FATTY OILS OF AQUATIC INVERTEBRATES. X. FATTY OILS OF *STICHOPUS JAPONICUS, ASTRICLYPEUS MANNI, CLYPEASTER JAPONICUS,* AND *GORGONOCEPHALUS CARYI*

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Of the echinoderms put in the heading, *Stichopus japonicus* belongs to the Holothurioidea, *Astriclypeus manni* and *Clypeaster japonicus* to the Echinoidea, and *Gorgonocephalus caryi* to the Ophiuroidea. Among the Holothurioidea, there are only three species whose sterol components have hitherto been studied; namely—*Cucumaria chronhjelmi*,[1] *Holothuria princepo*[2] and *S. japonicus* in this study. Among the Echinoidea, several species of the order Regularia have previously been studied on their sterol components. But the order Clypeasterida to which *A. manni* and *C. japonicus* belong have been little studied on their sterol components but that sterols from *Echinorachinus parma*[3] and *Laganum*[4] have been reported to contain chiefly cholesterol. As for the oil of *G. caryi*, though an oil extracted from the animal caught around Osaki-shimojima Island, Hiroshima Prefecture, was examined in the 6th report of this series, another specimen of oil extracted from the animal caught around Himagajima Island, Aichi Prefecture, is examined in this study.

Sterol from *S. japonicus* was considered to consist chiefly of sterol of the Δ^7-series from its optical rotation and the change of color with the period of reaction in the Liebermann-Burchard test. Sterols from *A. manni* and *C. japonicus* were demonstrated to contain chiefly cholesterol, while sterol from *G. caryi* was found to contain chiefly sterol of the Δ^5-series from its optical rotation, iodine value and the change of color with the period of reaction in the Liebermann-Burchard test.

The authors have previously noted that sterols of the Holothurioidea and Asteroidea contain chiefly sterols of the Δ^7-series, whereas sterols of the Echinoidea, Ophiuroidea and Crinoidea consist predominantly of sterols of the Δ^5-series. This characteristic feature of the correlation between the taxonomical location of an echinoderm and its sterol components is brought out also in the case of four species of the Echinoderm in this study.

The fraction obtained after removal of sterols from the unsaponifiable matter of *S. japonicus* showed a high acetyl value, and was considered to contain mainly alcohols of the batyl and selachyl series. The sterol content in the unsaponifiable matter of *S. japonicus* was found very small, 7.45%. Such a small sterol content in unsaponifiable matter was noted also in the case of *C. chronhjelmi* and *H. princepo* in previous studies. Accordingly this appears to be a characteristic feature of oils of the Holothurioidea.

It was noted in the 7th report of this series that oils obtained from one and the same species of aquatic invertebrate of different catching localities often show a considerable variance in their properties. Such is the case also with oils from

G. caryi. The oil in the present study has a very lower iodine value than the oil described in the 6th report of this series, even if it is taken into consideration that the former was extracted from the whole body while the latter was extracted from the central disc after removal of the arms.

Polyethenoid acids in the fatty acids from *S. japonicus* and *C. japonicus* were determined by ultraviolet absorption measurement of alkali-isomerized fatty acids. Relative proportions of polyethenoid acids were not alike for these two oils, and the dienoic and trienoic acids in the fatty acids of *C. japonicus* showed negative values though small. These results seem to indicate that ultraviolet absorption measurement of alkali-isomerized fatty acids is not yet fully established to determine accurately polyethenoid acids.

Experimental

1. Echinoderms used in this study. *S. japonicus* and *G. caryi* were received in the form of sun-dried material. *A. manni* and *C. japonicus* received in fresh state were dried in an oven at about 70°C. The dried materials were crushed into fine pieces and then extracted with ether. The ether-extract (lipid) was refluxed with about tenfold acetone for a while and the mixture was cooled to ordinary temperature. The acetone-insoluble matter (phosphatide) was removed by filtration, and the acetone-soluble oil (fatty oil) was recovered from the filtrate. Catching locality, date of receipt, and some data on the yields of ether-extract and acetone-soluble oil for four species of echinoderms used in this study are recorded in Table 1.

TABLE 1. List of the Echinoderms Used in This Study

Name of species	*Stichopus japonicus* Selenka	*Astriclypeus manni* Verrill	*Clypeaster japonicus* Döderlein	*Gorgonocephalus caryi* (Lyman)
Catching locality	Osaki-shimojima, Hiroshima Pref.	Onizaki, Aichi Pref.	Onizaki, Aichi Pref.	Himagajima, Aichi Pref.
Date of receipt	August, 1954	March, 1955	March, 1955	October, 1953
Number	—	1	52	1
Wt. of dried material (g)	116	74	2,100	140
Ether-extract (g)	2.9	0.80	3.5	3.2
Ether-extract (%)	2.5	1.1	1.7	2.3
Acetone-soluble oil (g)	2.5	0.69	27	1.9
Acetone-soluble oil (%)	86	86	77	59

Notes: Percentage yield of acetone-soluble oil is expressed on the basis of ether-extract.

2. Properties of oils. Characteristics of the acetone-soluble oils obtained above together with some properties of their fatty acids and unsaponifiable matter are given in Table 2.

3. Estimation of polyethenoid acids. The fatty acids from *S. japonicus* and *C. japonicus* were isomerized under the condition of 21% KOH-glycol, 180°C and 15 minutes with a current of nitrogen. Ultraviolet absorptions (Fig. 1) of the

TABLE 2. Properties of Oils

Oil	*S. japonicus*	*A. manni*	*C. japonicus*	*C. caryi*
Appearance at ordinary temperature	Orange yellow liquid with some solid	Orange yellow liquid	Reddish orange liquid	Reddish orange liquid with some solid
d_4^{20}	—	—	0.9318	—
n_D^{20}	1.4686	1.4776	1.4758	1.4702*
Acid V.	95.2	41.9	28.5	96.0
Saponif. V.	149.3	157.1	164.6	146.9
Iodine V.	106.2	158.6	190.3	91.6
Unsap. M. (%)	16.97	18.97	14.75	29.58
Neutr. V. of fatty acids	189.4	189.4	179.4	189.5
Iodine V. of fatty acids	117.2	167.0	202.1	97.0
Solid acids (%)	27.91	—	17.30	—
Iodine V. of solid acids	10.3	—	12.1	—
Sterol in unsap. M. (%)	7.45	64.7	—	62.4

Notes: Iodine values recorded in Table 2 were determined by the Wijs method. Solid acids were determined by the lead salt ethanol method.

* n_D^{30}.

TABLE 3. Polyethenoid Acids in the Fatty Acids from *Stichopus japonicus* and *Clypeaster japonicus*

	Fatty acids from *S. japonicus*	Fatty acids from *C. japonicus*
Sp. extinc. coeff. of alkali-isomerized fatty acids at		
235 mμ	14.43	23.00
270 mμ	14.74	26.50
316 mμ	10.60	31.85
348 mμ	5.33	19.10
376 mμ	1.67	1.91
Polyethenoid acids (%)		
Diene	3.6	−1.0
Triene	5.3	−0.9
Tetraene	7.7	17.2
Pentaene	7.5	34.3
Hexaene	5.7	6.5

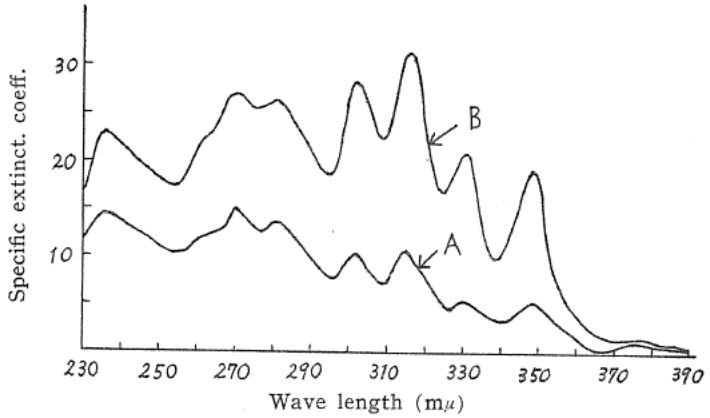


FIG. 1. Ultraviolet absorption curves of the alkali-isomerized fatty acids from *Stichopus japonicus* and *Clypeaster japonicus*.

Curve *A* for *S. japonicus*; curve *B* for *C. japonicus*.

alkali-isomerized product were measured, and polyethenoid acids were estimated by applying the formula given by Hammond-Lundberg[5] with the results shown in Table 3.

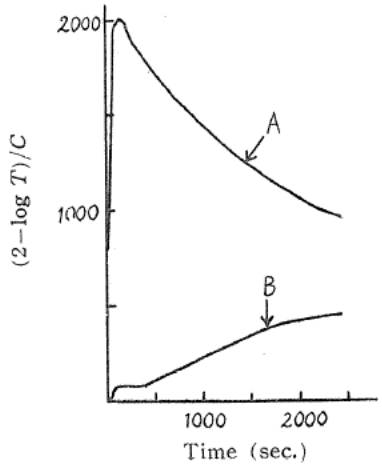
4. Sterol components. Digitonide (105 mg) prepared from the unsaponifiable matter of *S. japonicus* was refluxed with acetic anhydride, and the product was poured into water. The insoluble material was separated and extracted with ether. On refluxing the ether-extract (27 mg) with methanol, a small portion remained undissolved. After removal of the insoluble matter, the methanol solution yielded a steryl acetate fraction (20 mg) of m.p. 111°−113°C and $[\alpha]_D^{23} = \pm 0$. Liebermann-Burchard reaction for this fraction gave a curve (curve *A* in fig. 2), showing absorption at 620 mμ vs. reaction period, which lies close to the curve for a sterol of the Δ^7-series. The unsaponifiable component freed from sterol was a mixture of solid and liquid, and its acetylated product had n_D^{20} 1.4791, S.V. 236.6 and I.V. (pyridine sulfate dibromide method) 66.3.

Recrystallization of the unsaponifiable matter of *A. manni* from methanol gave a crystalline substance of m.p. 145°–146° C. Its acetate, recrystallized from methanol, had m.p. 115°C and $[\alpha]_D^{23} = -41°$. The free sterol and its acetate showed no depression of melting point when mixed with cholesterol and cholesteryl acetate, respectively.

Recrystallization of the unsaponifiable matter of *C. japonicus* gave a sterol fraction of m.p. 142°–143°C and $[\alpha]_D^{18} = -39.1°$. Its acetate had m.p. 114°−114.5°C, $[\alpha]_D^{18} = -42°$, S.V. 130.6 and I.V. (pyridine sulfate dibromide method) 62.6 (calcd. for cholesteryl acetate: S.V. 130.9 and I.V. 59.2). Its benzoate melted at 145°C to a turbid liquid and at 175°C to a clear liquid.

Recrystallization of the unsaponifiable matter of *G. caryi* from methanol gave a sterol fraction of m.p. 135°–136°C. Liebermann-Burchard reaction for this fraction gave a curve (curve *B* in fig. 2) which lies close to the curve for a sterol of the Δ^5-series. Its acetate, recrystallized from methanol, had m.p. 121°–123°C, $[\alpha]_D^{18} = -39°$ and I.V. (pyridine sulfate dibromide method) 69.1.

None of the unsaponifiable matter from the above mentioned echinoderms showed an absorption maximum in the region of 230 mμ to 300 mμ.



T: Transmittance
C: Concentration (10^{-3} mole)
Curve *A* for *S. japonicus*
Curve *B* for *G. caryi.*

FIG. 2. Absorption at 620 mμ vs. reaction period in Liebermann-Burchard reaction for sterol components of *Stichopus japonicus* and *Gorgonocephalus caryi.*

Summary

1. Fatty oils were extracted from *Stichopus japonicus*, *Astriclypeus manni*, *Clypeaster japonicus* and *Gorgonocephalus caryi*, and their properties were determined. Polyethenoid acids in the fatty acids from *S. japonicus* and *C. japonicus* were estimated by ultraviolet absorption measurements of the alkali-isomerized fatty acids. The oil obtained from *G. caryi* in the present study showed a very lower iodine value than the oil obtained from the same kind of animal in a previous study. This is possibly attributable to different catching localities.

2. The sterol components of *S. japonicus* contain sterol of the Δ^7-series, while those of *A. manni* and *C. japonicus* consist chiefly of cholesterol, and those of *G. caryi* consist chiefly of sterol of the Δ^5-series.

3. The sterol content of the unsaponifiable matter of *S. japonicus* is very small, 7.45%. Such a small sterol content in the unsaponifiable matter seems to be a characteristic feature of the oils from the Holothurioidea. The unsaponifiable components other than sterol in the oil of *S. japonicus* are considered to contain alcohols of the batyl and selachyl series.

References

1) Cf. the 8th report of this series.
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