INFRARED STUDIES ON SOME FEATURES OF METHYLENE-INTERRUPTED DOUBLE BOND IN AUTOXIDATION OF FATTY ACID ESTERS

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IN continuation of the previous studies[11] it is necessary to describe some specific features of the procedures for the isolation of methyl linoleate, and linolenate hydroperoxides. The investigation on autoxidation with and without agitation have been extended to methyl linoleate and linolenate. Infrared studies on the fatty acid esters and their hydroperoxides have revealed many clues to the mechanism of autoxidation reactions. On the basis of these data and the other experimental facts, the mechanism of the initial attack by oxygen molecule on methylene-interrupted double bonds (5-C system of methyl linoleate and linolenate) has been presented. Some light has been thrown on the catalysis of autoxidation by lipoxidase which is specific in autoxidizing only methylene-interrupted double bonds.

Methyl linoleate and linolenate were freshly prepared by bromination and debromination procedures. All samples were kept under nitrogen and vacuum below -50°C. Autoxidation of methyl linoleate and linolenate (50 g. each) to 10% peroxide contents, was carried out at -10°, 0°, and 24-26°C. in 500 ml. Erlenmeyer flasks without agitation. The same substances were autoxidized at -10°C. under constant agitation by oxygen as previously described.[11] The counter-current extraction between two immiscible solvents was found effective in concentrating the oxygenated products. Unfortunately, the standard procedures for the counter-current extraction[7] distribute the peroxides over several fractions, and under no conditions is the recovery quantitative. Hence, a scheme of manual counter-current extraction, (Fig. 1) was improvized to separate the oxidized from the unoxidized fraction quantitatively. Two immiscible solvents were first obtained by mixing absolute alcohol (distilled over KOH and zinc dust), light petroleum (30°-60°C., and free from unsaturates[14]), and water in the proportions 5:6:1. The resulting petroleum ether and alcohol phases were allowed to separate and used as stock solvents. Both the phases were then cooled to 0°C. In Fig. 1, the S-rows are twelve separatory funnels, each containing 200 ml. of the cold petroleum ether phase. The partially oxidized fatty acid ester was dissolved in the 200 ml. of petroleum ether in S_1. Twelve extractions, each with a 100 ml. portion (A) of the cold alcohol phase, removed all peroxides from S_1. The subsequent extractions, carried out successively in

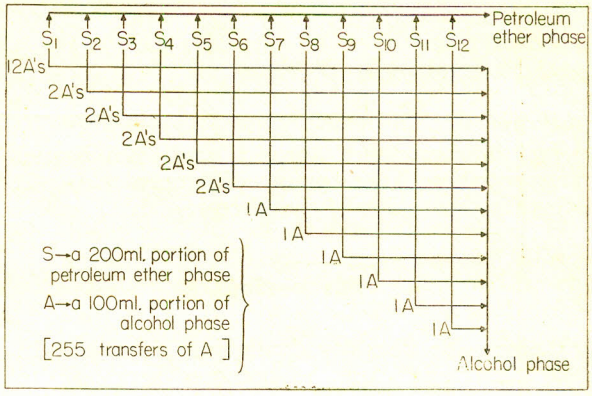

Fig. 1. A Scheme for the Manual Countercurrent Extraction.

S_2 through S_{12} by each of these alcohol portions (12A's, Fig. 1), recovered the majority of peroxides and distributed all unoxidized fatty acid ester together with some peroxides in the petroleum ether phase. These remaining peroxides, in S_2 to S_{12} inclusive, were extracted by the number of alcohol portions (2A's—1A—) indicated at the end of the vertical lines extending from the corresponding petroleum ether phase. This additional extraction of peroxides by fresh solvent is not feasible with the standard counter-current procedures.[7] Determination of peroxide values at different steps indicated the efficiency of separation. The peroxide-containing phase of alcohol was kept cooled below 0°C. The pre-cooled oxygen-free nitrogen was bubbled through the above alcohol phase as an added protection. The alcohol phase yielded in general 99.0-99.9% of the oxygenated products formed during the autoxidation of fatty acid esters. The unoxidized fraction obtained from the petroleum ether phase contained only 0.0-0.7% of oxygenated products. All precautions were taken to recover the oxygenated products entirely from the unoxidized fatty acid esters, so that the oxygenated products may represent the initial autoxidation process involved. For the convenience of analysis, an aliquot portion of the oxygenated products was reduced in a

1% alcohol solution by stannous chloride (5 moles/mole of peroxide), agitating with oxygen-free nitrogen.

Methyl linoleate autoxidized at 0° without agitation gave a peroxide concentrate representing 99.9% of the oxygenated products formed during the autoxidation reactions. The corresponding unoxidized fraction isolated was found to be pure methyl linoleate by the following analysis : hydrogen absorption, 1.98 mole/mole ; hydroxyl, 0.0 ; acid value, 0.0 ; carbonyl value, 0.0 ; and polymer, 0.0 ; elementary analysis, C, 77.47 and H, 11.52; calculated from methyl linoleate, C, 77.55 and H, 11.56. The polymer contents were measured by the special micro-technique in distillation.[16] The peroxide concentrate having the peroxide value of 6115 m.e./kg. (theory for methyl linoleate monohydroperoxide, 6125 milliequivalents per kilogram), added 2.92 moles H_2 to yield monohydroxystearic acids (m.p., 75.0-77.5°C;[3] hydroxyl, 1.04 moles/mole). The preliminary polarographic studies indicated that peroxide concentrate consists of 100.5% of hydroperoxide. The reduced peroxide concentrate gave the following analysis : hydrogen absorption, 1.99 mole per mole ; hydroxyl, 1.02 mole/mole ; acid value 0.0 ; carbonyl value, 0.0;[19] and polymer, 0.0 ; elementary analysis, C, 73.41, and H, 10.89 ; calculated for monohydroxy methyl linoleate C, 73.51, and H, 10.97. The infrared analysis of the above samples also indicated production of almost 100% *cis*, *trans*-conjugated isomer. Two other samples of peroxide concentrates obtained from methyl linoleate autoxidized under identical conditions gave peroxide values of 6100 and 6135 m.e./kg. respectively. It is, therefore, apparent that methyl linoleate forms monomeric monohydroperoxide with the conjugation of double bonds. Similar results were obtained with the autoxidation of methyl linolenate at -10°C. giving only *cis*, *trans*-conjugated hydroperoxides under otherwise identical conditions. Both methyl linoleate and linolenate involve only 5-C system during the autoxidation reactions in yielding hydroperoxides without any decomposition. The additional double bond in the latter enhances the reaction rate, emphasizing the role of π electrons (cf. arachidonate linolenate linoleate).

Under somewhat more drastic conditions, the hydroperoxides were found to decompose without initiating any polymer formation. The peroxide concentrates (99.0-99.7%) from methyl linoleate autoxidized below 0° under agitation (including one particular case in which 20% of the fatty acid ester was oxidized) gave peroxide values in the range, 5500-5650 m.e./kg. and the reduced peroxide concentrates showed polymer contents, 0.0. Methyl linolenate when oxidized at 0° without agitation yielded peroxide concentrates of 5690 m.e./kg. without any polymer formation. It may be possible that the peroxide decompositions under these conditions do not initiate active free radicals to give rise to polymers in conformity with the information available.[12]

In another series of experiments, the drastic conditions were used with the inevitable results of polymer formation. The peroxide concentrates from methyl linoleate and methyl linolenate, autoxidized at 24-26° without any agitation indicated peroxide values, 5400 m.e./kg. and 3450 m.e./kg. ; their reduced states had polymer contents, 16%, and 31% respectively. The agitation caused 20-25 times faster oxidation, accompanied by greater decomposition of peroxides and polymer formation. Bateman[2] and Bolland[4] with their co-workers have employed the conditions in these last series of experiments and consequently may not have taken into consideration the initial reactions of autoxidation that yield hydroperoxides quantitatively.

After establishing the proper conditions for study of the initial autoxidation processes, it is justifiable to obtain information on the structures of relevant fatty acid esters and oxygen and on their reactivity towards oxidation. With this end in view, the following attempts were made in order to throw some light on the mechanism of autoxidation.

The susceptibility of fatty acid esters to autoxidation, increasing with the number of methylene-interrupted *cis*-double bonds may be inferred from the infrared studies (LiF spectra) at the region of 3.30μ (Fig. 2.) on the isomers and derivatives of methyl linoleate. The amounts of absorption at this region for the different substances are : lino-elaidate (*trans*, *trans*-non-conjugated), nil (curve 1) ; conjugated isomers, equivalent to that of methyl oleate (curve 2) ; conjugated linoleate hydroperoxides (curve 3), conjugated isomer ; and methyl *cis*, *cis*-linoleate (curve 4), twice that of oleate. Following the observation of Adams and Auxier[1] in other fatty acids, the infrared absorption at 3.30μ for the linoleate isomers indicates consistently the reactivity of alpha methylenic hydrogen atoms and consequently arises due to $-CH_2-$. The stereochemical configurations play a great role in such reactivity. Herman and Reeves in their recent book on theoretical organic chemistry[9] have, on

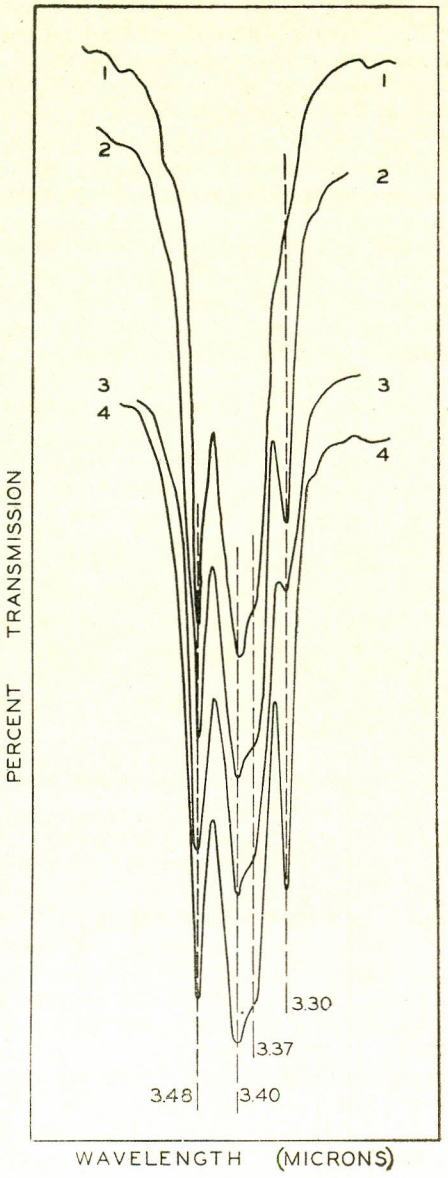

Fig. 2. Infrared Absorption Spectra. Active Methylene groups in the isomers and derivatives of Methyl Linoleate.

quantum mechanical basis, emphasized the non-localization of double bond and spread of electrons over 4-C in conjugated diene as in conjugated linoleate, affecting the alpha methylenic group just as a double bond. Similarly, the spread of π electrons over 5-C in the non-conjugated linoleate with an increased influence on alpha methylenic group is envisaged. The preliminary experiments with the linoleate isomers indicate the different rate of hydroperoxidation according to the alpha methylenic activity. The conjugated linoleate is already known to react with oxygen by ionic 1:4 addition (electrovalent bond) to form 6-membered ring peroxide and so is rubrene and anthracene by apparent covalent bonds. In between these extremes there should be many transition bonds, as it is known today about the cobalt complexes with oxygen molecule[5] and iron porphyrin complexes (oxyhemoglobin and methhemoglobin in particular).

On the other hand, O_2 contains an even number of electrons. However, the ground state of O_2 contains two unpaired electrons, which make O_2 paramagnetic. Though the oxygen molecule is very inactive, it can be attached as a ligand either in such a way that after pairing of those two electrons, a pair of electrons may be used in filling up the needs of substances to be oxidized, thus forming a 'dative bond' (cf. oxyhemoglobin), or in a binuclear complex with an oxygen bridge, each one of the unpaired electrons of O_2 may form a pair with those available from other reactants (anthracene, terpene, and rubrene and the conjugated dienes).

From the foregoing information, it seems probable that oxygen may act as a ligand to the five-carbon system of fatty acid esters through a number of transition complexes to overcome the energy barrier. These concepts in conjunction with the mechanism of oxygen-olefin interaction, previously reported,[12] the attack on 5-C system by oxygen molecule may be graphically represented as in Fig. 3. These processes (I-VII, Fig. 3) yield two positional isomers of linoleate conjugated hydroperoxides (9-, and 13-) which have been described as the products of autoxidation [3, 11, 12] The rearrangement of the non-conjugated peroxy radical to the conjugated form as proposed by Bateman[2] becomes open to question by the isolation of non-conjugated hydroperoxides from the photo-chlorophyll-oxidized linoleate[11,12] which probably involved free radicals. [12,20] Harrison and Wheeler[8] also isolated the non-conjugated products from the reactions of linoleate, supposedly involving free radicals. The limitation of the isomerization to one double bond next to the hydroperoxyl group [2,19] may also speak against allylic radical mesomerism. Furthermore, the complex formation may allow formation of *cis-cis*-conjugated products which have been reported by Sephton and Sutton.[18] It is difficult to predict the behaviour of the allylic radical on the surface of the protein moiety with many active centres in lipoxidase which also autoxidizes linoleate to the definitive products.

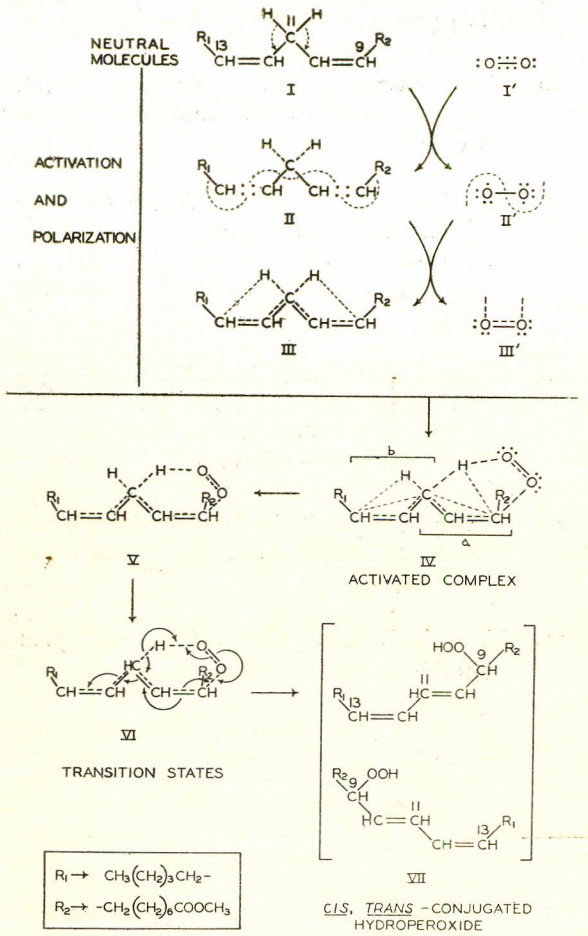

Fig. 3. Initial attack by oxygen molecule on methylene-interrupted double bonds.

However, the polar characteristics provided by the structures (I-IV, Fig. 3) may give rise to a certain specific charge distribution in the 5-C system of each molecule and explain the specificity[10] of lipoxidase-catalyzed autoxidation. Oxygen may act as a prosthetic group[10,15] in the ternary enzyme complex[6] consisting of enzyme, oxygen molecule and fatty substrate. The lipoxidase may, thus, incorporate each molecule of oxygen individually to a molecule of the fatty substance. The optical rotation of the lipoxidase-catalyzed autoxidation products is direct evidence of this organized individual attack on each molecule.

One particular batch of sodium linoleate was autoxidized in the presence of lipoxidase as described in the earlier publications.[13] The peroxide concentrate, isolated quantitatively by counter-current extraction, constituted 99.3% of the oxygenated products formed during the lipoxidase-catalyzed oxidation. On storage at -50°C. as a 1% solution in alcohol, the peroxide concentrate precipitated some gelatinous polymer materials (4.6 per cent of total peroxide concentrate by weight). The peroxides (94.7% of peroxide concentrate) were separated and reduced by stannous chloride. The reduced products were proved by chemical and infrared analyses to be same as the ordinary autoxidation products, except for the optical rotation. The polymer formation may be attributed to minor side reactions in a complicated system.

The mechanism proposed for methylene-interrupted double bonds (five-carbon system) may account for : (a) the energy requirements and the formation of only two positional conjugated isomers ; (b) the effects of different spatial and electronic configurations, and the possible olefin-oxygen interactions involving a ligand and thereby yielding the initial molecules of hydroperoxides ; (c) the independent hydroperoxidation in fatty acid esters below 0° for an indefinite length of time in complete darkness ; (d) the specific catalytic effects of lipoxidase in autoxidizing each individual molecule of fatty acids with the five-carbon systems and maintaining the plane of symmetry, responsible for optical rotation.

Further details will be published later.

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