The Sterol Composition of *Styela clava*

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미더덕의 스테롤 組成

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미더덕의 스테롤 조성을 가스크로마토그라피로 분석한 결과 22-트란스-24-놀 코레스타-22-디엔-3 β-올이 1.3%, 22-데하이드로콜레스테롤이 5.1% 콜레스테롤이 33.1%, 브라시카스테롤이 26.2%, 24-메칠렌콜레스테롤이 13.1%, 스티그마스테롤이 7.1%, β-시토스테롤이 10.9%, 푸코스테롤이 3.1% 였다.

Marine invertebrates contain very complex and interesting sterol mixtures. With an advent of refined technique as gas chromatography, and more recently the combined use of GLC and mass spectrometry, many new sterols were separated from them and identified.

Styela clava is a well-known tunicate(Urochorda), one of major natural enemies to oyster, and showing strong reproducibility, although the meat is very popular with the inhabitants in the south coast of Korea.

Few reports on the sterol composition of tunicates are seen;according to Bergmann,[1] *Styela plicata* contained mainly cholesterol with about 10% of a Δ^7-cholesterol, and Lee *et al.*[2] reported the free amino acid content in the meat extract of *Styela clava*.

The present study was carried out to examine the sterol composition of *Styela clvaa* prior to research on the sterol metabolism and steroid hormones in the tunicates.

Materials and Methods

Animals; 269 grams of *Styela clava* peeled-off collected on July 19, 1976, at Masan Fish Market, Masan, Korea, were used for this experiment.

Isolation of Sterols; Lipids were extracted with ether from the contents of *Styela clava*. The ether layer was evaporated on a rotary evaporator and the residue put on a vacuum dessicator overnight to constant weight. The lipids were saponified with a solution of 10% KOH in 90% ethanol for 30 minutes. Upon cooling, the solution was diluted with distilled water, the unsaponifiables were extracted with anhydrous ether, and the ether phase was washed several times with two volumes of distilled water. From the unsaponifiables was the crude sterol fraction obtained by a column chromatography, Silica Gel(Mallinckrodt, 60-100 mesh), with a mixture of petroleum ether(p.e.) and ether. The sterol fraction so obtained was checked on TLC(Wakogel B-5, Wako Pure Chemical Industries, Ltd., Osaka, Japan) by using the developing system of p.e: ether: acetic acid(80:20:1).

The purified sterol fraction was acetylated with a solution of anhydrous pyridine and anhydrous acetic acid(1:1) in a refrigerator overnight.[4]

GLC apparatus used in this study was a dual column Hitachi Model GLC—2C with a flame ionization detector. The coiled stainless columns 2 *m* x 3 *mm*, i.d.) packed with 1.5% OV-17 and SE-30 on Chromosorb W were used.[4][5]

The column temperature was 250°C. The iden-

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tification of sterols was achieved by comparing the relative retention times(to cholesterol) to those of authentic sterols.

Results and Discussions

The lipids content of *Styela clava*, was 4.2%, and its iodine value was 138(Table 1). In GLC on 1.5% OV-17, the sterol mixture isolated from *Styela clava*, were found to be composed of eight components, as shown in Fig. 1, all the peaks were identified in the relative retention time with the authentic 22-trans-24-norcholesta-5,22-dien-3β-01, 22-trans-dehydrocholesterol, cholesterol, brassicasterol, 24-methylenecholesterol, stigmasterol, β-sitosterol and fucosterol(Table 2).

The sterol components are very similar to those of pelecypods(Table 3).

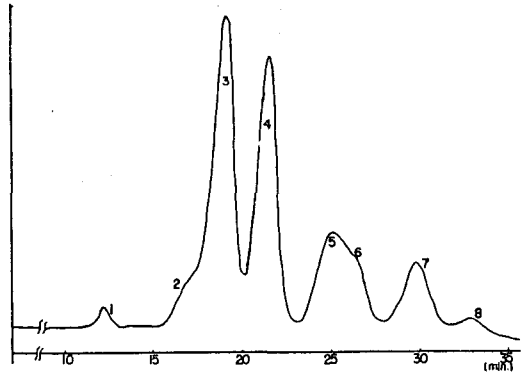

Fig. 1. Gas chromatogram of sterol esters from *Styela clava*.
Condition: Column 1.5% OV-17
Temp. 250°C
N_2 40 *ml/min*

Table 1. Lipids content in *Styela clava*

Sample wt. (*g*)	Lipids(ether soluble) wt. (*g*)	%	Iodine value (by Wij's method)
269	11.3	4.2	138

Table 2. The composition of the sterol methylated from *Styela clava*

Peak	Rt* (*min.*)	Rrt**	Sterol	%
1	12	0.63	22-trans-24-norcholesta-5, 22-dien-3β-ol	1.3
2	17	0.89	22-trans-dehydrocholesterol	5.1
3	19	1	Cholesterol	33.1
4	21.4	1.13	Brassicasterol	26.1
5	25	1.31	24-methylenecholesterol	13.1
6	26.1	1.37	Stigmasterol	7.1
7	29.7	1.56	β-sitosterol	10.9
8	32.5	1.71	Fucosterol	3.1

Rt* : Retention time on OV-17
Rrt**: Relative retention time (to cholesterol)

The major sterols of *Styela clava* were cholesterol 33.1%, brassicasterol 26.2%, 24-methylenecholesterol 13.1% which are typical of pelecypod, and as minor components, 22-trans-24-norcholesta-5, 22-dien-3β-ol, 22-trans-dehydrocholesterol, stigmasterol and fucosterol were contained 1.3%, 5.1%, 7.1% and 3.1%, respectively. Takagi *et al.*[6] indicaed the presence of Δ^7-dehydrocholesterol, poriferasterol and clionasterol in *Cynthia roretzi*, and Nomura *et al.*[7] reported the main sterols in *Halocynthia roretzi* of cholesterol, 24-methylenecholesterol, cholestanol and Δ^7-choles-

terol. According to Bergmann *et al.*[1], *Styela plicata* contained mainly cholesterol with 10% a Δ^7-dehydrocholesterol. But in this work, any kinds of Δ^7-dehydrocholesterols were not detected.

Table 3. The sterol composition of some pelecypods and *S. clava*

Sterols	Pelecypods(%) A*	B*	C*	*S. clava*(%)
1. 22-trans-24-norcholesta-5, 22-dien-3β-ol	3.0	3.9	4.6	1.3
2. 22-cis-dehydrocholesterol			1.1	
3. 22-trans-dehydrocholesterol	6.7	10.2	7.5	5.1
4. Cholesterol	39.6	48.6	34.1	33.1
5. Brassicasterol	14.1	13.8	28.1	26.2
6. 22-dihydrobrassicasterol or Campesterol			1.7	
7. Poriferasterol			1.5	
8. 24-methylenecholesterol	19.4	11.5	8.1	13.1
9. Stigmasterol.	2.4			7.1
10. β-sitosterol	10.5	11.9	3.7	10.9
11. Fucosterol	4.3		0.1	3.1
12. Iso-fucosterol			8.3	

* A, *Mactra sulcataria*[5] B, *Spisula sachalinensis*[5]
C, *Crassostrea virginica*[11]

The presence of 22-trans-24-norcholesta-5, 22-dien-3 β-ol was demonstrated in the pelecypods [5,8,9,10,11] and urochord.[7] Brassicasterol is widely distributed in the pelecypods,[5,8,9,10,11,14,17] other marine invertebrates[12,13,20] and diatoms.[8] *Crassostrea virginica*[14] was found to be conatined brassicasterol as much as 28.1%. The pelecypods and marine invertebrates with very diverse sterols, are most plankton feeder. This fact suggests that the sterols in marine animals be derived from their feed to some extent.

Little information is available concerning the origin of sterols in invertebrates with the exception of certain insects. In invertebrates like molluscs and echinoderms, endogenous syntheses of sterols have been found. The starfish, *Pisaster ochraceus*,[15] is capable of converting the double bond at C 5 to the C 7 position. According to Itoh,[16] after injection of 1-C^{14}-acetate into *Placopecten magellanicus*, little radioactivity was detected in the fractions of C26-sterol and C29-sterol. The clam, *Saxidomus giganteus*, injected with cholesterol-26-C^{14}, is able to introduce unsaturation into the side chain of cholesterol at C 22 and C 25, and to transform injected cholesterol-4-C^{14} into radioactive 24-methylenecholesterol. Joh[19] observed that fucosterol, 24-methylenecholesterol and brassicasterol were conversible to cholesterol in abalone, and that abalone could synthesize cholesterol from acetate. Many insects [21,22,23,24,25] are able to convert the C 29 sterol into cholesterol. Nomura *et al.*[7] suggested that endogenous C 27-sterol in phytophagous *Halocynthia roretzi* could be modified from exogenous C 27, C 28 and C 29 sterols.

By analogy with other invertebrates, in *Styela clava* in vivo modifications of exogenous sterols and sterol synthesis from lower carbon metabolites could not be ruled out.

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