XCVI. AUTOXIDATION OF THE FATTY ACIDS.

I. THE OXYGEN UPTAKE OF ELAIDIC, OLEIC AND STEARIC ACIDS.

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THE various experiments carried out in the past to elucidate the nature of the absorption of oxygen gas by substances containing the ethylene linkage, more particularly when present in the unsaturated oils, have shown that this oxidation process yields somewhat complex products. In the case of the drying oils, which, when exposed to the air in thin films, absorb oxygen rapidly and become gelatinous, the complex nature of the products may be largely due to the existence of different fatty acids each containing two or more double bonds. In the case of a mono-ene, such as oleic acid, the oxygen uptake is almost negligible at ordinary temperatures, but the products of autoxidation should be less complex. It has been found possible greatly to accelerate the autoxidation of this acid and of its *trans*-isomeride elaidic acid, particularly by high dispersion of the acid, but also by the use of a catalyst and by moderate increase of temperature. It was found, however, that even stearic acid was liable to an appreciable autoxidation under these conditions if the temperature exceeded 75°. To avoid, as far as possible, any autoxidation other than that concerned with the ethylene linkage, it was necessary to work below this temperature.

In the earlier stages of the present work it was observed that when oleic acid was intimately mixed with about 1% of a catalyst in the form of cobaltous oleate, at summer temperatures oxygen was taken up by the highly dispersed acid, causing a considerable reduction in the iodine value after a few days' exposure. An idea of the oxygen uptake of this acid, partially purified, is indicated by some of the results given in Table I (p. 798). The product was an oil and was liable to be contaminated by impurities in the original acid. For this reason elaidic acid, the *trans*-isomeride of oleic acid was chosen for investigation. It can be obtained readily in a pure state and does not absorb oxygen when in its crystalline form, but when it is kept at temperatures slightly above its melting-point of 45°, appreciable absorption takes place, the rate depending as mentioned above upon dispersion, the catalytic agency and temperature. Roscoe and Schorlemmer [1890] observe that this acid will absorb oxygen when kept molten, and in about 14 days will change to a

yellow oil. It will be shown below that this period has been reduced to as many hours.

Slow oxidation exposes the first product or products to further change and so leads to possible complications. For this reason alone a high degree of dispersion of the acid was desirable, so as to bring the autoxidation into line with the oxidation reactions of solutions. Attempts to bring about the absorption of oxygen by elaidic acid in solution have been made. A closed oxygen system of measured volume was caused to circulate for some days through solutions of the acid in different solvents and containing about 0.05 % of cobaltous elaidate either dissolved or in suspension. When boiling solutions were used a simple method for circulating the oxygen was adopted. The vapour from the boiling solution was led to the top of a condenser. From the bottom the condensed vapour fell in small drops from a constriction. These drops fell through the vertical portion of a T-piece with a tube of small bore. A Sprengel action was set up, and oxygen gas, entering by the third arm of the T-piece, was carried in bubbles through the boiling solution. Unabsorbed oxygen eventually found its way round the condenser to the T-piece. A mechanical device for circulating the oxygen through cold solutions at room temperatures was also employed. In neither case was the rate of oxygen uptake comparable with that obtained by surface dispersion of the acid. Although the former methods are still being pursued, the latter have yielded the results which form the subject of the present communication.

Preparation of elaidic acid and the catalyst.

Fairly pure oleic acid was converted into its sodium salt, and from its solution the lead salt was precipitated, washed, dried and extracted with ether. The filtered ethereal solution was shaken with dilute hydrochloric acid until all the lead was removed, washed, dried and evaporated. The oleic acid so obtained was placed in a shallow dish in a desiccator, which contained sodium nitrite in place of the drying reagent. A little nitric acid was poured on to the nitrite and the lid of the desiccator closed firmly. The fumes of nitrous acid were readily absorbed, and in less than 24 hours the oleic acid changed to a solid mass. This was spread on absorbent paper to remove adhering oil and nitrous fumes and was recrystallised from dilute alcohol until it gave a sharp melting-point of 44.5° . Previous to weighing the acid was dried in a vacuum desiccator over sulphuric acid.

The catalytic action of cobalt, manganese, lead and iron as "dryers" in the paint industry has been found to apply to the autoxidation of elaidic acid. Comparative experiments show that cobalt is appreciably more active than are the other metals. A number of estimations of the oxygen absorbed for different forms of these various catalysts and for different periods will be described elsewhere.

In the recent experiments of this work, cobaltous elaidate has been used to the extent of not less than 0.1 g. of the salt to 20 g. of the acid, corre-

sponding to an introduction of about 0.05 % of the metal. The salt was prepared by precipitation, a solution of cobaltous chloride being added in equivalent quantity to that of a solution of sodium elaidate. The pale pink powder is anhydrous and readily soluble in alcohol to form a brown solution. It is slightly soluble in other organic solvents. It is incorporated with the acid by mixing ethereal solutions containing a little alcohol, or by dissolving the salt in the molten acid. The small proportion used imparts a faint brown coloration to the otherwise colourless molten acid.

The use of a large proportion of sand as the dispersing medium in more recent experiments may have introduced other catalytic agencies. Although acid-washed, the sand is never free from iron, which appears to exercise a pronounced catalytic action, at any rate after a period of exposure, during which, perhaps, the iron assumes a suitable soluble form.

The apparatus employed to bring about autoxidation.

A number of different forms of apparatus have been made use of. A convenient method for keeping the acid just above its melting-point and in the presence of a closed oxygen system was to introduce it on to the inner surface of a large flask, the surface being increased by the aid of powdered glass. The heat was supplied from a small electric candle lamp introduced into the centre of the flask, connecting wires passing through the rubber cork. A glass tube connected the oxygen in the flask with an outside supply, and a surrounding tin cylinder served to retain the heat and keep the acid in a molten state, the temperature adjustment being made by movement of a partial covering to the cylinder. With a 2-litre flask this method proved suitable for the autoxidation of 3–5 g. of the acid. The heat was not well distributed and a more evenly oxidised product was obtained by dispersion of the acid on filter-papers, loosely folded and made to fill a flask immersed in a water- or oil-bath. The application of these methods has provided a number of facts to be recorded, but the most valuable results have been obtained from the more recent experiments in which sand was used as the dispersing medium. About one part by weight of the acid was mixed with 80 parts of purified sand. It was calculated, by counting the number of grains in a small weight and assuming their shape to be near that of a hemisphere, that the surface area over which 10 g. of acid was dispersed approximated to 8 m.² The acid, admixed with the catalyst as described above, was poured molten on to the sand contained in a dish over a water-bath. The acid and sand were thoroughly well mixed so that the latter had the appearance of an evenly distributed "dampness." After cooling the caked mass was powdered and introduced into the glass container in which the acid was to be oxidised. In some cases, when particular stress was laid on the determination of the rate of oxidation, a differential apparatus was employed, which corrects for variations in atmospheric conditions. It was introduced by Haldane [1920] and has been extensively used for measurements of oxygen uptake by a number of different

workers. It does not lend itself to the estimation of volatile products, nor to the removal of carbon dioxide liable to impede the rate of oxidation. The most satisfactory apparatus is one which includes a method of circulating oxygen through an oxidising chamber and through absorption tubes. The method adopted in the majority of the experiments to be referred to will be described briefly by reference to the accompanying diagram from which, for clearness, some details of the apparatus have been omitted. The glass cylinder *A*

Fig. 1. Autoxidation of fatty acids.

- A.* Autoxidising cylinder immersed in water-bath.
 - B.* Absorption apparatus for volatile products.
 - C.* Valves.
 - D.* Water column.
 - S.* Intermittent syphon.
 - H.* Oxygen gas holder.
 - I.* Recording drum.
- } Oxygen-circulating mechanism.

is of sufficient capacity to hold 800 g. of sand containing 10–20 g. of elaidic acid. The rubber corks are covered on their inner surfaces with tin foil. Layers of cotton-wool keep the sand from choking the inlet and outlet tubes at either end. This container is designed to ensure, as far as possible, an equal degree of oxidation throughout the mass. The oxygen and volatile products pass out by the upper small-bored tube and are led to the absorption tubes *B* containing calcium chloride and soda lime. The oxygen now passes to the circulating mechanism *C*. Two mercury valves *v* and *v'* will allow of gas passing only in the direction of the arrows. The mercury in the large bulb is given an

up and down motion by the water movements in *D*. The height of the water column in the wide tube *D* must be about 13·6 times the difference required in the mercury levels at *c*, *c'*. A water supply *E* flows continuously into *D*, which is emptied intermittently by the syphon tube *S*. The intermittent action, essential to the working of this circulator, is only possible when the bore of the syphon tube lies between 3 and 4 mm. Another necessary condition is that the bend of the syphon must not be constricted, but rather widened out. When *D* is emptying the syphon tube is full, when it is filling the inner portion of the syphon is a broken column of water, which on passing over the bend must flow down the slanting outer portion of the syphon as a continuous stream. This circulator if properly constructed will work continuously without any trouble. Temporary stoppage of water or slight variations in its pressure do not impede its efficiency. The oxygen is now forced into the holder *H*, which consists of a bell-jar moving up and down an annular space containing mercury. The mercury displacement is accurately compensated so that the holder delivers oxygen at the existing atmospheric pressure, and adjusts itself to all pressure changes. It is graduated to deliver known volumes of the gas. A T-piece at *T* enables the holder to be filled with oxygen as required, without interfering with the gas measurements involved in the experiment. This holder is described elsewhere [Ellis, 1931]. Finally the oxygen is returned to the bottom of the oxidising cylinder. A revolving drum *I* gives a graph of the oxygen uptake. The pen is attached to the weight balancing the bell-jar of the oxygen gas holder.

Over 40 different experiments have been undertaken with apparatus such as has been described above. Many of them were chiefly for the purpose of obtaining the oxidised product for chemical investigation but a number have furnished information on the rate of oxidation and on the amounts of oxygen uptake by elaidic, oleic and stearic acids. Table I contains the more important results obtained.

The rate of oxygen uptake.

In the absence of a catalyst, other than that afforded by absorbent paper, there is an induction period characteristic of the autoxidation of the drying oils. This period may last a day or two at temperatures below 55°, but above 70° it tends to disappear. Further, the induction period is lessened if the acid is heated for a short period to about 70° and then cooled to 55°. In an experiment particularly designed to show the difference of ordinary and catalytic autoxidation a differential apparatus comprising three bulbs was constructed. One of these bulbs acted as the control, a second contained 0·68 g. of elaidic acid dispersed on filter-paper. The third contained a similar quantity of acid and paper with the addition of the catalyst. Although at 62°, after 24 hours, the acid without the catalyst was only just commencing to oxidise, and in 3 days' time only 13·8 cc. of O₂ had been absorbed, the velocity increased with time and on the 4th day a volume nearly equal to that obtained for the previous 3 days was absorbed. In the case of the catalytic oxidation over

70 cc. were absorbed during the 1st day and the rate fell after this period. Without the actual addition of a catalyst the rate of the autoxidation of elaidic acid increases with time. It is therefore autocatalytic as has been observed in studies on the autoxidation of linseed oil [Genthe, 1906]. In the presence of a catalyst such as cobalt the rate falls from the commencement. This was shown in another experiment on 0.7186 g. in a differential apparatus. The actual hourly readings taken from the start, which represent the cc. of oxygen absorbed were: 12.2, 9.3, 8.4, 7.2, 6.4, 5.1, 4.2 and 3.4. After this period the fall was slow.

The experiments mentioned above, together with other similar ones, gave results only of value up to the periods given, beyond those periods the results were vitiated owing to the accumulation of carbon dioxide, which was not readily removed in the apparatus employed. In the experiments giving the results set out in Table I absorption tubes for removing volatile products and a circulating oxygen system as above described were employed. In recent experiments with the latter apparatus it has been the object to allow 20 g. of the acid to autoxidise as quickly, and at as low a temperature, as possible. By this weight of acid at about 74° an absorption of about 300 cc. of oxygen per hour can be procured. In all these experiments it was observed that there was never any definite cessation of oxidation. There was always a persistent absorption which, although less than 15 % of the maximum rate, was more in regularity and amount than could be accounted for by the decrease in the product of the originally reacting masses of what is probably a bimolecular reaction.

Fig. 2. Curve showing the uptake of oxygen by 5 g. of elaidic acid at 67° – 70° . The ordinate represents cc. at room conditions (769 mm., 15.5°). Each atom of oxygen taken up per mol. of the acid is represented by a volume of 207 cc.

[From a photograph of an actual curve obtained by the apparatus shown in the accompanying diagram.]

The graph shown in Fig. 2, obtained as a result of Exp. 11 given in Table I, illustrates the above observations. In Part II it will be shown that a number of oxidation products are produced and that there is appreciable subsidiary oxidation to that which may be regarded as the main initial one.

The curve shown, although simple in its form, is that of a number of

processes, and shows, in particular, a high initial rate of autoxidation followed by a constant reaction velocity, for the latter part of the graph is a straight line except for a slight drop during the 46th hour. This was due to an accumulation of carbon dioxide following a temporary blockage in the soda lime absorption tube. From the results of other experiments extending for some days evidence goes to show that the autoxidation process or processes represented by the straight part of the graph will persist for many days. Oxidation that does not give rise to a reduction in the active mass is due probably to the gradual break-down of the fatty acid chain, and the continual evolution of water and carbon dioxide is in support of this view. It may be that this curve is a near representation of the biochemical oxidation of the fats in the animal organism.

The amount of oxygen absorbed.

Although there is an indefinite continuation in the autoxidation of elaidic acid, attempts have been made to judge of the amount of oxygen that might be concerned in an initial process. For this purpose the oxidation was brought about as rapidly as possible by high dispersion on sand and at a rate already intimated. It is to be realised, however, that the ethylene linkage is liable to produce an initial oxidation product highly susceptible to further change, and that even in the case of oxidation in solution, when both the acceptor and the oxidising reagent may be dispersed in molecular form, the amount of oxygen absorbed is generally in appreciable excess of that which may be required for an initial oxidation product.

In Table I the actual volumes of oxygen absorbed are set out in column 4 and in the next column the calculated percentages by weight of oxygen absorbed are given. The results of the first four experiments given are those obtained by deliberately stopping the oxidation before the marked decrease in the velocity of the reaction had taken place. It was possible to make an approximate determination of the unoxidised acid, and so arrive at the amount of oxygen absorbed, under conditions that would tend to eliminate any secondary oxidation process. The amounts of the unchanged acid, recovered in a crude form, are given in column 8. These results indicate that nearly 20 % by weight of oxygen was absorbed by the oxidised portion of the acid. It will be noticed that the periods set out in column 3 of Table I vary considerably for a given amount of oxidation. This variation is due to different degrees of dispersion of the acid and to different temperatures employed. A third cause may be variation in the effective incorporation of the catalyst. The results of Exp. 5 are in agreement with the above, for a small quantity of the unchanged acid was recovered. Exp. 6 shows the effect of oxidation at a slightly higher temperature. In Exp. 7 and the subsequent experiments traces of the unchanged acid were isolated, but oxidation had been allowed to proceed until a nearly constant, slow rate of oxygen uptake was reached. Exp. 9 shows the effect of prolonging the oxidation and Exp. 10 the effect of oxidation

Table I. *Oxygen uptake of fatty acids.*

No. of exp.	Weights taken g.	Duration and temperature	Observed oxygen absorbed cc. at N.T.P.	Per-centage weight	CO ₂ % by wt. as oxygen	H ₂ O % by wt. as oxygen	Remarks
Elaidic acid.							
1	5.1348	12 hrs. at 63°	358	10.0	—	—	51 % } 45 % } 26 % } 37 % } Impure elaidic acid recovered
2	8.1077	2 days at 60°	692	12.2	—	—	
3	0.7186	6 hrs. at 67°	60.0	13.7	1.8	—	
4	10.000	18 hrs. at 60°	1088	15.5	2.5	2.4	
5	10.050	36 hrs. at 65°	1198	17.0	3.5	3.2	
6	10.050	17 hrs. at 75°	1517	21.7	4.4	1.1	Higher dispersion
7	19.376	3 days at 60°	2748	20.3	3.6	—	—
8	20.000	5 days at 55°	3287	23.5	4.2	—	—
9	10.050	{ 2 days at 65°	1484	21.2	4.0	3.0	—
		{ 4 days at 65°	1824	26.0	5.3	4.7	
10	6.000	3 days at 60°	784	18.7	3.2	5.6	} No catalyst
		14 days at 60°	1224	29.2	5.5	12.1	
11	5.000	68 hrs. at 70°	1088	31.1	—	14.8	See graph
Stearic acid.							
12	1.5558	11 days at 80°	63.9	16.4	5.5	5.2	} Cobalt stearate as catalyst
13	0.2281	6 days at 75°	8.2	5.1	—	—	
Oleic acid.							
14	5.000	{ 3 days at 14°	97	2.8	0.1	0.7	} Two periods of one experiment. Impure acid with cobalt oleate as catalyst
		{ 2 days at 65°	692	19.8	6.3	3.4	
15	10.091	23 hrs. at 37°	498	6.4	0.4	—	Fairly pure acid

in the absence of any other catalyst than may be afforded by the sand used for dispersion.

If 3 atoms of oxygen are concerned in the oxidation of each molecule of elaidic acid the uptake would amount to 17.0 %. If 4 atoms were so concerned a percentage of 22.7 would be expected. When the disturbing factors dealt with under the headings of the next two paragraphs have been considered, it will be realised that the lower percentage is all that can be expected to represent any initial oxidation process.

A comparative autoxidation of stearic acid.

It does not follow that any oxidation of elaidic acid, taking place elsewhere than at the ethylene linkage, would take place at a similar position in stearic acid. The comparison, however, is of interest. It was necessary to work at temperatures above 69°; otherwise the same technique was applied. At 75° and in the absence of a catalyst stearic acid did not take up oxygen in more than insignificant amounts, but in the presence of a small proportion of cobalt stearate the autoxidation became appreciable.

A comparison of the results obtained in Exps. 12 and 13 shows that there is a considerable difference in the rates of oxidation at the two temperatures 75° and 80°. At 80° the rate is comparable with that of elaidic acid, but it will be noticed that longer periods of exposure for both experiments were made. The nature of this oxidation has not been fully investigated. The products appear to represent a complex mixture, which is, however, largely

crystalline; and may lend itself to separation more readily than the products obtained by Salway and Williams [1922] and by Zorner [1923], who have oxidised the acid at 120–130° by passing oxygen through in the presence of manganese stearate. The large proportion of carbon dioxide and water evolved would indicate a degradation of the chain, and it must be supposed that this would happen even more so with elaidic acid under the same conditions. In the latter case however the lower temperature and much shorter periods employed have probably largely eliminated what would appear to be an extra autoxidation process to that concerned with the ethylene linkage. Further investigations of the autoxidation of stearic acid seem desirable. The results of such might throw further light on the nature of biochemical processes in fat metabolism.

The carbon dioxide and water formed.

In columns 6 and 7 of Table I are set out the approximate percentages of carbon dioxide and water formed. The figures represent the respective weights of oxygen contained in the two products, so that the sum of their weights deducted from the total oxygen uptake, as given in column 5, represents the weight absorbed by the main product. The results were obtained, as described above, by the continual circulation of oxygen through the system which included a calcium chloride tube and either potash bulbs or a soda lime tube. The circulation was continued for at least 24 hours after the autoxidation had been brought to a termination by removal of the source of heat. The figures obtained for water are only approximate owing to the presence in the absorption tube of volatile products. When sulphuric acid was used in the earlier experiments, this darkened considerably. That carbon dioxide was the main product absorbed by the potash bulbs was shown in the case of Exp. 9. The gas was liberated after its absorption and also from a control amount of the potash used: hence it was ascertained that at least 95 % of the increase in weight of the potash bulbs was due to this gas. In earlier experiments, before it was realised that appreciable volumes of this gas were being formed, the autoxidation was brought to an apparent cessation owing to its accumulation. If 1 atom of carbon were removed as carbon dioxide from a molecule of elaidic acid, the percentage of oxygen absorbed and evolved as this gas would amount to 11.3.

There is no doubt about the formation of quantities of water approximately represented by the figures shown; every precaution has been taken to ensure dry initial conditions. This product collects in the communicating tubes when the rate of autoxidation is at its height, and is only admixed with a small proportion of other volatile products. If from 1 molecule of the acid there was an evolution of 2 atoms of hydrogen as water, the oxygen concerned would represent 5.66 %.

Neither product seems to be present in an amount which points to its being concerned in the main initial autoxidation process, but both are formed in

appreciable quantities and may represent a degradation of the chain, comparable with that which stearic acid appears to undergo.

Deducting the oxygen evolved in these volatile decomposition products from the total absorbed, it is seen that 2 or 3 atoms are taken up by a molecule of the acid to form the non-volatile portion of the oxidation products. The results are lower than those obtained by Skellon [1931] by the autoxidation of pure oleic acid at considerably higher temperatures and for longer periods.

SUMMARY.

1. Whereas oleic and elaidic acids do not absorb oxygen gas at ordinary temperatures except in negligible amounts, conditions have been devised under which autoxidation proceeds at such a rate as to cause an uptake by either acid of over 20 % of oxygen in from 24 to 48 hours. High dispersion of the acids is an important factor. Purified, dry sand has been employed as a successful dispersing medium. Autoxidation of elaidic acid in solutions of different solvents has not, as yet, been observed. The presence of about 0.1 % of the cobaltous salt of the acid provided an efficient catalyst, which has either reduced, or caused to disappear altogether, a more or less prolonged induction period. Warm conditions are essential. Below 55° the oxygen uptake by elaidic acid is slow. Above 75° stearic acid shows appreciable autoxidation under similar conditions of dispersion and catalytic agency. Hence oleic and elaidic acids have been allowed to autoxidise below 75° in order to avoid the possible introduction of an autoxidation process secondary to that concerned with the ethylene linkage.

2. Water and carbon dioxide are evolved during the autoxidation. In the case of elaidic acid, a number of determinations show that these are produced in amount considerably below that corresponding with a molecular proportion to the acid. Their formation appears to be due to secondary reactions, which continue indefinitely and probably represent a gradual degradation of the fatty acid chain.

3. The observations on the oxygen uptake of elaidic acid seem to show that under the most advantageous conditions for bringing about only an initial oxidation process, 3 to 4 atoms of oxygen are absorbed, and that 2 or 3 of these are retained by the non-volatile autoxidation products. The chemical nature of the latter will be dealt with in Part II.

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