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$\Delta^{5,7}$-Sterols of Some Gastropods and Pelecypods

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Abstract

This paper presents the compositions of $\Delta^{5,7}$-sterols and other sterols of 2 gastropods and 6 pelecypods. Nine $\Delta^{5,7}$-sterols were detected in these molluscs in addition to the Δ^{5}-sterols commonly occurring in marine molluscs. As the most abundant sterols, the gastropods, *Neverita didyma* and *Tonna luteostoma*, contained cholesta-5.7-dienol, whereas the pelecypods, *Scapharca broughtonii, Glycymeris vestita, Cyclina sinensis, Metretrix petechialis, Mactra chinensis,* and *Sinonovacula constricta,* involved 24-methylcholesta-5,7,22-trienol and/or cholesta-5,7-dienol. A C_{26} sterol, 24-norcholesta-5,7,22-trienol, was present in all molluscs examined. *N. didyma* and *M. chinensis* contained 24-methylenecholesta-5,7-dienol and (24Z)-24-ethylidenecholesta-5,7-dienol. 24-Methylenecholesta-5,7-dienol was also detected in *T. luteostoma.*

Sterols of molluscs have been extensively investigated by earlier[1] and recent workers[2,3] from the viewpoint of comparative biochemistry and natural product chemistry due to the complexity and novelty of sterols. Aside from the findings of new C_{26} and C_{30} sterols in the pelecypods[4-7], salient aspects of molluscan sterols are the occurrence of considerably large amounts of $\Delta^{5,7}$-sterols in some molluscs[2,3]. Although much information has shown the presence of $\Delta^{5,7}$-sterols in molluscan sterol mixtures by u.v. spectal analysis, only a few studies have dealt with the characterization of $\Delta^{5,7}$-sterol components. Previously, we have demonstrated that the oyster *Crassostrea virginica* contained a variety of types of $\Delta^{5,7}$-sterols including a new sterol, 24-norcholesta-5,7,22E-trienol[8]. The present study is planned to obtained further knowledge on the $\Delta^{5,7}$-sterols occurring in molluscan species in the viewpoint of comparative biochemistry. This paper presents the compositions of sterols from 8 molluscs.

Materials and Methods

Specimens of the molluscs except *Cyclina sinensis* were obtained at the fish-market in Kagoshima, 1981. The pelecypod *C. sinensis* was collected at the mouth of River Yatsufusa, Kushikino City, Kagoshima Prefecture. Lipids were extracted from the alive molluscs (Table 1) with chloroform-methanol-water[9] and saponified with 10% ethanolic KOH at 80°C for 2 hrs to give unsaponifiable matters in the usual manner. Sterols were isolated from the unsaponifiable matters by alumina column chromatography with hexane-ether as described previously[8] and

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Table 1. The molluscs examined and their taxonomy

Class	Order	Species	Japanese name	Sampling
Gastropoda	Mesogastropoda	*Neverita didyma*	Tsumetagai	September
		Tonna luteostoma	Yatsushirogai	September
Pelecypoda	Eutaxodonta	*Scapharca broughtonii*	Akagai	February
		Glycymeris vestita	Tamakigai	September
	Heterodonta	*Cyclina sinensis*	Okishijimi	July
		Metretrix petechialis	Shinahamaguri	September
		Mactra chinensis	Bakagai	May
		Sinonovacula constricta	Agemakigai	September

then acetylated with pyridine-acetic anhydride (1:1). Sterol components were identified by comparision of the data on relative retention times (RRT) in gas-liquid chromatography (GLC) and mass spectra with those of reference steryl acetates as follows: (1) the preliminary analysis of sterol composition (%) by analytical GLC on 1.5% OV-17 (3m×3 mm i.d., column temp. 260°C)[10] of stery acetate mixtures, (2) the separation of steryl acetate mixtures into sub-fractions by thin-layer chromatography (TLC) on 10% $AgNO_3$-Kieselgel G with chloroform (ethanol-free or 2-3% ethanol containing) or by column chromatography on $AgNO_3$-silicic acid with hexane-benzene[8,10], (3) the combined GLC and mass spectrometry (GLC-Mass) of sub-fractions obtained by chromagraphy.

GLC-Mass was carried out by using JEOL JGL-20K gas-chromatograph with a column of 3.0% OV-1 (2 m×2 mm i.d., column temp. 285°C) and JEOL JMS-D300 mass-spectrometer as mentioned previously[10]. U.v. spectra were recorded to determine $\Delta^{5,7}$-sterol content in sterol mixtures on the basis of the molecular extinction coefficient of cholesta-5,7-dienol ($\varepsilon = 11,400$).

Results and Discussion

In the present study, sterols of 2 gastropods belonging to the order Mesogastropoda and 6 pelecypods belonging to the the orders Eutaxodonta and Heterodonta were characterized. The sterol content (% of wet weight) ranged from 0.02 to 0.16% (Table 2). The sterols of 8 molluscs examined were mainly composed of Δ^5-series of sterols (Table 3) which occur widely in molluscs[2,3]. As pointed out in many pelecypods[2,3,11-13], the pelecypods, *Scapharca broughtonii, Glycymeris vestita, C. sinensis, Metretrix petechialis, Mactra chinensis,* and *Sinonovacula constricta,* contained complex mixtures of C_{26} to C_{29} sterols; the major sterols being choleserol (30.4-56.1%), 24-methylcholesta-5,22E-dienol (11.1-19.8%), cholesta-5,22E-dienol (4.5-13.6%), 24-methylcholest-5-enol (6.5-25.7%), 24-methylenecholesterol (0.5-25.7%),and 24-ethylcholest-5-enol (2.4-7.2%). Generally, gastropods contain cholesterol as the exclusively major sterol. However, the gastropods, *Neverita didyma* and *Tonna luteostoma,* contained relatively large amounts of sterols other than cholesterol. The C_{28} and C_{29} sterols occurring in *N.*

Table 2. Lipid and sterol contents of the molluscs

Species	Content (% of fresh weight)		
	Lipids	Unsap. matters	Sterols
N. didyma	1.46	0.25	0.16
T. luteostoma	1.28	0.23	0.13
S. broughtonii	0.11	0.03	0.02
G. vestita	0.67	0.08	0.05
C. sinensis	0.36	0.11	0.07
M. petechialis	0.39	0.07	0.05
M. chinensis	0.58	0.10	0.08
S. constricta	1.15	0.16	0.12

Table 3. Composition of sterols except $\Delta^{5,7}$-sterols in the molluscs

Mollusc	Composition (%)*1 of sterol										
	A	B	C	D	E	F	G	H	I	J	K
N. didyma	1.3	2.3	7.1	53.3	15.7	5.2	9.8	1.3	—	2.6	1.2
T. luteostoma	0.6	—	4.7	61.0	11.4	5.7	9.2	—	—	7.2	0.2
S. broughtonii	2.3	—	11.8	37.1	19.8	20.1	0.5	1.9	—	6.4	0.1
G. vestita	0.5	2.4	12.1	36.8	16.8	25.7	0.5	1.2	0.2	3.0	0.8
C. sinensis	1.0	3.1	7.5	56.1	18.2	8.1	3.8	1.6	0.2	5.1	0.3
M. petechialis	7.9	—	9.7	30.4	19.6	6.5	12.0	3.0	—	4.2	6.7
M. chinensis	2.9	—	13.6	42.4	17.7	7.2	12.0	—	—	2.4	1.8
S. constricta	1.0	—	4.5	52.8	11.1	8.2	13.7	2.3	0.2	5.0	1.2

*1 Percentage of the sterols except $\Delta^{5,7}$-sterols. A, 24-norcholesta-5, 22-dienol; B, occelasterol; C, cholesta-5, 22E-dienol; D, cholesterol; E, 24-methylcholesta-5, 22E-dienol; F, 24-methylcholesta-5-enol; G, 24-methylenecholesterol; H, 24-ethylcholesta-5, 22E-dienol; I, unidentified; J, 24-ethylcholest-5-enol; K, (24Z)-24-ethylidenecholest-5-enol.

didyma and *T. luteostoma* may be assumed to come partly from their food such as pelecypods, because both gastropods are carnivorous.

In addition to the above Δ^5-series of sterols, various $\Delta^{5,7}$-sterols were present in the gastropods and pelecypods examined (Table 4). The acetates of sterols N, P, and S with a saturated side cain gave the molecular ions (M^+) at m/e 426, 440, and 456, and the intense ions [M^+−AcOH] at m/e 366, 380, and 394. The mass spectra of these 3 steryl acetates also afforded the diagnostic ions [M^+−AcOH-C(1) to C(3)] at m/e 325, 339, and 353 for $\Delta^{5,7}$-sterols[14,15], and other ions at m/253 (M^+−R−AcOH, R=side chain), 227 (M^+−R-26-AcOH), 226 (M^+−R−27−AcOH), 211 (M^+−R-42-AcOH), 157, and 143. The ions at m/e 157 ana 143 were indicative of $\Delta^{5,7}$-sterols[16]. The acetates of sterols L, M, O, and R with a Δ^{22}-bond gave the molecular ions at m/e 410, 424, 438, and 452, and the ion [M^+−AcOH] at m/e 350, 364, 378, and 392, respectively. The mass spectra of these 4 steryl acetates afforded the

Table 4. Composition of $\Delta^{5,7}$-sterols in the molluscs

Mollusc	$\Delta^{5,7}$-sterol (%)*1	Composition (%)*2 of sterol								
		L	M	N	O	P	Q	R	S	T
N. didyma	10.6	1.3	10.8	46.5	13.0	5.2	9.0	2.1	8.5	3.0
T. luteostoma	9.7	1.0	7.7	63.9	8.4	6.7	2.5	—	9.8	—
S. broughtonii	1.1	2.4	—	—	94.4	—	—	—	3.2	—
G. vestica	13.2	5.1	13.2	7.4	68.1	—	—	6.2	—	—
C. sinensis	3.0	4.3	4.4	38.1	38.0	5.3	—	2.1	7.3	—
M. petechialis	8.1	8.2	11.5	—	43.0	—	18.0	—	3.7	15.6
M. chinensis	0.5	5.9	24.3	35.9	25.1	3.2	—	2.8	2.8	—
S. constrica	8.6	1.7	7.2	16.2	49.4	—	—	29.4	2.1	—

*1 Percentage of total sterols.

*2 Percentage of $\Delta^{5,7}$-sterols. L, 24-norcholesta-5, 7, 22E-trienol; M, cholesta-5, 7, 22E-trienol; N, cholesta-5, 7-dienol; O, 24-methylcholesta-5, 7, 22E-trienol; P, 24-methylcholesta-5, 7-dienol; Q, 24-methylenecholesta-5, 7-dienol; R, 24-ethylcholesta-5, 7, 22E-trienol; S, 24-ethylcholesta-5, 7-dienol; T, (24Z)-24-ethylidenecholesta-5, 7-dienol.

ion at m/e 251 (M^+-R-2H-AcOH) suggestive of an unsaturated side chain[17,18], and other ions such as [M^+-AcOH-CH_3], [M^+-AcOH-C(1) to C(3)], [M^+-R-AcOH], [M^+-R-26-AcOH], [M^+-27-AcOH], and [M^+-R-42-AcOH] along with the ions at m/e 157 and 143. The mass spectral fragmentation patterns of steryl acetates N, P, S, L, M, O, and P were identical with those of reference steryl acetates which were isolated from the oyster *C. virginica*[8]. Therefore, these 7 sterols were indentified as cholesta-5,7-dienol (sterol N), 24-methylcholesta-5,7-dienol (sterol P), 24-etylcholesta-5,7-dinol (sterol S), 24-norcholesta-5,7,22-trienol (sterol L), cholesta-5,7,22-trienol (sterol M), 24-methylcholesta-5,7,22-trienol (sterol O), and 24-ethylcholesta-5,7,22-trienol (sterol R). The most polar $\Delta^{5,7}$-steryl acetates obtained by argentic chromatography were identified as the acetates of 24-methylenecholesta-5,7-dienol (sterol Q) and (24Z)-24-ethylidenecholesta-5,7-dienol (sterol T). The mass spectrum of the acetate of sterol Q showed the molecular ion at m/e 438, the ions at m/e 354 (M^+-C(23) to C(28)-1H) and 294 (m/e 354-AcOH) due to the McLafferty rearrangement[18], and other ions at m/e 378 (M^+-AcOH), 363 (M^+-AcOH-CH_3), 337, 253, 251, 227, 226, 211, 157, and 143. The mass spectrum of the acetate of sterol T also gave the diagnostic ions at m/e 354 and 294 besides the molecular ion at m/e 452 and other ions at m/e 392 (M^+-AcOH), 377, 351, 253, 251, 227, 226, 211, 157, and 143.

As mentioned above, the present study shows the occurrence of 9 $\Delta^{5,7}$-sterols, 24-norcholesta-5,7,22-trienol, cholesta-5,7,22-trienol, cholesta-5,7-dienol, 24-methylcholesta-5,7,22-trienol, 24-methylcholesta-5,7-dienol, 24-methylenecholesta-5,7-dienol, 24-ethylcholesta-5,7,22-trienol, 24-ethylcholesta-5,7-dienol, and (24Z)-24-ethylidenecholesta-5,7-dienol in the gastropods and pelecypods examined. Cholesta-5,7-dienol, 24-methylcholesta-5,7,22-trienol, 24-methylcholesta-5,7-dienol,

24-ethylcholesta-5,7,22-trienol, and 24-ethylcholesta-5,7-dienol have been widely encountered in various molluscs[2,3]. The C_{26}-sterol, 24-norcholesta-5,7,22-trienol, was first isolated from the oyster, *C. virginica*[8]. Later, this sterol has also been found in the sponge *Tethya amamensis*[19]. 24-Methylenecholesta-5,7-dienol has been found in a terrestial fungus *Phycomyces blakesleeanus*[20] and the sponge *Dysidea herbacea*[21]. (24Z)-24-ethylidenecholesta-5,7-dienol has been occurred in the sponges *D. herbacea*[21] and *T. amamensis*[19]. But, neither 24-methylenecholesta-5,7-dienol nor (24Z)-24-ethylidenecholesta-5,7-dienol have been found in molluscs.

The composition of $\Delta^{5,7}$-sterols varied among the molluscan species examined, the major $\Delta^{5,7}$-sterols being cholesta-5,7-dienol and 24-methylcholesta-5,7,22-trienol (Table 4). As the most abundant $\Delta^{5,7}$-sterols, the gastropods contained cholesta-5,7-dienol, whereas the pelecypods involved 24-methylcholesta-5,7,22-trienol and/or cholesta-5,7-dienol. These aspects of $\Delta^{5,7}$-sterol compositions may reflect the dietary habits and abilities for modification of dietary sterols in molluscs. In fact, a possitive correlation (correlation efficient, $r=0.85$; $P<0.05$) was observed between the percentage composition of cholesterol of total sterols and that of cholesta-5,7-dienol of total $\Delta^{5,7}$-sterols. This assumes that the occurrence of cholesta-5,7-dienol is closely related with that of cholesterol. The origin of $\Delta^{5,7}$-sterols in molluscs will be interesting subjects in future studies.

The $\Delta^{5,7}$-sterol content varied with the molluscan species (Table 4). YASUDA[13] has also observed the wide variation in the $\Delta^{5,7}$-sterol content among 11 species of the snail and bivalves. In the present study, we noticed that the $\Delta^{5,7}$-sterol content determined by u.v. spectrometry did not always coincide with that measured gravimetrically. The adequate methods should be worked out for reliable quantification of $\Delta^{5,7}$-sterols in molluscs.

References

1) BERGANN, W.: in "Comparative Biochemistry" (ed. by FLORKIN, M. and S. MASON), Vol. 3, Academic Press, New York, 1962. pp. 103-162.
2) IDLER, D.R. and P. WISEMAN: *J. Fish. Res. Bd. Can.*, **29**, 385-398(1972).
3) GOAD, L.J.: in "Marine Natural Products, Chemical and Biological Perspectives in Marine Biology" (ed. by SCHEUER, P.J.), Vol. II, Academic Press, New York, San Fransisco, and London, 1978, pp. 75-172
4) IDLER, D.R. and P. WISEMAN: *Steroids*, **16**, 451-461 (1970).
5) TESHIMA, S., A. KANAZAWA, and T. ANDO: *Comp. Biochem. Physiol.*, **41B**, 121-126 (1972).
6) IDLER, D.R., L.M. SAFE, and E.F. MACDONALD: *Steroids*, **18**, 545-553 (1971).
7) IDLER, D.R., M.K.W. KHALIL, J.D. GILBERT, and C.J.W. BROOKS: *Steroids*, **27**, 155-166 (1976).
8) TESHIMA, S., G.W. PATTERSON, and S.R. DUTKY: *Lipids*, **12**, 1004-1011 (1980).
9) BLIGH, E.G. and W.J. DYER: *Can. J. Biochem. Physiol.*, **37**, 911-917 (1959).
10) TESHIMA, S., A. KANAZAWA, S. HYODO, and T. ANDO: *Comp. Biochem. Physiol.*, **64B**, 225-228 (1979).
11) TESHIMA, S. and A. KANAZAWA: *Bull. Japan. Soc. Sci. Fish.*, **38**, 1299-1304 (1972).
12) IDLER, D.R. and P. WISEMAN: *Int. J. Biochem.*, **2**, 516-528 (1971).
13) YASUDA, S.: *Yukagaku*, **20**, 479-483 (1971).
14) JAURÉGUIBERRY, G., J.H. LAW, J.A. MCCLOSKEY, and E. LEDERER: *Biochemistry*, **4**, 347-353 (1965).

15) SMITH, T.J. and E.D. KORN: *J. Lipid Res.*, **9**, 405-408 (1968).
16) GALLI, G. and S. MARONI: *Steroids*, **10**, 189-197 (1967).
17) KNIGHTS, B.A.: *J. Gas*-chromatog., **5**, 273-282 (1967).
18) WYLLIE, S.G. and C. DJERASSI: *J. Org. Chem.*, **33**, 305-313 (1968).
19) TESHIMA, S.: A. KANAZAWA, I. YAMADA, and Y. TANAKA : *Lipids*, in press.
20) GOULSTON, G. and E.I. MERCER: *Phytochem.*, **8**, 1945-1948 (1969).
21) DELSETH, C., L. TOLELA, P.J. SCHEUER, R.J. WELLS, and C. DJERASSI: *Helv. Chim. Acta*, **62**, 101-109 (1979).