CXCVI. ABSORPTION SPECTRA IN RELATION TO THE CONSTITUENTS OF FISH OILS.

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IN comparison with other organic materials the vegetable fats and oils exhibit, as a rule, a high degree of transparency to ultra-violet rays. Thus in many instances, *e.g.* olive oil and groundnut oil, a thickness of several millimetres of oil is necessary for appreciable absorption to be shown, and, even so, little of a selective nature is exhibited. From this it follows that the glycerides of the common fatty acids, saturated and unsaturated, are relatively transparent. The same is true of mammalian- and fish-body oils which do not contain vitamin A.

In the case of fish-liver oils the greater part of the selective absorption is usually due to vitamin A, but in a number of cases, *e.g.* codling-liver oils [Lovern *et al.*, 1931] the gross absorption is so low, sometimes only recorded by the use of undiluted oil, that it becomes reasonable to conclude that the absorption shown by the glycerides of the polyethylenic acids occurring in nature is inconsiderable and exhibits but little selectivity.

In these circumstances it is somewhat surprising to find that some constituents of the total acids obtained by saponification of many fats, particularly fish-liver oils, exhibit relatively intense absorption of a highly selective nature, a long series of sharply defined narrow bands, about $10m\mu$ wide, being regularly observed, together with a few inflexions and fainter bands which are not always seen. After allowing for the vitamin A content of the richer oils it is found that the "acid" curve invariably lies well above the "oil" curve, showing the absorption to be not only more selective, but also considerably more intense, than that of the glycerides and other esters in the oils.

Table I summarises the spectral absorption of the total acids from a number of fish-liver and fish-body oils. The last three in the table are interesting in that, although they are not liver oils, they contain appreciable quantities of vitamin A.

Before attempting to interpret the above data it is essential to recall the main facts concerning the spectral absorption of those constituents of natural fats which do not belong to the fatty acids, namely glycerol, cholesterol, ergosterol, vitamin A, vitamin D, certain higher alcohols, *e.g.* oleyl, batyl, selachyl, chimyl and in certain cases (shark, dog-fish) the hydrocarbon squalene, which, however, shows no selective absorption and may be dismissed here. Many oils also contain small quantities of coloured substances not unlike carotenoids [*cf.* Lovern and Morton, 1931]. Glycerol, although nearly always the predominant non-acid constituent, amounting to 4–5 % of the original oil, does not absorb

Table I. *Absorption maxima of total acids from saponification of fish oils.*

Maxima and inflexions (italics) are given in $m\mu$, with the $E_{1\text{ cm.}}^{1\%}$ values immediately beneath. Thus $\frac{378}{0.12}$ implies an absorption band with a maximum at $378m\mu$, and an $E_{1\text{ cm.}}^{1\%}$ value (i.e. $\log I_0/I$ for a 1 cm. layer of 1 % solution) of 0.12.

Monk (angler-fish)-liver oil	—		(420 0.2	400 0.3	377 0.4)		347 1.5	328 2.0	315.5 9.0	301 10.0	—	281 68	269 82	260 74	234 $m\mu$ 116 $E_{1\text{ cm.}}^{1\%}$
Haddock-liver oil	—	445 0.032	423 0.038	400 0.052	377 0.075	362 0.13	350 0.225	330 0.285	316.5 0.63	301 0.72	—	282 1.32	269 1.55	—	Tail-off 22
Turbot-liver oil	460 0.07	442 0.1	422 0.14	402 0.21	378 0.31	360 0.45	350 0.7	333 0.9	317 1.18	301 1.45	288 2.3	282 2.72	270 2.76	—	Tail-off 26
Pollack-liver oil	460 0.017	—	422 0.027	401 0.055	377.5 0.1	360 0.11	349 0.22	331 0.27	316.5 0.55	302 0.67	290 1.1	282 2.4	270 2.7	—	Tail-off 28
Ling-liver oil	—	—	422 0.1	402 0.14	377 0.2	360 0.25	349 0.42	332 0.55	316.5 0.76	301 0.97	294 1.4	283 2.2	270 2.3	—	Tail-off 24–25
Dab-liver oil	—	—	423 0.02	402 0.07	378 0.12	360 0.16	348 0.28	333 0.72	316.5 0.97	302 1.2	290–5 1.7	282 2.36	270 2.4	—	Tail-off 18
Cod-liver oil	—	—	—	392 1	375 1.5	360 2	350 14	330 15	316 80	302 85	—	281 210	270 250	259 205	234 200
Sturgeon peritoneal fat	—	—	—	400 0.025	377 0.05	361 0.065	348 0.15	331 0.2	316 0.31	301.5 0.35	—	282 0.93	269 1.2	259 1.25	Tail-off 22–23
Sturgeon pancreatic fat	—	—	—	400 0.042	377 0.08	359 0.12	348 0.21	332 0.3	317 0.55	300 0.65	—	282 1.1	270 1.14	259 1.1	Tail-off 18–20
Conger eel peritoneal fat	—	—	423 0.12	401 0.19	377 0.5	361 0.65	349 1.0	332 1.1	316.5 1.4	302 1.42	—	283 2.9	270.5 3.1	—	Tail-off 20

selectively over the region concerned. The remainder, known collectively as the “unsaponifiable matter,” usually accounts for about 1–1.5 %¹ of the original oil, and (in an average cod-liver oil) has approximately the composition:

Cholesterol and higher alcohols	...	About	96 %
Vitamin A	...	..	3.4 %
Ergosterol	...	(Order)	0.1 %
Vitamin D	...	..	0.02–0.03 %

Cholesterol is practically diatinctic; a 10 cm. layer of 4 % solution shows no selective absorption and only very little general absorption in the extreme ultra-violet. Similar considerations apply to the higher alcohols, as would be expected from their saturation; in fact neither cholesterol nor the higher alcohols can be detected spectroscopically in natural oils. The remaining three substances all show strong selective absorption.

Vitamin A, which is responsible for most of the absorption of liver oils in the near ultra-violet, has a characteristic broad band with a maximum at $328m\mu$. A portion of the observed intensity at $328m\mu$ is not due to vitamin A, as is shown by discrepancies between the vitamin A potency calculated from this value and from feeding tests and antimony trichloride blue values. However, since these discrepancies largely disappear when the unsaponifiable matter instead of the oil itself is used in the estimation [Coward *et al.*, 1931] it would seem that the interfering absorption is due to certain of the acid constituents, which are considered later. On the basis of the values: $E_{1\text{ cm.}}^{1\%}$ 617 $m\mu$ 5000, 580 $m\mu$ 2600, and 328 $m\mu$ 1600, Carr-Price, 78,000, for apparently pure vitamin A [Carr and Jewell, 1933] an average good cod-liver oil contains about 0.04 % of vitamin A. The figure varies considerably from fish to fish and even more so from species to species, the range covered within our experience by mature fish

¹ This applies to the liver oil from most fishes, including the average cod, but certain fish-liver oils are so rich in one constituent (*e.g.* vitamin A in halibut; squalene in elasmobranch fishes) as to increase this figure considerably, up to 10 % or more in special cases.

being from 0.004 % in haddock-liver oil to about 10 % in a sample of halibut-liver oil representing the mixed oil from several livers. No typical value can therefore be given. We have considered these variations in a separate paper [Lovern *et al.*, 1933].

Ergosterol exhibits intense, highly selective absorption in the middle ultra-violet, with maxima at 293.5, 281.5, 270.6, 260, 250.3, 242 $m\mu$, $E_{1\text{cm.}}^{1\%}$ max. being of the order 290 (in alcohol). Thus with 0.001 % of ergosterol (a high figure for a fish oil) the absorption intensity ($\log I_0/I$) in this region for a 1 cm. layer of a 1 % solution of the oil would be about 0.003, an obviously insignificant contribution to the u.v. absorption at 280 $m\mu$ usually observed, which ranges from 0.4 upwards in the typical fish-liver oils examined. This explains the observation of Morton *et al.* [1931] that the intensity of absorption in this region cannot be correlated with the sterol content.

Vitamin D (calciferol) in the pure state also absorbs strongly in the middle ultra-violet, having a single broad band, free from fine structure, with a maximum at 265 $m\mu$ ($E_{1\text{cm.}}^{1\%}$, 265 $m\mu$, 485). Askew *et al.* [1932] found a mean anti-rachitic value of 40,000 International Units per mg. of purified calciferol; typical cod-liver oils range from 25 to 200 units per g. Hence the vitamin D content of a cod-liver oil is, on this basis, of the order 0.00006–0.0005 %, giving for a solution of the oil an $E_{1\text{cm.}}^{1\%}$ value of 0.0003–0.0025, which obviously cannot influence the measured absorption curve of the oil. Even in puffer fish-liver oil, which it is stated [Bills, 1927] may be 15 times as rich in vitamin D as cod-liver oil, the effect would be inconsiderable.

Vitamin A, then, is the only non-acid material contributing appreciably to the ultra-violet absorption of most fish-liver oils. This, of course, is to be expected from the facts that it is both intrinsically more strongly absorbing than either ergosterol or vitamin D, and is also usually present in vastly greater proportions.

Upwards of a dozen different fatty acids combined as triglycerides are present in most fish-liver oils, a feature of which is their high content of unsaturated acids with 20–22 carbon atoms and four or more double bonds in the molecule. Though the proportions vary somewhat, saturated acids account on the average for 15–20 % of the total weight, mildly unsaturated (1–2 double bonds) about 70 %, and the highly unsaturated, including the so-called clupanodonic acids, 12–15 %. The precise composition of the more unsaturated fractions remains indeterminate by ordinary methods of analysis, in the sense that only the average degree of unsaturation is ascertained (expressed in terms of number of H atoms lacking as compared with the corresponding saturated acid) and not the individual unsaturation of the various members. Thus, to take a simple case, ($-2n\text{H}$) might imply one acid with n double bonds, or an equimolecular mixture of acids with $n-1$ and $n+1$ double bonds. In addition, the extent of conjugation of these double bonds is often a matter of uncertainty, particularly in the more unsaturated members. The latter, incidentally, have also been detected in several different kinds of algae, though in much lower proportions than in fish oils [Tsujiimoto, 1925], and in the fat of birds which feed on fish, but not in birds which feed exclusively on insects or grain [Grün and Halden, 1929]. They are thus apparently connected in some way with aquatic life.

Turning now to spectral characteristics, oils which are known to consist mainly of saturated glycerides show either no selective absorption or at most only feeble indications of a group of three bands near 270 $m\mu$. Oleic acid, with one double bond, shows traces of the 270 $m\mu$ group which become fainter as the acid is purified. Linseed oil, which largely consists of the glycerides of

linoleic acid (order 50 %, F_2) and linolenic and *isolinolenic* acids (order 30–35 %, F_3) has an $E_{1\text{ cm.}}^{1\%}$ 270 $m\mu$ value of 29, which is a mere fraction of that shown in this region by some fish-liver oil acids (*cf.* cod, monk) containing, as is usual, a rather smaller proportion of di- and tri-ethylenic acids than do the acids from linseed oil. It is therefore impossible to associate straight chain di- and tri-ethylenic acids with the highly selective absorption regularly observed at 270 $m\mu$, and we are forced to the conclusion that the acid responsible for this group, if indeed it is a straight chain acid, must have more than three double bonds. This is not inconsistent with other considerations to be mentioned later, which further indicate that the remaining narrow bands exhibited nearer the visible region of the fish-liver oil acids must be associated with a still higher degree of unsaturation.

The C_{20} – C_{22} clupanodonic and higher unsaturated acids remain to be considered as possible causes of selective absorption. Here the mean unsaturation as determined by hydrogenation and iodine values is of the order F_{4-5} , but little appears to be definitely known concerning the extent of conjugation of these double bonds, without which little absorption of a selective nature is to be expected, in the absence of ketonic or other absorbing groups. According to Tsujimoto [1928] there is no conjugation of double bonds in a C_{20} acid which he terms clupanodonic, and which is known to possess a straight chain of carbon atoms from its conversion into behenic acid by hydrogenation; but the term “clupanodonic” is used somewhat loosely in the literature, sometimes being confined to the C_{20} and C_{22} acids with 4 or 5 double bonds, and sometimes including everything from C_{20-22} upwards¹. The highly absorbing acids are evidently to be found somewhere among, or else are derived from, the polyethylenic higher acids. There are three possibilities: (i) that they are the “clupanodonic” *etc.* acids themselves; (ii) that they belong to a series of as yet unidentified compounds which, although perhaps very highly unsaturated, are present in such small quantities that they contribute little to the general unsaturation; (iii) that they are derived from the “clupanodonic” acids by isomerisation, cyclisation or other chemical change. In case (i), even if in some of the acids all the double bonds were conjugated, one would expect a series of relatively broad absorption bands, one from each acid, superposed to form an ill-defined summation curve, rather than the sharply defined narrow bands actually observed. Further, since the typical fish-liver oil of low free fatty acid content but rich in clupanodonic glycerides—using the term “clupanodonic” in its widest sense—is devoid of narrow bands, and since synthetic glycerides prepared from the highly absorbing total acids, including clupanodonic, *etc.*, exhibit the same fine structure as these acids, though at a slightly lower intensity [Gillam *et al.*, 1931] the interesting position arises that either the clupanodonic acids are not responsible for any of the observed fine structure, or the configuration of the acidic portion of the natural clupanodonic glycerides or esters differs from that of the clupanodonic acids themselves as prepared by thorough saponification of these glycerides [Morton *et al.*, 1931]. The same thing applies, of course, to any even more highly unsaturated acids which might be present.

Against case (ii) is the extraordinarily high extinction coefficients which must be postulated for the highly absorbing acids if they are present only in very small quantities.

Case (iii) provides the best working hypothesis, although it is not yet known with certainty whether the change in configuration (which appears to be irreversible) occurs during the saponification process or during the subsequent

¹ Inone and Sahashi [1932] give a $C_{22}F_5$ acid as containing an acetylenic linkage.

acidification. The evidence available points strongly to the former alternative and is supported by the recent work of Dann and Moore [1933], the results of these authors suggesting the existence of some connection between the length of time during which the alcoholic KOH solution is boiled (usually much longer than is strictly necessary for saponification) and the quantity of highly absorbing acids produced.

The possibility of an isomeric change in the acidic portion of certain glycerides giving rise to the narrow bands may account for a phenomenon which has been observed on more than one occasion. Sturgeon peritoneal fat containing an appreciable amount of vitamin A ($E_{1\text{ cm.}}^{1\%}$ 602 $m\mu$, 0.34; 568 $m\mu$, 0.3) when first examined showed only two bands in the ultra-violet, one at 328 $m\mu$ $E_{1\text{ cm.}}^{1\%}$ 0.28, and one at 281 $m\mu$, $E_{1\text{ cm.}}^{1\%}$ 0.26, with no detectable fine structure. On re-examination some 4 months later bands were found at 460, 399 and 377 (inflexions), 325, 292, 282, 271 $m\mu$, the 325–330 $m\mu$ intensity remaining unchanged within experimental error ($E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$, 0.27) while the rest of the bands ranged from $E_{1\text{ cm.}}^{1\%}$ 0.0065 (460 $m\mu$) to 0.54 (271 $m\mu$). Similarly, a fresh solution of ling-liver oil (5 %) in heptane exhibited a broad band at 321 $m\mu$ ($E_{1\text{ cm.}}^{1\%}$ 0.5) and very faint traces of selective absorption at 398, 282 and 270 $m\mu$. Two days later, unmistakable signs of selective absorption at 400, 377, 360, 348, 315, 282 and 270 $m\mu$ were observable, together with a general increase in in-

Fig. 1. Ling (*Molva vulgaris*). Solvent: heptane. SbCl_3 blue value of oil: $E_{1\text{ cm.}}^{1\%}$ 603 $m\mu$, 0.54; 578 $m\mu$, 0.44.

— — — Liver oil, fresh solution.
 Liver oil, same solution two days later.
 - - - - - Acids from saponification of liver oil.

tensity of absorption throughout (see Fig. 1). The new curve could in fact be accounted for by superposition of a low intensity ling-liver oil acid curve upon the original ling-liver oil curve.

Two interpretations are possible: either spontaneous hydrolysis takes place, or the fine structure-producing change can occur, without hydrolysis, in the

acidic portion of certain glycerides. It is probable that most of the fine structure observed in stale fish oils is due to one or both of these causes. Which cause predominates can be decided by washing with sodium carbonate and re-examining spectroscopically. That the change can occur within the glyceride molecule as a result of some process other than hydrolysis is proved by the fact that the narrow bands exhibited by a skate-liver oil were not eliminated as a result of washing with sodium carbonate. Further, the experience with ling-liver oil suggests that this non-hydrolytic change is favoured by dilution with a solvent, since the oil itself, which had only just started to show selective absorption apart from the vitamin A band, was by no means fresh.

It has not been possible to trace any parallelism between the vitamin A potency of an oil (as measured by the SbCl_3 colour test) and the intensity of absorption exhibited by the total acid fraction. Moreover, with increasing vitamin content the absence of such correlation becomes increasingly evident. It is therefore inferred that the fine structure acids are not decomposition products of vitamin A, a conclusion already arrived at by Morton *et al.* [1931] and supported by the appearance of narrow bands in the above-mentioned sturgeon peritoneal fat without sensible loss of intensity at $328m\mu$. There is likewise no evidence to connect the acids in any way with vitamin D, and no direct connection has been traced between the acids and the sterol content [Morton *et al.*, 1931]. That the acids are directly associated with the synthesis of either vitamin A or D seems equally improbable for the same reasons, and also because a really fresh fish oil shows very little selective absorption apart from the $328m\mu$ band, but the possibility of their being indirectly connected in some way with vitamin synthesis (*e.g.* as degradation or by-products of intermediate stages in the conversion of carotene) cannot at this stage be definitely excluded. Two significant facts stand out: first that liver oils are an incomparably better source of the highly absorbing acids than vegetable oils (*e.g.* olive, cottonseed); second that, except when the diet contains minimum quantities of carotene, the conversion of carotene into vitamin A is a very wasteful process. It has not yet been explained what happens to the "wasted" carotene.

It will be recalled that the typical total acid absorption curve exhibits the following maxima: 230–235 $m\mu$ (always at a high intensity, sometimes a genuine maximum, more often a tail-off), (259), 270, 282, 288–295 (inflexion), 301.5, 316.5, 332, 348, 360 (inflexion), 377, 401 (422, 442, 460 inflexions or very weak bands), the figures in brackets being not always strictly reproducible. The question arises whether the whole of the fine structure is due to one acid or whether it is the result of superposition of a number of absorption curves, each arising from a different acid.

Evidence adduced from various sources all points to the conclusion that there are several absorbing entities. This evidence is summarised in Table II, in which the bands are segregated into discrete groups as far as the data warrant. Two different lines of attack have been followed, one based on an examination of the intensities of the various bands, to see whether there is any tendency for certain groups of bands to vary together in intensity, and the other based on frequency differences. The intensity method was applied to (i) the methyl esters of the more unsaturated cod-liver oil acids in which a partial separation had been effected by high vacuum distillation; details of this have already been published [Gillam *et al.*, 1931]; (ii) monk (angler-fish)-liver oil acids, which were partially separated (a) by adsorption on fibrous alumina, (b) by the Twitchell lead salt process, and (c) by distillation of the esters; (iii) the total acids in Table I. Incidentally, the separation of the monk acids by fibrous alumina

Table II. *Grouping of the maxima exhibited by the highly selectively absorbing acids.*

Former work*		Present work			
Fractionation of cod-liver oil acids (from intensity of maxima shown by different fractions)	Fractionation of monk- liver oil acids	Acids from several fish-liver and body oils. Theoretical considerations of			
		Intensity of maxima		Frequency differences	
		230-5 $m\mu$ (259) 270 282		230-5 $m\mu$ 259 270 282	
234 $m\mu$ 259 270 281	234 $m\mu$ 260 270 281			295	288
(301)	301	290 (inflexion)		301.5	317.5
(316)	316.5	301.5		316.5	332
	(331)	316.5		333	348
	(331)	332			365.5
	(349)	348			357
	—	360 (infl.)			377
	375	377			401
	394	401			427

$\Delta \text{ cm.}^{-1} = 1580, 1570, 1380, 1590$

* Gillam *et al.* [1931].

gave the highest all round values yet recorded for the intensities of the narrow bands $E_{1 \text{ cm.}}^{1\%}$, 269 $m\mu$, 380; 234 $m\mu$, 440.

When a broad absorption band can be resolved into a number of narrow bands, the separation (in wave-numbers) of the components is often nearly constant, the $\Delta \text{ cm.}^{-1}$ value corresponding with a vibrational frequency. The provisional grouping of the maxima shown by our acids can be tested by applying this criterion. Thus if the 270 and 282 $m\mu$ bands are physically related ($\Delta \text{ cm.}^{-1}$, 1580), bands would also be expected at 259 and 295 $m\mu$; and similarly for the other groups in Table II. The figures in heavy type represent the dominant regularly observed bands, while the others are the calculated wavelengths of the fainter bands which one would expect to be associated with the main bands. This particular segregation into groups agrees very well with the main facts and provides a plausible explanation of a number of otherwise apparently anomalous features.

(i) The 259 $m\mu$ band is not always observed. This is to be expected if it is both intrinsically faint and occurs on a steep part of the curve due to the 230-5 $m\mu$ substance, by which it is likely to be masked or at most reduced to a faint inflexion. It should perhaps be explained that a weak band superposed on a steep absorption curve due to another substance appears as an inflexion on that curve.

(ii) A long inflexion, varying in position, is often found about 290 $m\mu$. This can be attributed to the joint effects of the 288 and 295 $m\mu$ faint bands, the middle of the inflexion being nearer to 288 or 295 $m\mu$ according to which substance predominates.

(iii) The overlapping of the two middle groups similarly explains variations of 0.5-1.0 $m\mu$ in the position of the 316.5 and 332 $m\mu$ bands according to the source of the oil from which the total acids were derived.

(iv) The 360 $m\mu$ inflexion is apparently due to the superposition of the two faint 365.5 and 357 $m\mu$ bands, and, forming a link between two sets of bands, accounts for the tendency to group them together as one when intensity data only are considered.

(v) By calculation, a band should appear at $427m\mu$. In practice, one is observed, either as an inflexion or as a weak band, near $423m\mu$. This may be due to interference by a trace of something analogous to the monk pigment [Lovern and Morton, 1931] one of the absorption bands of which lies at $422m\mu$. It should be remembered that the intensity of this part of the "total acid" absorption curve is very low and the curve correspondingly more susceptible to such influences.

There are thus probably at least five highly absorbing entities contributing to the complex absorption curve of the total acids. At this stage we can only speculate as to their inter-relationship and structure, but there are certain general conclusions which can be regarded as not improbable. In common with the other acid constituents, they all appear to be monobasic. This is suggested by the behaviour of the total unsaturated acids on esterification with methyl alcohol and sulphuric acid. In general, monobasic acids esterify readily and almost completely (95–98 %), while dibasic acids show a tendency to stop short at one carboxyl group (*i.e.* 50 %). The acids from fish-liver oils esterify with the same ease and to about the same extent as normal monobasic acids, showing that in the main, at least, the acids are monobasic. Further, the small "un-esterified" portion, in which any partially esterified dibasic acids would collect if present, shows no increase in unsaturation or absorption, thus indicating that the fine structure acids themselves also are monobasic.

Concerning the absolute quantities of the highly absorbing acids present, little can be said at the moment. The percentage of so-called highly unsaturated acids, C_{20-22} , with four or more ethenoid linkages, shows no correspondence with the observed range of $E_{1\text{ cm.}}^{1\%}$ values. Thus the $270m\mu$ group of bands has usually an $E_{1\text{ cm.}}^{1\%}$ value of 2 or 3 in the acids from the liver oils of most of the species studied, and yet in certain cod-liver oil acids, with about a normal percentage of C_{20-22} , f_{4-5} upwards, the E value was 250. Since the cod-liver oil acids evidently contained 100 times the normal amount of these fine structure acids, and this was not reflected in a measurable increase in the general unsaturation of the polyethylenic fraction, the inference is either that the fine structure acids are present only in minute quantities, or that the change from relatively diacetic unsaturated acids to highly absorbing unsaturated acids leaves the iodine value practically unchanged.

The monk-liver oil acids, which were examined in considerable detail, support these general conclusions. Their approximate composition is as follows:

<i>Saturated acids:</i>			%
Myristic...	...	...	4.9
Palmitic...	...	...	9.6
Stearic ...	...	...	1.3
<i>Unsaturated acids:</i>			
Myristoleic	...	...	0.4 (– 2.0 H)
Palmitoleic	...	...	12.1 (– 2.0 H)
C_{18} group	...	...	30.9 (– 3.3 H)
C_{20} group	...	...	24.9 (– 5.9 H)
C_{22} group	...	...	15.9 (– 8.6 H)

They thus fall in line with the fatty acids from the majority of marine fish-liver oils as regards general composition, but are unusually interesting in that they are associated with a red pigment in the liver oil [Lovern and Morton, 1931] and are from 10 to 30 times as highly absorbing in the ultra-violet as the usual

total acids from most fish-liver oils. Like the cod-liver acids, their high absorption is not reflected in the measured unsaturation, and they give no colour reaction with antimony trichloride. Distillation of the methyl esters under 0.1 mm. pressure results in a very imperfect separation of the highly absorbing constituents, but there is a marked tendency for them to accumulate in the high-boiling fractions and in the residues, a fact which suggests a high molecular weight, probably of the order 350.

Table III summarises spectroscopic data on certain of the simpler unsaturated compounds with conjugated ethenoid linkages, the data on the acids being due to Kuhn (private communication) and on the hydrocarbons to

Table III.

<i>Acids</i>		λ max. <i>mμ</i>	$\log \epsilon$ max.
Crotonic	$\text{CH}_3\text{CH}=\text{CHCOOH}$	204	4.085
Sorbic	$\text{CH}_3(\text{CH}=\text{CH})_2\text{COOH}$	254	4.398
Octatrienecarboxylic	$\text{CH}_3(\text{CH}=\text{CH})_3\text{COOH}$	294	4.556
Decatetraenecarboxylic	$\text{CH}_3(\text{CH}=\text{CH})_4\text{COOH}$	329	4.690
<i>Hydrocarbons</i>			
<i>xx'</i> -Diphenylbutadiene	$\text{C}_6\text{H}_5(\text{CH}=\text{CH})_2\text{C}_6\text{H}_5$	325	3.65
<i>xx'</i> -Diphenylhexatriene	$\text{C}_6\text{H}_5(\text{CH}=\text{CH})_3\text{C}_6\text{H}_5$	371	3.8
		355	3.83
		333	3.7
		396	3.87
<i>xx'</i> -Diphenyloctatetraene	$\text{C}_6\text{H}_5(\text{CH}=\text{CH})_4\text{C}_6\text{H}_5$	372	3.86
		357	3.80
		441	3.73
<i>xx'</i> -Diphenyldodecahexaene	$\text{C}_6\text{H}_5(\text{CH}=\text{CH})_6\text{C}_6\text{H}_5$	418	3.73
		380	3.7
		317	3.2

Rădulescu and Bărbulescu [1931]. Two general tendencies are observable: (i) an increase in the number of conjugated double bonds causes a shift of the ultra-violet absorption maxima, or group of maxima, towards the visible; (ii) this shift is usually accompanied by an increase in absorption intensity. The groups of bands exhibited by the series of hydrocarbons due to the combined influence of ring systems and conjugated double bonds show a qualitative overlapping similar to that already mentioned in connection with the "fine structure" acids, indicating the possibility of a similar series relationship between the acids, the quantity present decreasing as the extent of conjugation increases. This would account for the general shape of the absorption curve of the total acids, which falls as the visible is approached, instead of rising as would be expected if equal quantities were present.

Table IV records the absorption maxima of a number of unsaturated substances connected with the whole question of fat-soluble vitamins. It will be observed that the bands in the visible associated with the highly unsaturated acids are reminiscent of those shown by carotenoids and polyene acids, whilst the ultra-violet bands resemble those shown by polycyclic, partially hydrogenated substances, the resemblance being closest for "dihydro" carotene (prepared by the action of aluminium amalgam on carotene) and the cyclised product derived from vitamin A and hydrochloric acid [Edisbury *et al.*, 1932]. The highly unsaturated acids probably contain appreciable quantities of substances with tricyclic skeletons, a view which receives some support when the absorption spectra of anthracene and phenanthrene derivatives are considered.

Table IV.

Total acids	—	460	445	422	401	—	377	360	348	332 $m\mu$
Vitamin A + HCl	—	—	—	420	—	392	—	369	350	333
"Dihydrocarotene"	—	461	426-45	—	—	392	—	369	350	332
Carotene	492	461	436	—	—	—	—	—	348	—
Bezssonoff's extract*	476	—	448	426	408	386	—	—	349	330
α -Croctetin	—	—	448	420.5	399	—	—	—	—	—
Dihydrocroctetin	—	—	—	—	—	—	382	361.5	344	—
Monk (angler-fish) pigment	480	—	450	422	—	—	—	—	—	—
Ergosterol	—	—	—	—	—	—	—	—	—	—
Dehydro-ergosterol	—	—	—	—	—	—	—	—	342	325.5
Ergosterol D	—	—	—	—	—	—	—	—	—	—

(continued)

Total acids	316.5	301.5	290	282	270	259	—	—	230-5 $m\mu$	
Vitamin A + HCl	313-20	—	292	280	272	261	254	—	—	
"Dihydrocarotene"	316	301	—	—	—	—	—	—	—	
Carotene	—	—	—	280	—	—	—	—	—	
Bezssonoff's extract*	—	300	—	283	—	—	—	—	—	
α -Croctetin	310	—	—	—	—	—	249.5	—	—	
Dihydrocroctetin	320-6	—	—	—	274.5	—	—	—	—	
Monk (angler-fish) pigment	—	—	—	—	—	—	—	—	—	
Ergosterol	—	—	293	281.5	270	260	250.3	242	—	
Dehydro-ergosterol	311	—	296	—	—	—	—	—	—	
Ergosterol D	—	—	—	—	—	—	252	243	235	

* A residue, kindly furnished by Prof. Bezssonoff, from carotene extracts.

The position now reached may be stated briefly.

1. The well-defined narrow absorption bands owe their origin to unsaturation in the absorbing molecules.

2. The highly absorbing acids are not present in the original oils as the corresponding glycerides.

3. The hypothesis that the absorbing acids are straight chain compounds with conjugated double bonds is not in harmony with the evidence.

4. A change, unsaturated acids (low absorption) $\rightarrow$ unsaturated acids (high and selective absorption) occurs during saponification.

5. Cyclisation is the most plausible explanation of this change.

Further work is proceeding on these lines.

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