CCII. SPECTROSCOPIC CHANGES IN FATTY ACIDS

VI. GENERAL

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IN the course of the research described in the preceding papers of this series some interesting questions arose as side issues of the problems immediately under consideration. An account of additional evidence obtained in the investigation of these points may serve to throw further light on the general significance of spectroscopic changes in fatty acids.

EXPERIMENTAL

The effect of hydrogenation on the spectroscopic properties of the acids of tung oil. The very intense absorption shown by elaeostearic acid at $270\text{ m}\mu$ is presumably to be related to the presence of three conjugated unsaturated linkages. In view of the finding that in the hen and rat the administration of tung oil leads to the appearance of an acid absorbing at $230\text{ m}\mu$ it seemed desirable to see whether a similar change could be effected by chemical means. Hydrogenation to an extent sufficient to saturate one of the three linkages appeared to be the treatment most likely to produce the desired effect.

A sample of tung oil was kindly hydrogenated by Lever Brothers Ltd. The hydrogenation was done at 180° with 0.64 % of Crosfield's powdered catalyst. Since tung oil gives an abnormal I_2 value (164 instead of the theoretical value of about 246) the degree of saturation was estimated by the H_2 uptake, which during the early stages of the hydrogenation was much greater than would be calculated from the fall in i.v. The results obtained are given in Table I. H_2 to an extent of 2/6 saturation caused a fall in i.v. from 162 to 147. The spectroscopic absorption ($E_{1\text{ cm.}}^{1\%}$) at $270\text{ m}\mu$ fell from about 1400 to 250, but $E_{1\text{ cm.}}^{1\%}$ at $230\text{ m}\mu$ rose from 75 to 280. Further hydrogenation to 3/6 saturation with the i.v. at 103 caused a reduction in $E_{1\text{ cm.}}^{1\%}$ at $230\text{ m}\mu$ to 62, and at $270\text{ m}\mu$ to 6. Still further hydrogenation resulted in the spectroscopic absorption falling to negligible levels at both wave-lengths.

Attempts to fractionate the acids obtained by 2/6 saturation of the tung oil with a view to obtaining a product showing absorption only at $230\text{ m}\mu$ were unsuccessful. A solid fraction which could be removed after keeping the mixed acids in the cold showed absorption in both positions. A similar result was obtained with the liquid fraction, even after the residual solid acids had been crystallized out by keeping for 12 months in a refrigerator.

The change in absorption from 270 to $230\text{ m}\mu$ on the hydrogenation of tung oil has also been reported by Hulst [1935].

Table I. *Hydrogenation of tung oil*

Fraction of H ₂ absorption	Litres H ₂ per 100 g. calc.	Litres H ₂ per 100 g. found	Observed i.v.	$E_{1\text{ cm.}}^{1\%}$ of mixed acids	
				230 m μ	270 m μ
Nil	0	0	164	75	1400
2/6	8.2	8.2	147.2	280	250
3/6	12.3	12.3	103	62	<6
4/6	16.4	16.4	59.9	9	<2
5/6	20.5	20.0	6.8	—	—
6/6	24.6	22	3.8	—	—

The difference between the theoretical and observed H₂ absorptions for the 6/6 saturated fraction is probably due to leaks in the gas meters, which become significant over long hardening periods.

Oxidation of the solid acid obtained by refluxing the acid of linseed oils with KOH (pseudo-elaeostearic acid). Two small specimens of the solid acid described in Part II [Moore, 1937] were sent to Prof. T. P. Hilditch, who kindly undertook a preliminary study of the products produced by oxidation with alkaline KMnO₄. The only dibasic acid formed in each case was sebacic acid. Small amounts of monobasic acids having, in impure condition, an apparent equivalent of about 106 were also obtained.

The spectroscopic properties of storage fat from rats receiving different dietary fats. Mixed intraperitoneal and subcutaneous fat was taken from rats which for 4 weeks had received diets containing 30 % of coconut oil, lard, linseed oil and cod liver oil respectively. The fatty acids were examined spectroscopically before and after refluxing for 48 hr. with alcoholic KOH. Except in the specimen from rats given coconut oil considerable amounts of "pro-absorptive" acids were present.

Table II

Fatty acids from rats given 30 % of	i.v. of fat (rat)	$E_{1\text{ cm.}}^{1\%}$			
		2 min. saponification		48 hr. saponification	
		230 m μ	270 m μ	230 m μ	270 m μ
Coconut oil	37	10	0.6	12	1.7
Lard	65	5.5	0.4	17	4
Linseed oil	78	11	<1	50	20
Cod liver oil	79	7	0.5	50	50

The spectroscopic properties of the acids of dried grass. Preparation of acids. 500 g. dried grass, supplied by the kindness of Mr H. J. Page of Imperial Chemical Industries, were shaken five times with 1500 ml. ethyl alcohol. The extracts were combined, the alcohol evaporated off and the green residue saponified rapidly with alcoholic KOH. After dilution with water the soaps were extracted six times with ether, the extracts being discarded. The soaps were acidified, and extracted with ether. The ether extract, which was still bright green, was washed with dil. H₂SO₄ partially to decolorize it, washed with water, dried with Na₂SO₄ and given a preliminary treatment with norite charcoal. The ether was evaporated and the residue extracted with light petroleum (B.P. 50–60°). Much gummy material was left undissolved. The solution was again shaken with norite, which caused almost complete decoloration, the final colour on evaporation being very faintly yellow. About 90 mg. of acids were obtained. They partially solidified on cooling and had an odour suggestive of linseed cake.

Spectroscopic examination. The grass acids prepared as above had $E_{1\text{ cm.}}^{1\%}$ at 230 m μ = 20. There was no maximum at 270 m μ . The i.v. (Rosenmund & Kuhnenn method) was about 160, which is in reasonably good agreement with

the value of 170 found by Smith & Chibnall [1932]. When the spectroscopic absorption is compared with the i.v. it is apparent that not more than a small proportion of the polyethylenic acids was present in the "absorptive" form.

DISCUSSION

The chemical change underlying increased absorption in polyethylenic acids. It was suggested [Moore, 1937] that the most probable cause of increased absorption was the re-orientation of unsaturated linkages into conjugated positions. The production of sebacic acid, $\text{COOH}(\text{CH}_2)_8\text{COOH}$, from the solid absorptive acid obtained by refluxing the acids of linseed oil would suggest that the three unsaturated linkages have been changed from 9 : 10, 12 : 13, 15 : 16 to 10 : 11, 12 : 13, 14 : 15, thus producing a length of saturated chain sufficiently long for the production of sebacic acid. Working on the same acid in Burr's laboratory Kass [1938] has independently reached the conclusion that the first double bond has changed from the 9 : 10 to the 10 : 11 position. [See Kass *et al.* 1939.]

The effect of hydrogenation in reducing absorption at $270\text{ m}\mu$ and increasing adsorption at $230\text{ m}\mu$ agrees with the conclusion that absorption in the former position is attributable to three conjugated unsaturated linkages, in the latter to two conjugated unsaturated linkages.

The spectroscopic properties of the fatty acids in the rat. Differences were reported [Moore, 1937] in the spectroscopic properties of acids from various fats: vegetable fats and the fats of marine animals were found to have their polyethylenic acids in the "pro-absorptive" state, whereas those of land mammals were in the "absorptive" state. The results of the present work indicate that while differences in the spectroscopic properties of polyethylenic acids certainly exist between various animals, no consistent generalization can be made. Thus for acids from rats which had received large amounts of unsaturated dietary fat the spectroscopic properties were substantially those of the ingested acids in unchanged state. The spectroscopic absorption of the acids of the storage fats after feeding coconut oil was, however, greater than that of the acids of coconut oil, which are almost saturated and spectroscopically "non-absorptive", and was little affected by refluxing with KOH. It appears probable therefore that the former conclusion that land mammals have their polyethylenic acid in "absorptive" form may apply when the intake of polyethylenic acid is low. When the intake is high the acids may be laid down unchanged in the fat depots. When large amounts of a fat differing widely from the usual composition of the fat characteristic of the animal are ingested it is obvious that the properties of the latter may be readily obscured.

The spectroscopic absorption of grass acids. Since the acids of grass were found to be in the spectroscopically "pro-absorptive" form it is clear that absorptive acids found in the fats of herbivorous animals must be changed into this condition in the animal.

SUMMARY

1. Catalytic reduction of tung oil caused a change in the position of the absorption maximum of the mixed acids from 270 to $230\text{ m}\mu$, i.e. the same change which occurs *in vivo* when tung oil is fed to a hen or rat. This finding supports the conclusion that absorption in polyethylenic acids at $270\text{ m}\mu$ is caused by three conjugated unsaturated linkages, absorption at $230\text{ m}\mu$ by two such linkages.

2. Evidence has been obtained supporting the view that the change underlying increased absorption in polyethylenic acids when submitted to prolonged

refluxing with KOH is a re-orientation of the unsaturated linkages from unconjugated to conjugated positions.

3. When large amounts of "pro-absorptive" polyethylenic acids are included in the diet of the rat they are stored in the fat deposits in unchanged spectroscopic state. The predominance of absorptive acids found in the fats of some mammals, e.g. the cow, cannot therefore be made the basis of a general rule. The absorption of the acids from rats receiving an almost saturated fat (coconut oil) suggests however that when the intake of polyethylenic acids is low these are largely converted into the absorptive form.

4. Polyethylenic acids from dried grass were found to be in the "pro-absorptive" form. It appears therefore that "absorptive" acids present in the fats of herbivorous animals such as the cow must be converted in the animal.

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REFERENCES

- Hulst (1935). *Rec. trav. Chim.* **54**, 639, 644.
Kass (1938). Private communication by Prof. G. O. Burr.
— Miller and Burr (1939). *J. Amer. chem. Soc.* **61**, 482.
Moore (1937). *Biochem. J.* **31**, 138.
Smith & Chibnall (1932). *Biochem. J.* **26**, 218.