

FATTY OILS OF AQUATIC INVERTEBRATES.  
 XI. FATTY ACIDS AND UNSAPONIFIABLE  
 MATTER OF SEA-ANEMONE

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Fat components of sea-anemone have not yet been much studied. There is scarcely any literature on the fatty acid components of sea-anemone. As for the sterol components of sea-anemone, the presence of cholesterol<sup>1)</sup> was reported by Dorée. Actinasterol,<sup>2)</sup> which was stated to occur in the sea-anemone, *Anemonia sulcata*, was found later to be a mixture of sterols of one double bond and two double bonds.<sup>3)</sup> Recently three kinds of sea-anemone<sup>4)</sup> were studied on their sterol components with the results that sterol of *Palythoa mammilosa* contains palysterol which is possibly an optical isomer of clionasterol at C-20, while sterols of *Zoanthus proteus* and *Condylactis gigantea* contain chalinasterol as a chief component. Besides these sterols, cetyl palmitate occurs in *P. mammilosa* and *Z. proteus*, while saturated monohydric alcohols of C<sub>14</sub>, C<sub>16</sub> and C<sub>18</sub> series occur in *C. gigantea*. The provitamin D contents of the crude sterols from *Actina equina*, *Actinoloba dianthus* and *Anemonia sulcata*<sup>5)</sup> were reported to be 5.0%, 2.03-10.6% and 1.6% respectively.

The present paper records the results of our studies on the fatty acids and unsaponifiable matter of the oil extracted from the sea-anemone caught at Hokkaido. The composition of fatty acids was estimated by the methyl ester fractionation method. The results showed that the composition of fatty acids of this oil had no remarkable feature as compared with the composition of fatty acids of an ordinary aquatic animal oil. Sterol components in the unsaponifiable matter of this oil was found to consist chiefly of sterol of the  $\Delta^5$ -series. Recrystallization of the steryl acetate mixture yielded a fraction consisting of  $\beta$ -sitosteryl acetate (see Table 1). This is the third instance preceded by the instances of *Spisula sachalinensis* in the 4th report and *Gorgonocephalus caryi* in the 6th report of this series, in which  $\beta$ -sitosterol was separated as a sterol component of aquatic invertebrates. The unsaponifiable components other than sterol were considered to contain mainly monohydric alcohols such as cetyl and oleyl alcohols. Also alcohols

TABLE 1. Properties of  $\beta$ -Sitosterol and its Derivatives

	Free sterol		Acetate		Benzoate	
	m.p. (°C)	$[\alpha]_D^\circ$	m.p. (°C)	$[\alpha]_D^\circ$	m.p. (°C)	$[\alpha]_D^\circ$
Sitosterol from sea-anemone .....	137	-40.8	126	-42.2	145-146	-15.1
$\beta$ -Sitosterol <sup>6)</sup> .....	136-137	-36.6	125-126	-41.0	146-147	-13.8 <sup>7)</sup>
$\beta$ -Sitosterol <sup>8)</sup> .....	139-140	-38.0	130-132	-42.5	144-145	-13.9
$\beta$ -Sitosterol <sup>9)</sup> .....	140	-37.1	129.5	-42	147	—

of other series, alcohols of batyl series for an instance, seem to be present in a lesser amount since higher fractions obtained by the fractional distillation of the acetate mixture showed somewhat higher saponification values than lower fractions (see Table 5).

### Experimental

**1. Fatty oil extracted from sea-anemone.** The sea-anemone used in the present study was received by the courtesy of Dr. M. Yamada, Faculty of Fisheries, Hokkaido University, in the form of sun-dried material (1,688 g), which was prepared from the fresh sea-anemone (12.5 kg in total) by light boiling followed by sun-drying, in a yield of 14.0%. The fresh sea-anemone was caught in Hakodate Bay, Hokkaido, in June, 1952 and consisted mostly of *Anthopleura japonica* Verrill with some *Anthopleura stella* Verrill and *Anthopleura pacifica* Uchida. The sun-dried material was reduced to powder and extracted with ether, giving 103 g of ether-extract (6.1% of sun-dried material). The ether-extract was then treated with tenfold acetone, the acetone-insoluble matter (phosphatide) was removed, and the acetone-soluble oil (95 g, 5.6% of sun-dried material) was obtained. This oil was a dark greenish orange liquid with some solid at ordinary temperature:  $d_4^{20}$  0.9199,  $n_D^{20}$  1.4810, A.V. 56.1, S.V. 117.8, I.V. 162.0\* and Unsap. M. 34.01%.

**2. Fatty acids.** The acetone-soluble oil was saponified, and the fatty acids and unsaponifiable matter were separated by extraction of the soap solution with ether in the usual way. The fatty acids had  $n_D^{20}$  1.4714, N.V. 189.2 and I.V. 193.2. Bromination of the fatty acids yielded 69.87% of ether-insoluble bromide with Br-content 69.71%.

The methyl esters prepared from the fatty acids were fractionated with the results shown in Table 2.

TABLE 2. Fractionation of the Methyl Esters

Fraction	Yield		b.p. (°C/5 mm)	$n_D^{20}$	Saponif. equivalent	S.V.	I.V.
	(g)	(%)					
1	3.9	8.7	-160	1.4471	264.2	212.4	69.2
2	4.0	9.0	160-165	1.4496	272.6	205.8	77.9
3	4.6	10.3	165-170	1.4522	281.5	199.3	93.1
4	3.1	6.9	170-175	1.4539	291.5	192.5	112.3
5	1.6	3.6	175-180	1.4601	295.8	189.7	146.8
6	1.7	3.8	180-185	1.4612	299.9	187.1	159.0
7	1.5	3.4	185-190	1.4624	302.1	185.7	182.3
8	1.8	4.0	190-195	1.4653	305.1	183.9	193.8
9	2.6	5.8	195-200	1.4688	310.7	180.6	229.3
10	4.7	10.5	200-205	1.4745	321.2	174.7	253.8
11	5.3	11.9	205-210	1.4762	328.9	170.6	264.8
12	3.3	7.4	210-215	1.4793	338.8	165.6	273.0
13	3.5	7.8	215-	1.4812	343.8	163.2	295.9
Residue	3.1	6.9	—	—	—	142.1	173.3

\* Unless stated otherwise, iodine values recorded in this paper were determined by the Wijs method for the oil and fatty acid components, and by the pyridine sulfate dibromide method for unsaponifiable components.

Saturated esters in each fraction were estimated by the permanganate oxidation method, and the saponification equivalent of the saturated esters was determined. From the observed data, the saponification equivalent and the iodine value of the unsaturated esters together with the carbon number and the unsaturation of the unsaturated acids in each fraction were deduced by calculation. The results are shown in Table 3. Since the fractions 8-13 contained only a minor proportion of saturated esters, the saponification equivalent of the unsaturated esters in these fractions was assumed to be the same with the corresponding value for the respective fractions. Since the residue was contaminated with unsaponifiable matter and polymerized methyl esters, saturated esters in the residue were determined with the methyl esters of hexane-soluble fatty acids which were prepared from the residue after removal of the unsaponifiable matter and hexane-insoluble fatty acids.

TABLE 3. Saturated and Unsaturated Esters in Each Fraction

Fraction	Saturated esters		Unsaturated esters			
	Yield (%)	S.E.	S.E.	I.V.	Carbon number of fatty acid	Unsaturation (-H)
1	45.7	261.2	266.7	127.4	15.93	2.68
2	41.1	269.4	274.8	132.3	16.52	2.86
3	34.9	275.1	284.9	143.0	17.26	3.21
4	22.5	282.2	294.2	144.9	17.94	3.36
5	17.3	286.7	297.7	177.5	18.25	4.16
6	13.1	292.0	301.1	183.0	18.50	4.35
7	8.8	294.0	302.9	199.2	18.66	4.75
8	3.8	—	305.1	201.5	18.82	4.84
9	3.5	—	310.7	237.6	19.29	5.82
10	2.7	—	321.2	260.8	20.09	6.60
11	2.5	—	328.9	271.6	20.67	7.04
12	2.4	—	338.8	279.7	21.41	7.47
13	2.0	—	343.8	301.9	21.81	8.18
Residue	1.7	—	—	—	—	—

Notes: Unsaturation (-H) is expressed by the atom equivalent of hydrogen required to a complete saturation of one mole of unsaturated esters. Saturated esters in the residue were determined with the methyl esters prepared from the hexane-soluble fatty acids (1.7 g, N.V. 170.1).

The composition of the saturated and unsaturated esters in each fraction was calculated by assuming each of these ester fractions contains two adjacent homologous esters of even acid. The unsaturated esters in the residue were regarded wholly as those of C<sub>22</sub> acids. The results are shown in Table 4. The average unsaturation for the unsaturated esters of C<sub>16</sub>, C<sub>18</sub>, C<sub>20</sub> and C<sub>22</sub> acids were obtained by plotting the carbon number against the unsaturation for the unsaturated acids in each fraction.

**3. Unsaponifiable matter.** The unsaponifiable matter was found to contain 44.7% of sterol by the digitonide method. The acetylated product (30 g, S.V. 164.6) prepared from the unsaponifiable matter was recrystallized from 600 cc of methanol, yielding a crude steryl acetate (10.2 g) of m.p. 118°-120.5° C. Its ultraviolet absorption spectra exhibited  $k_{282} = 0.491$ ,  $k_{277} = 0.456$  and  $k_{250} = 0.421$ , from which the provitamin D content in the crude steryl acetate is found to be 0.59%.

Two recrystallizations of the crude steryl acetate from acetone gave a fraction

TABLE 4. Composition of the Methyl Esters

Fraction	Saturated esters						Unsaturated esters							
	C <sub>14</sub> acid		C <sub>16</sub> acid		C <sub>18</sub> and higher member		C <sub>18</sub> acid		C <sub>18</sub> acid		C <sub>20</sub> acid		C <sub>22</sub> acid	
	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)
1	32.9	0.58	67.1	1.20	—	—	96.4	2.04	—	—	—	—	—	—
2	3.6	0.06	96.4	1.58	—	—	73.9	1.74	26.1	0.62	—	—	—	—
3	—	—	83.2	1.34	16.8	0.27	36.8	1.10	63.2	1.89	—	—	—	—
4	—	—	57.9	0.41	42.1	0.29	3.2	0.08	96.8	2.32	—	—	—	—
5	—	—	41.8	0.12	58.2	0.16	—	—	87.9	1.16	12.1	0.16	—	—
6	—	—	20.0	0.04	80.0	0.18	—	—	75.0	1.11	25.0	0.37	—	—
7	—	—	15.7	0.02	84.3	0.11	—	—	67.1	0.92	32.9	0.45	—	—
8	—	—	—	—	—	0.07	—	—	59.3	1.03	40.7	0.70	—	—
9	—	—	—	—	—	0.09	—	—	35.7	0.90	64.3	1.61	—	—
10	—	—	—	—	—	0.13	—	—	—	—	95.4	4.36	4.6	0.21
11	—	—	—	—	—	0.13	—	—	—	—	66.4	3.43	33.6	1.74
12	—	—	—	—	—	0.08	—	—	—	—	29.3	0.94	70.7	2.28
13	—	—	—	—	—	0.07	—	—	—	—	8.9	0.31	91.1	3.12
Residue	—	—	—	—	—	0.03	—	—	—	—	—	—	—	1.67
Total (g)	0.64	—	4.71	—	1.61	—	4.96	—	9.95	—	12.33	—	9.02	—
Total (%)	1.5	—	10.9	—	3.7	—	11.5	—	23.0	—	28.5	—	20.9	—
Unsaturation (-H)	—	—	—	—	—	—	2.7	—	3.5	—	6.5	—	8.4	—

of m.p. 124.5°–126°C,  $[\alpha]_D^{15} = -42.0^\circ$ , S.V. 122.7 and I.V. 68.3 (by the pyridine sulfate dibromide method) and 64.4 (by the perbenzoic acid method). Further recrystallizations of this fraction from acetone and ethanol brought about only a slight rise in its melting point. The final recrystallization yielded a small amount (0.1 g) of a fraction of m.p. 126°–127°C,  $[\alpha]_D^{15} = -42.0^\circ$  and I.V. 76.1. Several acetate fractions having melting points above 123°C were recovered from the mother liquors of recrystallizations, and these were united and recrystallized three times from methanol, giving a fraction of m.p. 126°C. The melting point of this fraction was unaltered by further recrystallizations from ethanol, acetone, and ethanol-ether, and the fraction recovered from the mother liquor of the final recrystallization had the same melting point, 126°C.

The acetate fraction of m.p. 126°C had  $[\alpha]_D^{25} = -42.2^\circ$  and I.V. 62.1. Its absorption curve showed no peak in the region of 230–300 m $\mu$ . The free sterol obtained by saponification of this acetate had m.p. 137°C and  $[\alpha]_D^{15} = -40.8^\circ$  after recrystallization from methanol. The benzoate had m.p. 145°–146°C,  $[\alpha]_D^{15} = -15.1^\circ$  and S.V. 107.5 (calcd. for C<sub>36</sub>H<sub>54</sub>O<sub>2</sub> : 108.1). The 3,5-dinitrobenzoate, recrystallized from acetone, had m.p. 201°–203°C (Calcd. for C<sub>36</sub>H<sub>52</sub>O<sub>6</sub>N<sub>2</sub> : N, 4.60%. Found : N, 4.48%)\*.

The methanol filtrate separated from the crude steryl acetate was concentrated, and the crystalline solid (2 g) formed was removed by filtration. From the final filtrate, a reddish orange liquid of S.V. 201.6 and I.V. 53.3 was recovered. Fifteen g of this liquid material was fractionated as shown in Table 5.

The fractions 1 and 2 were united and the united fraction was saponified. The product was recrystallized from methanol, yielding a crystalline substance of m.p.

\* Scarcity of the material did not permit us to measure its optical rotation. The corresponding derivative of  $\beta$ -sitosterol was reported to have m.p. 202°–203°C.<sup>(7)</sup>

TABLE 5. Fractionation of the Liquid Material Separated from Acetylated Product of Unsaponifiable Matter

Fraction	Yield (g)	b.p. (°C/4 mm)	$n_D^{15}$	S.V.	I.V.
1	3.0	175-180	1.4495	192.8	24.6
2	2.5	180-190	1.4497	194.4	27.0
3	1.5	190-200	1.4518	199.5	37.4
4	1.3	200-210	1.4537	202.7	41.8
5	4.0	210-215	1.4643	205.1	57.6
Residue	2.7	—	—	—	—

49°C and acetyl V. 198.4 (calcd. for  $C_{16}H_{34}O$ : 197.2). This substance was identified with cetyl alcohol by the mixed melting point test. The methanol filtrate was concentrated in order to separate a further quantity of crystalline substance. From the final filtrate, an oily liquid was obtained. Its acetylated product had  $n_D^{15}$  1.4521, S.V. 183.7 and I.V. 89.4 (calcd. for oleyl acetate  $C_{20}H_{38}O_2$ : S.V. 180.7 and I.V. 81.7).

### Summary

1. Fatty acids and unsaponifiable matter were separated from the oil extracted from sea-anemone, consisting mostly of *Anthopleura japonica* with some *Anthopleura stella* and *Anthopleura pacifica*. The composition of the fatty acids were estimated by the methyl ester fractionation method with the following results. Saturated acids (in the form of methyl esters):  $C_{14}$  acid 1.5%,  $C_{16}$  acid 10.9%,  $C_{18}$  acid and higher acid 3.7%; unsaturated acids (in the form of methyl esters):  $C_{16}$  (-H, 2.7) acid 11.5%,  $C_{18}$  (-H, 3.5) acid 22.6%,  $C_{20}$  (-H, 6.5) acid 28.9%,  $C_{22}$  (-H, 8.4) acid 20.7%.

2. The unsaponifiable matter contains 44.7% of sterol. The sterol components consist chiefly of sterol of the  $\Delta^5$ -series.  $\beta$ -Sitosterol forms its main component. The unsaponifiable components other than sterol contain cetyl alcohol and also unsaturated alcohol of the oleyl series.

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