FATTY OILS OF AQUATIC INVERTEBRATES

XX. FATTY OIL OF THE SHELLFISH *BRACHIDONTES SENHOUSIA* WITH A PARTICULAR REFERENCE TO ITS STEROL COMPONENTS

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The shellfish *Brachidontes senhousis* (Benson) of the family *Mytilidae* is a small-sized bivalve of a shell-length of about 2 cm and lives on the sandy or muddy sea bottom, getting intertwined with one another by its byssi. Its propagation is sometimes so tremendous as to cover the entire area of the sea bottom and damage the culture of clams and other edible shellfish such as *Venerupis philippinarum*. It appears sometimes to be used locally as fertilizer and feed. No literature on the fatty oil of this shellfish has been known to us. In this study, the fatty oil was extracted from three lots (No. 1–No. 3) of this shellfish, characteristics of each oil sample and its fatty acids were determined, and its sterol components were particularly investigated.

The provitamin D ($\Delta^{5,7}$-conjugated sterol) content in the total sterol, determined spectrophotometrically with the sterol digitonide prepared from three oil samples, showed a value above 30% in each case which is higher than the values for Japanese shellfish hitherto reported. The sterol digitonide from No. 1 oil exhibited the absorption maxima, though small, at 311 mμ, 324 mμ and 339 mμ besides the absorption maxima corresponding to $\Delta^{5,7}$-conjugated sterol. As these wave lengths of the absorption maxima accord with those of $\Delta^{5,7,9(11)}$-conjugated sterol,[1] the occurrence of $\Delta^{5,7,9(11)}$-sterol in No. 1 oil is indicated. Since, however, these absorption maxima were not discernible in the case of No. 2 and No. 3 oils, these oils appear to contain $\Delta^{5,7,9(11)}$-conjugated sterol only in an extremely minor amount if any.

In an attempt to separate $\Delta^{5,7}$-conjugated sterol, the steryl acetate mixture from three oil samples was first subjected to repeated recrystallizations, but it was found that while the first recrystallization served more or less to concentrate $\Delta^{5,7}$-conjugated component, the second and succeeding recrystallizations resulted in a diminution of $\Delta^{5,7}$-conjugated component. Therefore, the steryl acetate from No. 3 oil was recovered and subjected to chromatographic fractionations using an adsorption column of alumina, by which a fraction *A* of m.p. 124°–125°C was eventually obtained from relatively later eluate fractions. Its ultraviolet absorption accorded with that of $\Delta^{5,7}$-conjugated steryl acetate. The free sterol from this acetate had m.p. 119°–120°C and its infrared absorption spectra indicated the presence of a *trans* double bond in the side chain. A steryl acetate fraction *B* of m.p. 123°–125°C, adjacent to the fraction *A* but somewhat inferior in its purity, was hydrogenated in ether with platinum black catalyzer. The purified material

of hydrogenation product accorded with Δ^7-cholestenyl acetate in its melting point and optical rotation and also in its curve showing the relation between the absorption at 620 mμ and the reaction period in the Liebermann-Burchard reaction. From these results, the $\Delta^{5,7}$-conjugated sterol in the oil of *Brachidontes senhousia* is considered to be $\Delta^{5,7,22}$-(or $\Delta^{5,7,23}$-)cholestatrienol.

The $\Delta^{5,7}$-conjugated sterol from *Brachidontes senhousia* is characterized by a relatively low melting point of both free sterol and its acetate. As for $\Delta^{5,7}$-conjugated sterols resembling this sterol from *Brachidontes senhousia*, a sterol (m.p. 122°–123°C, its acetate m.p. 120°–122°C) separated from *Pinna pectinata japonica*[2] and a sterol (m.p. 118°–119°C, its acetate m.p. 115°–116°C) separated from *Tonna luteostoma*[3] have been known to us. The infrared spectroscopy* indicated that both sterols have a *trans* double bond in the side chain and that the sterol from *Tonna luteostoma* has a terminal methylene group. The sterol from *Tonna luteostoma* was prematurely considered to be $\Delta^{5,7,22,25}$-cholestatetraenol. But the observed value of the absorption corresponding to the terminal methylene group is considerably small as compared with the value in the literature, and it is suspected that the sterol from *Tonna luteostoma* is not a uniform sterol but a mixture of $\Delta^{5,7,22}$-(or $\Delta^{5,7,23}$-)cholestatrienol and $\Delta^{5,7,25}$-sterol having a terminal methylene group. This point will be reinvestigated shortly.

Experimental

1. Fatty oil

Three lots of the shellfish *Brachidontes senhousia* used in this study were caught in Shimmaiko, Chita-gun, Aichi-ken. Each lot contained byssi to which seaweeds and other contaminants were clung. In the case of No. 1 lot, byssi and other contaminants were thoroughly removed by a careful selection. In the case of No. 2 and No. 3 lots, byssi and other contaminants were roughly removed. The selected shellfish were dried in an infrared drying oven and the dried material was crushed and then extracted with ether. The ether-extract was refluxed with about ten times its weight of acetone, the mixture was cooled to the ordinary temperature, and the acetone-insoluble matter was removed by filtration. The acetone-soluble oil (fatty oil) was recovered from the filtrate. The date of catch, the weight of shellfish, the yield of fatty oils and the characteristics of fatty oils and their fatty acids and unsaponifiable matter are given in Table 1. Iodine values recorded in Table 1 were determined by the Wijs method, solid acids in the total fatty acids by the lead salt ethanol method, and the sterol content by the digitonide method. The $\Delta^{5,7}$-conjugated sterol content was calculated from the ultraviolet absorption value of the sterol digitonide which was separated for the determination of the total sterol,[4] the mean molecular weight of sterol being taken as 384.6, the molecular weight calculated for a diunsaturated C_{27}-sterol.

* In a previous report[1] on the sterol of *Pinna pectinata japonica*, data on the infrared spectroscopy were not recorded. A later examination indicated that while an unrefined sterol sample showed a small absorption corresponding to the terminal methylene group besides an absorption corresponding to the *trans* double bond in the side chain, a refined sterol sample showed only an absorption corresponding to the *trans* double bond.

TABLE 1. Fatty Oils from the Shellfish *Brachidontes senhousia*

Sample No.	1	2	3
Date of catch	July 29, 1956	Aug. 11, 1956	Oct. 21, 1957
Weight (kg)	1	15	20
Wt. after selection (kg)	0.644	10.5	13.2
Wt. of dried material (kg)	0.265	4.4	5.0
Ether-extract (g)	7.0	75	48
Ether-extract (%)	2.6	1.7	1.0
Acetone-soluble oil (Fatty oil) (g)	4.0	37	30
Acetone-soluble oil (Fatty oil) (%)	1.5	0.8	0.6
Fatty oil			
Acid value	79.5	72.8	85.1
Saponification value	164.3	155.7	145.2
Iodine value	150.1	152.9	150.5
Unsaponif. matter (%)	20.4	24.1	31.0
Fatty acids			
n_D^{30}	1.4715	1.4724	1.4728
Neutralization value	189.5	191.4	190.3
Iodine value	163.9	174.5	177.8
Solid acids (%)	18.4	17.5	18.0
I.V. of solid acids	13.1	10.9	14.2
Unsaponifiable matter			
Sterol (%)	34.9	40.1	40.3
$\Delta^{5,7}$-conjugated sterol in the total sterol (%)	30.5	32.7	34.7

Notes: Each oil is a viscous liquid of dark reddish orange color with a dash of green. It deposits some solid at the ordinary temperature. Percentage yields of ether-extract and fatty oil are expressed on the basis of dried material.

2. Sterol

The ultraviolet absorption curve of sterol digitonide from the unsaponifiable matter of No. 1 oil, in anhydrous ethanol, is indicated in Fig. 1. Besides the characteristic absorption maxima of $\Delta^{5,7}$-conjugated sterol at 272 mμ, 282 mμ and 294 mμ, the absorption maxima, though small, corresponding to $\Delta^{5,7,9(11)}$-conjugated sterol are exhibited at 311 mμ, 324 mμ and 339 mμ; $k_{311}=1.29$, $k_{324}=1.41$ and $k_{339}=0.88$. The content of $\Delta^{5,7,9\,11)}$-conjugated sterol in the total sterol of No. 1 oil is calculated as about 4% on the basis of the absorption value of $\Delta^{5,7,9(11)}$-sterol;[5] $\varepsilon_{312}=10{,}100$, $\varepsilon_{325}=12{,}800$ and $\varepsilon_{339}=7{,}950$. Sterol digitonides prepared from the unsaponifiable matter of No. 2 and No. 3 oils exhibited only the absorption maxima

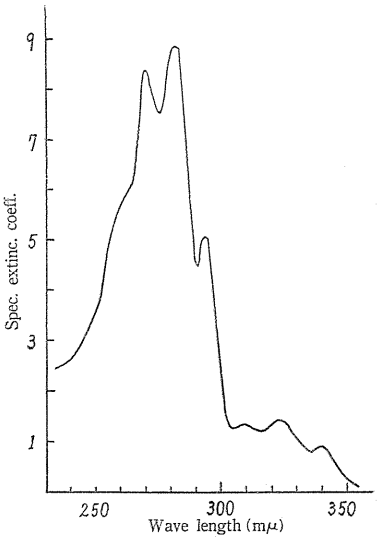

FIG. 1. Ultraviolet absorption curve for the sterol digitonide from No. 1 oil.

corresponding to $\Delta^{5,7}$-conjugated sterol, no absorption maxima corresponding to $\Delta^{5,7,9(11)}$-conjugated sterol being observed.

The united unsaponifiable matter, 9 g, from No. 1 and No. 2 oils was refluxed with 500 cc of methanol, and a small amount of methanol-insoluble matter was removed by filtration. Concentration and cooling of the filtrate gave a crystalline precipitate (crude sterol), 3.5 g. It showed m.p. 125°–127°C and $\Delta^{5,7}$-conjugated sterol content 31% by the ultraviolet absorption. Its acetate had m.p. 114°–120°C. The acetate recrystallized from acetone showed m.p. 118°–122°C and $\Delta^{5,7}$-conjugated component 37%, but a further recrystallization resulted in a diminution of $\Delta^{5,7}$-conjugated component. After four recrystallizations the acetate showed m.p. 127°–133°C and $\Delta^{5,7}$-conjugated component 16%.

The crude sterol, 3.4 g, obtained in the same way from the unsaponifiable matter, 9 g, of No. 3 oil showed m.p. 123°–129°C and $\Delta^{5,7}$-conjugated sterol content 33%. Its acetate of m.p. 112°–118°C showed, after two recrystallizations, m.p. 130°–132°C and $\Delta^{5,7}$-conjugated component 17%. Thus $\Delta^{5,7}$-conjugated component is decreased by recrystallization in this case, too. Hence, the steryl acetate from No. 3 oil was recovered and chromatographed. The recovered material, 3.2 g, was dissolved in 400 cc of hexane and the solution was poured into an adsorption column, 5.5 cm in diameter and 35 cm in height, packed with 800 g of Merck Alumina for chromatographic use. Development and elution with hexane and ethanol-hexane (1:200) followed by removal of solvent from each eluate gave nine fractions shown in Table 2.

TABLE. 2. Chromatography of Steryl Acetate from No. 3 Oil.

Fraction	m.p. (°C)	Yield (g)	$\Delta^{5,7}$-Conjugated component (%)
1	135–137	0.4	1
2	132–135	0.7	6
3	129–133	0.4	16
4	125–128	0.5	31
5	120–124	0.3	59
6	118–122	0.2	68
7	114–119	0.15	83
8	114–118	0.15	75
9	137–140	0.10	14

The fractions 5–8 in Table 2 were united and subjected to a further chromatography. Eluate fractions of $\Delta^{5,7}$-conjugated component 96–98% were collected, and the united material was fractionally crystallized giving two fractions as shown below.

Fraction *A*: 0.09 g, m.p. 124°–125°C, $[\alpha]_D^{18} = -75°$, $k_{272} = 26.44$, $k_{282} = 28.18$ and $k_{294} = 15.81$.

Fraction *B*: 0.12 g, m.p. 123°–125°C, $k_{272} = 23.17$, $k_{282} = 25.35$ and $k_{294} = 15.10$.

The melting point of the fraction *A* was not altered by recrystallization from acetone. The free sterol obtained by saponification of the fraction *A* showed m.p. 119°–120°C, $k_{272} = 26.49$, $k_{282} = 28.49$ and $k_{294} = 15.68$ after recrystallization from methanol, and its infrared absorption spectra (Fig. 2) exhibited a strong absorption at

970 cm^{-1} corresponding to the *trans* double bond but no absorption at 890 cm^{-1} corresponding to the terminal methylene group. The fraction *B* appears to contain more or less non-conjugated component from its ultraviolet absorption values. After hydrogenating the fraction *B* in an ether solution using platinum black as catalyzer, the product was chromatographed using alumina as adsorbent and hexane as eluant. The eluate, after removal of solvent, was then recrystallized from methanol. The recrystallized material showed m.p. 117°–118°C and $[\alpha]_D^{22} = \pm 0$ and was free from $\Delta^{5,7}$-conjugated component. The curve showing the absorption at 620 mμ vs. the reaction period in the Liebermann-Burchard reaction for this material accorded with the curve for Δ^{7}-cholestenyl acetate, the maximum absorption (2-log *T*)/*C* being 1,990, where *T* and *C* denote transmittance (%) and concentration (10^{-3} mole), respectively. The free sterol obtained by saponification of this material showed m.p. 121°–122°C and no depression of melting point on admixture with Δ^{7}-cholestenol, m.p. 122°–123°C, in various proportions.

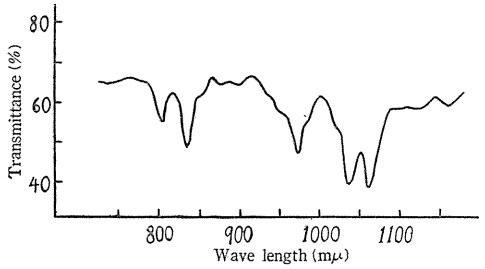

FIG. 2. Infrared spectra for the free sterol from the fraction *A*.

Summary

Fatty oils were extracted from three lots of the shellfish *Brachidontes senhousia* (Benson) of the family *Mytilidae*, and their characteristics were determined. The content of $\Delta^{5,7}$-conjugated sterol in the total sterol was found to be more than 30% for each oil. The ultraviolet absorption indicated the presence of a minor amount of $\Delta^{5,7,9(11)}$-conjugated sterol besides $\Delta^{5,7}$-conjugated sterol in the sterol mixture from one oil. The chromatography of steryl acetate mixture using an adsorption column of alumina yielded a $\Delta^{5,7}$-conjugated steryl acetate having a *trans* double bond in the side chain. Hydrogenation of an unpurified fraction of this steryl acetate in ether using platinum catalyzer yielded a product which, after purification, gave Δ^{7}-cholestenyl acetate. Therefore, the $\Delta^{5,7}$-conjugated sterol in the shellfish *Brachidontes senhousia* is presumably regarded as $\Delta^{5,7,22}$-(or $\Delta^{5,7,23}$-) cholestatrienol.

References

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