XXX. STUDIES ON THE CHEMICAL NATURE OF THE UNSAPONIFIABLE FRACTION OF FISH-LIVER OILS.

II. COD-LIVER OIL.

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IN 1926 Gunnar Weidemann published a paper, "Studies on the chemical nature of the unsaponifiable fraction of fish oil" [Weidemann, 1926], in which he describes a method for the isolation of selachyl alcohol and batyl alcohol contained in the unsaponifiable fraction of the liver oil of the Greenland shark (*Somniosus microcephalus*). Weidemann transformed the alcohols into acid esters of phthalic acid, the salts of which can be purified. This and similar methods Weidemann later used on the unsaponifiable fraction of cod-liver oil, in which he assumed four alcohols and one hydrocarbon to be present.

The untimely death of Weidemann put an end to his research work, but notes and a short description of the applied methods were preserved. On this basis I have tried to reconstruct the procedure of his work, and I shall in this paper give an account of the results which Weidemann had obtained. The difficulties especially consisted in getting a survey of the numerous fractions which, when investigated, appeared to be more or less identical. Weidemann's results seem to me to be valuable for future investigations regarding the unsaponifiable matter of cod-liver oil and the vitamins contained therein. I wish to emphasise that Weidemann himself regarded these investigations as preliminary work for future researches.

On the basis of the iodine value and the equivalent weight of the phthalic ester acids of the alcohols and from the barium content of the corresponding barium salts Weidemann calculated the formulae of these alcohols. Complete analyses, however, were not made, accordingly the formulae calculated must be regarded as hypothetical and will not be given here.

For the hydrocarbon only refractive index and iodine value are given. The analogy between two of the alcohols (G_1 and G_2) may indicate that they have not been in a pure condition, and perhaps only a single alcohol is concerned. As the hydrocarbon fraction had only been prepared as an oil, it is difficult to have any idea about its composition. Since tertiary alcohols can react with

phthalic anhydride only under special conditions [Tiemann and Krüger, 1896] such alcohols may probably be contained in the hydrocarbon fraction.

Weidemann supposes that the alcohols belong to the aliphatic and cyclic group respectively.

In the unsaponifiable fraction of cod-liver oil were found:

(1) A hydrocarbon differing considerably from spinacene, its refractive index being $n_D^{20} = 1.5257$.

(2) A monohydric alcohol very sensitive to acids, and probably related to cholesterol.

(3) A dihydric alcohol.

(4) Two dihydric alcohols of the aliphatic group (G_1 and G_2).

The method of treatment of the unsaponifiable fraction is shown in the following scheme:

The unsaponifiable fraction of the oil, from which most of the cholesterol has been removed, is treated with a mixture of phthalic anhydride and pyridine, whereby the alcohols are transformed into phthalic ester acids, while the hydrocarbon and the unsaponified glycerides remain unchanged. The pyridine and the excess of phthalic anhydride are removed by shaking first with dilute sulphuric acid and then with water. A yellowish red oil remains, which contains hydrocarbon, unsaponified glycerides and phthalic ester acids of cholesterol and of unknown alcohols.

The reaction product is dissolved in ether and transferred to a separating funnel and repeatedly shaken out with fresh light petroleum. In solution are (A₁) the hydrocarbon, glycerides and ester acids of cholesterol and the two unknown cyclic alcohols (mono- and di-hydric respectively);

(A₂) the ester acids of the dihydric aliphatic alcohols remain undissolved. The two fractions A₁ and A₂ are worked out separately.

From fraction A₁ the cyclic ester acids are isolated by shaking with an aqueous solution of sodium bicarbonate, which only dissolves the ester acid of the corresponding dihydric alcohol. By subsequent shaking with sodium hydroxide the ester acids of the monohydric alcohol and of cholesterol are dissolved. The residue of fraction A₁, consisting of glycerides and hydrocarbon, yields the hydrocarbon upon saponification.

From A₂ the phthalic esters of the two aliphatic alcohols are isolated by shaking the fraction with an aqueous solution of sodium bicarbonate, freeing the salts from bicarbonates and then precipitating with barium chloride. From the precipitated barium salts the acids are liberated with dilute mineral acid. By saponification of the acids with alcoholic potassium hydroxide the corresponding alcohols are subsequently isolated.

EXPERIMENTAL.

The preparation of the phthalic ester acids.

30 g. of the unsaponifiable part of the cod-liver oil still containing 16.1 % cholesterol were dissolved in 315 cc. of freshly distilled pyridine, and to this solution 66 g. of pulverised and dried phthalic anhydride were added. The solution remained for 3½ days in an opaque flask filled with nitrogen. 800 cc. of water were added and, after solution, 600 cc. of peroxide-free ether and a mixture of 210 cc. of sulphuric acid and 1000 cc. of water were added with stirring. The ether layer was removed and repeatedly washed with water until the washing water showed a neutral reaction. The ethereal solution was dried (Na₂SO₄), and evaporated in an atmosphere of carbon dioxide, and finally *in vacuo*. The residue, a yellowish red oil, weighed 50.4 g. This oil was dissolved in a small quantity of ether and then treated as indicated on p. 265. After repeated shakings of the oil with cold light petroleum the following fractions were obtained.

Fraction A₁, easily soluble in light petroleum; weight 13.4 g.

Fraction A₂, insoluble in light petroleum; weight 27.76 g.

Of these two fractions the first contains the mono- and the second the diphtalic ester acids¹.

Fraction A₁ was again esterified with phthalic anhydride according to the method previously described. The reaction product was treated with cold

¹ According to Weidemann [1926] the diphtalic ester acids differ from monophthalic ester acids in their solubility in cold light petroleum, the first being insoluble, the second easily soluble in this solvent.

light petroleum, which dissolved most of it. After evaporation of the light petroleum the soluble fraction weighed 12.20 g. It was dissolved in ether and first shaken out with a 5 % solution of sodium bicarbonate, which takes up the ester acid of the dihydric cyclic alcohol (a_1). The ether solution was then shaken with a weak sodium hydroxide solution, which dissolved the ester acid of the monohydric cyclic alcohol (a_2) and of cholesterol (a_3). From the sodium salts the ester acids themselves (a_1 , a_2 and a_3) were liberated by means of dilute sulphuric acid. The cholesterol hydrogen phthalate, which is less soluble in absolute alcohol and ether than the cyclic ester acid a_2 , is separated from this by crystallisation.

The ethereal solution, now free from ester acids, was evaporated and the residue saponified. The hydrocarbon remained. From the ester acids or their Ba salts respectively the alcohols were isolated in the usual way by saponification.

The following figures were found:

Ester acid a_1	{ Eq. weight	353.5
	{ $n_D^{20^\circ}$	1.5199
	{ Ba	16.26 % in the Ba salt of the ester acid
	{ $n_D^{20^\circ}$	1.4851 for the corresponding alcohol

This alcohol does not give the Lifschütz oxycholesterol reaction. It yields a weak blue colour with $SbCl_3$.

As to the ester acid a_2 , this substance was contaminated by cholesterol hydrogen phthalate, therefore the figures found for equiv. weight, *etc.*, are not given. The alcohol contained in a_2 was prepared in a crystalline state, M.P. 144° . A negative Liebermann's reaction and a very weak Salkowski's reaction were found. With antimony trichloride a feeble blue colour appeared.

Cholesterol phthalic ester acid (a_3)	{ Eq. weight	533.1. Calculated	534.
	{ M.P.	162°	

That this substance really was cholesterol hydrogen phthalate was proved by esterification of pure cholesterol with phthalic anhydride. The hydrogen phthalate so prepared melted at $161-161.5^\circ$. Determination of the melting-point of a mixture of the two identical ester acids has not been made.

Hydrocarbon K	{ $n_D^{20^\circ}$	1.5257
	{ Iodine value	180 (?)

The hydrocarbon gave no condensation product with picric acid. It gave an intense blue colour with $SbCl_3$, which was about 250 times as strong as that given by cod-liver oil and also stronger than the colour given by any of the other substances isolated.

Weidemann distilled the hydrocarbon at 0.2–0.3 mm. pressure and a temperature below 140° . The distilled hydrocarbon was a light yellowish oil. The hydrocarbon was brominated in ethereal solution, and the product treated with ether and ethyl alcohol. In this way a white substance, M.P. $164-165^\circ$, was obtained.

After having been treated with cold light petroleum, which removed a small quantity, the rest of fraction A_2 (21 g.) was dissolved in 200 cc. of ether and

shaken 3 times with a 5 % sodium bicarbonate solution in a carbon dioxide atmosphere. Only a small part, consisting of neutral products, remained in the ether solution. These products were not examined more closely. The faintly yellowish bicarbonate solution was acidified with HCl and the liberated acids were dissolved in NaOH and treated with a barium chloride solution. The precipitated barium salts were filtered and dried in a vacuum desiccator over sulphuric acid. One of the barium salts was slightly, the other easily, soluble in alcohol. Weidemann called these salts G_1Ba and G_2Ba respectively. The barium salts were shaken with 10 % HCl and ether for 1–2 hours, whereby the ester acids were liberated and taken up by the ether. The ether solution was washed, dried, and at last evaporated *in vacuo*. The residue consisted of free diphtalic ester acids¹, which were saponified at first with cold alcoholic potassium hydroxide in a nitrogen atmosphere for 24 hours, and then on a boiling water-bath for an hour. The alcohols liberated were extracted with ether. These alcohols were given the names gaddiol, gaddiol 1 and gaddiol 2 respectively.

The examination of fraction A_2 gave the following result:

G_1Ba (slightly soluble in alcohol)...18.31 % Ba.

G_2Ba (easily soluble in alcohol)...18.30 % (18.44 ?) % Ba.

The iodine values for the two alcohols were:

G_1 : 64 ?

G_2 : 160 ?

These figures are, presumably, calculated on the basis of the determination of the iodine value in the two salts G_1Ba and G_2Ba .

According to Weidemann both alcohols belong to the aliphatic group; they are, further, dihydric, a fact which can be concluded from their forming diphtalic esters.

Further researches on the unsaponifiable matter of fish-liver oils will be carried out by the present writer according to the method previously described.

In conclusion the writer wishes to thank Prof. Poulsson for helpful criticism and advice.

¹ See footnote, p. 266.

REFERENCES.

- Tiemann and Krüger (1896). *Ber. deutsch. chem. Ges.* **29**, 902.
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