FATTY OILS OF AQUATIC INVERTEBRATES

XIV. FATTY OILS FROM ONE SPECIES OF THE HOLOTHURIOIDEA, NINE SPECIES OF THE GASTROPODA AND TWO SPECIES OF THE ANTHOZOA

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In this paper the results of our study on the fatty oils extracted from the following kinds of aquatic invertebrates are recorded: one species of the Holothurioidea, *Stichopus japonicus*; nine species of the Gastropoda, *Actinocyclus japonicus*, *Philine japonica*, *Turris unedo*, *Asprella sieboldi*, *Fusinus perplexus*, *Chicoreus sinensis*, *Ficus subintermedius*, *Tonna tessellata* and *Apollon perca*; two species of the Anthozoa, *Pterolides sparmanni* and *Calliactis japonica*. Among these oils, no one excepting the oil from *S. japonicus* has hitherto been studied. Referring to the oils from *A. japonicus* of the order Acoela, *P. japonica* of the order Pleurocoela and *P. sparmanni* of the order Pennatularia, no literature on oils from any species of the same orders, not to mention these three oils, has been known to us.

Some properties of the oil from *S. japonicus* was studied by Tsujimoto,[1] and the unsaponifiable components of the oil from *S. japonicus* caught around Osaki-Shimojima in the Inland Sea of Seto was reported in the 10th report[2] of this series. In the present study, the oil from *S. japonicus* caught off the coast of Atsumi, Aichi Prefecture was examined, since it was thought to be of interest to know whether oils of *S. japonicus* of different catching localities show a notable difference in their properties as it was the case with some aquatic invertebrates previously studied in this laboratory. However, the oil in the present study was found to resemble the oil of the 10th report in its properties, having a common characteristic feature that the unsaponifiable matter contains a relatively small amount of sterols in which the presence of Δ^{7}-sterol is indicated.

Although sterol components from the oils of the Gastropoda and Anthozoa were not closely studied due to scarcity of the material, some noticeable results obtained are given below. Ultraviolet absorption curves (Fig. 2) of the sterols from *A. japonicus* and *P. japonica*, unlike those from the other kinds of animal used in this study, showed a single maximum at 277–278 mμ without the three maxima characteristic to $\Delta^{5,7}$-sterol. Although $\Delta^{6,8}$-sterols ($\Delta^{6,8}$-cholestadienol and -coprostadienol)[3] are known to have a single maximum at 270–280 mμ, it could not been established in this study whether any $\Delta^{6,8}$-sterol is really present in the sterols from *A. japonicus* and *P. japonica*. Since *T. tessellata* in this study belongs to the same genus as *T. luteostoma* reported in the 9th report[4] of this series, the sterol mixture of the former was expected to have a high content of $\Delta^{5,7}$-sterol and a low melting point like the sterol mixture of *T. luteostoma*. However, the results of this study showed that the crude sterol mixture of *T. tessellata* contained approximately 1% of $\Delta^{5,7}$-sterol and its melting point was considerably higher than that of the sterol mixture of *T. luteostoma*. Its ultraviolet absorption curve (Fig. 3) showed another maximum near 240 mμ along with the three maxima characteristic to $\Delta^{5,7}$-sterols. While the sterol components of the oil of sea-anemone, mainly *Anthopleura japonica* Verrill, reported in the 11th report[5] of this series were found to consist mainly of Δ^{5}-sterols in which β-sitosterol is present, the sterol components from *C. japonica* in this study were found to contain cholesterol whose occurrence in sea-anemone was reported long ago by Doreé.[6] The presence of β-sitosterol in *C. japonica* could not be established.

Experimental

1. Samples used for extraction of oil

Among the invertebrates used in this study, the raw material of *S. japonicus* was previously heated on a water bath in order to remove the bulk of water and then dried in an electric oven at a temperature below 80°C. The Gastropoda excepting *A. japonicus* and *P. japonica* were shelled and their meats alone were dried, while the whole bodies of *A. japonicus, P. japonica, P. sparmanni* and *C. japonica* were dried without any previous treatment. Each dried material was reduced to powder and extracted with ether. The ether-extract was refluxed with about tenfold acetone for a while and then cooled to the ordinary temperature, the acetone-insoluble matter was filtered off, and the acetone-soluble oil was recovered from the acetone filtrate. Name of species and some data on the yield of oil are shown in Table 1.

TABLE 1. List of Samples

Sample No.	Species	Number	Weight (g)	Dried material (g)	Ether-extract (g)	Ether-extract (%)	Acetone-soluble oil (g)	Acetone-soluble oil (%)
1	*Stichopus japonicus*	7	1,323	131	1.4	1.1	0.96	69
2	*Actinocyclus japonicus*	1	58	5	0.25	5.0	0.18	72
3	*Philine japonica*	16	62	9	0.40	4.4	0.22	55
4	*Turris unedo*	43	542	43	3.1	7.2	2.2	71
5	*Asprella sieboldi*	15	327	22	1.2	5.5	0.74	62
6	*Fusinus perplexus*	2	133	9	0.46	5.1	0.37	80
7	*Chicoreus sinensis*	4	245	25	1.4	5.6	0.99	71
8	*Ficus subintermedius*	4	182	30	1.5	5.0	1.1	73
9	*Tonna tessellata*	1	97	14	0.66	4.7	0.42	64
10	*Apollon perca*	26	400	25	1.1	4.4	0.80	73
11	*Pterolides sparmanni*	2	85	12	1.1	9.2	0.80	73
12	*Calliactis japonica*	50	1,938	254	9.6	3.8	5.9	61

Notes : *A. japonicus* was caught at Sugashima, Mie Prefecture in late July, 1954, while all other animals were caught by the sea-bottom seine at the south-east off Atsumi, Aichi Prefecture in late August, 1954. Dried material of the sample Nos. 4-10 was obtained from shucked shellfish (meat). Percentage yield of ether-extract is expressed on the basis of dried material while that of acetone-soluble oil is expressed on the basis of ether-extract.

2. Properties of oils

Acetone-soluble oils extracted from each sample are a viscous liquid of dark yellowish or reddish orange color with some solid at the ordinary temperature.

The properties of each oil are shown in Table 2. The sterol content in Table 2 was determined by use of digitonin. The sterol was prepared in the following way: the digitonide was refluxed for one hour with tenfold acetic anhydride, the product was poured into hot water, the mixture was agitated, and the insoluble matter was separated and dried. The dried material was extracted with ether, and the ether-extract (steryl acetate) was saponified to give free sterol. The provitamin D ($\Delta^{5,7}$-sterol) content was calculated from the extinction coefficients at 277 mμ, 282 mμ and 290 mμ determined in ethanol.

TABLE 2. Properties of Oils

Sample No.	n_D^{40}	A.V.	S.V.	I.V. (Wijs)	Unsap. M. (%)	Fatty acids Neutr. V.	Fatty acids I.V.	Unsap. M. Sterol (%)	Sterol m.p. (°C)	Sterol Pro-V.D. (%)
1	1.4769	23.5	152.6	108.2	17.04	184.1	108.4	10.46	103–106	0
2	1.4878	—	125.7	—	—	—	—	34.44	108–111	0
3	1.4802	—	142.0	—	—	—	98.7	29.20	117–120	0
4	1.4753	32.7	159.1	116.4	19.76	194.4	120.5	55.92	126–128	0.56
5	1.4836	85.8	133.4	133.2	30.33	183.9	128.8	43.63	135–137	0.85
6	1.4841	57.9	143.8	144.1	25.12	186.9	139.5	33.53	122–124	2.3
7	1.4803	48.4	133.3	142.7	—	185.5	136.7	36.41	133–136	3.4
8	1.4913	50.2	141.2	126.1	—	187.0	116.9	50.95	134–136	1.1
9	1.4879	76.4	129.9	130.3	—	183.9	133.5	29.25	120–123	0.99
10	1.4873	82.9	142.4	134.6	26.80	—	142.8	39.94	127–129	2.4
11	1.4860	—	137.5	168.3	—	191.8	—	31.59	116–118	0.17
12	1.4847	35.8	127.4	166.9	32.20	186.3	179.0	54.46	132–134	0.07

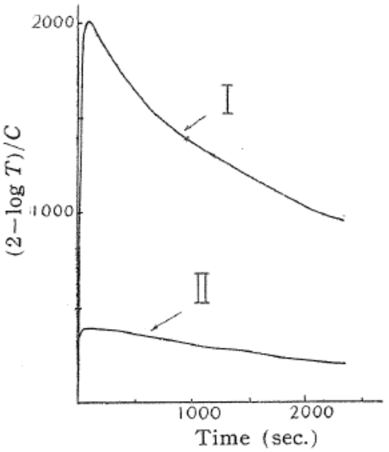
Notes: The oil of *S. japonicus* reported by Tsujimoto[1] and that described in the 10th report of this series have the following characteristics: S.V. 145.3, 149.3; I.V. 127.5, 106.2; Unsap. M. 21.3%, 16.97%; N.V. of fatty acids 187.5, 189.4; I.V. of fatty acids 128.2, 117.2; respectively.

3. Unsaponifiable matter and sterols

The unsaponifiable matter of the oil from *S. japonicus* in Table 2 showed no characteristic absorption maximum in the region of 230–320 mμ as in the case of the unsaponifiable matter of *Cucumaria chronhjelmi*[7] and *S. japonicus* in the 10th report of this series. The crystalline solid obtained by recrystallization of the unsaponifiable matter from methanol had m.p. 60°–93°C. The relatively low melting point is possibly due to the presence of alcohols of the batyl alcohol series along with sterols, since alcohols of the batyl alcohol series were found in the unsaponifiable components from *C. chronhjelmi* and *S. japonicus* previously studied. Liebermann-Burchard test for the crystalline solid of m.p. 60°–93°C gave a curve (Fig. 1), showing the absorption at 620 mμ vs. the reaction period, which indicates the presence of Δ^7-sterol. In this respect, it is quite similar to the case with *C. chronhjelmi* previously studied.

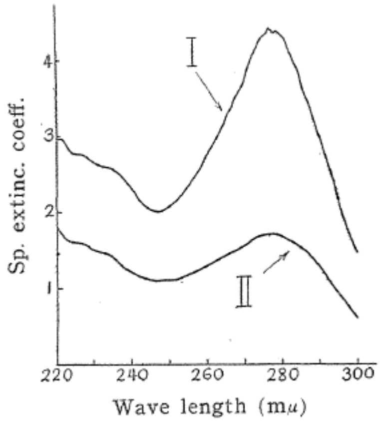
Ultraviolet absorption curves (Fig. 2) of the sterols from *A. japonicus* and *P. japonica* showed a single absorption maximum at 277–278 mμ without the three maxima characteristic to $\Delta^{5,7}$-sterol. This single absorption maximum was marked, especially in the case of *A. japonicus*.

Ultraviolet absorption curves of the sterols from the seven kinds of shellfish (sample Nos. 4–10) indicated a small content of provitamin D ($\Delta^{5,7}$-sterol) in these sterols. However, the ultraviolet absorption curve (Fig. 3) of the crude sterol obtained by recrystallization of the unsaponifiable matter of *T. tessellata* from methanol showed another absorption maximum in the proximity of 240 mμ along with the maxima corresponding to $\Delta^{5,7}$-sterol.



T: Transmittance; C: Concentration (10^{-3} mole).
Curve I for a typical Δ^7-sterol.
Curve II for *S. japonicus*.

FIG. 1. Liebermann-Burchard reaction for the crystalline solid from the unsaponifiable matter of *S. japonicus*.



Cuve I for *A. japonicus*.
Curve II for *P. japonica*.

FIG. 2. Ultraviolet absorption curves for the sterols from *A. japonicus* and *P. japonica*.

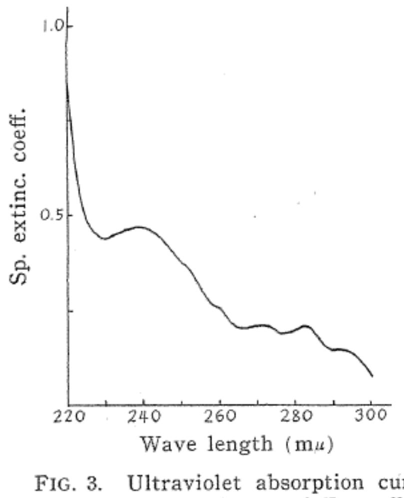


FIG. 3. Ultraviolet absorption curve for the crude sterol mixture of *T. tessellata*.

The crystalline solid obtained by recrystallization of the unsaponifiable matter of *C. japonica* from methanol was acetylated, and the acetate (about 1 g) was separated into seven fractions by fractional crystallization. The first fraction (0.07 g) had m.p. 124°–125°C, and the succeeding fractions had lower melting points in turn, giving the sixth fraction (0.42 g) of m.p. 115°–116°C and the seventh fraction (0.16 g) of m.p. 113°–114°C. Saponification of the first fraction yielded a free sterol which showed after recrystallization from methanol m.p. 142°–143°C, $[\alpha]_D^{14} = -46.5°$ (in chloroform) and I.V. (pyridine sulfate dibromide method) 85.5, and it was found from the iodine value that this free sterol contains F_2-sterol besides F_1-sterol. The sixth fraction had $[\alpha]_D^{14} = -44.1°$, S.V. 130.9 and I.V. 64.8. The free sterol prepared from this acetate showed, after recrystallization from methanol, m.p. 145°–146°C, $[\alpha]_D^{14} = -39.0°$ and I.V. 70.8, and the melting point was not depressed on admixture with authentic cholesterol (calcd. for cholesteryl acetate: S.V. 130.9, I.V. 59.2; for cholesterol: I.V. 65.6).

Summary

1. The fatty oils were extracted from one species of the Holothurioidea (*Stichopus japonicus*), nine species of the Gastropoda (*Actinocyclus japonicus, Philine japonica, Turris unedo, Asprella sieboldi, Fusinus perplexus, Chicoreus sinensis, Ficus subintermedius, Tonna tessellata* and *Apollon perca*) and two species of the Anthozoa (*Pterolides sparmanni* and *Calliactis japonica*), and their properties were determined.

2. The fatty oil from *S. japonicus* was found to have similar properties as the oil from the same kind of animal of a different catching locality described in the 10th report of this series. It showed a low content of sterol in the unsaponifiable matter and contained Δ^7-sterol as a sterol component.

3. Ultraviolet absorption curves of the sterols from *A. japonicus* and *P. japonica* had a single maximum at 277–278 mμ.

4. Cholesterol is present as a main component in the sterols of *C. japonica.*

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