. Therefore, these four $\Delta^{5,7}$-sterols are assumed not to be directly formed from dietary sources of corresponding Δ^{5}-sterols. As pointed out by KHAN and GOAD,[9] there is the possibility that although molluscs are capable of *de novo* synthesis of Δ^{5}-sterols by the usual route, they accumulate $\Delta^{5,7}$-sterols because the reduction of $\Delta^{5,7}$-sterol to Δ^{5}-sterol is rate limiting. However, there is no evidence for the above hypothesis. Considering the data available, we think that $\Delta^{5,7}$-sterols occurring in molluscs may originate directly from dietary organisms and/or be formed from dietary sources of some sterols by the interconversion of Δ^{5}-sterol to $\Delta^{5,7}$-sterol.

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