V. SPECTROGRAPHIC DATA OF NATURAL FATS AND THEIR FATTY ACIDS IN RELATION TO VITAMIN A.

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(Received November 21st, 1930.)

THE observation that vitamin A concentrates yield, on treatment with sodium ethoxide, acids of which the absorption spectra exhibit characteristic fine structure [Morton, Heilbron and Thompson, 1931] has prompted us to commence a detailed investigation of the absorption spectra of natural fats and their related acids.

In the present paper certain broad aspects of the problem are considered.

Fig. 1.

- (1) Total acids from cod-liver oil 0.08 % [2 mm.].
- (2) " " 1.00 % [2 mm.].
- (3) Saturated and less unsaturated acids from cod-liver oil 0.4 % [2 mm.].
- (4) Synthetic glycerides of cod-liver oil acids 0.08 % [2 mm.].
- (5) " " 1.0 % [2 mm.].

Attention has not been solely confined to vitamin A-containing oils but has been extended to embrace typical vegetable fats. In the first place the total

acids¹ from cod-liver oil have been examined. The absorption spectra of these are characterised by sharp bands with heads at about 392, 375, 350, 330, 316, 302, 281, 270, 259 and 235 $\mu\mu$ (Fig. 1, curves 1 and 2), and correspond closely with those of the selectively absorbing acids from the vitamin A concentrates [Morton *et al.* 1931, p. 24]. Fig. 2 (curve 2) gives the absorption spectrum of a

Fig. 2.

- (1) ——— Total acids from halibut-liver oil 0.2 % [2 mm.].
 (2) Halibut-liver oil 0.2 % [2 mm.].

halibut-liver oil rich in vitamin A (180 Carr and Price units) and here the smooth characteristic vitamin band at 328 $\mu\mu$ is clearly shown with, in addition, a second small band at about 225 $\mu\mu$. The acids obtained from this oil on saponification reveal on the other hand a wholly different type of absorption, bands with heads at identically the same positions as those of the cod-liver oil acids are now shown although a difference is observed in their relative intensities (Fig. 2, curve 1).

In Fig. 3 the absorption curve obtained for butter acids is given, butter being chosen to represent a non-liver animal fat containing vitamin A. It will be seen that five definite maxima appear, indicating the presence of small quantities of substances having the same bands as those shown by the cod- and halibut-liver oil acids. Turning now to butter-fat itself, this has been examined in chloroform solution and it is important to observe that in this case the spectrum shows distinct selective absorption at 322, 309, 284, 274 and 231 $\mu\mu$. There is little doubt that these bands correspond with those of the acids in alcohol, the shift towards the red being a solvent effect quite similar to that observed by Morton and Heilbron [1930] in the carotene bands of the non-saponifiable matter from butter. It is important to note however that, in striking contrast to butter itself, its non-saponifiable fraction does not give rise to narrow ultra-violet absorption bands.

¹ Unless otherwise specified, total acids include unsaponifiable matter originally present in the oil, the acids being prepared in the usual way by alkaline hydrolysis of the fat followed by liberation of the acids and extraction of the latter with ether. In the case of the cod-liver acids, the removal of the unsaponifiable matter brought about no alteration in the spectrum of the acids.

Fig. 3.

Total butter acids. (1) 1 % [1 cm.] alcohol. (2) 0.2 % [2 mm.].

Fig. 4.

- (1) Whale oil 0.234 mm.
 (2) Acids from whale oil 0.25 % alcohol [5 mm.].
 (3) " " 1.0 % alcohol [1 cm.].

The case of whale oil was next considered, this being selected as a typical marine animal non-liver oil deficient in vitamin A. The absorption spectra of the mixed acids from this oil are, as shown in Fig. 4 (curves 2 and 3), qualitatively, and roughly quantitatively, comparable with the cod- or halibut-liver acids. The oil itself (curve 1) like butter, also has selective absorption similar to that of the acids, but different in intensity. This specimen of whale-oil contained nearly 1 % of free fatty acids; in order to determine whether these were responsible for the observed selective absorption they were removed and the neutral oil was again submitted to spectrographic examination. The same banded structure was again observed, but the intensity of absorption in the region 260–280 $\mu\mu$ appeared to be increased. It is thus clear that, as with butter, the selective absorption of the whale oil is not exclusively due to hydrolytic products of the fat.

In Fig. 5 (curves 1 and 2) the absorption spectra of the mixed fatty acids

Fig. 5.

- (1) Thresher shark-liver acids 0.2 % [4 mm.].
- (2) " " " 0.2 % [2 cm.].
- (3) Methyl esters of Thresher shark-liver acids 0.2 % [4 mm.].
- (4) " " " 0.04 % [4 mm.].

of a Thresher shark-liver oil, from which vitamin A is absent, are reproduced. A comparison of the curves with those given in Figs. 1, 2 and 4 affords evidence of marked differences in definition and intensity. In place of the numerous sharp bands in the latter curves there are here only ill-defined maxima at 270, 280 and 315 $\mu\mu$; on the other hand, the prominent band at 230 $\mu\mu$ still persists¹.

Finally we have selected two examples from the vegetable oil group. The absorption spectra of olive oil and its mixed acids are shown in Fig. 6, while

¹ It should be noted that a band of high intensity in the extreme ultra-violet occurs with great regularity in a wide range of unsaturated organic compounds.

Fig. 6.

- (1) — Olive oil acids [1 cm.] 1 % alcohol.
 (2) - - - Olive oil [2 cm.] 1½ % alcohol.

Fig. 7.

- (1) — Cottonseed acids 0.2 % [3 mm.].
 (2) - - - Cottonseed oil 1.0 % [2 mm.].

the curves for cottonseed oil and its acids are represented in Fig. 7. The curves differ markedly from those shown by the vitamin A-containing liver-oils and the corresponding acids of the latter; the vegetable oils exhibit a triplet group of bands with heads at 260, 271 and 281 $\mu\mu$ and indications of other absorption in the region 300–350 $\mu\mu$, but the typical vitamin A band is absent. The curves for the corresponding mixed acids somewhat surprisingly show less fine structure between 260–280 $\mu\mu$ than those for the respective oils, and thus stand in striking contrast with those of the fish-liver oil acids with which this discussion was opened.

Although the present communication is mainly preliminary in nature and confines itself for the most part to a statement of experimental observations, it is nevertheless desirable to consider the evidence available as to the nature of the compound or compounds which give rise to the characteristic elaborate spectrum with 8–10 sharply defined absorption bands. It seemed possible at one time that the source of the banded absorption might lie in some of the highly unsaturated fatty acids common to marine animal oils but the experiments now to be described lend little support to this view.

The total mixed fatty acids from cod-liver oil (Fig. 1) were separated into

Fig. 8.

- (1) ——— Highly unsaturated acids from cod-liver oil 0.06 % [2 mm.].
(2) - - - - Methyl esters of above acids 0.05 % [2 mm.].

two groups by crystallisation of their lithium salts from acetone. The acids from the salts insoluble in acetone, which consist almost entirely of the

saturated and monoethylenic derivatives, have similar absorption (Fig. 1, curve 3) to that of the total mixed acids (Fig. 1, curves 1 and 2) but the intensity has been decreased by about 80 %. On the other hand with the acids from the soluble lithium salts which comprise the very highly unsaturated acids together with any unsaponifiable matter soluble in acetone, although the type of selective absorption is the same (Fig. 8, curve 1) the intensity is greater than with the total mixed acids. Fig. 8 (curve 2) shows the absorption curve of the corresponding methyl esters from the soluble lithium salts; this is in all respects similar to the curve of the free acids. These esters were submitted to fractionation under high vacuum as follows:

Fraction	Wt (g.)	B.P. (1 mm.)	Sap. equiv.	Iodine value
1	15.5	80-160°	283.8	138.2
2	41.6	160-177°	294.8	208.1
3	31.9	177-185°	307.0	266.4
4	46.2	185-190°	319.9	271.3
5	59.3	190-195°	333.7	285.4
6	25.4	195-200°	345.7	271.0
*Residue	84.0			

* Mainly polymerised highly unsaturated esters.

and the absorption spectrum of each of these primary fractions was observed. The following table gives the approximate relative intensity of the various bands calculated to 1 % solution in alcohol and 1 cm. thickness.

Wave length of maxima ...	235 $\mu\mu$	270 $\mu\mu$	281 $\mu\mu$	316 $\mu\mu$
Fraction 1	100	42	25	8
" 2	150	25	16	2
" 3	200	35	28	?
" 4	212	75	53	2
" 5	250	35	28	3
" 6	325	36	25	4

It will be observed that whereas the intensity of the band at 235 $\mu\mu$ (*cf.* footnote, p. 33) rises steadily throughout the series to a maximum, the bands at 270 and 281 $\mu\mu$ reach maximum intensity in fraction 4, but this intensity is already relatively much lessened as compared with that of the undistilled esters. Fraction 4 was re-distilled and the two main fractions having B.P. respectively 160-170° and 170-172°, and corresponding iodine values 279.8 and 301.5, were again examined. The absorption curves of these fractions are reproduced in Fig. 9 (curves 2 and 3). Comparison with the absorption of the esters prior to fractionation (curve 1) shows that a pronounced decrease in both absorption and selectivity has been brought about, presumably by decomposition. In view of these results it is improbable that the recorded fine structure can be attributed to esters of highly unsaturated acids ("clupanodonic" type). Moreover, a similar even more unsaturated re-fractionated ester from the acids of Thresher shark-liver oil (sap. equiv. 327.3, iodine value 320.3) fails to show (Fig. 5, curves 3 and 4) the characteristic fine structure under discussion.

It might be argued that these results leave open the bare possibility that the observed selective absorption may be due to the presence of those highly

unsaturated esters of cod-liver oil which polymerise entirely [*cf.* Guha, Hilditch and Lovern, 1930], and which are left in the residue (84 g.) from the primary fractionation. If these were indeed the cause it is difficult to understand why the original oil is wholly devoid of the banded spectrum for if the acids were actually present in the oils themselves in the form of glyceryl esters there is no reason to suppose that, with glyceryl as with methyl esters, the same absorption should not be shown. We have in fact observed that the neutral triglycerides prepared from glycerol and the total mixed fatty acids of cod-liver oil by heating the alcohol with an excess of the free fatty acids under reduced pressure exhibit qualitatively the same absorption characteristics as the original fatty acids (Fig. 1, curves 4 and 5).

Fig. 9.

- (1) Methyl esters of highly unsaturated cod-liver oil acids 0.05 % [2 mm.].
 (2) After refractionation; iodine value = 279.8] 0.05 % [2 mm.].
 (3) " " " = 301.5]

DISCUSSION OF RESULTS AND SUMMARY.

In considering the data, a true perspective must be maintained. From the physiological angle vitamin A, vitamin D and ergosterol loom large, but from the point of view of quantitative analysis they are merely contaminants of minor constituents. This background cannot be dispensed with when the investigation of liver oils broadens out to embrace the indirect as well as the direct attack on the nature of the substances to which we attach special biological significance. Whilst the results now recorded form a logical sequence of the study of vitamin A, and it is natural to hope that a not very remote

connection may emerge, there seems to be no justification for allowing the discussion to be dominated by vitamin A.

It appears to us at the moment that the simplest explanation in consonance with all the facts so far observed is that under hydrolytic conditions a substance (or substances) accompanying vitamin A gives rise to acid decomposition products which display intense selective absorption. We suggest that even under the ordinary mild conditions of saponification, sufficient of this acidic decomposition product is formed to give rise to the observed spectra. That the acid decomposition products are in some way connected with the presence of vitamin A is indicated by the distinctly different absorption curves obtained with the acids from the non-vitamin A-containing olive or cottonseed oils.

It must again be emphasised that the absorbing acidic substances could not have been present originally in the form of simple esters for, were this the case, the liver oils should exhibit similar selective absorption. Indeed, once the acidic substances have been developed their characteristic spectra persist after their esterification with either methyl alcohol or glycerol. We are thus forced to the conclusion that their formation is the result of some form of molecular breakdown.

A most interesting corollary lies in the observations connected with the whale (blubber) oil examined and also with butter. With whale oil we are dealing with a vitamin-free fat which nevertheless originates in the liver, from which organ it is highly improbable that the vitamin is absent. If it be assumed that products of both hydrolytic and synthetic actions taking place in the liver are deposited in the tissue fat, an explanation is immediately forthcoming of the selective absorption of the oil as well as its derived acids. The same reasoning appertains in the case of butter, although here vitamin A itself is also present to a small extent in the milk fat.

The correctness of the foregoing speculations remains to be tested by the extended experimental work which we are undertaking on this subject. The main object of the present communication has been to record the evidence which we have collected showing that the fatty acids (or esters prepared therefrom) produced in the ordinary hydrolysis of vitamin A-containing liver oils, or of some other fatty oils from animals whose liver oils contain vitamin A, yield highly characteristic banded absorption spectra which are absent from the corresponding acids of vitamin A-free oils.

We desire to thank Dr G. Collin, Mr K. D. Guha and Dr A. Thompson for assistance in the preparation of a number of the compounds studied in the course of this work and the Department of Scientific and Industrial Research for assistance in various directions.

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