CVIII. THE ABSORPTION SPECTRA OF ACIDS FROM FISH-LIVER OILS¹.

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It has already been demonstrated [*cf.* Gillam *et al.*, 1931; Edisbury *et al.*, 1933] that although most naturally occurring glycerides, whether saturated or unsaturated, or of animal or vegetable origin, are relatively diactinic, the mixed acids derived from them by prolonged saponification often show intense and highly selective absorption in the ultra-violet. This was found to apply in a particularly striking manner to fish-liver oils and their acids. In a series of acids from several species, narrow absorption bands some 10 $m\mu$ wide were repeatedly recorded at or near 270, 282, 290 (inflection), 301.5, 316.5, 332, 348, 360 (inflection), 377 and 401 $m\mu$, and less frequently near 234, 259, 422, 445 and 460 $m\mu$. It was shown that (i) the bands owe their origin to unsaturation in the absorbing molecules; (ii) these highly absorbing acids, of which there appear to be at least five, are not present as the corresponding glycerides in the original freshly extracted oils; (iii) the hypothesis that the acids are open chain compounds is not in harmony with the spectrographic evidence; (iv) a change,

unsaturated acids (low absorption) $\rightarrow$ unsaturated acids (high and selective absorption),

occurs during the saponification treatment; (v) cyclisation to form polycyclic hydroaromatic compounds is the most plausible explanation of the change; (vi) this change can also occur, spontaneously and without hydrolysis, within the more unsaturated glyceride molecules and is considerably accelerated when the oil is dissolved in a hydrocarbon solvent; but it occurs at best extremely slowly and to a limited extent, involving only a minute proportion of the available material, even when the oil is allowed to stand indefinitely. The change is however quite definite, and is easily detectable if a light source giving a continuous spectrum is employed (*e.g.* hydrogen vacuum tube, or high frequency discharge under water).

We have since found, on the other hand, that the selective absorption of the mixed free acids as usually prepared undergoes little if any change with time; thus, a sample of ling-liver oil acids, which had been kept in nitrogen in a sealed tube for 1½ years, showed little difference between the absorption spectra at the beginning and end of that period, apart from a slight decrease in the persistence of the bands, due probably to the gradual development of general absorption. Richer preparations (*vide infra*) are apparently not so stable. It is entirely unknown what causes the initial development of narrow absorption bands in a neutral liver oil, or what inhibits this spontaneous cyclisation process

¹ The substance of this paper was read at the 166th meeting of the Biochemical Society (Liverpool, Dec. 15th, 1934).

and prevents the production of more than minimum quantities of cyclised material. For the present, therefore, our attention must be confined to the acid products.

Dann and Moore [1933] and Dann *et al.* [1935] showed that although, under suitable conditions, saponification of cod-liver oil was practically complete in a few minutes, the acids so produced exhibited only weakly selective absorption, whilst by prolonging the heating with alcoholic potash the intensity and selective nature of the absorption could be greatly enhanced. They confined their preliminary observations to the middle and further ultra-violet regions. We have confirmed their observations and extended them to the near ultra-violet and the edge of the visible region, and we have found, incidentally, that herring-body oil exhibits the effect quite as strikingly as cod-liver oil. This is interesting because in our earlier work we noted that although the development of highly selective absorption by the saponification products was predominantly a characteristic of oils containing vitamin A, no quantitative connection could be traced between this absorption and the vitamin content. The herring-body oil which we examined (kindly supplied by Prof. Channon) was highly coloured, but gave no blue colour with antimony trichloride, and therefore contained no β -vitamin A. Nevertheless, the results of saponification are in no way inferior to those we have already obtained with a number of fish-liver oils and pancreatic and peritoneal fats, all of which contained vitamin A. Little selective absorption was exhibited by the oil itself in the ultra-violet. The acids obtained as a result of saponification for one minute formed a yellow oil, showing clear bands (though of small persistence) at 270 and 283 $m\mu$ and several others, more poorly defined, nearer the visible. Vigorous saponification for some hours destroyed much of the yellow colour and resulted in greatly increased persistence and definition all round, with an increase in intensity in the middle ultra-violet and a decrease in the region 300–400 $m\mu$. Some substance (possibly an acid pigment exhibiting general or weakly selective absorption in this region and giving rise to the yellow colour of the oil and of the mildly saponified product) was largely destroyed during prolonged saponification. Similar remarks apply, *mutatis mutandis*, to the 270 $m\mu$ portions of the curves for the original oil and the one minute saponification product. In any event, the generally increased persistence of the bands again proves that, despite the reduction in overall intensity above 300 $m\mu$, prolonged saponification has resulted in an increased yield of the highly absorbing acids.

In view of the success attained in securing increased yields by prolonged heating with ethyl alcoholic potash, it was decided to try the effect of saponifying cod-liver oil at different temperatures. Methyl, amyl and octyl alcohols were used as solvents, giving under reflux at atmospheric pressure, reaction temperatures of approximately 70°, 135° and 180° respectively, after allowing for the presence of oil *etc.*, in solution. The spectrographic results are summarised in the first part of Table I.

At this juncture it may be well to explain the system of notation we have adopted for this type of investigation. The figures in Table I record both the absorption intensity and the persistence of the various bands, reduced to a common basis of comparison, namely $E_{1\text{ cm.}}^{1\%}$, which represents the extinction ($\log_{10} \frac{\text{incident light}}{\text{emergent light}}$) of a 1 cm. layer of a 1 % solution at the particular wavelength concerned. The upper figure in each pair gives the $E_{1\text{ cm.}}^{1\%}$ value at the head or maximum of an absorption band, whilst the lower figure shows approximately the persistence of that band, a quantity which is most conveniently

Table I.

Wave-length ($m\mu$)	234	245	260	270	282	290	302	317	332	348	360	377	400
Cod-liver oil saponified for: 1 hour in methyl alcohol	17 Tail-off at 220 $m\mu$. No band	—	2.1 ~	1.85 0.12	1.75 0.15	0.77 ~	0.57 ~	0.29 0.02	0.16 ~	0.08 ~	0.06 ~	0.04 0.005	0.02 ~
1 hour in amyl alcohol	180 70	—	180 20	185 55	150 25	60 ~	31 7	28 12	4.7 ~?	2.7 0.9	0.75 ~	0.5 0.13	0.12 0.025
6 hours in amyl alcohol	300 90	—	240 10	285 55	245 20	150 ~	75 15	65 15	23 Very shallow	17 8	3.3 ~	3 1	0.25 ~
1 hour in octyl alcohol	320 130	—	210 20	250 55	220 25	150 ~	95 25	87 24	47 6-7	29 11	7.3 ~	5.7 1.8-2	0.8 ~
6 hours in octyl alcohol	270 <i>ca. 150</i>	—	125 ~	145 20	137 12	95 ~	90 20	83 20	53 10	43 15-17	14 ~	8.7-9 3.5	0.8 ~
Almost pure $C_{20}F_8$ acids, saponified 6 hours in amyl alcoholic KOH	800 240	—	670 <i>ca. 100</i>	830 200	730 100	<i>ca. 350</i> ~	295 85	290 110	105 15	90 40	<i>ca. 25</i> ~	17.5 8	<i>ca. 2.5</i> ~
Fairly pure $C_{22}F_8$ acids, 6 hours amyl alcoholic KOH	920 280	—	800 <i>ca. 50</i>	960 230	840 110	<i>ca. 500</i> ~	500 <i>ca. 150</i>	460 <i>ca. 150</i>	220 50	200 100	45 ~	50 25	3.3 ~
Liquid acids, C_{20}	1030 300	850 ~	920 110	1040 240	910 110	<i>ca. 600</i> ~	240 60	200 50	55 <i>ca. 3-4</i>	40 17	8 ~	5.2 1.2	<i>ca. 1.5</i> ~
Semi-solid acids, C_{20}	720 200	600 ~	700 <i>ca. 50</i>	940 200	800 150	<i>ca. 500</i> ~	460 <i>ca. 130</i>	460 <i>ca. 150</i>	170 30	155 90	<i>ca. 25</i> ~	20 8	1.6 ~
Liquid acids, C_{22}	930 270	870 ~	770 60	870 150	800 100	500 ~	400 125	400 150	150 <i>ca. 30</i>	96 40	20 ~	18 7	<i>ca. 1.5</i> ~
Solid acids, C_{22}	500 150	420 ~	460 <i>ca. 10</i>	600 30	660 100	—	860 200	930 300	750 220	730 350	<i>ca. 220</i> ~	220 120	<i>ca. 15</i> ~
Acids from methyl alcohol saponifi- cation heated 1 hour at 135° in N_2	Tail-off 30	—	—	6.2 0.3	6 0.1	4.4 ~	3.3 ~	2.7 0.2	1.6 ~	—	0.6 ~	0.3 ~	0.15 ~
Dry neutral soaps heated 6 hours at 135° in N_2	Tail-off 300	—	300 10	300 20	280 15	—	—	—	General absorption	—	—	—	—
Original cod-liver oil	Tail-off 10.5	—	—	—	276 $m\mu$ 0.29 0.02	287 $m\mu$ 0.29 0.02	313 $m\mu$ 0.27 ~	332 $m\mu$ 0.32 0.09	365 $m\mu$ 0.16 ~	—	—	—	400 $m\mu$ 0.035 ~

Note. ~ denotes inflection. Figures represent $E_{1\text{cm}}^{1\%}$ values; intensity above, persistence (approx.) beneath, in italics, taken as difference between max. and nearest related minimum.

taken as the difference between the $E_{1\text{cm.}}^{1\%}$ values at the maximum and at the nearest related minimum (*i.e.* $E_{\text{max.}} - E_{\text{min.}}$). Inflections, by this definition, have no persistence. Owing to the unknown extent to which irrelevant absorption of a general (unselective) nature contributes to the observed total absorption curve, it is necessary to take into account persistence as well as gross intensity of absorption in order to form even a rough estimate of the relative quantities of selectively absorbing material present in different samples. In these experiments, many normally well-defined bands are so completely masked by other absorption as to be rendered imperceptible or reduced in some cases to an inflection with a much higher gross $E_{1\text{cm.}}^{1\%}$ value than that of the original band, the net intensity of which may remain indeterminate apart from the fact that an extreme upper limit is set by the observed $E_{1\text{cm.}}^{1\%}$ value of the inflection.

It will be seen from Table I that saponification at relatively elevated temperatures rapidly results in a surprisingly good yield of the highly absorbing acids and that temperature is an even more effective factor in their formation than time, so far at least as the acids responsible for selective absorption below $300\text{ m}\mu$ are concerned; a rise in reaction temperature from 70° to 135° accounts for a hundredfold increase in the yield of these acids. Further prolonged heating at the same temperature causes a greater percentage increase in the acids absorbing in the region above $300\text{ m}\mu$ than in those below $300\text{ m}\mu$, and an increase in temperature to 180° again favours those over $300\text{ m}\mu$. On still further prolonging the heating at 180° , a marked decrease is observed in the absorption below $300\text{ m}\mu$, with a roughly corresponding increase above $300\text{ m}\mu$. In the present state of knowledge it seems probable that the acids responsible for the latter are of higher molecular weight and either more complex as regards ring structure and/or more unsaturated than the acids with maxima below $300\text{ m}\mu$.

The precise mechanism whereby cyclisation is induced is by no means clear, but it is worth noting that when the acids from the methyl alcohol saponification were heated in nitrogen for an hour at 135° —the amyl alcohol saponification temperature—the principal change was an all-round but not very marked increase in general absorption, reflected in a perceptible deepening in colour, with an increase in selective absorption only in the case of two bands, 270 and $317\text{ m}\mu$.

An attempt was made on similar lines to cyclise the dry neutral soaps. The product showed practically the same intensity of absorption as the corresponding material from amyl alcohol saponification, but such bands as could be detected were of poor persistence, and some were entirely masked by general absorption. During the heating considerable charring occurred, which would account for much of the general absorption. The presence of a solvent evidently prevents this charring, whilst the alkali (possibly assisted in some way by the solvent) apparently plays an important if at present obscure part in the cyclisation process.

As would be expected, the iodine value steadily decreases during the cyclisation process, from 175.0 for the original oil to 137.6 , 129.6 and 115.1 for the products of saponification in amyl alcohol (6 hours) and octyl alcohol (1 and 6 hours) respectively. It is interesting that the iodine value remains as high as it does, suggesting that large quantities of aliphatic unsaturated material must still be present, both in the form of unchanged uncyclised acids and also, probably, as long side-chains attached to partially hydrogenated aliphatic nuclei resulting from the cyclisation process. From the absorption data, it seems unlikely that direct polymerisation is responsible for more than a fraction of the observed decrease in iodine value, although the possibility cannot be altogether excluded that subsequent combination of already cyclised molecules may

occur and give rise to polycyclic nuclei absorbing in the near ultra-violet and visible regions. This of course is pure conjecture; nevertheless, the hypothesis is attractive and is not inconsistent with the spectrographic evidence.

Most of the constituents of fish-liver oils are obviously not cyclisable to give acids of the type under discussion. Only the highly unsaturated C_{20} and C_{22} ("clupanodonic") acids, with four and five ethenoid linkages, appear to have a sufficiently long and sufficiently unsaturated carbon chain to provide a suitable starting material. Accordingly it was decided to prepare these C_{20} and C_{22} acids in as pure a state as possible and then to cyclise them. 800 g. of cod-liver oil were saponified in the normal manner, the unsaponifiable matter removed and the acids, still largely uncyclised, recovered. These were submitted once to a lithium salt/acetone separation, yielding 140 g. of highly unsaturated acids which were esterified and distilled *in vacuo*. The following fractions were collected:

Fraction	Weight g.	Boiling-point	Saponification equivalent	Iodine value
1	27.5	130–155°	285.5	212.8
2	42.2	155–165°	313.6	322.5
3	21.7	165–180°	328.1	333.0
4	35.7	180–186°	348.5	340.0
Residue	—		Not examined	

Fractions 2 and 4 were respectively fairly pure C_{20} and C_{22} esters, and were separately cyclised for 6 hours with amyl alcoholic potash; octyl alcohol might have increased the yield, but was not used since it is apt to give exceedingly intractable emulsions. On recovering the acids, the iodine values had fallen to 227.1 for the C_{20} and 251.0 for the C_{22} acids. This would represent a loss in each case of between 1 and 2 double bonds. Polymerisation is again not likely to have been extensive, and it is probable that mixtures of monocyclic, dicyclic and polycyclic products with one or more side-chains were formed. The cyclised C_{20} and C_{22} acids gave qualitatively similar absorption spectra (Table I). This may plausibly be taken to indicate that, so far at least as the major light-absorbing constituents are concerned, there is little essential difference in the nuclei derived from the two fractions—possibly the substitution of an ethyl or one or two methyl groups in the C_{22} product for hydrogen atoms in the C_{20} . The available evidence is not inconsistent with the view that hydronaphthalene derivatives with fairly long side-chains, and also, probably, polycyclic compounds with a low degree of unsaturation, predominate among the absorbing constituents and are distributed roughly equally between the two fractions. The absorption above $300 m\mu$ further suggests that the C_{22} material is considerably richer in the more unsaturated polycyclic bodies than the C_{20} acids. These latter polynuclear substances appear, however, to be present in relatively small proportions.

There is abundant evidence that several absorbing entities are responsible for the complex absorption curve of the acids. In view of the occurrence of cyclised products it seemed possible that a repetition of the lithium salt/acetone process might effect some separation of the unchanged acids from the others, or of those less highly cyclised (hydronaphthalenic) from those more so (tricyclic *etc.*). The following fractions were obtained:

	Weight in g.	Iodine value
C_{20} acids (liquid)	14.65	231.8
„ (semi-solid)	11.85	225.7
C_{22} acids (liquid)	21.16	259.9
„ (solid)	1.95	203.2

The absorption spectra of these fractions are also shown in Table I, and it will be seen that a very material increase in the concentration of (presumably) polycyclic acids was achieved in the solid C_{22} fraction. Otherwise the degree of separation attained, while by no means insignificant, was disappointing.

Further separation was attempted on both the C_{20} fractions, but the products showed signs of decomposition and polymerisation. For example, although the products, initially clear liquids soluble in light petroleum, were kept under nitrogen, many became gummy and insoluble in the course of a week. Details of this fractionation are given in Tables II *a* and II *b*, to illustrate the type of

Table II *a*. Attempted separation of C_{20} acids. Table II *b*. Absorption maxima of C_{20} fractions—cf. Table II *a*.

Frac- tion	234	260	270	282	290	302	317	332	348	360	377 $m\mu$	Intensity and persistence in $E_{1\text{cm.}}^{1\%}$
B	530 200-250	230 20	280 35	260 30	180 ~	150 10	140 35	65 1-2	43 13	20 ~	13 2-5	
B^1	Tail-off 300	Weakly selective $E=120$ at ca. 270 and 285 $m\mu$				$\left(\begin{smallmatrix} \sim 320 m\mu? \\ 35 \end{smallmatrix} \right)$					14 ~	
C	570 ca. 100	630 80	730 150	660 100	400 ~	240 80	220 90	65 7-8	50 25-28	6 ~	4 1-5	
D	570 200	500 ca. 30	630 130	570 60	240 ~	210 60	180 50	65 ca. 3	45 20	9 ~	4-5 ~	
E	Tail-off 460	Wide ~ 267 $m\mu$ 130		Wide ~ 310 $m\mu$ 60		$\left(\begin{smallmatrix} \sim 320 m\mu? \\ 33 \end{smallmatrix} \right)$						
F	800 ca. 400	700 ca. 20	800 150	720 ca. 70	240 ~	150 55	140 70	30 ca. 10	22 10	4-5 ~	3 1	
G	?	300 10	320 20	300 10	200 ~	95 5	70 6	37 Very low	20	—	?	

Note. Fraction F also exhibited an ill-defined inflection at 249 $m\mu$, intensity $E_{1\text{cm.}}^{1\%}=700$.

behaviour encountered. Fraction B, for example, would be expected to be richer in the highly absorbing acids than fraction C. This may possibly have been the case initially, but by the time (2 or 3 days) that it could be examined spectrographically it had deteriorated markedly and contained a number of flexible tubular bodies, not unlike small worms in appearance and insoluble in alcohol (B¹). These, like the other fractions, refused to crystallise, remaining flexible even at -20° . Little apparently is to be gained by repeating the lithium process on the C₂₀ acids, and there was insufficient of the C₂₂ solid acids available to render an attempt on these profitable. The C₂₂ liquid acids polymerised like the C₂₀ material.

Owing partly to this instability and partly to a desire to prepare solid derivatives, it was decided to brominate samples of the cyclised acids. In sharp contrast to the uncyclised acids, no precipitate was obtained on brominating the cyclised product in ethereal solution. The bromination was therefore carried out in light petroleum. The C₂₀ and C₂₂ acids were not in this case freed from monoethylenic esters. 76 g. of cyclised C₂₀ acids, and 80 g. of cyclised C₂₂ acids, were brominated. In each case, dark green sticky precipitates were obtained, and all attempts at crystallisation failed. A considerable amount of material remained in solution in the light petroleum and was recovered, 90 g. from the C₂₀ and 53 g. from the C₂₂ acids. Both these petroleum-soluble fractions had a strong camphor-like odour. On debromination with zinc and alcoholic hydrogen chloride, they yielded respectively 25 g. (iodine value 122.5) and 12.5 g. of ethyl esters. The C₂₀ insoluble bromides weighed 111 g. and on debromination gave 43.3 g. of ethyl esters (iodine value 171) with an aromatic odour: those from the C₂₂ acids resulted in a black tarry product which was not examined. The iodine values mentioned indicate respectively a mean unsaturation of two double bonds and a mixture of mono- and di-ethylenic substances, but spectrographic examination of all the available material showed that very little separation of the selectively absorbing acids had been effected, and that a considerable amount of decomposition had occurred. Bromination as a means of attacking the problem was accordingly abandoned, and other methods of preparing solid derivatives are being attempted.

With a view to confirming the presence of polynuclear ring systems in the structure of the selectively absorbing acids, attempts have been made to identify some of the products of selenium dehydrogenation (Diels's method). The acids from 1 kg. of cod-liver oil were subjected to a lithium salt/acetone separation, and 164 g. of crude highly unsaturated acids obtained. These were converted into ethyl esters and distilled, and distillation was stopped when the esters below C₂₀ had come over. The residue (C₂₀ and C₂₂ esters) was cyclised by saponification for 6 hours in amyl alcohol; the mixture of C₂₀ and C₂₂ was used in order to provide an adequate quantity of representative starting material. The cyclised product weighed 109 g. During dehydrogenation there was considerable evolution of volatile oily matter (215–250°), presumably alkyl selenides resulting from removal of side-chains from the nuclei. The action then apparently ceased until the temperature was raised to 290–300°, when evolution of hydrogen selenide commenced. The product after heating for 26 hours was taken up with ether, excess selenium filtered off and the filtrate shaken with sodium carbonate to remove unchanged acid. A considerable amount of colour was removed. After washing thoroughly and removing the solvent, a dark brown tarry liquid remained, weighing 78 g. and having an iodine value of 53.8. This was extracted three times with hot alcohol, giving a brown solution with a strong green fluorescence. No solid matter could be recovered from the alcoholic extract, and

the solvent was removed, leaving an oily brown liquid. The material insoluble in hot alcohol was a black tarry solid which was not further examined.

Attempts to prepare insoluble picrates from the alcohol-soluble material failed. The picric acid, however, evidently reacted with some constituent of the material, for the green fluorescence disappeared and a red colour developed. On removal of the picric acid with ammonia the original colour and fluorescence were restored. The material was next distilled at 2 mm. pressure and the fractions shown in Table III *a* collected. It seems probable that the true fluorescence of the residue is violet, the green effect arising from a combination of this violet with the natural brown colour of the residue.

All the fractions were examined spectrographically, with the results shown in Table III *b*. An attempt has been made here to classify the types of absorption bands encountered in these fractions—the “narrow” carotenoid type, some 10–20 $m\mu$ wide (*n* in Table), the “fine” band about 5–10 $m\mu$ wide, as exhibited by anthracene and phenanthrene (*f* in Table), and the “aromatic” type, 2–5 $m\mu$ wide (*a* in Table), characteristic of naphthalenic hydrocarbons.

The characteristic absorption maxima of some naphthalenic hydrocarbons are given in Table IV. The similarity between the wave-lengths of the various

Table IV. *Hexane solutions of naphthalene derivatives (cf. Table III b).*

Naphth.	1-Me	2-Me	2:6-diMe	1-Me:6-Et	1-Me:7-Et	Δ_2 -diH
—	—	325.8	—	—	—	—
320.5	322.2	—	322.8	322	321.5	324.5
—	318.7	319	316.2	—	317.2	320.7
~	—	—	—	—	—	~
314.8	314	314	—	314.9	315	315
311	—	310.8	310.1	—	—	311
306.8	307	—	—	307	307	306.5
304.5	—	304	—	—	—	303.7
302	302.8	—	303.1	—	—	301
299.8	299	—	—	—	—	—
297.5	—	297.8	296.3	—	—	297
—	293.2	294	—	291	291.8	293
286.5	289	287	—	—	—	287
283.5	281	—	—	280.8	281.8	—
275.5	—	275.1	—	273.5	274	274
272.2	—	—	272 (broad)	—	—	—
—	270.5	—	—	—	—	—
265.5	—	266	—	—	—	267
—	—	—	—	—	—	~
256.5	—	—	—	—	—	262

materials is noteworthy, and whilst amply confirming the presence of a bicyclic aromatic ring system among the dehydrogenation products, emphasises the difficulty of specifically identifying the compound(s) responsible for the absorption. From available data on the relative intensities of absorption at the various maxima, it seems highly probable that a mixture of dimethylnaphthalenes predominates amongst the selectively absorbing products (though present only to the extent of some 5 %), but that naphthalene itself and several of its other derivatives are also likely to be present, together with upwards of 90 % extraneous material exhibiting general absorption. From the well-known inefficiency of the selenium dehydrogenation process (order of yield 3–10 %) the proportion of hydroaromatic bodies in the material before dehydrogenation must have been considerable, though it is conceivable that cyclisation might

also have occurred during the early stages of the dehydrogenation process, which appeared to have two well-defined phases.

In any event, however, the presence of aromatic compounds demonstrates that the ability to cyclise is an inherent property of the more unsaturated higher acids. The product is too complex to be worked up by the usual means of picrate or styphnate preparation, owing probably to the presence of partially dehydrogenated products and decomposition products, and also to the presence of several different nuclei in the starting material. Fraction 1, for example, probably contains something of the order of 5 % of mixed dimethylnaphthalenes.

The absence of selective absorption in the region 295–303 $m\mu$ is explicable on the grounds that the absorption curve is here relatively steep, the bands overlapping and masking each other. The bands in the region 330–400 $m\mu$ almost certainly owe their origin to more complex polynuclear systems of the anthracene, phenanthrene, or some higher type. Here again it is at present impossible to identify the substances responsible, for much work remains to be done on such compounds and their derivatives, and the bands here observed do not exactly correspond in position with those given by any polynuclear material for which spectrographic data are at present available. The absorption spectra of aromatic polynuclear materials present, however, a characteristic and unmistakable appearance on the photographic plate, and while this generic similarity increases the difficulty of specific identification of the components of a mixture, it leaves little doubt concerning the type of compound responsible for the absorption.

SUMMARY.

It was shown by earlier work that, although most natural glycerides are relatively diactinic, the acids prepared from them by saponification often show intense, highly selective absorption, particularly in the case of fish oils. Attempts have now been made to concentrate and isolate the absorbing acids and to elucidate their structure. The attempts have been only partially successful, but although the acids have not yet been isolated in a pure state, they have been obtained in much higher concentration than hitherto by prolonged saponification at fairly high temperatures. Thus, saponification for one hour at 135° increased the yield a hundredfold, compared with similar treatment at 70°. Of the methods of separation attempted, the lithium salt/acetone process has given the best results. The solid C_{22} acids from this process represent the best source at present known of the acids absorbing near 350 $m\mu$, and the liquid C_{20} acids of those absorbing near 270 $m\mu$, the concentrations achieved over the original mixed acids (70° saponification) being of the order 10,000 to 1 and 500 to 1 respectively. Cyclisation of the natural C_{20} and C_{22} polyethylenic acids to form hydroaromatic polycyclic derivatives is the most plausible explanation of the changes in absorption, and now receives further support from (1) a marked decrease in iodine values with increasing absorption, and (2) the identification of definite aromatic nuclei, mainly naphthalenic, but some undoubtedly more complex, among the products of selenium dehydrogenation.

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