

# ON THE FATTY OIL FROM EGG MASS OF THE SEA-HARE, *APLYSIA KURODAI*, AND ITS UNSAPONIFIABLE MATTER

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The fatty oil extracted from the sea-hare, *Aplysia kurodai*, and its unsaponifiable matter were reported in a recent paper<sup>1)</sup> by the authors. The sterols of this oil contained cholesterol together with about 30% of a  $\Delta^{5,7}$ -sterol whose acetate had a comparatively low melting point. The non-sterol fraction which constituted more than half of the unsaponifiable matter was found quite different from that of any other oil and contained a bromophenol derivative,  $C_{16}H_{21}OBr$ , of m.p. 69°–71°C and another bromine containing substance of m.p. 146.5°–147.5°C.

This paper is concerned with the fatty oil and its unsaponifiable matter extracted from egg mass of this animal. The egg mass is of a stringy shape and has a bright yellow color. The oil extracted from this material has a low iodine value and its content of unsaponifiable matter is small as compared with the oil extracted from adult animals. The unsaponifiable matter also has a comparatively low iodine value. The  $\Delta^{5,7}$ -sterol in the total sterol is less than 1%. Cholesterol is found to be the principal component of the sterol mixture since the acetate of the crude sterol mixture gives readily cholesteryl acetate by recrystallization. The non-sterol fraction obtained after removal of sterol from the unsaponifiable matter has a comparatively low refractive index,  $n_D^{40}$  1.4721, and is negative for the flame reaction for halogen. Its acetate has a high saponification value, 239.4. These properties differing from those of the non-sterol fraction from adult animals appear to indicate that the non-sterol fraction from egg mass is of a similar nature as that from common shellfish.

## Experimental

### 1. Fatty oil and its fatty acids

The egg mass used in this study was collected around Sugashima, Mie-ken in late May 1959. From 1690 g of fresh material was obtained 153 g of dried material by infrared drying. The dried material was reduced to powder and extracted with ether. The ether-extract (8.4 g, 5%) was refluxed with ten times its weight of acetone for a while and the insoluble matter (phosphatide) was removed by filtration at the ordinary temperature. The acetone filtrate yielded 6.9 g of an orange yellow liquid (fatty oil). The oil was saponified and the fatty acids were refluxed with ten times their weight of hexane for a while and a small amount of dark brown insoluble matter was removed by filtration at the ordinary temperature. The hexane-soluble fatty acids were analyzed for their characteristics. The results are shown in Table 1. For comparison, the characteristics of the previously reported oils from adult animals<sup>1)</sup> are also shown in Table 1.

TABLE 1. Fatty Oil

	Oil from egg mass	Oil from adult animals	
		A	B
$d_4^{40}$	0.9607	—	—
$n_D^{40}$	1.4773	1.4953	1.4833
Acid value	15.5	42.0	22.2
Saponification value	178.9	127.3	137.6
Iodine value	110.1	157.1	—
Unsaponifiable matter (%)	10.92	41.9	61.7
Fatty acids			
$n_D^{40}$	1.4607	1.4747	—
Neutralization value	198.7	180.7	—
Iodine value	117.2	138.9	173.8
Saturated acids (%)	39.4	—	22.9
N.V. of sat. acids	211.9	—	203.3
I.V. of sat. acids	1.6	—	1.9
Unsaponifiable matter			
Iodine value	66.9	166.8	157.8
Sterol (%)	24.31	26.10	4.73
$\Delta^5,7$ -Sterol in total sterol (%)	0.75	28.3	33.0

Notes: Iodine values for fatty oils and fatty acids were determined by the Wijs method and those for unsaponifiable matter and its components by the pyridine sulphate dibromide method. Saturated acids (%) were calculated from the results of the determination of the saturated methyl ester in the methyl ester of total fatty acids by the permanganate acetone oxidation method.  $\Delta^5,7$ -Sterol (%) was calculated from the ultraviolet absorption data of the digitonide obtained in the determination of total sterol in the unsaponifiable matter, assuming the mean molecular weight of sterols as 387 (Mol. Wt. of monounsaturated  $C_{27}$ -sterol).

TABLE 2. Polyethenoid Acids

Polyethenoid acid	Wave length ( $m\mu$ )	Specific extinc. coeff.	%, if pentaethenoid acids are taken as	
			$C_{22}$	$C_{20}$
Hexaethenoid	374	0.30	1.02	1.02
Pentaethenoid	346	3.98	7.33	4.22
Tetraethenoid	315	9.26	7.91	9.05
Triethenoid	268	13.20	6.03	7.20
Diethenoid	235	16.74	7.78	8.31

Notes: Characteristic wave lengths were observed as shown in Table 2, and these wave lengths were adopted in place of 374, 346, 315, 268 and 233  $m\mu$  in the formula given by Hammond and Lundberg.<sup>21</sup> Monoethenoid acids (%) can be calculated by subtracting polyethenoid acids (%) and saturated acids (%) from 100. Assuming the iodine value of monoethenoid acids as 89.9 (calculated value for oleic acid), the iodine value of the total fatty acids are calculated as 117.9, if pentaethenoid acids be taken as  $C_{22}$ , and 115.6, if pentaethenoid acids be taken as  $C_{20}$ , as compared with the observed value 117.2 in Table 1.

The hexane-soluble fatty acids were isomerized under the condition of 21% KOH-ethylene glycol, 180°C and 15 minutes with a current of nitrogen, and the composition of the polyethenoid acids were calculated from the ultraviolet absorption values of the isomerized fatty acids as shown in Table 2.

## 2. Unsaponifiable matter

The unsaponifiable matter was a mixture of yellowish orange liquid and crystalline solid. Recrystallization of the unsaponifiable matter (0.6 g) from methanol gave the crude sterol (0.08 g) of m.p. 144.5°–145.5°C which contained a very small amount (0.22%) of  $\Delta^{5,7}$ -sterol. The sterol remaining in methanol was separated in the form of digitonide from which sterol was regenerated by treating with pyridine.<sup>3)</sup> The combined sterol fraction (0.13 g) was converted to the acetate which, after recrystallization from methanol showed m.p. 113°–114°C,  $[\alpha]_D^{27} = -46.8^\circ$  (in chloroform) and saponification value 131.1 (Calcd. for  $C_{29}H_{48}O_7$ , 130.9). Saponification of this acetate gave the free sterol which, recrystallized from methanol, showed m.p. 147°–148°C and iodine value 66.3 (Calcd. for  $C_{27}H_{46}OF_1$ , 65.6) and showed no depression of melting point on admixture with cholesterol.

The fraction (0.41 g) freed from sterol was a yellowish orange soft solid. It was negative for the flame reaction for halogen and showed no characteristic absorption in the ultraviolet region (220–400  $m\mu$ ). The acetylated product was a yellowish orange liquid of  $n_D^{40}$  1.4636, saponification value 239.4 and iodine value 48.8.

## Summary

Characteristics of the oil extracted from egg mass of the sea-hare, *Aplysia kurodai*, have been determined. The sterols from this oil consist chiefly of cholesterol. The  $\Delta^{5,7}$ -sterol content of sterols is less than 1%. The non-sterol fraction of the unsaponifiable matter, unlike that from the previously reported oil of adult animals, does not contain bromine-containing substance.

## References

- 1) T. Tanaka and Y. Toyama: *Memoirs Faculty of Engineering, Nagoya Univ.* **11**, 187 (1959).
- 2) E. G. Hammond and W. O. Lundberg: *J. Am. Oil Chemists' Soc.* **30**, 433 (1953).
- 3) W. Bergmann: *J. Biol. Chem.* **132**, 471 (1940).