Infrared studies on initiation of the autoxidation of some fatty acid esters with and without light-sensitized chlorophyll, ultraviolet light and lipoxidase*

The studies by application of tracer techniques involving particularly deutero-oleate have indicated that the induction period is not basically due to trace impurities, but rather to the role played by the double bonds together with the alpha methylene groups during the transition states of activation in the initial stages of autoxidation reactions. The removal of the trace impurities[1,2] has added further support to the same concept. The recent investigations employing the infrared spectra[3-6] have also brought forth more conclusive experimental evidence for the initiation processes in autoxidation of methyl oleate, linoleate and linolenate. The purpose of the present preliminary report is to show by means of the infrared absorption analysis that in the above initiation step, isomerization and molecular rearrangement of the substances used occurs at the intermediate transition states of their reactions with oxygen.

Methyl oleate was prepared from olive oil methyl esters by a combination of low-temperature crystallization and fractional distillation at reduced pressure[7]. The product was subjected to mild hydrogenation to remove the traces of polyethenoid impurities and finally to low-temperature crystallization[2]. Methyl oleate (Iodine number = I.N., 85.6) showed no absorption at 10.36 μ characteristic of *trans* olefines. Linoleic acid was first prepared by bromination and debromination procedure and then recrystallized twelve times for petroleum ether at —65° to —60° C according to the method of MATHEWS *et al.*[8] *cis, cis*-methyl linoleate (I.N., 172.2 and free of *trans* double bond) was prepared by first esterifying such linoleic acid and then distilling under reduced pressure. Methyl linolenate (I.N., 259.4) prepared by the usual bromination and debromination procedure showed some absorption characteristic of *trans* double bond in the infrared region.

Methyl oleate and linoleate were autoxidized to 10% peroxide content and methyl linolenate o 5%. Oxygen-free nitrogen was bubbled through all the samples as a precaution against further oxidation. The solvents, ether, alcohol, petroleum ether (b.p., 30°–60°), heptane were purified by the usual methods. All analytical determinations in this report were carried out on the substances isolated from solutions as the necessity arose due to experimental conditions.

Of four different samples (each, 25–30 g) of methyl oleate (a–d), two samples (a, b) were autoxidized in darkness at room temperature (25–30°) and the other two (c, d) under irradiation with ultraviolet at 35°. However, the degree of agitation in some of these samples was varied by employing a and c as a layer in a shallow dish in the still atmosphere of air and by agitating b and d contained in narrow cylindrical tubes (each, 1″ × 10″) by oxygen bubbled through a gas dispenser (Corning fritted glass). The peroxides formed were isolated by counter-current extraction[9]. The solvent pair used was, 87% alcohol and petroleum ether saturated with the same alcohol. Infrared analyses on (i) Peroxide concentrates (5540–5780 m.equiv./kg), (ii) recovered unoxidized materials revealed that the former contained all the *trans*-isomer absorbing at 10.36 μ and the latter none. The peroxide concentrates from the four autoxidized samples, a–d, contained respectively, 61–66%, 74–76%, 65–68% and 87–90% of *trans*-form. These estimates are based on comparisons with the absorption of methyl elaidate, assuming a constant molecular extinction coefficient for an isolated *trans* double bond. These variations in isomerization indicate the primary role played by oxygen itself during autoxidation under different environmental conditions.

In our initial experiments[10], the photo-chlorophyll oxidation of methyl linoleate was found to form linoleate hydroperoxide with an isolated *trans* double bond. Since certain percentage of the original linoleate also had an isolated *trans* double bond[10], it was not known definitely whether this gave rise to the peroxide with an isolated *trans* double bond in spite of much experimental evidence. In the present work, photo-chlorophyll oxidation of pure *cis, cis*-linoleate (25–30 g at a time) as a 30% solution in heptane below o° C yielded decisive results. Methyl linolenate (25–30 g at a time) has also been oxidized using the same technique in the presence of chlorophyll with similar results. Due to the instability of the peroxides, modifications in the procedures for isolation had to be introduced. The peroxides were concentrated by counter-current extraction[9] without letting the temperature rise above 10°. The peroxide concentrates (5980 m.equiv./kg in the case of linoleate and 5560 m.equiv./kg in that of linolenate products) were reduced by stannous chloride. The reduced products were extracted by cold ether. The ether extract was first washed by 3% HCl solution and then thoroughly by water and dried over anhydrous sodium sulfate. On removal of ether, the products were dissolved in precooled petroleum ether and passed through a 3″ × 12″ column packed with 1″ charcoal over 2″ dry sugar (dried in vacuum at 90° for 1 h) to remove any chlorophyll products and finally eluted by 1% ether in petroleum ether. The temperature was kept below 20° for linoleate and below 10° for linolenate.

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Chemical and physical analyses including infrared indicated that the products were monohydroxy linoleate and linolenate. Both types of products consisted of the similar types of isomers, absorbing moderately at 10.55 μ (*cis, trans* conjugated), strongly at 10.12 μ (*trans, trans* conjugated) and moderately at 10.36 μ (isolated *trans*).

The formation of the peroxides with the isolated *trans* double bond by the *cis, cis*-linoleate should indicate the formation of 11-hydroperoxide. Similarly, the isolated *trans* double bond in the case of linolenate peroxides should mean the formation of either 11-, or 14-hydroperoxide that has not been reported before. These observations establish one missing link in the field of autoxidation of methyl linoleate and linolenate. In no other autoxidation reactions for linoleate[10] and linolenate except this photo-chlorophyll oxidation, was such peroxide obtained. Peroxides, from autoxidation of linolenate[5], from catalyzed autoxidation by ultraviolet irradiation (3% oxidation being accomplished below 0° C), and from lipoxidase catalyzed oxidation carried out according to the previous methods[4,11,12] below zero degree (5% oxidation) in the case of linolenate soap, or in that of emulsion of methyl linolenate (by means of gum gutty) in the present investigation, did show small amounts of *cis,trans* and majority of *trans, trans* conjugated products by infrared absorption analyses. Great pains were taken in controlling factors, such as, temperature, pH, duration and extent of oxidation which have a profound influence on the final products[9].

It is, thus, evident that the hydroperoxide with the isolated *trans* double bond, once formed, is stable, undergoes no rearrangements and can be easily detected. It seems, therefore, probable that the rearrangement of the non-conjugated linoleate and linolenate almost entirely to conjugated products by autoxidation reactions in general should have occurred during the intermediate transition states involved in the reactions with oxygen. The changes in the geometric configurations of the different peroxidic products indicate isomerization under the same circumstances. These concepts are also borne out by the fact that the extent of isomerization during the autoxidation reactions varied under different environmental conditions for the different fatty acid esters, especially methyl oleate in the present work. Hence, the doubt expressed by SEPHTON AND SUTTON[6] regarding the presence of 11-hydroperoxide in the autoxidized methyl linoleate is confirmed by our experimental results. Similarly, the 11-, or 14-hydroperoxide has been proved to be absent in the autoxidized methyl linolenate. Anomalous results in literature on autoxidation of methyl linolenate obtained by various workers may be attributed to the extreme difficulties in isolating the highly unstable primary hydroperoxides.

The foregoing data on the initial stages of autoxidation reactions offer experimental evidence for, (a) the isomerization and other molecular changes occurring only in the autoxidized products of methyl oleate, linoleate and linolenate and none in their unoxidized fractions; (b) the occurrence of such isomerization (geometrical) during the intermediate activated stage involving energy transfer and leading to the formation of monomeric monohydroperoxides; (c) the activity of alpha methylenic groups (especially, that in 11-C in linoleate and 11-C, or 14-C in linolenate) in all autoxidation reactions in close proximity with the double bonds; and (d) the absence of hydroperoxides at Carbon, 11 in autoxidized linoleate, as well as the absence of those at Carbon, 11 or 14 in the autoxidized linolenate products. These findings may demand some modifications in the mechanistic concept of the initiation steps of autoxidation reactions.

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