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# STUDIES ON THE MECHANISM OF AUTOXIDATION OF FATS. PART I KINETICS OF CATALYTIC OXIDATION OF OLEIC AND LINOLEIC ACIDS

By S. Mukherjee

The mechanism of autoxidation of unsaturated fatty acids by use of catalyst has been studied up to a primary stage of oxidation.

Although the autoxidation of fats and simple esters of unsaturated fatty acids have been the subject of a number of recent communications (Farion, *Chem. Ztg.*, 1904, 28, 1196; Goldschmidt *et al.*, *Ber.*, 1934, 67, 1588; Rieche, *Z. angew. Chem.*, 1937, 50, 520; Farmar and co-workers, *Trans. Faraday Soc.*, 1946, 42, 228; 1942, 38, 340; *J. Chem. Soc.*, 1943, 119; 1946, 10), it has not been possible to explain the primary process of autoxidation and to completely establish the nature of the products formed. Instead, the observations of the later investigators stand largely in contradiction to those of the earlier workers in the field, and as such the non-critical acceptance of any of these theories presents certain difficulties which must be clarified before arriving at a decision in favour of any of the hypotheses. The early theories which have been evolved with regard to autoxidation of fats have been founded on some concept concerning the initial addition of oxygen to the unsaturated linkage and the majority of workers have accepted the theory of formation of a heterocyclic peroxide of the formula ( Farion, Goldschmidt, *loc. cit.*)

$$-\underset{\displaystyle O}{\overset{|}{CH}}-\underset{\displaystyle O}{\overset{|}{CH}}- \quad (1:2)$$

as the primary step in the autoxidation process. The existence of fatty acid peroxide containing such a configuration has been assumed on the basis of certain analytical data (viz. iodine and thiocyanogen value, peroxide, hydroxyl, carbonyl values, etc.) which have been interpreted as substantiating the existence of such a configuration in autoxidised unsaturated acids; but the more recent work of Farmer and co-workers (*loc. cit.*) has led to a considerable questioning concerning the validity of the cyclic peroxide theory. It appears to have been well established by Farmer that the autoxidation of all unconjugated olefinic compounds proceeds by addition of a molecule of oxygen to the carbon atom adjacent to the double bond to form a hydroperoxide having an intact double bond thus,

$$-CH_2-CH=CH+O_2 \longrightarrow -CH(OOH)-CH=CH-$$

The most fundamental advance in the chemistry of autoxidation of fats was made by Farmer and Sutton when they isolated for the first time a moderately

pure peroxidised methyl oleate by molecular distillation of a partially oxidised sample of methyl oleate. It was further shown from spectrophotometric measurements that autoxidation of polyethenoid substances, whose double bonds are separated by methylenic groups, resulted in the formation of conjugated diene isomers (Farmer, Koch and Sutton, *J. Chem. Soc.*, 1943, 541; Bergstrom, *Arkiv Kemi Min. Geol.*, 1945, 14, Bd. 21A, 1). Further substantiation of the hydroperoxide theory was obtained when Atherton and Hilditch (*J. Chem. Soc.*, 1944, 105) isolated substantial proportion of suberic and octoic as well as azelaic and nonanoic acids, products predicted by Farmer as the fission products of hydroperoxido-oleic acid from the oxidative disruption of an autoxidised sample of oleic acid, thus proving to a large extent (though not necessarily exclusively) that peroxidation had occurred at the $-CH_2-$ group adjacent to the ethenoid bond. However, on the basis of comparative studies of the autoxidation of methyl oleate and linoleate, Hilditch and co-workers (*ibid.*, 1945, 836; 1946, 1022; Hilditch, *J. Oil Col.-Chem. Assoc.*, 1947, 30, 1) disputed the concept that the attack by oxygen occurs only at the α-methylenic positions. Hilditch has suggested a mechanism in which oxygen adds directly to the double bond, forming a transitory cyclic peroxide, which then rearranges to give hydroperoxides. But nevertheless, the isolation of the methyl hydroperoxido-oleate by Farmer appears to render the cycloperoxide theory of autoxidation untenable, and if we once accept the hydroperoxide theory, a reinterpretation of a considerable amount of previous experimental work becomes necessary and much of the speculation and conclusions concerning subsequent stages of autoxidation is vitiated.

Now, there are two major points of difference between the formulation as proposed by Hilditch and the α-methylenic reactivity concept of Farmer *et al.* According to the former, a double bond shift must occur in all cases, even with a mono-olefines, if a hydroperoxide is the end product. The α-methylenic concept, on the other hand, permits the formation of hydroperoxides with or without the shift of double bond from its original position. Another point of difference is that whereas the Farmer's postulation provides for the propagation of the chain reaction, no such mechanism is provided by the Hilditch's formulation. Then again, acceptance of Farmer's hydroperoxidation mechanism as the primary course of autoxidation apparently presents certain difficulties. Firstly, it must be borne in mind that any detachment of an α-methylenic hydrogen atom from the olefin system to leave an active radical $-\dot{C}H \cdot CH=CH-$ (the first likely stage in the production of an α-methylenic -OOH group) will require the expenditure of 80 K. Cal. of energy which must be supplied from some not very obvious source; secondly, there exists numerous direct experimental evidences to show that conjugated compounds, including those in which the unsaturated centres are flanked by α-methylenic groups, undergo peroxidation additively at the double bond systems, leaving the adjacent α-methylenic group intact and iodine values of various olefinic substances inculding linoleates

(Paschke and Wheeler, *Oil & Soap*, 1944, 21, 52) have been found to fall during autoxidation to an extent that cannot be readily accounted for if it is assumed that all of the initial peroxides formed are hydroperoxides. For these reasons it has been considered of importance to make further studies on the mechanism of the autoxidation process to arrive at a more definite conclusion.

It may be stated that the complete understanding of the early stages of the autoxidation process has by no means been achieved and the two main points regarding which confusion still exists are (*i*) whether any cyclic (& polymeric) peroxides are formed and (*ii*) whether any appreciable amount of unconjugated diene hydroperoxides is formed. The data presented in the paper are not as clear cut as they might be, but these preliminary experiments reflect some light on the structure of the peroxides formed in the early stages of autoxidation of oleic and linoleic acids. A detailed study of the catalytic oxidation of the unsaturated acids, oleic and linoleic, has been carried out with special reference to peroxide formation and saturation of double bonds in direct relationship to the amount of oxygen absorbed.

It has been a common observation that if a sample of oleic or linoleic acid is shaken in a Warburg instrument, there is a gradual absorption of oxygen, and if aqueous KOH is placed in the inner cup, a fall in the manometer reading takes place. But the speed of the reaction is extremely slow such that it takes a week or so for a small quantity of the acid to be oxidised completely. The speed of the reaction can be highly increased by raising the temperature to 98°, but at this temperature it is very difficult to maintain the temperature of the bath rigorously constant owing to a large radiation losses. For this reason recourse was taken to a method similar to that of Banks (*J. Soc. Chem. Ind.*, 1944, 63, 8) used for the rapid testing of antoxidants for fats, where it was demonstrated that the presence of a mere trace of haematin (1/7000th mg.) was able to accelerate the oxygen absorption of aqueous suspensions of linoleic acid at ordinary temperature.

## EXPERIMENTAL

*Preparation of the Substrates.*—In such studies as mechanism and kinetics of oxidation the purity of the substance plays a prominent part. Hence, attention was paid to the purification of the substrates (viz. oleic and linoleic) employed in these experiments

*Oleic Acid.*—The U. S. P. grade oleic acid (I. V. 89.0) was distilled at reduced pressure and the middle portion of the fraction distilling at 232°-234°/15 mm. was collected. This was subjected to a further distillation at 0.1 mm. when the fraction coming out at 153° was collected. The I. V. of this sample was 89.9, identical with the theoretical value.

*Linoleic Acid.*—The B. D. H. linoleic acid (I. V. 178.0, theoretical value 181.2) when subjected to a similar distillation in vacuum at 17 mm. (b.p. 224°) and again at 1mm. at 147°, yielded an acid with I. V. 179 25. For further purification, the modern chromatographic adsorption separation method was taken recourse to. In applying chromatography to the preparation of pure linoleic acid in the present work, appropriate, reproducible and experimentally determined conditions have been employed to remove the complication introduced by the lack of visual means of differentiation between the adsorbed bands. The results of preliminary experiments indicated that the activity of adsorbent alumina was the most important factor. Brochmann's alumina (E. Merck) diluted with 4 times of its weight of inactivated alumina, prepared by the method of Fisk (*Paint Tech.*, 1945, 10, 85, 107), was found most suitable for the present work ; as the use of pure Brochmann's alumina resulted in poor separation it was found necessary to make such a dilution, determined by a set of preliminary experiments. By trial experiments the quantity of developing and eluting solvents to remove the fraction of low I. V. was determined and the proportions used in the following experiment have been found to be quite satisfactory for the present work ; 15 g. of linoleic acid, obtained by distillation in vacuum, as detailed above, were dissolved in 100 c.c. of petroleum ether (E. Merck) and adsorbed on a column of Brochmann's alumina using 1:4 dilution (100 g. in a tube of 7/8th inch dia.). The chromatogram was developed with 500 ml. of petroleum ether in course of one hour, which eluted a fraction of I V. 179.8. The pure linoleic acid fraction was obtained by rapidly eluting with 1500 c.c. of petroleum ether applying vacuum at the bottom, at the same time maintaining a positive pressure of $CO_2$ at the top. During the process of removing the solvent by distillation in vacuum, a slow stream of $CO_2$ was maintained throughout to prevent oxidation. The product obtained has an I. V. of 181.1. The acid can be taken as sufficiently pure for all practical purposes. The recovery of acid was 86%. By repeating this procedure of chromatographic separation a considerable amount of the pure acid was prepared and stored under $CO_2$.

*Measurement of Oxygen Absorption.*—The fatty acid emulsions were prepared after the method of Banks (*loc. cit.*) and were used directly for estimation of oxygen absorption using haematine catalyst (0.2 c.c. of a solution containing 7 mg./100 c.c.). Oxygen absorption was measured in the Warburg manometric apparatus in a manner similar to that described by Johnston and Frey (*Ind. Eng. Chem., Anal. Ed.*, 1941, 13, 479). The flask containing 500 mg. of the acid in suspension was flushed with oxygen for 3 minutes and connected to the manometer and shaken for 15 minutes in the thermostat at 37°±0 1° after which the haematine solution was tipped off from the side bulb and oxygen absorption followed manometrically. With both the acids a static method was resorted to.

In Tables I and II are shown the c.c. of oxygen absorbed by oleic and linoleic acids respectively with time.

TABLE I

*Oxygen absorption in the catalytic oxidation of oleic acid at 37°.*

| Time. | C.c. $O_2$ absorbed at N. T. P. No. 1. | No. 2. | No. 3. | Time. | C.c. $O_2$ absorbed at N. T. P. No. 1. | No. 2. | No. 3. |
|---|---|---|---|---|---|---|---|
| 1 hr. | 4.06 | 4.0 | 3.86 | 8 min. | 15.0 | 15.0 | 14.98 |
| 2 | 5.0 | 5.0 | 4.85 | 9 | 15.5 | 15.5 | 15.4 |
| 3 | 8.10 | 7.95 | 7.85 | 10 | 16.05 | 16.0 | 16.0 |
| 4 | 10.0 | 9.92 | 9.85 | 11 | 17.5 | 17.3 | 17.45 |
| 5 | 12.9 | 12.90 | 12.85 | 24 | 20.0 | 19.92 | 19.85 |
| 6 | 12.95 | 13.0 | 12.96 | 36 | 25.0 | 24.6 | 24.85 |
| 7 | 14.0 | 14.0 | 13.99 | 48 | 30.0 | 29.3 | 29.75 |

TABLE II

*Oxygen absorption in the catalytic oxidation of linoleic acid at 37°.*

| Time. | C.c. $O_2$ absorbed at N. T. P. No. 1. | No. 2. | No. 3. | No. 4. | Time. | C.c. $O_2$ absorbed at N. T. P. No. 1. | No. 2. | No. 3. | No. 4. |
|---|---|---|---|---|---|---|---|---|---|
| 15 min. | 2.00 | 2.00 | 2.00 | 2.00 | 120 min. | 20.0 | 20.0 | 20.0 | 19.9 |
| 30 | 4.85 | 4.80 | 4.80 | 5.00 | 180 | 25.10 | 25.0 | 25.1 | 25.0 |
| 45 | 6.45 | 6.40 | 6.45 | 6.45 | 210 | 30.2 | 30.0 | 30.0 | 30.0 |
| 60 | 8.02 | 8.0 | 8.0 | 8.0 | 300 | 40.95 | 41.0 | 41.0 | 40.86 |
| 75 | 10.05 | 10.2 | 10.0 | 10.1 | 600 | 43.9 | 44.0 | 44.0 | 43.90 |
| 90 | 15.0 | 15.0 | 14.93 | 14.95 | 1200 | 46.0 | 46.1 | 46.0 | 46.0 |
| 105 | 17.6 | 17.5 | 17.5 | 17.46 | 1500 | 46.8 | 46.9 | 47.0 | 46.45 |

These results are shown graphically in Fig. 1.

At each stage of oxidation carried out with a number of flasks the oxidised fatty acids were recovered by extraction with ether - absolute alcohol mixture (4 : 1), the solvent removed under vacuum, and the peroxide and iodine value determinations were made in the usual way. In Tables III and IV are shown the relationship between iodine and peroxide values with oxygen absorption, all the results being expressed in percentages for easy comparison.

TABLE III

*Catalytic oxidation of oleic acid at 37°.*

| Time. | $\%O_2$ absorbed. | Proxide value (obs.). | % Peroxide formation on basis of $O_2$ consumn. | % Peroxide formation on wt. basis. | Iodine value (obs.) | % Iodine No. | Drop in I. V. Obs. | Calc. |
|---|---|---|---|---|---|---|---|---|
| 2 hours. | 10 | 173 | 90 | 5.6 | 84.5 | 95 | 4.59 | 5.02 |
| 4 | 20 | 372 | 48 | 12.3 | 81.0 | 80 | 8.9 | 11.06 |
| 8 | 30 | 345.6 | 30 | 11.5 | 73.0 | 80 | 16.0 | 10.3 |
| 15 | 35 | 400 | 35 | 13.3 | 63 | 70 | 23.9 | 12.0 |
| 24 | 40 | 364 | 21.8 | 12.1 | 61.2 | 68 | 28.7 | 10.9 |
| 36 | 50 | 282 | 18.5 | 9.4 | 49.6 | 55 | 40.4 | 8.5 |
| 48 | 60 | 260 | 17.5 | 8.9 | 46.0 | 49 | 43.9 | 8.0 |

TABLE IV

*Catalytic oxidation of linoleic acid at 37°.*

| Time. | % $O_2$ absorbed. | Peroxide value (obs.) | % Peroxide formation on basis of $O_2$ consumn. | % Peroxide formation on wt. basis. | Iodine value (obs.) | % Iodine No. | Drop in I. V. Obs. | Drop in I. V. Calc. |
|---|---|---|---|---|---|---|---|---|
| 0.5 hr. | 10 | 1000 | 100 | 16 | 166.6 | 92.0 | 14.4 | 28.8 |
| 1.0 | 16 | 1650 | 100 | 27 | 156.3 | 86.7 | 24.2 | 48.6 |
| 1.25 | 20 | 2056 | 100 | 33.7 | 144.0 | 80.0 | 37.0 | 66.6 |
| 1.50 | 30 | 2985 | 98 | 49 | 129.6 | 72.0 | 51.4 | 88.2 |
| 1.75 | 35 | 5492 | 90 | 90 | 117.0 | 65.0 | 64.0 | 102.0 |
| 2.0 | 40 | 4650 | 70 | 80 | 95.4 | 52.0 | 85.6 | 144.0 |
| 3.0 | 50 | 3200 | 48 | 52.4 | 82.8 | 46.0 | 98.2 | 94.3 |
| 3.5 | 60 | 2750 | 40 | 45 | 72.6 | 40.0 | 108.4 | 81.0 |
| 4.0 | 80 | 2100 | 30 | 34.4 | 54.3 | 30.0 | 126.7 | 61.8 |

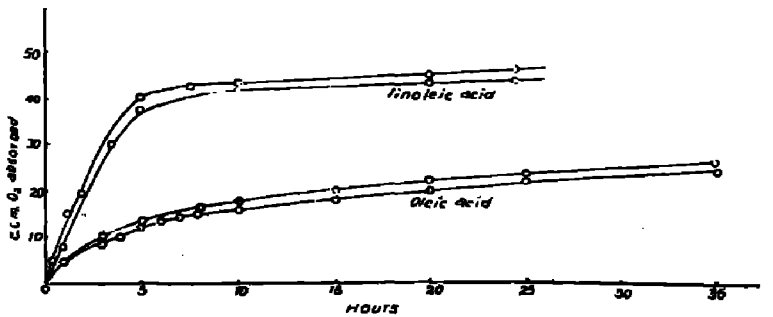
The figures in columns 4 and 7, viz. the percentage peroxide formation and iodine number are plotted against percentage oxygen absorption (column 2) in Figures 2 and 3.

In column 3 the peroxide values as determined experimentally are given; column 4 shows the theoretical peroxide value calculated on the basis of weight of oxygen absorbed, and on the assumption that peroxidation takes place entirely at the double bond; column 5 gives the percentages of peroxide formed calculated from the full theoretical peroxide value of 3050 and 6090. Column 7 gives the percentage iodine value, expressing the experimentally determined value as percentage of the theoretical iodine values, viz. 89.9 in case of oleic acid and 181.1 in case of linoleic acid, these being designated as 100%. Column 9 shows the drop in Iodine value calculated on the assumption that peroxide is formed at the double bond and its iodine value is nil.

## DISCUSSION

Figure 1 shows the oxygen absorption curves of the two fatty acids and it

FIG. 1




may be seen from the shape of the curve that in both the cases catalytic autoxi-

dation proceeds without any induction period, the velocity of autoxidation increasing with the double bond. In the presence of catalyst, the rate of absorption of oxygen is very vigorous at the beginning and falls off with time. It can also be observed that in no case even at the end of 48 hours the end-point in oxygen absorption is reached, although theoretically 100% oxygen has been consumed by the fat. The mechanism of oxidation of the two acids is discussed below.

*The Behaviour of Oleic Acid.*—From the results in Table III and the peroxide curve (Fig. 2) it can be observed that the absorbed oxygen is probably taken up only in the peroxide form at the initial stages, which corresponds to the oxygen absorption below 10%. The percentage peroxidation decreases afterwards and the curve becomes flatter in the later stages of oxidation. From the iodine number figures (columns 8 and 9), viz. drop in iodine value observed and that calculated from full peroxidation of the double bonds, it is clear that at the initial stage up to 20% oxygen absorption the double bond disappears quantitatively (Fig. 2) during autoxidation. Later on, the double bond does not disappear in this proportion of $O_2$ consumption which proceeds at a much enhanced rate than can be expected from saturation of double bond alone. The only logical conclusion is that oxygen apart from attacking the double bond probably also brings about further oxidation at some other place in the acid molecule, possibly of the reactive methylene group.

FIG. 2

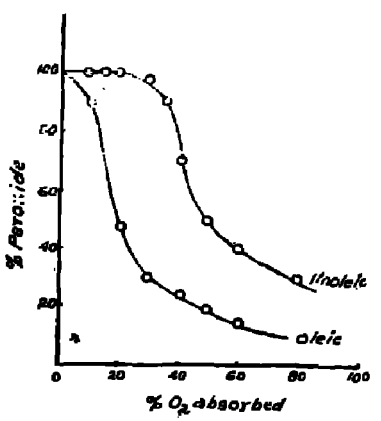
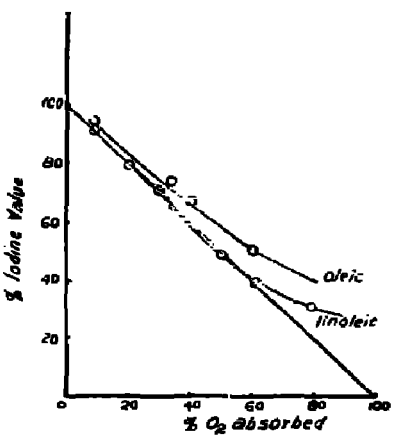



FIG. 3




*The Behaviour of Linoleic Acid.*—In the case of the more unsaturated linoleic acid, the absorbed oxygen can be seen to be utilised quantitatively in peroxidation of double bonds (Fig. 2) up to 35%, after which a sharp fall in peroxidation occurs and at 80% oxygen absorption, only one-third of the oxygen absorbed is used up in the formation of peroxides. The drop in the iodine value observed in this case is exactly half the theoretical value at the initial

stage where it can be seen from the peroxidation curve (Fig. 2), the oxygen is quantitatively used up in the peroxide formation. One can therefore at best conclude that for absorption of 1 molecule of oxygen only one double bond disappears. This relationship persists roughly up to 20% oxygen absorption (Table IV), after which the difference in the observed and calculated drop in iodine value is not very regular to arrive at a definite conclusion, though it may be sufficiently accurate to say from the intermediate position of the observed drop that further saturation of the other double bond proceeds at later stages in oxidation. But, it is clear from the iodine number curve (Fig. 3) that the rate of decrease of iodine number with oxygen absorption remains fairly constant far above the region of 100% peroxide formation (20% $O_2$-absorption) and this linear relationship is broken nearly at 50% $O_2$-absorption, indicating that an intensive oxidation proceeds simultaneously with the addition of one oxygen molecule at the double bond.

With both the oleic and linoleic acids, however, in the final stages of oxidation the observed drop in iodine value has been greater than the calculated value which apparently seems anomalous. But if one considers the corresponding peroxide curves showing quite appreciable break or inflexion, signifying considerable and abrupt decomposition of primary products in the course of oxidation, it will be logical to assume that the observed discrepancy is due to the disappearance of the double bonds by polymerisation or condensation reactions which accompanies decomposition reactions.

Thus far it can be concluded that during the initial stages of oxidation (catalytic) of the unsaturated fatty acids, the oxygen absorbed is used totally in peroxide formation, and that the peroxides are formed entirely at the double bond and with diethenoid compounds only one of the double bonds is saturated by oxygen with a considerable velocity resulting in the formation of peroxides. Indications have also been obtained during the period of intensive oxidation following the initial stage of full saturation of double bonds by peroxidation, of the probable oxidation at the active methylene group, such that the observed drop in iodine value is less than that can be expected from complete saturation of double bonds by the formation of cyclic peroxides, and further work is needed to find out the nature of the products of oxidation when enhanced oxidation is taking place.

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DEPARTMENT OF APPLIED CHEMISTRY,
UNIVERSITY COLLEGE OF SCIENCE,
CALCUTTA.

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