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Studies on the Lipids of "Bugbangjohgae" *Spisula sachalinensis****

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북방 조개의 유지에 관한 연구

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북방 조개는 우리나라 동해안과 일본 동북 지방에 널리 분포하는 백합과에 속하는 조개로서 일본에서는 식용으로도 이용되나 여기에 대한 지질학적 연구(脂質學的硏究)가 없으므로, 이 조개에 대한 지질학적 연구를 행하여 그 결과를 보고한다.

1) 총 지질중 인지질(燐脂質)이 43.1%로 제일 많았고, 다음 트리그리 세라이드가 36.2% 스테롤이 10.3%였다.

2) 인지질의 구성을 보면, 포스화티질코린, 포스화티질에나놀아민, 포스화티달에타놀아민, 포스화티질세린이 중요한 것이었다.

3) 중성지질의 지방산은 20 : 5, 16 : 1, 16 : 0이 제일 많았고, 에타놀아민 함유(含有) 인지질에는 20 : 5, 18 : 0, 22 : 6, 코린함유(含有) 인지질에는 16 : 0, 20 : 5, 22 : 6이 중요한 것이었다.

4) 프라스마로겐은 대부분 에타놀아민 함유 인지질에 편재해 있었고, 코린함유 인지질에는 소량 함유되어 있었다.

5) 중요한 스테롤은 22-트란스-24-놀코레스타-5. 22-디엔-3β-올, 22-데하이드로콜레스테롤, 코레스테롤브라시카스테롤, 24-메치렌코레스테롤 및 β-시토스테롤로 구성되어 있었다.

Introduction

"Bugbangjohgae", *Spisula sachalinensis* belong to Eulamellibranchia like *Meretrix meretrix lusoria*, is widely distributed on rather cold area of both the East Sea and the Pacific Ocean.

Recently, the comparative study of fatty acid composition of gastropoda taking sea weeds as their diet and pelecypoda known as plankton feeder, showed a recognizable difference between above tow groups[1]. De Koning[2] and the authors[3] made a comparison between the fatty acid composition of the neutral lipids and that of the phospholipids from abalone and recognized a significant difference among both the fractions.

On the other hand, the sterols of some shell fish were isolated by sveral workers[4-9]. Idler and Wiseman [10] isolated 11 sterols from scallop on gas-liquid chromatography and identified them using mass spectrometry. The authors[11] reported the sterols from the tissues of abalone were mainly composed of cholesterol, and then brassicasterol, desmosterol and 24-methylenecholesterol as minor components. Some workers[12] reported that cholesterol was present in gastropoda as a sole component, while complex mixture of sterols in pelecypoda.

According to Thompson and Lee[14], marine

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molluscs contain plasmalogen to the high level. Rapport and Alonzo [15] indicated high content of plasmalogen in the lipids of many marine invertebrates. The authors[16] reported the composition of aldehyde ether-linked to α-position of glyceryl moiety of plasmalogen in the phospholipids from gastropoda and pelecypoda.

Few papers on "Bugbangjohgae" lipids study are shown except Toyama report[17]. They reported the physical properties of the lipids ether-extracted from fried "Bugbangjohgae", the fatty acid composition of the lipids acetone-extracted and the sterol composition.

However, it is suspicious whether the satisfiable results could be obtained by the methods they used or couldn't. And, still less, they have no mention of the phospholipids.

As a series of studies on the lipid metabolism of shell fish in a view of food chain, the authors investigated the fatty acid composition of the neutural lipids and the phospholipids in detail, and identified the sterols isolated from the clam, *Spisula sachalinensis*.

Materials and methods

1. Materials

Whole content(173.1*g*) except shell of "Bugbangjohgae", *Spisula sachalinensis*, purchased on the fish market of Kitasendai, Miyagi Prefecture, Japan, on July 6 in 1974, were used for this study. The characteristics of the sample are given in Table 1.

2. Lipid Extraction and Fractionation of Total Lipids

Total lipids were extracted from the muscle tissue with a mixture of chloroform-methanol solvents according to the procedure of Bligh and Dyer [18]. Small quantities of the total lipids(157.5*mg*) were loaded on 50*g* of silicic acid, activated at 120℃ for 5 hours, and were eluted with petroleum ether, 1%, 5%, 10%, 15%, 20% and 30%(V/V) ether-peteroleum ether, ether and 20% chloroform-methanol and methanol, in sequence.

3. Preparation of Fatty Acid Methyl Esters(Fame)

FAME were obtained by methanolysis of the lipids with 5% Hcl-methanol at 85℃ for 3 hours, and purified on TLC, if in danger of contamination of dimethyl acetal possibly derived from plasmalogen.

4. Isolation of Sterols

The lipids were saponified with 10% of alcoholic potassium hydroxide at 80℃ for 2 hours, and then the unsaponifiables were extracted with ether in the usual manner. The sterols were isolated from the unsaponifiables by column chromatography on silicic acid with petroleum ether-ether[19]. The sterols so obtained were recrystallized several times from methanol.

5. Acetylation of Sterols

Sterols were acetylated by addition of acetic anhydride-dry pyridine(1 : 1) and standing for 48 hours at room temperature. After elimination of reagents under the stream of nitrogen. The steryl acetates were purified by recrystallization from methanol.

6. Analysis of FAME and Steryl Acetates on GLC

Analytical GLCwas made on a Hitachi Model GC Chromatograph unit, and the columns were 10% DEGS on Chromosorb W (3*m*×3*mm*) and 1.5% OV-17.3% SE-30 on Chromosorb W(2*m*×3*mm*) for FAME and steryl acetates, respectively.

7. Separation of Phospholipids on TLC

Thin layer chromatoplates(20*cm*×20*cm*) coated with Kieselgel H(Merck) were activated for 3

hours at 110℃ and stored in stock box. Small volume of crude phospholipids dissolved in chloroform-methanol(1 : 1) were spotted at the origin and developed with chloroform-methanol-acetic acid(65 : 25 : 4) until the front went up to the level 3*cm* below the top. This chromatoplate was air-dried for several minutes and was developed with chloroform-ethyl acetates (6 : 3) to the top to eliminate the less polar lipids.

The one-dimensionally developed lane of this chromatogram was sprayed with 5% $HgCl_2$ solution and desiccated in vacuum desiccator for 1 hour. Two-dimensional procedure was performed on the system of chloroform-methanol-ammonia(230 : 90 : 15).

8. Detection of Sports

The plates were air-dried at room temperature for 20 minutes. The spots were detected with iodine vapor and encircled with a fine dissecting needle. Most of the iodine was allowed to evaporate before removal of the spots.

Other detection methods were employed to confirm the identity of the spots, a) ninhydrin for phospholipids containing free amino groups, b) Dragendorff reagent for choline[21], c) Dittmer reagent for phospholipids[22].

A drop of water was placed on the spot to be removed for analysis, and the silica gel was transferred to a centrifuge tube. Three areas on different levels, where no lipid materials was applied, were taken as controls.

9. Elution of Phospholipids

Each sample was eluted from the silica gel by suspending the powder in eluting solvent by gently tapping the tube. The first and second elutions were performed with developing solvent by using 3 and 2 *ml* portions, respectively. After centrifuging, the solvent was removed with a capillary pipette. The third elution was made with 2 *ml* of methanol, and the fourth with 2 *ml* of methanol-acetic acid-water(95 : 1 : 5, by volume). Sample with an expected phosphorous content within the standard curve range(0.2-5μg)were transferred to the digestion tube directly; those with a much greater phosphorus content were transferred to 10*ml* volumetric flask and an appropriate s ample was taken for analysis.

10. Phosphorus Determination

Phosphorus content was determined on Bartlett's method[23].

Results and Discussion

1. Lipid Content and Properties of Total Lipids

The total lipids content and properties are given in Table 1. The total lipids content amounted to 1.3%, higher than 0.82% of *Meretrix meretrix lusoria* oil content[1]. The density and iodine value(Wijs) were d_{15}^{15} 0.9324 and 155.8.

2. The Lipid Composition of Total Lipids

The lipid composition of total lipids is given in Table 2. The most predominant phospholipids and the triglyceride content were 43.1% and 36.2%. The phospholipids content was lowerthan that of abalone mecle, 67%[24], higher compared to hake muscle oils, 30%[25].

3. The Fatty Acid Composition of the Neutral Lipids

Ethanolamine-and choline phospholipids are given in Table 3. In the neutral lipids, fatty acids were mainly composed of C20 : 5, C16 : 1, C16 : 0. The content of C16 : 0(12.4%), C16 : 1 (15.3%) of acetone-solubles from this species by Toyama [17] was similarto our result, but they reported C22 unsaturated fatty acids content was 10.4%, which was two times as much as our result.

The discrepancy of fatty acid composition

Table 1. Characteristics of "Bugbangjohgae", *Spisula sachalinensis*

Sampling Site	Date	Shell Numbers	Shell(*cm*) Length	Width	Height	Shell (*g*) Flesh Weight
Kitasendai Fish Market	July 6	5	6.7-8.9	7.8-11.1	4.8-6.4	173.6
Sendai, Japan.	1974		(7.9)	(9.7)	(5.6)	

( ) means average

Table 2. Properties of Total Lipids

Oil Content(%)	Density	Iodine Value (Wijs)
1.3	α_{15}^{15} 0.9324	155.8

Table 3. The Lipid Composition of "Bugbangjohgae", *Spisula sachalinensis*

Eluent	Volume (*ml*)	Fractions eluted	Weight eluted (*mg*)	%
100% Petroleum Ether(P. E.)	400	Hydrocarbon	4.2	3.2
1% Ether-P. E. (V/V)	200	Sterol ester, Wax	0.4	0.3
5% Ether-P. E. (V/V)	450	Triglyceride	48.8	36.2
10% Ether-P. E. (V/V)	200	Free fatty acid	1.3	0.9
15% Ether-P. E. (V/V)	300	Sterol	13.9	10.3
20% Ether-P. E. (V/V)	200	Monoglyceride	2.5	1.9
30% Ether-P. E. (V/V)	200	Monoglyceride	2.5	1.9
Ether	200	Pigments	5.6	4.1
Chloroform : Methanol (1 : 4)	500	Phospholipids	58.1	43.1
Total weight			134.8	

Total lipids 157.5*mg* loaded on 50*g* of silicic acid, activated at 120℃ for 5 hours Recovery 85.6%.

appeared between the ethanolamine phospholipids and choline ones. The content of C16 : 0 fatty acid in the choline phospholipids was compatible to 32% of that of hake flesh[25], lower than 43.5% of lecithin from chum salmon heart[23]. In the ethanolamine phospholipids, the main components of fatty acid were C20 : 5, C18 : 0. The fatty acids of the ethanolamine phospholipids were much more unsaturated than those of the choline phospholipids. This result is in agreement with that of De Koning[2].

Oleic acid content of each fraction was very small compared to one of fish oil[25] and gastropoda[2,25]. This observation was indicated by Shima and Taguchi[1]. It is very interesting whether this result can be ascribed to difference of food or low ability to convert C16 : 0 to C18 : 1 via C18 : 0 compared to fish and gastropoda.

4. The Sterol Composition

On 1.5% OV-17 of GLC, the sterols isolated from the clam, *Spisula sachalinensis* were found to contain six components as shown in Fig. 1.

The peaks 2, 3, 4, 5 and6 were identical in the retention times with authentic 22-dehydrocholesterol, cholesterol, brassicasterol, 24-methylenecholesterol and β-sitosterol, respectively. Peak I corresponded to the relative retention times of 22-trans-norcholesta-5, 22-dien-3β-ol on references. Furthermor, peak 1, 2, 4 and 5 were confirmed by GLC-mass spectrometry.

Table 4. Fatty Acid Distribution in "Bugbangjohgae", *Spisula sachalinensis*(as % of methyl ester)

	Ether Fraction	EP	CP*
C14:0	6.0	2.2	6.0
1	0.2	trace	trace
C15:0	1.2	0.5	1.3
1	0.3	0.2	0.3
C16:0	13.3	4.7	29.6
1	18.1	3.2	7.6
2	1.0	0.7	1.2
C17:0	1.6	1.5	0.7
1	0.6	—	trace
C18:0	4.6	17.5	2.6
1	8.1	3.0	5.5
2	0.7	0.3	0.3
3	0.6	0.7	0.3
4	9.5	5.6	2.0
C19:0	1.1	0.2	0.2
1	0.2	0.2	—
C20:0	0.2	trace	0.4
1	4.2	6.0	7.4
2	0.5	trace	0.3
4	2.0	5.3	3.0
5	19.2	31.4	14.0
C21:0	1.5	0.7	0.7
1	0.8	0.3	0.3
C22:1?	0.5	0.4	trace
4	0.9	1.6	2.4
5	trace	1.7	2.4
6	3.1	11.5	10.8
C24:1	trace	0.4	0.5

*EP;ethanolamine phospholipids
CP;choline phospholipids

The mass spectrum of peak I gave prominent ion peaks at 394, 352, 348, 337, 255 and 253. The peak at m/e 412 corresponding to the molecular ion of this acetate was not observed. However, a relativley intensive peak was seen at m/e 352 corresponding to that for the loss of acetic acid(m. w. , 60) from the molecular ion (M^+) of the sterol acetate. From this result, this sterol was considered to possess one hydroxy group in the molecule. The other peaks were interpreted as follows;m/e 394(M-HOH), 348[M−(HOH+3CH_3+H)], 337[M−(CH_3COOH +CH_3)], 255[M−(CH_3COOH+R, R=side chain)], 253[M+(CH_3COOH+R+2H)].

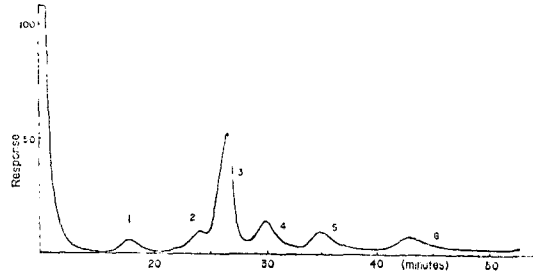

Fig. 1. Gas-Liqid Chromatogram of the sterols (acetates) from "Bugbangjohge", *Spisula sachalinensis*
1.5% OV-17 on Chromosorb W;Column Temperature, 250℃; Column length 3*mm*×2*m*; N2, 42*ml/min.*

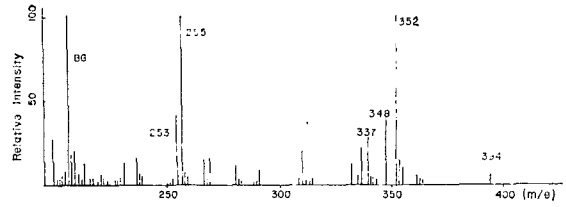

Fig. 2. The Mass Spectrum of 22-trans-norcholesta-5, 22-diene-3β-ol from "Bugbangjohgae", *Spisula sachalinensis.*

According to Toyama[17], the sterols of this clam, *Spisula sachalinensis*, were composed of β-sitosterol, clionasterol and two unknown sterols. The mleting points and specific rotary powers of two unknown sterols didn't correspond with those of any kind of sterols of references[27].

The percentage of individual sterols isolated is given in Table 4. The main sterol was cholesterol, 48.6% and then brassicasterol, 24-methylenecholesterol β-sitosterol and 22-trans-dihydrocholesterol content amounted to 13.8%, 11.5%, and 10.2%, respectively. This result shows a close resemblance to the sterol composition of hen-clam, *Mactra sulcataria*[28], and many other pelecypoda[4].

Recently, the authors indicated that the sterol components of some diatoms[29], *Thalassiossira decipiens*, *Chactoceros gracilis* were very similar to those of pelecypoda. According to Kanazawa, Yoshioka and Teshima[30], the sterols

from the diatomas from *Cyclotella nana* and *Nitzchia closterium* were composed of only brassicasterol. Since the diatoms are conceivable to be utilized as a diet for the marine invertebrates, it seems reasonable to consider that the sterols from the diatoms may be used as the exogenous sources in the marine invertebrates.

5. The Phospholipids Composition

The phospholipids of "Bugbangjohgae", *Spisula sachalinensis*, were mainly composed of phosphatidyl choline(45.4%), phosphatidyl ethanolamine(15.4%) phosphatidal ethanolamine(14.9%) and phosphatidyl serine (13.2%). The phospholipids composition was listed in Table 6.

Table 5. The Composition of the Sterols Isolated from "Bugbangjohgae", *Spisula sachalinensis*

Peak No.	Rt*	Rrt	Sterol	%
1	17	0.65	22-trans-norcholesta-5, 22-diene-3β-ol	3.9
2	24.4	0.94	22-trans-cholesta-5, 22-diene-3β-ol	10.2
3	26.0	1.00	Cholesterol	48.6
4	29.8	1.15	Brassicasterol	13.8
5	34.2	1.32	24-methylenecholesterol	11.5
6	42.6	1.64	β-sitosterol	11.9

* minutes.

Table 6. Phospholipid Composition of "Bugbangjohgae", *Spisula sachalinensis*

Spot	Phospholipid	Phosphorus content (%)
1	Cardiolipin	1.1
2	Unidentified	0.5
3	Phosphatidly Ethanolamine	15.4
4	Phosphatidal Ethanolamine	14.9
5	Phosphatidyl Serine	13.2
6	Phosphatidyl Choline	45.4
7	Phosphatidal Choline	0.2
8	Sphingomyelin	6.7
9	Lysophosphatidyl Choline	2.5

Total Lipids (26.25μg-P) were applied to 3 sheets of TLC. After Two-dimensional development, spots detected by iodine vapor were scraped off into centrifugal tubes and extracted by the solvents.

This result showed a very resemblance to that of abalone, but according to according to Yasuda[31], the phospholipids from Japanese little neck, *Tapes japonicus* Deshayes, were richer in the ethanolamine phospholipids than any other fraction.

Rapport and Alonzo[15], some workers [2,14] indicated that marine animal tissues were rich sources of plasmalogen, and almost all the plasmalogen were present in the etanolamine phospholipids, small amount of plasmalogen in the choline phospholipids. This was in agreement with our result. In contrast to those results, Dumont[32] demonstrated the posterior gills of *Eriocheir sinensis* contained only choline plasmalogen, and Bergmann and Landowne[33] observed that only choline plasmalogen was present in *A. elegentissim*, was further the evidence that the lipid composition of this animal was exceptional.

Summary

The present investigation was performed to find the lipid composition of the total lipids, the fatty acid components of the neutral lipids and the phospholipids, and the composition of sterols, from *Spisula sachalinensis*. The results obtained are as follows;

1) The main components of the total lipids are phospholipids(43.1%), triglyceride(36.2%), and sterol(10.3%).
2) The phospholipids are mainly composed of phosphatidyl choline, phosphatidyl ethanola, mine, phosphatidal ethanolamine and phosphatidyl serine.
3) The main fatty acids of the neutral lipids, the ethanolamine phospholipids and choline phospholpids, are C20:5, C16:1, C16:0: C20:5, C18:0, C22:6, and C16:0, C20:5, C22:6, respectively. Oleic acid content of all fractions is very small compared with one of gastropoda lipids and fish oil.
4) Most of plasmalogen are present in the ethanolamine phospholipids and only trace of plasmalogen in the choline phospholipids.
5) Sterols to be found are 22-trans-norcholesta-5, 22-diene-3β-ol, 22-dehydrocholesterol, cholesterol, brassicasterol, 24-methylenecholesterol and β-sitosterol.

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