FATTY OIL OF SNAIL, *EUHADRA HERKLOTSI* (MARTENS)

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The lipid components of snail have hitherto been studied only a little. According to Bock and Wetter[1] the crude sterol mixture from a species of snail, *Helix pomatia* contains 9.3–10.1% provitamin D ($\Delta^{5,7}$-conjugated sterol) which consists chiefly of ergosterol together with cholesterol and a sterol resembling sitosterol. The authors[2] reported the characteristics of oil from a species of snail, *Bradybaena similaris stimpsoni*, and found 9.4% $\Delta^{5,7}$-conjugated sterol in its crude sterol mixture. The present paper records the results of our study on the components of oil extracted from *Euhadra herklotsi* (Martens), most common snail distributed in Tokai district.

The snails used in this study were caught in three different regions, but the oils therefrom did not show a marked difference in their properties. Polyethenoid acids of these oils were found to differ from those of aquatic shellfish oils in some respects. Namely, the former contained a relatively large proportion of diethenoid acid while the proportion of pentaethenoid acid was relatively small and hexaethenoid acid was not found in a detectable amount. The fractionation of the bromination products of the fatty acids gave a tetrabromostearic acid which melted at 113°–114°C and was identified with the tetrabromostearic acid from linoleic acid. Thus the presence of linoleic acid as a diethenoid component of polyethenoid acids was demonstrated. Sterol components in these oils were found to consist chiefly of cholesterol and did not contain $\Delta^{5,7}$-conjugated sterol in an appreciable amount. Although Bock and Wetter[1] reported the presence of a sitosterol-like sterol besides cholesterol in the sterol mixture from the snail, *Helix pomatia*, and Tanaka and Toyama[3] also found β-sitosterol as a chief component of the sterol mixture from the slug, *Incillaria confusa* Cockarell, the results of the present study showed that the sterol mixture from *Euhadra herklotsi* consists chiefly of cholesterol and contains β-sitosterol only in a minor proportion, if any.*

Experimental

1. Snails used in this study and their oils

Snails, *Euhadra herklotsi*, used in this study were caught in Middle July, 1957 in three different regions. One or two days after their catch, they were dried to

* It was the authors' careless mistake that the results of their study on the fatty oil from a species of snail, *Bradybaena similaris*, were recorded in the 5th report under the heading "Fatty oils of aquatic invertebrates" and also that *Arion empiricorum* (a species of slug) was mentioned as a species of snail in a previous paper.[3]

some degree in an infrared drying oven and then shucked. The shucked shellfish were dried further. The dried material was reduced to powder and extracted with ether. The ether-extract (lipid) was refluxed with ten times its weight of acetone for a while and then the mixture was cooled to room temperature. The acetone-insoluble matter (phosphatide) was removed by filtration and the greenish orange-red oil was obtained from the acetone-soluble fraction. Some data on the snails and their oils are given in Table 1.

TABLE 1. Snails and Their Oils

Sample No.	1	2	3
Catching locality	Higashiyama, Nagoya-shi	Tsushima-shi, Aichi-ken	Enokido, Chita-gun, Aichi-ken
Number	281	105	47
Weight (g)	1,515	481	225
Dried material of shucked shellfish (g)	293	69	36
Ether-extract (g)	12.9	3.1	2.2
Ether-extract (%)	4.4	4.5	6.1
Acetone-soluble oil (fatty oil) (g)	8.5	2.7	1.7
Acetone-soluble oil (fatty oil) (%)	2.9	3.9	4.7
Fatty oil			
Acid value	54.4	33.2	62.4
Saponification value	165.5	168.2	166.3
Iodine value	117.3	115.6	111.7
Unsaponifiable matter (%)	26.93	29.62	25.18
Fatty acids			
Yield (%)	65.1	62.8	64.0
n_D^{40}	1.4581	1.4570	1.4568
Neutralization value	198.5	197.7	198.1
Iodine value	123.7	128.8	125.9

Notes: Percentage yields of ether-extract and acetone-soluble oil are expressed on the basis of dried material of shucked shellfish. The yield of fatty acids is expressed on the basis of fatty oil. Unless stated otherwise, iodine values are determined by the Wijs method. In comparison with the content of unsaponifiable matter in oil, the yield of fatty acids appears to be somewhat too low for all samples. This is presumably ascribed to the contamination of oil with more or less phosphatide components.

2. Fatty acids

The methyl esters prepared from the fatty acids of each oil were subjected to the oxidation with potassium permanganate in acetone, and the saturated methyl esters in the total methyl esters were determined as 19.0%, 21.4% and 20.2%, respectively, for the oil samples No. 1, No. 2 and No. 3. The saponification values of saturated methyl esters were found to be 193.9, 195.6 and 193.5, respectively, for the samples No. 1, No. 2 and No. 3. These values are a little higher than the value, 188.0, calculated for methyl stearate. The saturated methyl esters from each sample were united, and the united material was saponified to give a fatty acid mixture which had m.p. 62°–64°C after a recrystallization from 85% ethanol and m.p. 65°–67°C after a further recrystallization, and showed no depression of melting point when mixed with a pure specimen of stearic acid (m.p. 70°C) in various proportions.

The fatty acids from each oil were isomerized under the condition of 21%

KOH-ethylene glycol, 180°C and 15 min. with a current of nitrogen, and the ultraviolet absorption for the isomerized fatty acids was measured. The composition of polyethenoid acids was calculated from the absorption data by assuming the formula given by Hammond and Lundberg[4] to be applicable to this case. The results are shown in Table 2.

TABLE 2. Composition of Polyethenoid Acids

Sample No.	1	2	3
Sp. extinct. coeff. of isomerized fatty acids at			
233 mμ	28.2	28.6	29.7
268 mμ	7.5	10.4	10.0
316 mμ	3.3	4.8	4.7
346 mμ	1.1	1.1	1.2
Polyethenoid acids (%)			
Diethenoid	25.5	24.0	25.3
Triethenoid	5.3	7.2	6.8
Tetraethenoid	3.4	5.9	5.5
Pentaethenoid	2.2	2.2	2.4

Notes: The figures for polyethenoid acids are calculated by taking pentaethenoid acid as C_{22}.

The united material of the fatty acids from each sample was fractionated into the crystallized fractions I and II and the filtrate fraction by the urea adducts method in methanol. The fatty acids from the fraction I had I.V. 9.1 and consisted chiefly of saturated acids. The fatty acids from the fraction II had N.V. 198.1 and I.V. 93.7 which approximated the corresponding values for oleic acid (Calcd.: N.V. 198.6 and I.V. 89.8.) The fatty acids from the fraction II were oxidized by Hazura's method. The oxidation product had m.p. 132°–133°C after recrystallization from ether-hexane and showed no depression of melting point when mixed with a pure specimen of dihydroxystearic acid, m.p. 132°–133°C, from oleic acid. The fatty acids from the filtrate fraction had N.V. 196.8 and I.V. 179.2. Bromination of these fatty acids in ether gave a small amount of ether-insoluble bromide. After removing the ether-insoluble bromide by filtration, the ether-soluble bromide was recovered from the filtrate and then treated with hexane to separate the hexane-insoluble bromide. Fractionation of this hexane-insoluble bromide with ethanol-hexane gave a major fraction which had m.p. 113°–114°C and showed no depression of melting point when mixed with a specimen of tetrabromostearic acid, m.p. 114°C, from linoleic acid.

3. Sterols

The sterol content of the unsaponifiable matter from each sample was determined by the digitonin method. The $\Delta^{5,7}$-conjugated sterol content of the unsaponifiable matter was estimated by the ultraviolet absorption measurement.[2] Properties of the crude sterol mixture obtained by recrystallization of the unsaponifiable matter from methanol and the steryl acetate obtained by acetylation of the crude sterol mixture were determined. The results are given in Table 3.

The united material of steryl acetate from each sample had S.V. 129.7 (Calcd. for cholesteryl acetate: S.V. 130.9 and I.V. 59.2). This was brominated in ether, and the bromide crystallized from the solution under cooling was separated by

TABLE 3. Sterols

Sample No.		1	2	3
Sterol content of Unsaponif. matter (%)		71	68	75
$\Delta^{5,7}$-Conjugated sterol content of the total sterol (%)		1.5	0	5.5
Crude sterol	m.p. (°C)	143–145	142–145	143–145
	$[\alpha]_D^{28}$	−36	−37	−36
Steryl acetate	m.p. (°C)	112–113	111–112	111–113
	$[\alpha]_D^{28}$	−40	—	—
	I.V.*	62.0	59.3	—

Notes: The $\Delta^{5,7}$-conjugated sterol content was determined with unsaponifiable matter, and the percentage of $\Delta^{5,7}$-conjugated sterol in the total sterol was calculated from the sterol content of unsaponifiable matter. The steryl acetate had been recrystallized once from methanol. All optical rotation were measured in chloroform.

* Iodine value in this case was determined by the pyridine sulphate dibromide method.

filtration. The crystallized bromide and the bromide recovered from the filtrate were separately debrominated, and the debromination products were recrystallized from methanol, giving crystals (steryl acetate) of m.p. 114°-115°C and m.p. 111°-112°C, respectively. These crystals were subjected to a further recrystallization from methanol, but their melting points were not raised above 115°C, suggesting that sitosteryl acetate is not present in an appreciable amount, if any, in these crystals.

Summary

Fatty oils were extracted from three lots of the snail, *Euhadra herklotsi* (Martens), caught in different regions, and their characteristics were determined. The fatty acids and sterol components were examined. The fatty acids contained approximately 20% saturated acids consisting chiefly of stearic acid. Ultraviolet absorption measurements of the alkali-isomerized fatty acids indicated that differing from the polyethenoid acids of aquatic shellfish oils, the polyethenoid acids of snail oil contained a remarkably large proportion of diethenoid acid while pentaethenoid acid was present in a relatively small proportion and hexaethenoid acid was not present in a detectable amount. Oleic and linoleic acids were identified in monoethenoid and diethenoid acids, respectively. Sterol components consisted chiefly of cholesterol without noticeable amount of sitosterol. The $\Delta^{5,7}$-conjugated sterol content of the total sterol was found to be less than 5.5%.

References

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