# ON THE UNSAPONIFIABLE MATTER OF CALAMARY OIL.

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The general properties of calamary oil obtained chiefly from the liver of *Ommastrephes sloani pacificus* (Steentrup) have already been reported in a previous paper.(1) The oil contained more than 4% of unsaponifiable matter. As no investigation on the unsaponifiable matter of the oils of Cephalopoda has yet been found, the present experiment was made in order to compare its composition to those of the liver oils of Elasmobranch fishes and Crustacea ("taraba-kani"), which contain the alcohols of the selachyl alcohol group (monoglyceryl ethers) as invariable constituents.(2)

## Experimental Part.

1. **Material.** The calamary oil used for this experiment was prepared at Hokkaidô, and had the following properties:

| | | | |
|---|---|---|---|
| $d_4^{15}$ | 0.9298 | Saponification value | 175.8 |
| $n_D^{20}$ | 1.4828 | Iodine value | 184.1 |
| Acid value | 20.5 | Unsaponif. matter | 4.50% |

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(1) Tsujimoto and K. Kimura, *J. Soc. Chem. Ind. Japan*, **30** (1927), 865.

(2) Tsujimoto, ibid., **31** (1928), 1191; **32** (1929), 1139.

The unsaponifiable matter was separated by saponifying the oil with alcoholic potash, and extracting the soap solution with ether. The total amount of ether used was 3200-3400 c.c. per 100 gr. of the oil. By this operation 68 gr. (3.4%) of the unsaponifiable matter were obtained from 2000 gr. of the oil.

**2. Examination of the Composition of the Unsaponifiable Matter.** The unsaponifiable matter was warmed with methanol. On cooling the deposited crystals were separated, and the mother liquor concentrated; by repeating this treatment several times the final solution was cooled with ice to separate solid constituents as completely as possible. Thus the following three parts were obtained: (1) Solid part A, 35 gr., (2) Solid part B, 8 gr., (3) Liquid part, 20 gr.

(*1*) *Solid Parts.* The solid part A was that part which deposited firstly from the methanol solution, and amounted more than the half of the unsaponifiable matter. It formed white crystals of m.p. above 100°C. After recrystallisation it melted at 148.5°C., and by mixed test it was confirmed to be cholesterol. The amount of cholesterol determined by digitonin method was 48.0% of the unsaponifiable matter.

The solid part B was obtained by concentrating the mother liquor from cholesterol. The crude product formed fine orange-yellow crystals and melted below 100°C. By recrystallising it from methanol, 5.5 gr. of fine white crystals were obtained. This was acetylated by heating it with five times by weight of acetic anhydride, and 6.3 gr. of the acetyl product were fractionated under 4 mm. pressure as follows (the temperature soon rose up to above 200°C.):

| | Yield (gr.) | Appearance | M. p. | Saponif. value |
|---|---|---|---|---|
| (1) Until 230° | 1.9 | White solid | 30° | 272 |
| (2) 230-238° | 2.1 | ,, | 30° | 270 |
| (3) Residue | 2.1 | Dirty orange-yellow solid | — | — |

From the above result, it appears that the fractions consist mainly of the acetyl derivatives of chimyl and batyl alcohols, as may be seen from the following numbers:

| | M.p. | Saponif. value |
|---|---|---|
| Chimyl acetate, $C_{19}H_{38}O(OCOCH_3)_2$ | ca. 22-22.5° | 280.3 |
| Batyl acetate, $C_{21}H_{42}O(OCOCH_3)_2$ | 33.9-34.4° | 262.0 |

The boiling point of these compounds is 235–249°C./5mm.

As the fractions had nearly identical properties, they were mixed for further examination. When calculated from saponification value, the substance contained nearly equivalent parts of chimyl and batyl alcohols.

The free alcohols obtained by saponification were fractionally crystallised from 95% alcohol, and separated into the following three parts: (1) First crop, 1.8 gr., white, lustrous crystals, m.p. 64–65°C., (2) Second crop, 0.7 gr., similar crystals, m.p. 61°C., (3) Third crop, m.p. 59.5–60°C.

By recrystallisation the first crop yielded 1.1 gr. of crystals of m.p. 65°C.; so no elevation of m.p. was observed. This substance contained no cholesterol, and the mixed test (equal parts) with batyl alcohol (m.p. 70–70.5°C.) was 63.5°C., showing a little depression.

Anal. Subst. = 0.1092; $CO_2$ = 0.2870; $H_2O$ = 0.1268 gr.

Found: C = 71.68; H = 12.99%. Calc. for $C_{21}H_{44}O_3$: C = 73.18; H = 12.88%.

Thus the substance mainly consisted of batyl alcohol, but it probably contained some impurity of smaller number of carbon atoms.

The second and third crops probably consisted of chimyl and batyl alcohols; the mixed test of the latter with chimyl alcohol (m.p. 60-60.5°C.) was 58°C. Although not isolated, the presence of chimyl alcohol is highly probable from the high saponification value of the acetyl compound.

(2) *Liquid Part.* Although this was mentioned as "liquid" part, it really still contained solid constituents, and formed an orange yellow semisolid at the ordinary temperature. It had iodine value 73.0, and acid value 4.3. It was acetylated, and 22.4 gr. of the acetyl compound were firstly distilled under 4.5 mm. pressure until the boiling point rose to 250°C. (bath temperature 290°C.), the orange-yellow coloured distillate amounting to 18 gr. (80.4%). The residue (4 gr.) formed a dark brown crystalline semisolid; this contained vitamin substance (liver resin). The distillate was then fractionated under ca. 4 mm. pressure as the following table.

The high saponification values of the higher fractions pointed the presence of the compounds of dihydric alcohols, and the comparatively low iodine values indicated the admixture of the compounds of saturated alcohols. The each fraction contained also small amount of free acids.

Fractions (2) and (3). These were mixed and saponified. On extracting the alkaline soap solution with ether, the free alcohol was obtained; yield, 1.8 gr., an orange-yellow crystalline mass. This was dissolved in 90% acetone, and cooling with ice, the deposited crystals were separated. The liquid part obtained from the filtrate was dissolved in 80% acetone, and cooling with ice and salt, further amount of solid part was separated. So finally 0.5 gr. of liquid part was obtained.

| | Yield (gr.) | Appearance | Acid value | Saponif. value | Iodine value | $n_D^{20}$ |
|---|---|---|---|---|---|---|
| (1) Until 180° | 2.0 | Orange-yellow liquid | 5.2 | 164 | 45 | 1.4528 |
| (2) 180–190° | 1.3 | ,, | 6.3 | 188 | 33 | 1.4504 |
| (3) 190–200° | 1.1 | ,, | 6.8 | 199 | 37 | 1.4510 |
| (4) 200–210° | 1.3 | ,, | 10.2 | 218 | 41 | 1.4520 |
| (5) 210–220° | 1.2 | ,, | 7.9 | 224 | 45 | 1.4540 |
| (6) 220–230° | 2.8 | ,, | 7.0 | 246 | 48 | 1.4550 |
| (7) 230–245° | 5.3 | ,, | 5.5 | 248 | 53 | 1.4571 |
| (8) Residue | 3.0 | Dirty brownish yellow semi-solid | — | — | — | — |

The solid part thus separated, on recrystallisation from 90% acetone, melted at 49.5–50°C., and showed no depression of m.p. on the mixed test with cetyl alcohol.

Anal. Subst. = 0.1432; $CO_2$ = 0.4131; $H_2O$ = 0.1823 gr.

Found: C = 78.68; H = 14.25%. Calc. for $C_{16}H_{34}O$: C = 79.25; H = 14.14%.

The substance was therefore confirmed to be cetyl alcohol.

The liquid part was of an orange-yellow colour, and had iodine value 76.4 and $n_D^{20}$ 1.4696. On brominating it in petroleum ether solution, a somewhat appreciable amount of precipitate was formed. On hydrogenation the yield of solid product was small, so that the exact determination of m.p. was not possible. It appears, however, very probable that the liquid part contained oleyl alcohol.

Fractions (6) and (7). These were mixed and refractionated under 5 mm. pressure. The second fraction (ca. 3 gr.) boiling at 230–245°C. was an orange-yellow liquid of saponif. value 240.2, iodine value 53.9, and $n_D^{20}$ 1.4572. The free alcohol (semi-solid) obtained by saponification was dissolved in 90% acetone, and by cooling with ice the deposited solid (probably batyl alcohol) was separated. The liquid part obtained from the filtrate amounted to 1.3 gr. It was an orange-yellow liquid of iodine value 73.0 and $n_D^{20}$ 1.4725. A little precipitate was formed by brominating it in petroleum ether solution.

Anal. Subst. = 0.1240; $CO_2$ = 0.3326; $H_2O$ = 0.1362 gr.

Found: C = 73.15; H = 12.29%. Calc. for $C_{21}H_{42}O_3$: C = 73.61; H = 12.36%.

This substance was hydrogenated with platinum black as catalyser ; the hydrogenated product on recrystallisation from alcohol melted at 64°C. and showed no depression on the mixed test with batyl alcohol. So the original substance was confirmed to be selachyl alcohol.

The first fraction (until 230°C./5mm.) of the refractionation had saponif. value 239.4, iodine value 45.8, and $n_D^{20}$ 1.4550. The chief constituent of this fraction would also be selachyl alcohol.

The fractions (4) and (5) were not examined, but judging from their properties they consisted probably of mixtures of the acetyl compounds of cetyl, batyl and selachyl alcohols, and possibly also of oleyl and chimyl alcohols.

The compounds of alcohols of higher unsaturation were also present. The low saponification value of the fraction (1) appeared somewhat abnormal. Probably it contained not acetylated alcohols, or hydrocarbons, but the want of material prevented further examination.

## Summary.

(1) The unsaponifiable matter of calamary oil contained 48% of cholesterol.

(2) Besides cholesterol, the presence of cetyl, batyl and selachyl alcohols has been confirmed. Oleyl and chimyl alcohols were probably present. A small amount of the alcohols of higher unsaturation appeared also to be present.

(3) The occurrence of the alcohols of the selachyl alcohol group (monoglyceryl ethers) in calamary oil indicates the wide distribution and vital importance of these compounds as constituents of liver oils of various marine animals.

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