FATTY OILS OF AQUATIC INVERTEBRATES. XXIII.

FATTY OILS AND THEIR UNSAPONIFIABLE COMPONENTS OF THE MOLLUSCS—*DENDRODORIS RUBRA VAR. NIGROMACULATA, LITTORINA BREVICULA, MYA ARENARIA JAPONICA* AND *PINNA PECTINATA JAPONICA*

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This paper is concerned with the fatty oils extracted from four species of molluscs; two species of gastropod, *Dendrodoris rubra var. nigromaculata* and *Littorina brevicula*, and two species of bivalve, *Mya arenaria japonica* and *Pinna pectinata japonica*. Of these fatty oils, fatty oils from *M. arenaria japonica*[1)2)] and *P. pectinata japonica*[2)3)] have been more or less studied by previous authors and the occurrence of a $\Delta^{5,7}$-sterol of m.p. 122°–123°C and clionasterol (γ-sitosterol) in the fatty oil from *P. pectinata japonica* has been reported, whereas there seems no literature on the fatty oils from *D. rubra var. nigromaculata* and *L. brevicula*.

In this study, fatty oils were extracted from each species of molluscs and analyzed for their characteristics. The fatty acids and unsaponifiable matter were separated from the oil in the usual way. The composition of polyethenoid acids in the total fatty acids of each oil, except that from *D. rubra var. nigromaculata* was estimated by the spectrophotometric measurements of alkali-isomerized fatty acids.

The unsaponifiable matter was recrystallized from methanol to separate a sterol mixture. The sterol mixture from *D. rubra var. nigromaculata* could not be closely examined due to scarcity of the material. In the case of *L. brevicula* the sterol mixture was found to contain only a small amount of $\Delta^{5,7}$-sterol. Recrystallization of its acetate gave readily a fraction which was recognized to be cholesteryl acetate, indicating that the principal component of the sterol mixture is cholesterol. The sterol mixture from *M. arenaria japonica* was acetylated, the acetate was recrystallized, and there was obtained a fraction which appeared to consist mainly of C_{29}-diunsaturated steryl acetate on the basis of its saponification value and iodine value. The acetate of the sterol mixture from *P. pectinata japonica* was chromatographed to separate a non-conjugated fraction. This fraction was brominated, the bromide was subjected to fractional crystallization, and it was found that the non-conjugated sterol components contain poriferasterol as a diunsaturated sterol and cholesterol besides previously reported clionasterol as monounsaturated sterols. Properties of the sterols separated in this study are summarized in Table 1.

TABLE 1. Properties of the Sterols

	Free sterol		Acetate		Benzoate	
	M.p. (°C)	$[\alpha]_D^\circ$	M.p. (°C)	$[\alpha]_D^\circ$	M.p. (°C)	$[\alpha]_D^\circ$
P. pectinata japonica						
Sterol from bromide I	152–153	−50.6	146–147	−53.0	140–141	−21.6
Poriferasterol[4]	155–156	−49.7	146.5–147	−53	139.5–140.5	−21.9
Sterol from bromide II	137.5–138.5	−37.4	135.5–136.5	−42.6	137–138	−17.0
Clionasterol[4]	137.5–138.5	−37	137	−41.9	134.5–135	−16.8
Clionasterol[5]	139	−35	137	−40	140	−16
Sterol from bromide III	145–146	−38.5	113–114	—	—	—
Cholesterol	148	−39.5	114–115	−47.5	—	—
L. brevicula						
Sterol obtained by recrystallization of acetate mixture	144–145	—	114–115	−47.0	—	—

In the preceding study[6] on the unsaponifiable matter of fatty oil from *Aplysia kurodai*, a species of the order Opisthobranchia, the authors separated bromine-containing substances from the non-sterol fraction. Since the possibility is not quite excluded that such substances escaped their notice in previous studies on the fatty oils from various kinds of molluscs, the unsaponifiable matter from each oil used in this study and also from oils of various kinds of molluscs used in previous studies[7] were tested for halogen. However, halogen was detected in no case. Since *D. rubra var. nigromaculata* used in this study belongs to the same order Opisthobranchia as *A. kurodai*, the non-sterol fraction was separated from the unsaponifiable matter of the fatty oil of *D. rubra var. nigromaculata*. However, this fraction showed no characteristic absorption in the ultraviolet region and had a low refractive index (n_D^{40} 1.4747). These properties are different from those of the non-sterol fraction from *A. kurodai*, suggesting that the non-sterol fraction from *D. rubra var. nigromaculata* unlike that from *A. kurodai* consists of substances which are generally found in the non-sterol fraction from shellfish.

Experimental

1. Animals used for extraction of fatty oil

Among the molluscs used in this study, *D. rubra var. nigromaculata*, *L. brevicula* and *M. arenaria japonica* were received in the form of fresh whole animals, while *P. pectinata japonica* was received in the from of a refrigerated product of viscera separated from the whole animals. *D. rubra var. nigromaculata* and *P. pectinata japonica* were first heated on a water bath for the removal of water to some extent and then dried in an electric oven at a temperature below 80°C. *L. brevicula* with shells was roughly crushed and then dried in an electric oven. *M. arenaria japonica* was shelled and the meat alone was dried in an electric oven. Each dried material was reduced to powder and then extracted with ether. The ether-extract (lipid) thus obtained was refluxed with about ten times its weight of acetone for a while and then cooled to the ordinary temperature, and the acetone-soluble oil (fatty oil) was recovered from the acetone filtrate. The catching locality, the yields of dried material, ether-extract and acetone-soluble oil and some other data are shown in Table 2.

TABLE 2. Some Data on the Molluscs Used for Extraction of Oil

Name of species	*Dendrodoris rubra var. nigromaculata*	*Littorina brevicula*	*Mya arenaria japonica*	*Pinna pectinata japonica*
Catching locality	Sugashima	Sugashima	Mouth of the river Nabeta	Fukue
Date of catch	Late July, 1958	Late July, 1958	Middle Oct., 1956	Early Jan., 1957
Number	8	—	42	—
Weight (g)	45	612	7,350	6,700
Dried material (g)	—	—	363	1,091
Ether-extract (g)	0.41	4.0	29.0	76.5
Ether-extract (%)	—	—	8	7
Acetone-soluble oil (g)	0.33	3.6	17.7	51.3
Acetone-soluble oil (%)	80	90	61	67

Notes: All catching localities except Sugashima lie in Aichi-ken. Sugashima is an islet in Mie-ken. Percentage yield of ether-extract is expressed on the basis of dried material. The percentage yield of acetone-soluble oil is expressed on the basis of ether-extract.

2. Fatty oils and their fatty acids

All fatty oils are a dark yellowish orange viscous liquid with some solid at the ordinary temperature. The fatty oil from *L. brevicula* has some greenish cast. Each oil was saponified with alcoholic potassium hydroxide, and the fatty acids and unsaponifiable matter were separated in the usual way. Properties of fatty oils and their fatty acids and unsaponifiable matter are recorded in Table 3.

TABLE 3. Fatty Oils

	D. rubra var. nigromaculata	*L. brevicula*	*M. arenaria japonica*	*P. pectinata japonia*
n_D^{40}	1.4739	1.4749	1.4843	—
Acid value	—	31.5	6.9	52.5
Saponification value	141.7	174.7	171.0	169.4
Iodine value	142.6	155.3	178.2	183.6
Unsaponifiable matter (%)	—	13.55	17.50	18.79
Fatty acids				
Neutralization value	—	197.4	193.3	184.3
Iodine value	136.3	155.8	185.7	198.0
Saturated acids (%)	—	25.9	30.1	28.4
N.V. of sat. acids	—	213.1	215.5	209.4
I.V. of sat. acids	—	2.7	6.6	3.6
Unsaponifiable matter				
Iodine value	—	—	129.3	131.1
Sterol (%)	26.08	46.23	56.65	67.04
5, 7-Diene in total sterol (%)	11.0	2.2	12.6	15.8

Notes: Fatty oils from *L. brevicula* and *M. arenaria japonica* had d_4^{40} 0.9184 and 0.9317, respectively. Iodine values recorded in this paper were determined by the Wijs method for fatty oils and fatty acids and by the pyridine sulphate dibromide method for unsaponifiable matter and its components. For the determination of saturated acids (%) in the total fatty acids, the methyl esters of total fatty acids were subjected to the permanganate acetone oxidation method. For the determination of 5, 7-diene ($\Delta^{5,7}$-sterol) in the total sterol, the digitonide obtained in the determination of the total sterol in unsaponifiable matter was used for ultraviolet absorption measurements, and the percentage of 5, 7-diene in the total sterol was calculated by taking the mean molecular weight of sterols as 399 (Mol. Wt. of diunsaturated C_{28}-sterol).

The fatty acids from *L. brevicula*, *M. arenaria japonica* and *P. pectinata japonica* were isomerized under the condition of 21% KOH-ethylene glycol, 180°C and 15 minutes with a current of nitrogen, the ultraviolet absorptions of the isomerized fatty acids were measured, and the polyethenoid acids were estimated by applying the formula given by Hammond and Lundberg.[8] The results are shown in Table 4.

TABLE 4. Polyethenoid Acids in the Fatty Acids

Polyethenoid acid	*L. brevicula*				*M. arenaria japonica*				*P. pectinata japonica*			
	Wave length (mμ)	Specific extinc. coeff.	%		Wave length (mμ)	Specific extinc. coeff.	%		Wave length (mμ)	Specific extinc. coeff.	%	
			A	B			A	B			A	B
Hexaethenoid	374	0.63	2.15	2.15	376	3.34	11.39	11.39	376	3.37	11.49	11.49
Pentaethenoid	347	4.71	8.17	4.70	348	11.91	17.44	10.04	348	9.01	11.64	6.70
Tetraethenoid	315	10.68	8.93	10.20	316	17.79	7.54	10.26	316	15.96	9.91	11.73
Triethenoid	269	20.80	12.83	14.09	270	21.12	4.40	6.76	271	27.70	13.28	14.67
Diethenoid	235	24.55	11.45	12.05	235	22.88	6.25	7.61	235	26.43	7.18	8.14

Notes: The figures in the columns A and B are those calculated by taking pentaethenoid acids as C_{22} and C_{20}, respectively. Since the absorption maxima were observed at the wave lengths shown in Table 4, these wave lengths were adopted in place of those in the formula given by Hammond and Lundberg, 374, 346, 315, 268 and 233 mμ, respectively. Monoethenoid acids (%) can be calculated by subtracting saturated acids (%) and polyethenoid acids (%) from 100. Assuming monoethenoid acids to have an iodine value of 89.9 (iodine value of oleic acid), the iodine values of the total fatty acids from *L. brevicula*, *M. arenaria japonica* and *P. pectinata japonica* can be calculated from the observed composition as 155.1, 190.8 and 197.6, if pentaethenoid acids be taken as C_{22}, and 152.5, 184.8 and 193.3, if pentaethenoid acids be taken as C_{20}, respectively. In the case of the fatty acids from *L. brevicula* and *P. pectinata japonica*, the calculated values, 155.1 and 197.6, obtained by taking pentaethenoid acids as C_{22} are close to the observed values, 155.8 and 198.0, in Table 3, respectively. In the case of the fatty acids from *M. arenaria japonica*, the calculated value 184.8 is close to the observed value 185.7 in Table 3.

3. Unsaponifiable matter

(i) Sterol. The unsaponifiable matter from each oil was recrystallized from methanol to separate crude sterol mixture.

The crude sterol mixture from *D. rubra var. nigromaculata* showed m.p. 121°–123°C and 5, 7-diene content 14.6%.*

The crude sterol mixture from *L. brevicula* had m.p. 137°–139°C and 5, 7-diene content 2.5%. The acetate mixture prepared from this crude sterol mixture by refluxing with acetic anhydride showed after recrystallization from methanol m.p. 114°115°C, $[\alpha]_D^{28} = -47.0°$,** and saponification value 131.2 (Calcd. for $C_{29}H_{48}O_2$, 130.9). The free sterol obtained by saponification of this acetate had m.p. 144°–

* 5, 7-Diene contents (%) recorded in this paper, except those recorded in Table 3, were estimated by ultraviolet absorption measurements of the samples by applying the formula given in the 5th report[9] of this series.

** Optical rotations recorded in this paper were measured in chloroform.

145°C and iodine value 71.0 (Calcd. for $C_{27}H_{46}OF_1$, 65.6) after recrystallization from methanol and showed no depression of melting point on admixture with cholesterol.

The crude sterol mixture from *M. arenaria japonica* had m.p. 134°–135°C and 5, 7-diene content 11.9%. Its acetate (1.5 g, I.V. 114.5) gave after repeated recrystallizations from ethanol 0.2 g of a crystalline fraction of m.p. 139.5°–141°C, $[\alpha]_D^{13} = -44.3°$, 5, 7-diene content 3.1%, S.V. 124.7 and I.V. 105.1. On saponification it gave a free sterol, m.p. 132.5°–133.5°C. A combined acetate fraction (1.0 g, melting range 124°–136°C) recovered from the mother liquor of recrystallization was dissolved in ether, brominated at about −10°C and the resulting insoluble bromide was separated. Recrystallizations of the ether-insoluble bromide from chloroform-methanol brought out a gradual raise of its melting point to 182°–183°C but failed to give a constant melting bromide fraction.

A 5.6 g portion of the crude sterol mixture, m.p. 135°–137°C, from *P. pectinata japonica* was acetylated to give an acetate of m.p. 122°–125°C and 5, 7-diene content 12.5%. This acetate was fractionated into seven eluate fractions by chromatography; adsorbent: alumina, solvent: ethanol-hexane (1 : 200). The first to fourth eluate fractions contained no 5, 7-diene. These were united and the united fraction (3.6 g, melting range 128°–133°C) was dissolved in 35 cc of ether, an excess of bromine in glacial acetic acid (1 : 3) was added dropwise to the solution under cooling at about −10°C, and after standing at the same temperature for three hours the resulting insoluble bromide (I, 0.81 g) of m.p. 173°–175°C (decomp.) was separated by filtration. The bromide remaining in the filtrate was fractionally precipitated with addition of methanol to give four bromide fractions. The second bromide fraction (II, 2.41 g) had m.p. 122°–124°C and the fourth bromide fraction (III, 0.75 g) had m.p. 113°–115°C.

The bromide I was recrystallized from chloroform-methanol and 0.39 g of a crystalline bromide of m.p. 190°–192°C (decomp.) was obtained. Debromination with zinc dust and glacial acetic acid gave a steryl acetate which showed after recrystallization from ethanol m.p. 146°–147°C, $[\alpha]_D^{27} = -53.0°$, S.V. 122.7 and I.V. 110.9 (Calcd. for $C_{31}H_{50}O_2F_2$: S.V. 123.4, I.V. 111.6). The free sterol from this acetate had m.p. 152°–153°C and $[\alpha]_D^{28} = -50.6°$ after recrystallization from methanol. The benzoate prepared from the free sterol showed m.p. 140°–141°C and $[\alpha]_D^{28} = -21.6°$ after recrystallization from ethanol.

The steryl acetate prepared by debromination of the bromide II gave a crystalline acetate fraction of m.p. 135.5°–136.5°C, $[\alpha]_D^{27} = -42.6°$, S.V. 121.6 and I.V. 57.0 (Calcd. for $C_{31}H_{52}O_2F_1$: S.V. 122.8, I.V. 55.6) after recrystallization from methanol-ethanol and acetone-methanol. Saponification of this acetate fraction yielded a free sterol which had m.p. 137.5°–138.5°C and $[\alpha]_D^{28} = -37.4°$ after recrystallization from methanol. Its benzoate had m.p. 137°–138°C and $[\alpha]_D^{28} = -17.0°$ after recrystallization from ethanol. The melted benzoate showed a bluish green color on its solidification.

The debromination product of the bromide III was fractionated into seven fractions by fractional crystallization from methanol-ethanol and acetone-methanol. The sixth and seventh fractions of the lowest melting point were united and the united material (0.15 g, melting range 112°–115°C) was fractionally crystallized from methanol to give an acetate fraction of m.p. 113°–114°C and S.V. 129.4,

The free sterol from this acetate fraction, after recrystallization from methanol, had m.p. 145°–146°C and $[\alpha]_D^{28} = -38.5°$ and showed no depression of melting point on admixture with cholesterol.

(ii) Non-sterol fraction of the unsaponifiable matter from *D. rubra var. nigromaculata.* * The non-sterol fraction was separated from the unsaponifiable matter using digitonin. It was a yellowish orange viscous liquid of n_D^{40} 1.4747 and showed no characteristic absorption in the ultraviolet region (220–320 mμ) in ethanol.

Summary

Fatty oils were extracted from two gastropods, *Dendrodoris rubra var. nigromaculata* and *Littorina brevicula,* and two pelecypods, *Mya arenaria japonica* and *Pinna pectinata japonica* (viscera). Their characteristics were determined and their sterol components were examined. The sterol mixture of the oil from *L. brevicula* was found to consist mainly of cholesterol and contain a small amount of $\Delta^{5,7}$-sterol. Among the non-conjugated sterol components of the unsaponifiable matter from *P. pectinata japonica,* poriferasterol, clionasterol and cholesterol were found.

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References

1) M. Tsujimoto and H. Koyanagi: *J. Soc. Chem. Ind. Japan* **37**, 998 (1934).
2) Y. Toyama and T. Takagi: *Memoirs Faculty of Engineering, Nagoya Univ.* **7**, 24 (1955).
3) T. Takagi and Y. Toyama: *Memoirs Faculty of Engineering, Nagoya Univ.* 8, 169 (1956).
4) F. R. Valentine, Jr., and W. Bergmann: *J. Org. Chem.* **6**, 452 (1941).
5) W. Bergmann: "Progress in the Chemistry of Fats and Other Lipids", Vol. 1, p. 45, Pergamon Press Ltd., London, 1952.
6) T. Tanaka and Y. Toyama: *Memoirs Faculty of Engineering, Nagoya Univ.* **11**, 187 (1959).
7) Y. Toyama, T. Tanaka and T. Maeda: *Memoirs Faculty of Engineering, Nagoya Univ.* **7**, 145 (1955); Y. Toyama and T. Tanaka: *ibid.* 8, 29, 40 (1956).
8) E. G. Hammond and W. O. Lundberg: *J. Am. Oil Chemists' Soc.* **30**, 433 (1953).
9) Y. Toyama and T. Takagi: *Memoirs Faculty of Engineering, Nagoya Univ,* **7**, 16 (1955).

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* The unsaponifiable matters of the oils recorded in this study and also of the previously reported oils[7] from *Hemifusus ternatanus, Tonna luteostoma, Turbo cornutus, Lunella coronata coreensis, Monodonta labio, Haliotis gigantea, Solen gouldi, Sanguinolaria olivacea, Dosinia japonica, Anadara subcrenata, Buccinum* (*Volutharpa*) *perryi, Tegula* (*Chlorostoma*) *argyrostoma sublaevis, Mytilus edulis, Actinocyclus japonicus, Turris unedo, Ficus subintermedius,* and *Apollon perca* were negative for the flame reaction for halogen.