CCCXXVII. THE OXIDATION PRODUCTS OF THE UNSATURATED ACIDS OF LINSEED OIL.

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The effect of the addition of linseed oil to a fat-free diet was the subject of an investigation in which one of us was co-operating and the chemical examination of the particular sample of oil was therefore undertaken. This examination has not yet been completed but in view of the communication of Green and Hilditch [1935] on the identification of linoleic and linolenic acids, it seems desirable to set forth the results at which we have so far arrived.

Green and Hilditch compared the effects of oxidising α- and β-linoleic acids with potassium permanganate solution using Hazura's conditions [1888] and found that whereas from 4·8 g. α-linoleic acid they obtained of the two tetra-hydroxy-acids 1·3 g. (M.P. 173°) and 2·6 g. (M.P. 155°) together with 1·1 g. of a viscous material, from β-linoleic acid only very small amounts of crystalline products were obtained, the main product being an oil (I.V. 13·7; equiv. wt. 76). They concluded that the β-acid had undergone profound disruptive oxidation under the conditions described by Hazura. In the course of the present investigation we had arrived at a somewhat similar conclusion since some form of labile tetra- or hexa-hydroxy-acid must have been formed and have undergone oxidative degradation to smaller molecules.

The linseed oil (I.V. 174) was saponified by alcoholic KOH, the free acids (245 g.) separated into saturated and unsaturated acids by the solubility of the lead salts in alcohol and the 226 g. unsaturated acid (I.V. 207) oxidised in portions of 7·5 g. using 1% alkaline permanganate under Rollett's conditions [1909] but keeping the vigorously stirred solution at a temperature of 0–5°. The cooled permanganate solution was added during a period of 25 min. and after standing for 10 min. the solution was decolorised by SO_2 and left overnight at 0°. The white precipitate of hydroxy-acids was filtered off, dried and extracted with benzene, which dissolved 12·5 g. acids. The filtrate was steam-distilled, concentrated to 250 ml. and cooled overnight. A sticky yellow precipitate separated (C) and the filtrate on further concentration deposited a brown oil (F): by continuous ether extraction of the residual filtrate, more oil and a few white crystals were obtained.

From the first precipitate the dihydroxy-acid (A) was separated by its insolubility in boiling water and purified by recrystallisation from alcohol: the filtrate on cooling deposited mainly the tetrahydroxy-acids (B) and a mixture of tetra- and hexa-hydroxy-acids remained in solution, from which treatment with a limited amount of boiling water extracted a further amount of hexa-hydroxy-acid (D) and left in solution a mixture of the tetra- and hexa-hydroxy-acids (E). Fractions (B) and (C) were purified by repeated recrystallisation from 96% ethyl alcohol; the chief constituent of (B) was sativic acid, of (C) *iso*linusic acid. Fraction (D) consisting mainly of linusic acid was recrystallised from butyl alcohol. Fraction (E) was first extracted with boiling water by which a further quantity of linusic acid was separated and the remainder repeatedly recrystallised from ethyl alcohol. The volatile acids were isolated by steam-distillation, but the distillation was stopped before the acids had been completely separated.

The proportions of products actually isolated are shown in Table I.

Table I. *Analysis of 245 g. acids from linseed oil containing approximately 189 g. carbon.*

% total carbon	Weight g.		M.P. °C.	Equiv. wt.	C %	H %
6·0	19	Saturated acids fraction				
—	224	Unsaturated acids fraction				
4·6	12·5	Acid solution in benzene, contains saturated acids	—	316	70·7	10·3
		Oxidation products				
13·0	36·0	A. Dihydroxystearic acid	133	316	—	—
7·9	14·0	B. Tetrahydroxystearic acid	156–165	348	61·0	9·8
	10·0	Approximate amount present in E. A specimen isolated	174–175	—	—	—
		C and D. Hexahydroxystearic acids				
15·3	19·0	C. *iso*Linusic acid	169–179	386	57·2	9·5
			170–175	—	57·1	9·5
	27·0	D. Linusic acid	180–192	382	56·2	9·5
		E. Mixture of tetra- and hexa-hydroxystearic acids	—	—	—	—
	5·5	Approximate amount linusic acid present in E. A specimen isolated	202–204	—	56·5	9·0
		Degradation products				
15·3	48	F. Brown viscous oil containing acid	—	238	59·7	8·1
		$C_{12}H_{22}O_6$ and probably the lactonic acid	—	250	59·5	8·0
		$C_{12}H_{20}O_5$	—	—	60·0	7·8
7·4	35	G. Volatile acids: contain *n*-hexanoic acid. C calculated as acetic acid	—	56	—	—
69·5						

Determination of constitution of degradation products.

The brown oil (F) contained a trace of manganese: it was insoluble in ether but readily soluble in 96 % alcohol. After leaving for six weeks in an evacuated desiccator its weight was approximately constant: it showed no tendency to gain in weight, so oxygen was not being absorbed, but it became more viscid and darkened very much in colour.

Found: July 31st, C 59·71, 59·5 %; H 8·14, 7·96 %. Sept. 2nd, C 60·0 %; H 7·77 %. $C_{12}H_{22}O_6$ requires C 55·96 %; H 8·40 %. $C_{12}H_{20}O_5$ requires C 59·02 %; H 8·19 %.

When a small amount was titrated with alkali in the cold an equivalent weight of 350 was indicated but on warming the final end-point agreed with an equivalent of 181. The deep colour of the solution prevented good end-points being obtained.

Treated with zinc carbonate and cold water, a small proportion of a soluble zinc salt was obtained which was less soluble in hot water and separated out on heating the solution.

Found: zinc salt, C 45·51; H 6·31; Zn 20·0 %. Equivalent of acid = 131. $C_{12}H_{22}O_6Zn$ requires C 44·3; H 6·15; Zn 20·0 %. Equivalent of acid = 131.

The zinc salt was therefore the salt of a dibasic acid $C_{12}H_{22}O_6$. The position of the hydroxy-groups was determined by oxidation with lead tetraacetate solution according to the method introduced by Criegee [1931] for the identification

of glycols: a small amount of azelaic acid was separated from the product. It appeared therefore that oxidation of a tetra- or hexa-hydroxy-acid had taken place at the 12–13 linkage and that two hydroxyl groups had been added at the 9–10 linkage. From the behaviour of the original oil on titration it seemed probable that most of the oil present was in the form of the lactonic acid $C_{12}H_{20}O_5$, the 9-hydroxyl group being in the γ-position to the carboxyl group.

The zinc salt when treated with lead tetraacetate in acetic acid solution at 37° gave a titration corresponding with the presence of about 27 % of the dihydroxy-dibasic acid: it is possible that this low percentage may be due to the formation of the relatively stable lactone so that the reaction was not complete.

Determination of the constitution of the hydroxystearic acids.

Since the hydroxy-derivatives of the higher unsaturated acids are crystalline compounds capable of purification by recrystallisation, they appear to be particularly suitable compounds in which to determine the position of the ethylenic linkage in the original unsaturated acids. The method of Criegee [1931] was therefore applied to determine the position of the adjacent hydroxyl groups. Both dihydroxystearic and sativic acids reacted almost quantitatively with the lead tetraacetate reagent at 37°. In both cases azelaic acid was isolated from the product and identified by a mixed melting-point with a specimen of pure azelaic acid (M.P. 106–107°). Efforts to prepare the 2:4-dinitrophenylhydrazones of the aldehydes simultaneously formed failed in the presence of acetic acid since even moderately dilute acetic acid decomposed them forming the acetyl derivative of 2:4-dinitrophenylhydrazine. The solution of the aldehydes in glacial acetic was therefore diluted and extracted with ether and the ethereal solution shaken with a solution of sodium carbonate. The aldehydes were then converted into the hydrazone derivatives and these analysed, and identified; from sativic acid, *n*-hexaldehyde dinitrophenylhydrazone, M.P. 97–100°; from dihydroxystearic acid, *n*-nonaldehyde dinitrophenylhydrazone, M.P. 106–106·4°.

From sativic acid a small amount of a deep red hydrazone was also isolated melting about 200°, possibly the dihydrazone of malonic dialdehyde. Oxidation of the aldehydes from the oxidation of sativic acid gave, besides *n*-hexanoic acid, a small amount of crystals, M.P. 125–127° (M.P. malonic acid 133°). The positions of the ethylene linkages in linoleic acid between the 9–10 and 12–13 carbon atoms were therefore confirmed.

Hazura [1888] observed that with dilute solutions of permanganate, which were however stronger than those which we employed, only small amounts of azelaic acid were isolated: with increasing strength of permanganate solution the quantity of dibasic acid separated was greater. So far we have failed to identify normal dibasic acids among the oxidation products, probably owing to the greater dilution of the permanganate solution used. The ethylenic linkage in the 12–13 position was more readily broken than that between the 9–10 carbon atoms, since an 11-carboxy-9:10-dihydroxyundecanoic acid was isolated.

SUMMARY.

1. From the product of the oxidation of the unsaturated acids of linseed oil with $KMnO_4$ in dilute solution a dibasic acid $C_{12}H_{22}O_6$ has been isolated as the zinc salt, and its constitution determined as 11-carboxy-9:10-dihydroxyundecanoic acid. A considerable proportion of the corresponding lactonic acid appeared to be also present.

2. Labile forms of the hexa- and especially of the tetra-hydroxystearic acids seem to have been formed as products of the oxidation, readily suffering further degradation.

3. The method of Criegee was applied to determine the position of the hydroxyl groups of sativic and dihydroxystearic acids, and of the acid $C_{12}H_{22}O_6$.

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