

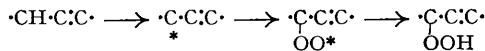
144. *The Course of Autoxidation Reactions in Polyisoprenes and Allied Compounds.
Part VII. Rearrangement of Double Bonds during Autoxidation.*

By E. HAROLD FARMER, H. P. KOCH, and DONALD A. SUTTON.

Ethyl linolenate and methyl docosahexaenoate, both displaying unsaturation of the methylene-interrupted type, $\cdot\text{C}:\text{C}\cdot\text{C}:\text{C}:\text{C}\cdot\text{C}:\text{C}\cdot$, are shown by spectrographic measurements to develop conjugated diene and conjugated triene unsaturation during the incorporation of molecular oxygen. The extent of double bond displacement is correlated with the degree of peroxidation occurring. Squalene and rubber, both displaying a different type of unsaturation, $\cdot\text{C}:\text{C}\cdot\text{C}\cdot\text{C}:\text{C}\cdot\text{C}\cdot\text{C}:\text{C}\cdot$, do not similarly develop conjugated units. The mechanism of autoxidative reaction in the two cases is discussed.

IN Part V (this vol., p. 122) it was reported that a typical drying-oil ester, methyl docosahexaenoate, showed a marked increase in its capacity to absorb ultra-violet light as the result of exposure to atmospheric oxygen in the illumination of the laboratory. The rapid peroxidation of the polyene system which set in during this

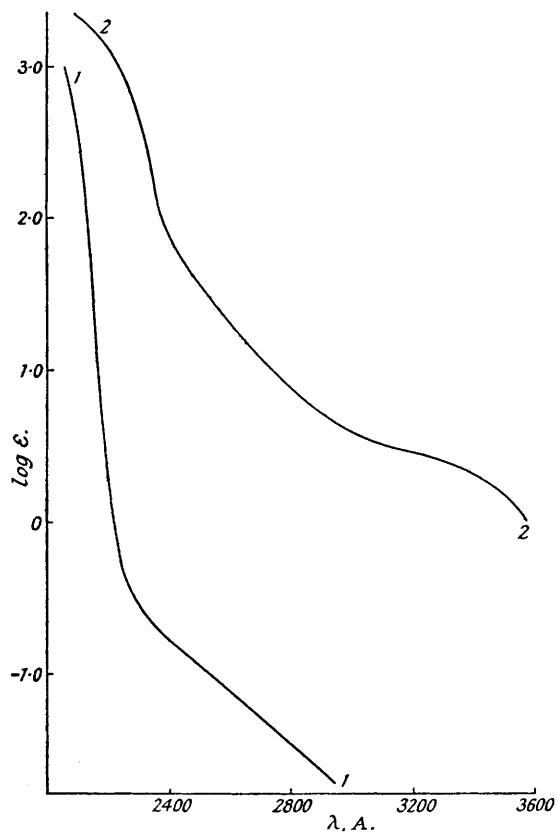
exposure promoted a structural change apparently of a conjugative nature. The nature of this change is now examined. Two types of unconjugated unsaturation are, however, taken into consideration, (1) the methylene-interrupted unsaturation, $\cdot\text{C}:\text{C}:\text{C}:\text{C}:\text{C}:\text{C}:\text{C}:\text{C}\cdot$, characteristic of numerous unconjugated drying-oil acids such as linoleic, linolenic, and various polyene fish-oil acids, and (2) the more widely spaced unsaturation found in rubber and in other unconjugated polyisoprene hydrocarbons. The structural changes that ensue on oxidation are of particular interest in relation to the free-radical mechanism of olefinic peroxidation already formulated (Farmer, Bloomfield, Sundralingam, and Sutton, *Trans. Faraday Soc.*, 1942, **38**, 348) in which the olefin is regarded as passing through the stages:



the asterisk representing the radical centre.

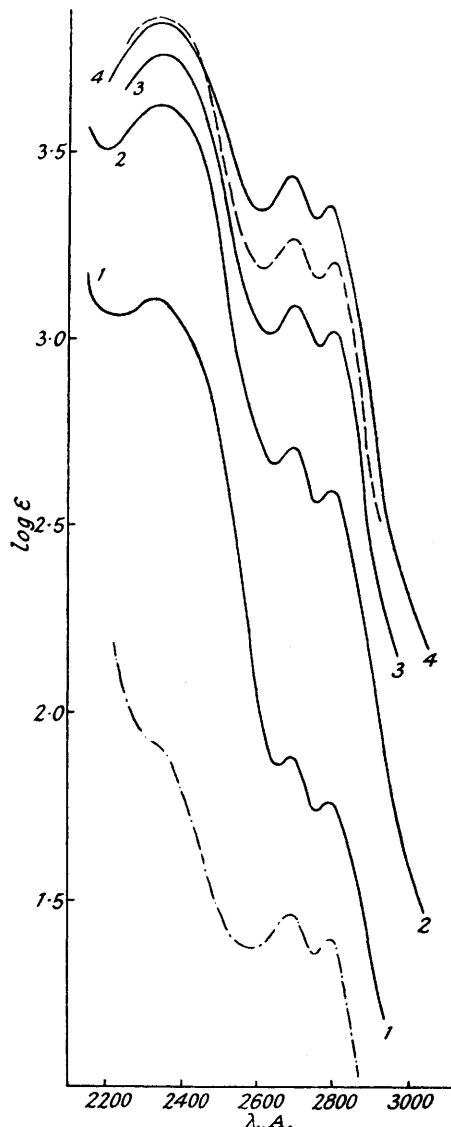
Materials.—For the purpose of differentiating quite clearly the effect of oxidation from that of (a) thermal treatment (cf. forthcoming paper) and (b) alkali treatment (cf. Farmer, *Trans. Faraday Soc.*, 1942, **38**, 357), it has been necessary to work with materials as nearly as possible spectroscopically pure. For this reason subjection of the materials during their preparation to temperatures above 115° , to prolonged treatment with alkali, or to

FIG. 1.



Spectra of cyclohexene (1) and cyclohexene hydroperoxide (2) in cyclohexane.

FIG. 2.
Spectra of ethyl linolenate (in ethanol).



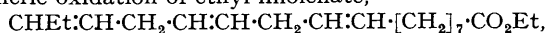
— — — — — Before oxidation.
— — — — — After 1.2% (1), 3.9% (2), 6.5% (3), and 11% (4) oxidation.
- - - - - After 8.5% oxidation in dark.

the action of atmospheric oxygen was highly undesirable. Ethyl linolenate represented a favourable case for study, the only undesirable feature attending its preparation being the necessity to subject the parent linseed oil to a short saponification with alkali. Methyl docosahexaenoate, which was selected to represent the unconjugated polyene esters derived from fish oils, had also to be submitted to short treatment with alkali at the first stage of its preparation, but the use of high temperatures, and exposure to oxygen during its laborious isolation were altogether avoided by a modification of Farmer and Van den Heuvel's original method (*J. Soc. Chem. Ind.*, 1938, **57**, 24). The yellow colour of the fish-oil ester, which developed rapidly in the

presence of oxygen, could only be completely removed by chromatographic treatment of the ester with alumina in an atmosphere of purified nitrogen. Squalene and rubber were employed to exemplify the second type of unsaturation. The former was rectified by molecular distillation below 112°, and then further purified by chromatographic treatment in purified nitrogen, and the latter was freed almost entirely from impurities by fractional dissolution of crepe rubber in petroleum-acetone in an atmosphere of nitrogen (Bloomfield and Farmer, *Trans. Inst. Rubber Ind.*, 1940, 16, 69).

Spectrographic Examination of Autoxidised Materials.—In determining the structural cause of the strong light-absorbing capacity of the autoxidised fish-oil ester, it was necessary at the outset to gain information concerning the peroxide chromophore. This has been examined in detail, and it is found that the strong light-absorption in the regions of 2400 and 2700 Å. is not to be attributed to the peroxido-olefin structure but must be associated with the formation of conjugated groups by double-bond shift in the carbon chain during its oxygenation. The spectrum of a typical olefin peroxide is compared with that of the parent olefin in Fig. 1. The most interesting features are the widely increased general absorption and the new broad band beyond 3000 Å., but the extinction values are low throughout the ultra-violet range. Simple alkyl peroxides appear to be even more transparent (Lederle and Rieche, *Ber.*, 1929, 62, 2573), so the influence of peroxidic absorption can certainly be neglected in the presence of typical organic chromophores.

Spectrographic analysis of the early stages of atmospheric oxidation of ethyl linolenate,



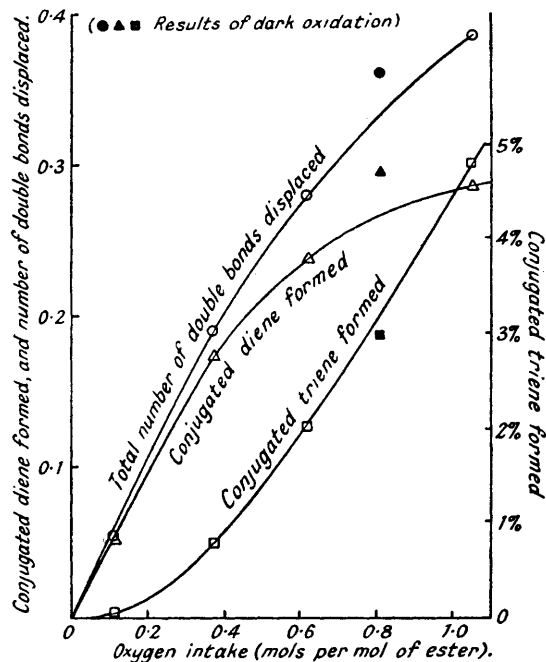
during which all the incorporated oxygen survives in peroxidic form, has revealed the emergence of intense selective absorption in two well-defined wave-length regions (cf. Fig. 2). In the absence of any possible chromophoric oxygen groups, maximum absorption near 2340 Å. is known to characterise the spectrum of a straight-chain conjugated diene (cf. spectrum of alkali-isomerised linoleic acid according to Mitchell, Kraybill, and Zscheile, *Ind. Eng. Chem. Anal.*, 1943, 15, 1), and the two narrow bands at 2690 and 2800 Å. are typical of straight-chain conjugated trienes such as elæostearic acid or alkali-isomerised linolenic acid (van der Hulst, *Rec. Trav. chim.*, 1935, 54, 639; Mitchell *et al.*, *loc. cit.*). The starting material (dotted curve, Fig. 2) evidently contains traces of similar absorbing compounds as impurities.

Assuming maximum molecular extinction coefficients of 24,400 and 47,300 for ethyl-alcoholic solutions of pure conjugated diene and triene respectively (figures calculated from the data of Mitchell *et al.*), and allowing for irrelevant absorption at 2690 Å. due to the ascending branch of the short-wave band at 2340 Å., we can estimate the actual amounts of conjugated material produced from ethyl linolenate during autoxidation. In Fig. 3 the number of conjugated diene groups per mol. of ester and the percentage concentration of conjugated triene formed are plotted against the observed intake of oxygen. It can be seen that, after the incorporation of 1 mol. of oxygen, rearrangement of double bonds has progressed to a stage at which *ca.* 28.5% of the ester taken contains two double bonds in conjugation, and 4.5% has all three double bonds conjugated. The top curve of Fig. 3 represents the total number of double bonds displaced, account being taken of the fact that two double bonds must be shifted for each conjugated triene system formed. The initial slope of this curve shows that during the early stages of autoxidation each molecule of oxygen absorbed is 50% effective in moving one double bond into conjugation.

The absorption spectra of two samples of methyl docosahexaenoate were also examined before and after autoxidation, and the occurrence of the same process of double-bond rearrangement during oxidation is revealed by the development of intense absorption in the originally feebly absorbing regions of 2340 and 2700 Å. (Fig. 4). The bands near 3015, 3165, 3290, and 3480 Å. exhibited by the unoxidised ester were also observed by Edisbury, Morton, and Lovern (*Biochem. J.*, 1935, 29, 899) in all their samples of fish-oil acids and are doubtless due to the presence of traces of higher conjugated polyenes. An important effect of autoxidation is the destruction of these conjugated systems implied by the decreased absorption at longer wave-lengths. The new band at 3190 Å. is probably associated with the triene spectrum (van der Hulst, *loc. cit.*).

The rearrangement phenomenon was overlooked by Treibs (*Ber.*, 1942, 75, 925), who studied the course of autoxidation of linoleic, linolenic, and liver-oil hexaene esters refractometrically; he found that there is no exaltation of the molecular refraction which could be ascribed to conjugated unsaturation after the incorporation of 1 mol. of oxygen. This finding agrees with our own observations on autoxidised methyl docosa-

FIG. 3.



hexaenoate (cf. Part V, *loc. cit.*), but in our experience the original ester always showed an exaltation of *ca* 1 unit, which disappeared as autoxidation (accompanied by rapid increase in the density and comparatively slow increase in the refractive index) proceeded.* Refractivity measurements are clearly unreliable as a guide to the presence of conjugation in the highly autoxidisable substances displaying methylene-interrupted unsaturation.

FIG. 4.
Spectra of methyl docosahexaenoate (in ethanol).

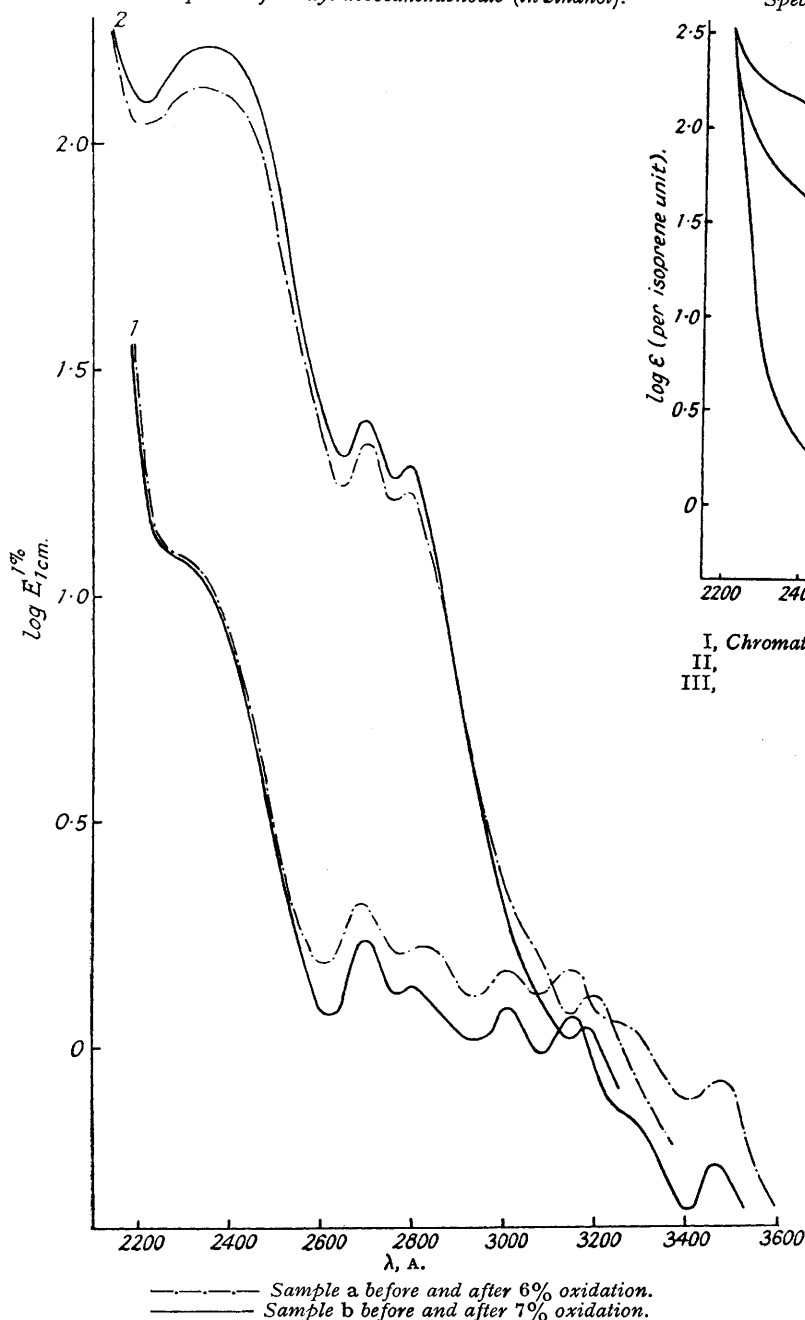
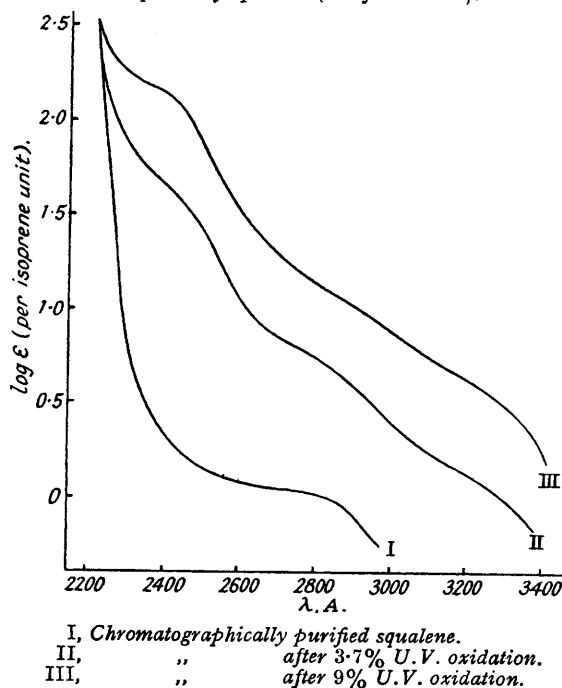


FIG. 5.
Spectra of squalene (in cyclohexane).

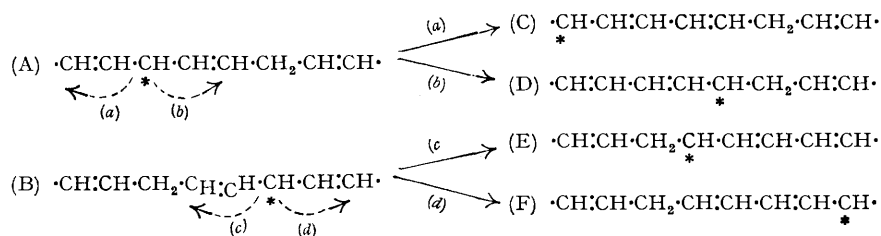


In contrast to the foregoing, when solutions of carefully purified squalene and of fractionated rubber were similarly examined before and after autoxidation, no strong increase in the absorption of light which could definitely be ascribed to the formation of conjugated units was observed. The spectra for squalene and two

* An early normal value of $[R_L]_D$ reported by Farmer and Van den Heuvel (J., 1938, 429) was due to unsuspected oxidation of the distilled ester.

of its oxidation products (3.7% and 9% oxygen-intake) are represented in Fig. 5. The small increases in absorption there shown may be due to small degrees of conjugation, or to the formation of peroxide groups (cf. the effect on light absorption of peroxide groups in the case of *cyclohexene*), or to a combination of both.

Course of Conjugative Change.—If the first stage of peroxidation consists in the severance (presumably by the aid of molecular oxygen) of a thermally or photochemically activated α -methylene C—H bond, so leaving an olefinic free radical, it may be expected that resonance between the two 3-carbon forms $\cdot\overset{3}{\text{C}}\text{H}:\overset{2}{\text{C}}\text{H}:\overset{1}{\text{C}}\text{H}\cdot$ and $\overset{3}{\text{C}}\text{H}:\overset{2}{\text{C}}\text{H}:\overset{1}{\text{C}}\text{H}\cdot$ will immediately follow, so that when in succession a molecule of oxygen and an atom of hydrogen (the latter probably detached from a nearby molecule) come to be incorporated, there will be an approximately equal tendency for the $\cdot\text{OOH}$ group to appear at the positions 1 and 3, and for the double bond to remain at the original position or to appear at the adjacent C—C bond. In the methylene-interrupted type of unsaturation considered above, the most reactive methylene groups are those flanked on either side by a double bond, and if the two methylene groups of this type in ethyl linolenate were to be attacked singly by oxygen the original radical forms (A) and (B) would be supplemented by (C)—(F) :



The hydroperoxides derived from (C)—(F) would then show diene conjugation. Similarly if *both* the reactive methylene groups were attacked consecutively by oxygen, which would happen frequently only in advanced stages of oxidation, then five other (di-)peroxido-forms would become possible, three of which would show diene conjugation, one triene conjugation, and the last the original state of unconjugation. Now if on each occasion that a hydrogen atom is detached from just one of the active methylene groups of a linolenic molecule the tendency for the radical centre so formed to migrate to left or right is assumed to be about equal to the tendency for it to retain its original position, then it will follow that only half the radical molecules that are ultimately successful in combining with oxygen and hydrogen to give mono-hydroperoxido-compounds will yield conjugated forms; if, however, all of the three resonance forms derivable by loss of a hydrogen atom from a pentadiene system $\cdot\overset{3}{\text{C}}\text{H}:\overset{2}{\text{C}}\text{H}:\overset{1}{\text{C}}\text{H}:\overset{3}{\text{C}}\text{H}:\overset{2}{\text{C}}\text{H}:\overset{1}{\text{C}}\text{H}\cdot$ are to be regarded as possessing equal stability, the proportion of conjugated to unconjugated forms will rise to *ca.* 2:1. Since the hydroperoxido-derivatives of ethyl linolenate undergo little decomposition during the absorption of the first molecule of oxygen by the ester, the quantitative relationship between the conjugated and the unconjugated forms produced is likely to have remained substantially unchanged by secondary reaction in the present experiments: the product, however, at the end of the "monoperoxidation" stage is likely to have contained diperoxido- and unchanged ester molecules as well as monoperoxido-derivatives.

In the case of the polyisoprene hydrocarbons, however, the reactive α -methylene groups are flanked only on one side by double bonds. This leads in practice to a much lower reactivity of the olefin, and, in terms of the hypothesis, to the necessity for two successful peroxidations to occur in any unconjugated diene unit of the chain (C:C-C-C:C) before diene conjugation can appear therein. Furthermore, the hydroperoxide groups formed in these compounds are very unstable, so that no very high (overall) percentage of peroxidic oxygen survives at the end of an experiment, and this applies whether the oxidation is restricted to an early stage or whether it is carried (in ultra-violet light) to an advanced stage; also the decomposition of the peroxide groups leads to a corresponding degree of destruction of unsaturation. It is not surprising, therefore, that in practice peroxidation does not produce any considerable degree of conjugation. The occurrence, however, of peroxidation at all in rubber, squalene, etc., may well cause change in the original arrangement of the double bonds, and so contribute to the observed heterogeneity of the final oxidation products, *e.g.*,



Presumably the easily oxidisable forms displaying methylene-interrupted unsaturation which ought thus to be produced by the first double-bond shift do not accumulate sufficiently in the earlier stages of autoxidation to cause by their further oxidation appreciable change in the direction of conjugation over the whole material; also in the more advanced stages where their numbers should increase their effect is nullified by the destruction of unsaturation accompanying peroxide decay.

If the free-radical mechanism of autoxidation is the correct one, double-bond shifts appear not unlikely to occur also in the autoxidation of mono-olefinic substances, since the 3-carbon grouping $\cdot\text{CH}:\text{CH}:\text{CH}\cdot$ is concerned here also, so that methyl oleate should give the peroxido-forms $\cdot\text{C}_{11}:\text{C}_{10}:\text{C}_9(\text{OOH})\cdot\text{C}_8$ and $\cdot\text{C}_{11}:\text{C}_{10}(\text{OOH})\cdot\text{C}_9:\text{C}_8$ as well as $\cdot\text{C}_{11}(\text{OOH})\cdot\text{C}_{10}:\text{C}_9:\text{C}_8$ and $\cdot\text{C}_{11}:\text{C}_{10}:\text{C}_9(\text{OOH})\cdot\text{C}_8$. This deduction has not been

tested experimentally, but the fully hydrogenated derivatives of hydroperoxido-oleic acid which were described in Part IV may well include 9- and 10-, as well as 8- and 12-hydroxystearic acid. In addition, since the production of conjugated forms from the above-mentioned substances has never been observed to occur in the entire absence of oxygen, even after many hours of illumination in ultra-violet light, the further deduction can hardly be resisted that molecular oxygen (in spite of various recorded observations to the effect that the rate of autoxidative reaction in olefins is independent of the oxygen concentration) plays a direct part in detaching sufficient hydrogen atoms * from the α -methylene C-H bonds to initiate the necessary reaction chains for bringing about successful autoxidation.

No distinction has hitherto been made in this series between the course of photochemical and that of thermal oxidation, since not only have experimental conditions in which both thermal and photochemical activation are possible usually been employed, but no results pointing to the functioning of two different mechanisms of oxygen incorporation have yet been obtained. It is now found that, apart from the induction period (see below), the result of conducting an oxidation of ethyl linolenate at room temperature in complete darkness is exactly the same with regard to efficiency of peroxide formation and the extent of double bond rearrangement as that obtained in (summer) daylight. The dotted curve in Fig. 2 represents the absorption spectrum of this thermally oxidised ethyl linolenate (8.5% oxygen uptake), and the three black points in Fig. 3 the extent of conjugation and the total double-bond shift produced by the oxidation. The free-radical mechanism of peroxidation, therefore, appears to apply to thermal as well as to photo-oxidation. It is of interest also that the analogy formerly pointed out by one of us (Farmer, *Trans. Faraday Soc.*, 1942, **38**, 340) between the mechanism of olefinic autoxidation and that of maleic anhydride addition to olefins at high temperatures clearly needs to be extended to include double-bond shifts in the latter reaction exactly comparable with those reported above to occur in the former. Alder (*Ber.*, 1943, **76**, 27) has recently observed that both maleic anhydride and azodicarboxylic esters unite with allylbenzene to give adducts of the type $\text{CHPh}\cdot\text{CH}\cdot\text{CH}_2\text{R}$ (not $\text{CHPhR}\cdot\text{CH}\cdot\text{CH}_2$), R representing the maleic or azodicarboxylic grouping, and similar results obtained by one of us will be published later.

EXPERIMENTAL.

cycloHexene.—*cycloHexene* was purified for optical examination by the method of Stücklen, Thayer, and Willis (*J. Amer. Chem. Soc.*, 1940, **62**, 1717), who record, over the range examined by them, an absorption curve identical with that given in Fig. 1.

cycloHexene Peroxide.—This was prepared by Farmer and Sundralingam's method (*J.*, 1942, 132) and twice distilled at $27^\circ/0.03$ mm. for spectrographic examination. Further distillation of the sample caused no change in the absorption curve.

Ethyl Linolenate.—Hexabromostearic acid (m. p. 185°) was debrominated according to Norris, Kass, and Burr (*Oil and Soap*, 1940, **17**, 123), and the resultant crude ester molecularly distilled at 78° (Found: C, 78.3; H, 11.3; I.V., 246. Calc. for $\text{C}_{20}\text{H}_{34}\text{O}_2$: C, 78.4; H, 11.2%; I.V. for $[\bar{3}]$ 249). The ester was kept sealed in a vacuum until required for use, and its absorption spectrum determined within 40 mins. of opening the tube.

Autoxidation.—Since no water is eliminated during the early stages of oxygenation (Farmer and Sutton, *loc. cit.*; Treibs, *Ber.*, 1942, **75**, 203), the oxygen intake was determined by direct weighing against a tare. Small beakers (2 cm. diameter) containing about 200 mg. each of the ester were exposed during June to a dust-free atmosphere at $20^\circ \pm 3^\circ$ near a window facing north. At intervals, successive samples were re-weighed, made up to a known volume in ethyl alcohol, and immediately used for spectrographic and peroxide determination, the latter by the method of Bolland *et al.* (*Trans. Inst. Rubber Ind.*, 1941, **16**, 29). The observations were completed within 2 hrs. of re-weighing. It was found that 1.1% of oxygen (P.O.C., 1.3%) was absorbed in 24 hrs., 3.7% (P.O.C., 4.1%) in 48 hrs., 6.3% (P.O.C., 6.7%) in 72 hrs., and 12.0% (P.O.C., 10.3%) in 110 hrs. In the dark, no oxygen was absorbed by the ester during the first 2 days, but 8.7% (P.O.C., 8.1%) was found to be absorbed after 7 days.

Methyl Docosahexaenoate.—The glycerides of cod-liver oil of good quality were converted by the action of methanolic hydrogen chloride into the corresponding methyl esters. From the mixed esters a fraction judged to contain the whole of the C_{23} -ester fraction was separated by a series of molecular distillations at temperatures below 115° . This fraction, containing some saturated and possibly some mono-olefinic esters in addition to the polyene esters, was rapidly saponified with a small excess of methanolic potassium hydroxide, and the potassium soaps converted *via* the free acid into the corresponding lithium soaps. By fractional crystallisation of the latter from acetone, the (relatively soluble) lithium soap of the crude hexaene acid was segregated, and thereafter converted *via* the free acid into the corresponding methyl ester. This was finally fractionated by molecular distillation. All operations were conducted in an atmosphere of purified nitrogen or in high vacuum. Two samples were employed: (a) one fraction distilled at 91° which still contained traces of the C_{21} ester ($[\bar{5}]$), and (b) another at 110° which gave satisfactory analytical values for the C_{23} ester ($[\bar{6}]$).

Autoxidation.—The oxidation technique was essentially similar to that outlined above. Sample (a) absorbed 6.3% (P.O.C., 5.8%) of oxygen in 72 hrs., and sample (b) (under rather more favourable conditions) 7.2% (P.O.C., 6.9%) in 28 hrs.

Squalene.—Portions of the purified squalene (Found: C, 87.85; H, 12.35. Calc. for $\text{C}_{30}\text{H}_{50}$: C, 87.72; H, 12.28%) were shaken (without solvent) with oxygen in ultra-violet light at *ca.* 35° until (a) *ca.* 3.5% and (b) *ca.* 9% of oxygen had been absorbed. The two products had: (a) C, 84.5; H, 11.85; O (diff.), 3.65; P.O.C., 1.7%, and (b) C, 79.8; H, 11.25; O (diff.), 8.95; P.O.C., 3.54%.

Rubber.—A sample of purified rubber, dissolved in spectroscopically pure *cyclohexane*, was shaken with oxygen at *ca.* 35° in ultra-violet light until *ca.* 1% of oxygen had been absorbed. The product had: C, 87.2; H, 11.75; O (diff.), 1.05%.

Spectrographic Measurements.—The Spekker photometer was used in conjunction with a tungsten-steel spark and a Hilger Small Spectrograph E 484 which gives a dispersion of 30 Å./mm. at 2700 Å. and 20 Å./mm. at 2340 Å. Spectral photographs exhibiting a high degree of contrast were obtained on Kodak B-10 plates. Two concentrations of ester solution normally sufficed to cover the entire range of the spectrum when two different cell lengths were used. *cyclo*-

* Probably in the form of $\text{HOO}\cdot$ radicals. Kinetic studies of the course of reaction will be published later by the authors' colleagues.

Hexane and ethyl alcohol were specially purified for absorption measurements (Twyman and Allsopp, "Absorption Spectrophotometry," p. 67).

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