THE FATTY ACIDS FROM OIL OF CANTHARIS (MYLABRIS PUSTULATA Fb., INDIA).

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In the previous communication entitled 'Cantharidin from *Mylabris Pustulata* Fb., India' it has been shown that a fatty oil could be obtained by boiling the ethyl acetate extract of the insects with petroleum ether. The cantharis oil so prepared does not appear to have been examined chemically before.

A casual reference to a cantharidin oil from *Lytta Vesicatoria* and to another from the Spanish Fly occurs in the literature (Lewkowitsch, Oils and Fats, 1922, Vol. II, p. 489; Beilstein, I, 455; Gossamann, *Annalen*, 1853, **86**, 317; 1854, **89**, 123); but no analytical data have been given for the oils or their component acids. It was therefore of interest to make a preliminary chemical examination of the oil with the quantity at our disposal.

The work described below shows that the fatty oil prepared as above retains much cantharidin in solution and that the separation of the pure fat, free from cantharidin, was attended with considerable difficulty. The fatty acids present in the oil appear to be a mixture of palmitic, stearic, Δ–9: 10-oleic and an isomeric oleic acid with the double bond probably in the 6:7 position. The unsaponifiable matter forming 5 per cent. of the oil is a liquid mixture from which a small portion of a waxy solid (m.p.60-65°) could be obtained. On account of the small quantity available it was not examined further.

EXPERIMENTAL.

The ethyl acetate extract from two kilos of the dried insects was boiled in about twenty lots with petroleum (b.p.40-60°) for several hours, under reflux, and the solution filtered hot each time. From the combined solution the petroleum was distilled, leaving 250 g. of crude fat, a yield of 12.5 per cent. of the dried insects. The fat was yellowish-brown and had an unpleasant odour.

ACID VALUE.

The crude fat on titration with standard caustic soda solution in the usual manner, showed the abnormally high acid value of 150. This high acidity led us to suspect the presence in solution of much cantharidin in the fat, and this was confirmed by a very strong orange-red coloration with a hydrochloric acid solution of vanillin, characteristic of cantharidin.

NEUTRAL FAT.

The crude fat (20 g.) was neutralised with dilute caustic potash solution and the mixture extracted with ether to remove the neutral fat. The ethereal solution was separated with great difficulty from the aqueous emulsion and yielded after removal of the solvent nearly 5 g. of a pale yellow fat melting at 30° to clear yellow liquid. The fatty oil had a specific gravity of 0·9106 at 30°/24° and its refractive index was 1·4714 at 30°. The preparation of much neutral oil from the crude fat was thus very tedious and the analytical data for the crude fat would only give misleading indications of its composition owing to admixture with much cantharidin. No further analysis of the oil was therefore made. Hence the crude fat was converted into the free fatty acids and these were examined further.

PREPARATION OF UNSAPONIFIABLE MATTER.

In a typical hydrolysis of the crude fat for the preparation of the mixed acids 100 g. of the fat was boiled for several hours under reflux with a solution of 40 g. of caustic soda in about 300 c.c. of 90 per cent. alcohol. The alcohol was then distilled and the resulting soap dried at 100°, powdered and extracted with ether in a Soxhlet apparatus. The ethereal solution after washing, drying and evaporation of the solvent gave 5·0 g. of unsaponifiable matter. After two or three crystallisations from a mixture of alcohol and ether a small amount of a white, waxy solid separated melting at 60–65°, the remainder being an oil which yielded no crystalline derivatives. On account of the small quantity available the substance was not examined further.

THE MIXED ACIDS.

The ether extracted soap was decomposed with boiling dilute (1 : 1) hydrochloric acid and the liberated fatty acids recovered in a pure condition in the usual manner; yield, 80 g.

REMOVAL OF VOLATILE ACIDS.

As the analytical data of the mixed acids (see Table I) show the possible presence of appreciable quantities of lower fatty acids, the former (80 g.) were subjected to steam-distillation during eight hours when the distillate was no longer acidic. The steam distillate was neutralised with baryta water, evaporated to small bulk and decomposed with hydrochloric acid, when an appreciable amount of cantharidin crystallised, and was identified by a mixed melting point with the pure substance. The residual mixed acids weighed 73 g. and still gave a positive vanillin test for cantharidin.

SEPARATION INTO SATURATED AND UNSATURATED ACIDS.

The steamed mixed acids were further separated into the solid and liquid acids, by the well-known lead salt alcohol method (*J. Ind. Eng. Chem.*, 1921, **13**, 806). In a trial separation of the unsteamed mixed acids the solid acids formed 30·2 per cent. and the liquid 69·8 per cent. In the steamed acids the respective proportions were 33·3 and 66·7 per cent. The analytical data of the above acids are summarised in Table I.

TABLE I.

	Unsteamed		Steamed	
	Equivalent	Iodine value	Equivalent	Iodine value
Mixed acids ...	220·0	42·6	251·2	43·4
Solid acids ...	258·8	2·1	285·0	6·3
Liquid acids ...	231·9	54·4	248·3	61·2

THE SOLID ACIDS.

As the quantity available was not quite sufficient for separation by fractional distillation of the methyl esters, a preliminary examination was made as follows. The solid acids (26 g.) prepared from the steamed mixed acids were dissolved in warm petroleum (40–60° b.p.) and the solution allowed to crystallise. The first crop of 4 g. was pure stearic acid (m. p. 68–70°), unchanged on mixing with pure stearic acid. Equivalent, 286, found: C, 76·2; H, 12·4 per cent. $C_{18}H_{36}O_2$ requires, equivalent, 284, C, 76·0; H, 12·7 per cent. The residual acids recovered from the mother liquor were crystallised from 90 per cent. alcohol giving 3 g. of a crystalline acid melting at 56–57° and having the equivalent 295. This corresponds to a mixture of stearic acid (60 per cent.) with a higher acid, probably arachidic (40 per cent.); but these could not be isolated in a pure condition. The alcoholic mother liquor from the second crop gave on evaporation to dryness 19 g. of a solid acid which had the equivalent 283·8 and an iodine value 8·6. Only stearic acid (equivalent 284) could be isolated from this solid acid which is therefore assumed to be a mixture of 17·1 g. of stearic and 1·9 g. of oleic acid. Thus 26 g. of the saturated acids appear to consist of 23·1 g. of stearic, 1·9 g. of oleic and 1 g. of (probably) arachidic acid. The solid acids as a whole did not give any reaction for cantharidin.

THE LIQUID ACIDS.

The low iodine value (61·2) of these showed the absence of acids with higher unsaturation than oleic; and the low equivalent (248·3) is mainly due to admixture with cantharidin, the presence of which was confirmed by the vanillin test. A preliminary examination of its composition was therefore attempted by the method of oxidation with alkaline permanganate solution, whereby dihydroxystearic acid formed from oleic acid could be easily separated from the other constituents which were further analysed as follows.

Oxidation.—The steamed liquid acids (35·5 g.) were neutralised exactly with alcoholic potash and the solution evaporated to dryness. The residual potassium salts dissolved in 3,500 c.c. of water were treated at 0° gradually with 700 c.c. of an ice-cold 1.5 per cent. solution of potassium permanganate with vigorous mechanical stirring during one hour. After standing for one more hour at 0°, the precipitated manganese dioxide was completely dissolved by sulphur dioxide, and a white crystalline precipitate of the hydroxy-acid resulted.

TABLE II.

<table>
<tr><td colspan="7">Liquid Acids (35·5 g.) oxidised by $KMnO_4$.</td></tr>
<tr><td colspan="3">(I)
Water insoluble (12 g.) treated with petroleum and filtered.</td><td colspan="4">(II)
Water soluble (12 g.) extracted by ether, treated with petroleum and filtered.</td></tr>
<tr><td>(IV)
Petroleum insoluble 2 g.</td><td colspan="2">(III)
Petroleum soluble 9 g. Prepared Pb-salts in alcohol and filtered.</td><td>(IX)
Petroleum insoluble 5 g.</td><td colspan="3">Petroleum soluble. Prepared Pb-salts in alcohol and filtered.</td></tr>
<tr><td rowspan="2"></td><td rowspan="2">(V)
Acid from insoluble Pb-salts 6·7 g.</td><td rowspan="2">(VI)
Acid from soluble Pb-salts 2·3 g.</td><td rowspan="2"></td><td rowspan="2">(VI)
Acids from soluble Pb-salts 1 g.</td><td colspan="2">Insoluble Pb-salts treated with ether and filtered.</td></tr>
<tr><td>(VII)
Acid from soluble Pb-salts 1 g.</td><td>(VIII)
Acid from insoluble Pb-salts 1 g.</td></tr>
</table>

Excess of sulphur dioxide was then expelled from the solution by heating and the precipitated acids (I) consisting of hydroxy-acids and any unchanged material were filtered off. The aqueous filtrate saturated with salt was extracted with ether several times to recover the water soluble as well as oxidation products mechanically carried over (II). The further treatment of (I) consisted in removing by means of petroleum unchanged oleic and any other higher monobasic acids (III, V and VI) from the dihydroxystearic acid (IV), while the further treatment of (II) consisted again in removing by means of petroleum the monobasic acid products of oxidation (VII and VIII) from the dibasic acids present including cantharidin (IX). Further separation of the monobasic acid constituents by means of their lead salts in alcohol or ether resulted in the removal of unchanged oleic acid (VI) from the saturated monobasic acids (V and VII) present. The whole scheme of the above separations is outlined in Table II.

ANALYSIS OF THE OXIDATION PRODUCTS.

Fraction (IV) which formed the crude dihydroxystearic acids (2 g.) gave on crystallisation from alcohol 0·4 g. of a white crystalline substance melting at 130–131° (unchanged on mixing with pure dihydroxystearic acid. Found: C, 69·2; H, 10·9, $C_{18}H_{36}O_4$ requires C, 68·4; H, 11·4 per cent. Equivalent, 320; calculated, 316).

The alcoholic mother liquor from the above yields what appears to be an isomeric dihydroxystearic acid, m.p. 120–122°.

Fraction (V) was practically pure palmitic acid melting at 60–62° (unchanged on mixing with pure acid. Equivalent, 254; calculated, 256).

Fraction (VI) is oleic acid having equivalent, 285; n_D^{25} 1·4585; iodine value, 53·3 after one hour. Oleic acid has equivalent 282; n_D^{25}, 1·4600; iodine value, 90·0.) The acid does not absorb bromine as readily as ordinary oleic acid.

Fraction (VII) is lauric acid, identified by its equivalent, 197·4 instead of 200, and melting point.

Fractions (VIII) and (IX) appear to be all cantharidin.

From the above analysis, allowing for loss due to the complete oxidation of a portion of the acids, the liquid acids (35·5 g.) appear to consist of palmitic acid (6·7 g.) and oleic acid (24·2 g.) from the original iodine value, and the remaining amount of 4·6 g. represents admixed cantharidin.

The occurrence of a small quantity of lauric acid among the oxidation products, the formation of a low-melting dihydroxystearic acid and the isolation of a small quantity of an oleic acid whose analytical values differ from those of the ordinary oleic acid, point to the probable existence in the liquid acids of an isomeric oleic acid with double bond in the 6 : 7 position, namely,

$$CH_3(CH_2)_{10}\cdot CH : CH\cdot(CH_2)_4\cdot CO_2H.$$

The occurrence of palmitic acid in the liquid acids is probably due to the incomplete precipitation of its lead salt from the original mixed acids. The case of lauric acid, however, seems to be different as no evidence of its presence was found in the original mixed acids, when they were steam distilled.

CONCLUSION.

The fatty acid mixture obtained by the hydrolysis of the crude fatty oil obtained from *Mylabris pustulata* Fb., India, has been shown to have the following approximate composition :—

Unsaponifiable matter	5·0	per cent.
Palmitic acid	11·0	,,
Stearic acid	27·0	,,
Oleic acid and isomeride	46·0	,,
Arachidic acid	1·0	,,
Dissolved cantharidin	10·0	,,

It is rather remarkable that the fatty oil tenaciously retains so large an amount of cantharidin in solution, the usual methods of extraction failing to recover this from the fatty oil. A new method must be evolved for complete extraction of cantharidin.

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