38. *The Course of Autoxidation Reactions in Polyisoprenes and Allied Compounds. Part V. Observations on Fish-oil Acids.*

By Ernest Harold Farmer and Donald A. Sutton.

The highly unsaturated acids of fish-oils and their esters are readily autoxidisable. Methyl docosahexaenoate, $C_{23}H_{34}O_2$ ($\lvert\overline{\overline{_6}}$), absorbs oxygen rapidly, the oxygen forming peroxide groups. These groups decompose spontaneously, causing chain scission in some molecules and forming oxygeno-groups in some others. Many of these oxygenated molecules appear to contain only one atom of absorbed oxygen per C_{23} chain. The unpleasant fishy odour of fish-oil acids and their esters appears to be due entirely to the scission products. Spectroscopic examination of autoxidised methyl docosahexaenoate reveals an intensity of absorption which is considerably greater than that of the original ester.

Oleic acid and its esters may be regarded as displaying a typically mono-olefinic degree of susceptibility towards autoxidation, but in passing from these to polyolefinic substances the susceptibility greatly increases : whereas oleic acid is attacked by oxygen only slowly at room temperature and in diffused daylight, the polyene acids of fish-oils undergo very rapid change under these conditions. The strong tendency to autoxidation amongst polyolefinic substances is encountered in both the conjugated and the unconjugated series, as exemplified by the carotene pigments and parinaric acid ($C_{17}H_{27}{\cdot}CO_2H$, $\lvert\overline{\overline{_4}}$; cf. Farmer and Sunderland, J., **1935**, 759) on the one hand and by rubber on the other. The autoxidative behaviour of the unconjugated fish-oil acids presents certain points of considerable interest in relation to the observations on oleic acid recorded in the preceding paper, and to similar observations on rubber (cf. succeeding paper), and hence brief consideration is given to them at this point, although owing to war conditions it has not been possible to work with freshly isolated specimens of the acids.

A specimen of methyl docosahexaenoate ($C_{23}H_{34}O_2$, $\lvert\overline{\overline{_6}}$) (91·3 g.) which had been isolated by Farmer and Van den Heuvel (*J. Soc. Chem. Ind.*, **1938**, **57**, 24; J., **1938**, 427) was found to have absorbed 5·75% of oxygen on standing (mostly in the dark) for about 4 years at atmospheric pressure in a stoppered flask to which air had been admitted on several occasions. This specimen had an insignificant peroxide content (<0·1%), indicating that any peroxide groups formed by the incorporation of oxygen had nearly all undergone secondary transformation. The autoxidised ester when submitted to distillation at 10^{-4} mm. in a Watermann-type molecular still, using only a few seconds' contact of the oil-film with the heating surface (maximum temperature, 110°), lost at once its most highly oxygenated components by volatilisation into the pump, leaving an ester having the average composition $C_{23}H_{34}O_{2\cdot28}$.* The material so lost was but little (5·0 g., including the still hold-up), and by reason of its volatility must be regarded as consisting of products of oxidative chain-scission : the remainder, on the other hand, was composed of undegraded (*i.e.*, unsevered) ester-molecules, most of which were in the original unoxidised condition, although some were oxygenated, doubtless being derived from peroxido-molecules by secondary transformation.

In the course of the molecular distillation the unchanged hexaene ester, as might be expected, showed

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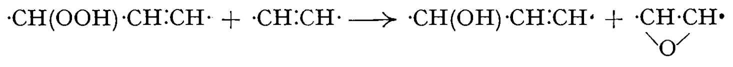
* The C/H atom ratio for this material and for its distillation fractions (below) is written as C_{23}/H_{34} to correspond with that of methyl docosahexaenoate, although the experimentally observed ratio (average of 10 analyses) was actually $C_{23}/H_{34\cdot66}$. An excess of $H_{0\cdot66}$ in an ester molecule $C_{23}H_{34}O_4$, although within usually accepted limits of microanalytical error (calc. for $C_{23}H_{34}O_2$: C, 80·64 ; H, 10·01%. Calc. for $C_{23}H_{34\cdot66}O_2$: C, 80·48 ; H, 10·19%), is nevertheless unexpectedly large in comparison with our usual error in hydrogen determination and may possibly be significant (see below). In the present paper, however, the feature of interest is the oxygen content of the autoxidised material, and this would be diminished by only a trivial amount if the higher hydrogen value were correct.

some tendency to distil preferentially to the oxygenated portion, but the separative efficiency of the still was insufficient to bring about a reasonably good separation of unoxygenated from oxygenated molecules unless these (respectively) differed in molecular weight by as much as 30 units (*i.e.*, by two atoms of oxygen). In practice it was found that after the material had been twice passed through the still no further useful degree of separation could be effected with the limited quantity of ester at disposal. The least oxygenated ester had then an average empirical composition of $C_{23}H_{34}O_{2\cdot16}$.

That peroxidation represents the first stage in the autoxidation of the unsaturated fish-oil acids, as it occurs spontaneously in the diffused light of the laboratory, was strikingly shown by exposing (in February) a sample of the least-oxygenated distillate to air in a shallow dish. In 7 days 5·2% of oxygen (1·2 atoms) had been absorbed, all of which, within the limits of accuracy of the method of determination employed, was in peroxidic form. This speed of oxidation is greater than has hitherto been observed in these investigations for thermal oxidations, and greatly exceeds that found for the polyisoprenes. Doubtless, the great speed is due to the particular susceptibility to oxidative attack of the methylene groups which stand between double bonds in the carbon systems $[\cdot CH{:}CH\cdot CH_2\cdot]_n$.

Account being taken of the known limits to the separative efficiency of the still, the oxygenated molecules which ultimately and unavoidably passed over with the unoxidised molecules may be concluded to be mono-oxygenated molecules ($C_{23}H_{34}O_3$). Such mono-oxygenated compounds could be either hydroxy- or epoxy-derivatives of the original hexaene ester, formed by the intermolecular reaction



$$\cdot CH(OOH)\cdot CH{:}CH\cdot + \cdot CH{:}CH\cdot \longrightarrow \cdot CH(OH)\cdot CH{:}CH\cdot + \underset{\diagdown O \diagup}{\cdot CH\cdot CH\cdot}$$

Careful hydrogenation of samples of the slightly oxygenated distillate showed an unsaturation of $|\overline{\overline{5{\cdot}7}}$, which is unexpectedly low compared with the anticipated value of $|\overline{\overline{5{\cdot}9}}$ * for a product possessing the observed oxygen content. This discrepancy in the unsaturation value seems rather too large and persistent to be attributed to analytical imprecision, and it may perhaps be due to the presence in the original fish-oil ester of a proportion of pentaene ester $C_{23}H_{36}O_2$ (clupanodonic ester) which the somewhat less accurate analytical technique in use at the time of Farmer and Van den Heuvel's study failed to reveal.† This seems the more probable in that the above-mentioned excess in the H/C ratio disappeared entirely during hydrogenation to give the correct ratio ($C_{22\cdot0}H_{44\cdot0}$) for the saponified, fully saturated product. A final decision, however, on the point must await the isolation of new samples of the highly unsaturated C_{22}-acid from fresh cod-liver oil.

The highest oxygen content observed for the less volatile part of the autoxidised ester had the average formula $C_{23}H_{34}O_{2\cdot41}$, but the quantity of this more highly oxygenated material available was insufficient for satisfactory fractionation, so the proportions of mono- and di-oxygenated ester and unoxidised and dimeric (oxygenated) ester contained in it cannot be stated.

An important effect of the introduction of peroxido-groupings into the carbon chain of the polyene fish-oil acids is the production of a marked increase in the capacity of the acids or their esters to absorb light—especially light of the shorter wave-lengths. In the diagram is shown the absorption curve (curve I) for the slightly oxygenated fraction of the C_{23}-ester (the data for which were obtained soon after its distillation, strict precautions being used against oxygen-access) together with that (curve II) for the same specimen after it had been exposed in a dish to air for 10 days. A similar oxidation effect is to be seen in the absorption curves for fresh and two-days-old samples of the liver-oil of ling (*Molva vulgaris*) published by Edisbury, Morton, and Lovern (*Biochem. J.*, 1933, **27**, **1455**), although the cause of the observed differences is not traced by these authors to autoxidation. Reproductions of the latter curves have been added to the diagram for comparison, curve III representing a fresh solution of the mixed (saturated and unsaturated) glycerides contained in the liver-oil, and curve IV the same solution after 2 days. Autoxidation, as is shown elsewhere, materially affects the density of polyene substances: consequently, it is of the utmost importance to take exceptional precautions against ingress of oxygen during physical studies of unconjugated as well as of conjugated polyene substances. The details of curve I will be discussed in a later paper.

Although the unconjugated polyene acids absorb oxygen very rapidly, and the whole of this oxygen up to at least 1·34 atoms uptake can survive in peroxidic form, yet a serious degree of peroxide decay is not long deferred. A sample of slightly oxygenated ester, when left open to the air for 24 days in the diffused light of the laboratory (in May), showed a net gain of 13·5% of oxygen (*i.e.*, an average of 3·7 atoms), of which only 5% was in peroxidic form. The sample gelled in the process, and doubtless had undergone a considerable degree of degradation (as well as molecular intercondensation). This change was reflected in the marked decrease in the H/C ratio from 0·1257 to 0·1174, probably denoting loss of water (and possibly also of carbon dioxide) from the oxidation product.

It is of interest that prolycopene, a *cis-trans*-conjugated isomeride of lycopene ($C_{40}H_{56}$, $|\overline{11}$) has recently

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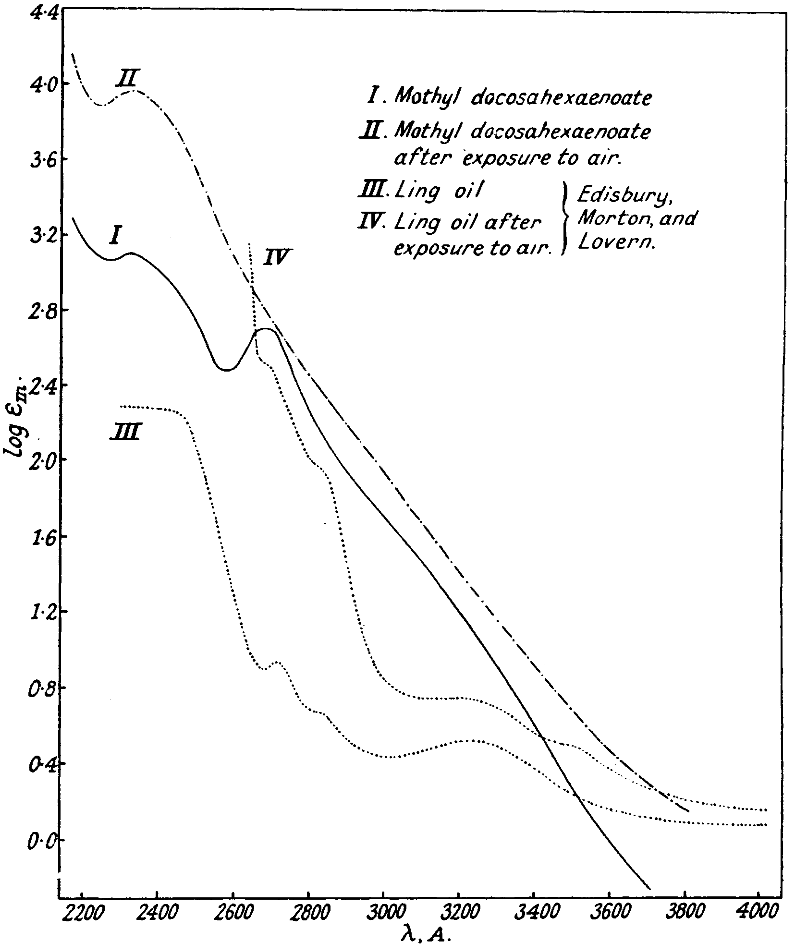
* This value assumes that the oxygenated impurity contained 50% of pentaene epoxy-ester and 50% of hexaene hydroxy-ester. In the very unlikely event of the impurity consisting wholly of epoxy-ester the overall unsaturation would still be $|\overline{\overline{5{\cdot}85}}$. The usual experimental error in determining the unsaturation of these easily hydrogenatable polyene esters corresponded to $< |\overline{\overline{0{\cdot}1}}$ per molecule.

† It was a matter of some surprise when docosahexaenoic acid was first isolated in a heat-unaltered condition that no C_{22}-pentaene acid appeared to be admixed with it, although a heat-altered (probably cyclised) pentaene acid forms an important constituent of liver-oil acids which have been strongly heated. No separation of C_{22}-acids of different degrees of unsaturation could be expected by molecular distillation.

been observed by LeRosen and Zechmeister (*J. Amer. Chem. Soc.*, 1942, **64**, 1075) to absorb 9 mols. of oxygen per mol., but since no determination of the peroxidic oxygen content has been made at this or at other stages of the autoxidation, it is not evident how much oxygen can be taken up in peroxidic form, and hence whether or not a greater proportion of peroxide groups than one per conjugated diene unit is formed.



Experimental.

Distillation of Air-exposed Methyl Docosahexaenoate.—The yellow 5-year-old specimen of the ester referred to above (Found : 75·25, 75·3 ; H, 9·6, 9·5%) had an intensely disagreeable fishy odour and contained 0·1% of peroxidic oxygen as determined by the method of Bolland, Sundralingam, Sutton, and Tristram (*Trans. Inst. Rubber Ind.*, 1941, **17**, 29). By submission to continuous molecular distillation with the distillation surface heated at 100° (cf. Farmer and Van den Heuvel, *loc. cit.*) this ester (91·3 g.) was divided into two parts, a colourless Distillate I, 48 g. (Found : C, 80·0, 79·95 ; H, 10·1, 9·85%), and a Residue I, 38·3 g. (Found : C, 79·15, 79·1 ; H, 10·05, 9·9%). Both distillate and residue had only very faint odours, with only the slightest suggestion of fishiness. The distillate was redistilled with the heating surface of the still at 100°, so giving Distillate II, 34·7 g. (Found : C, 79·75 ; H, 10·2%), and Residue II, 11·9 g. (Found : C, 79·1 ; H, 10·05%). Distillate II was redistilled at 80°, the small distillate so obtained being discarded (*ca.* 4 g.) and the large residue (30 g.) returned to the still and redistilled at the same temperature. Again, the small distillate was discarded and the larger residue (Residue III ; 26·3 g.) (Found : C, 80·05 ; H, 10·25% ; $n_D^{20^\circ}$ 1·4930 $d_{4^\circ}^{20^\circ}$ 0·9192 ; $[R_L]_D$ 108·24) distilled once again at 100° in order to obtain the middle fraction of the little-oxygenated ester. This had C, 80·0 ; H, 10·25% ; $n_D^{20^\circ}$ 1·4930 ; $d_{4^\circ}^{20^\circ}$ 0·9214 ; $[R_L]_D$ 108·0. The Eisenlohr value for $C_{23}H_{34}O_2, |\overline{\overline{}}_{6}$ is 107·2.

Hydrogenation of the Slightly Oxygenated Distillate.—This material, having on an average the formula $C_{23}H_{34}O_{2\cdot13}$ (0·7975 g.), was hydrogenated in *cyclo*hexane by means of Adams's catalyst. The hydrogen absorbed amounted to 297 c.c. at N.T.P., pointing to an unsaturation in the original ester of $|\overline{\overline{}}_{5\cdot7}$. On removal of the solvent a saturated ester was obtained. This was slightly impure methyl behenate, m. p. (without recrystallisation) 49—50° (correct m. p. 54°), and it gave on saponification with alcoholic sodium hydroxide impure behenic acid, m. p. (without recrystallisation) 74—75° (correct m. p. 81°). This partly oxygenated sample of behenic acid had C, 76·85 ; H, 12·9%, and so corresponded to $C_{22\cdot0}H_{44\cdot0}O_{2\cdot20}$. The increase in oxygen content (from $O_{2\cdot16}$ to $O_{2\cdot20}$) agreed exactly with that to be expected as the result of saponification.

Autoxidation of Methyl Docosahexaenoate.—A sample of the slightly oxygenated but peroxide-free methyl docosahexaenoate obtained above by distillation was kept in an open dish in the air for 7 days. It increased in oxygen content by 5·15% of the weight taken (then having C, 75·75 ; H, 9·6%), and was found to contain 5·2% of peroxidic oxygen ; its H/C ratio, however, remained unchanged (0·1267). This partly peroxidised ester had $n_D^{20^\circ}$ 1·4995 and $d_{4^\circ}^{20^\circ}$

0·9634, from which, the formula being assumed to be $C_{23}H_{34}O_2$ (*i.e.*, neglecting the fact that the sample has been exposed to air), the apparent $[R_L]_D$ is 106·8; *i.e.*, a nearly normal value for $[R_L]_D$ is erroneously obtained (Calc.: 107·2).

When a similar sample was exposed to the air for 24 days, it set to a stiff gel and then had: C, 69·0; H, 7·1; O (diff.), 22·9%; P.O.C., 5·0%. The increase in weight, calculated on the sample taken, was thus 15·1%.

We thank Dr. G. A. Jeffrey for carrying out the spectroscopic examination of our specimens.

THE BRITISH RUBBER PRODUCERS' RESEARCH ASSOCIATION,
48, TEWIN ROAD, WELWYN GARDEN CITY, HERTS.

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