Autoxidation of the Fatty Acids

3. THE OILY PRODUCTS FROM ELAIDIC AND OLEIC ACIDS. THE FORMATION OF MONOACYL DERIVATIVES OF DIHYDROXYSTEARIC ACID AND OF $\alpha\beta$ -UNSATURATED KETO ACIDS

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In Part 1 (Ellis, 1932) it was shown that finely dispersed elaidic and oleic acids, when exposed to oxygen at temperatures of about 65°, showed an uptake of over 20 % by weight in a few hours. About one-fifth of this oxygen was evolved as carbon dioxide and an appreciable but uncertain amount of water vapour was also given off. In Part 2 (Ellis, 1936) it was shown that the main crystalline product of these autoxidations for both acids was elaidic acid epoxide. Suberic, azelaic, heptane-1-carboxylic and octane-1-carboxylic acids were also isolated, but of the 120 % yield of products of a typical autoxidation there still remained some 68–76 % of oily products unidentified. The components of these oils have now been largely identified. They contain the monoacyl derivatives of dihydroxystearic acid, $\alpha\beta$ -unsaturated keto acids, a colourless oil regarded as a dimeride of the latter acids, and coloured polymerized products of the unsaturated keto acids, together with small yields of peroxide and, apparently, of β -ketone-containing materials. (This term is used to denote acids in which the olefinic linkage is $\alpha\beta$ to the keto group—thus, C:C.C:O—not $\alpha\beta$ to the carboxyl group.) Further observations have been made on the amounts of carbon dioxide evolved at different stages of the autoxidation process and on the oxygen uptake in the more recent and lower-temperature experiments. Also, in the Experimental part, some account is given of improved methods whereby the autoxidations are brought about at appreciably lower temperatures than previously, resulting in very considerable changes in the composition of the products formed.

Formation of monoacyl derivatives of θ -dihydroxystearic acid (isomer of m.p. 132°). These probably occur in the oils as pairs of structural isomers and are of the type shown in the formulae:

Evidence given below shows the readiness with which elaidic acid epoxide combines additively with acids present, also in the free state, as autoxidation

products. This is especially so at the higher temperatures and during the longer periods employed in the non-catalytic autoxidations. In the more recent experiments which have been conducted at temperatures of about 50° these monoacyl derivatives have almost disappeared from the oily products.

Formation of $\alpha\beta$ -unsaturated keto acids. The sharp, but not unpleasant, odour which invariably accompanied the saponification of the oily products and which had hitherto been attributed to traces of non-aldehyde due to oxidative fission, has now been shown to be due to a mixture of methyl heptyl ketone and nonaldehyde which could be isolated quantitatively. The source of the methyl heptyl ketone was at first thought to be a β -ketonic acid. Enol reactions were given very distinctly by some of the oily fractions but not by the light petroleum-soluble fractions, which gave the higher yields of the ketone. The presence of a 1:3-diketone was considered. This at first seemed the more probable since the pyrazoline colour reaction for such compounds was very marked. It was ascertained, however, that this reaction could be obtained by omitting the reduction process as applied to the test. It became apparent that an unsaturated ketone grouping would explain both this colour reaction and the hydrolysis not only to the methyl heptyl ketone, but also to the nonaldehyde. There evidently existed in the oily products of autoxidation, presumably in the *cis*-form from oleic acid and in the *trans*-form from elaidic acid, the two $\alpha\beta$ -unsaturated keto acids:

These compounds may be regarded as Schmidt and Claisen condensation products. (I) would be formed by the action of sodium hydroxide on azelaic semialdehyde, and methyl heptyl ketone and (II) would be formed from nonaldehyde and octan-8-one-1-carboxylic acid. The reversed reactions have been conveniently effected in this work by hydrolysis with 0.05N-sodium hydroxide, as compared with the 10 % solutions used in condensations described by Schmidt (1880). Two of the four hydro-

lysis products of (I) and (II) have been mentioned above. Evidence for the presence of the other two has been obtained, the aldehyde rather as a polymerized product.

The formation of these hydrolysis products fixes the positions of the keto groups. There is no evidence for supposing any movement of the double bond from the original position. Many unsaturated ketones do show $\alpha\beta \rightleftharpoons \beta\gamma$ tautomerism, but generally owing to asymmetrical grouping about the double bond, as has been demonstrated in the extensive researches on these compounds by Kon and his co-workers (see Birch, Kon & Norris, 1923). Straight-chain unsaturated ketones have been studied by Blaise (1905) and later by Eccott & Linstead (1930), who showed that the butyrideneacetones exist as independent, static isomers. The catalytic action of acids and alkalis interconverts the isomers, and accordingly the same mixture of products is formed from either isomer (Farrow & Kon, 1926).

As a result of these observations it was decided to attempt the preparations of the unsaturated ketones that might be obtained by oxidation of the *cis*- and *trans*-forms of ricinoleic acid. These preparations have been effected (Ellis, 1950) and a quantitative study of the hydrolysis product of the unsaturated

at temperatures between 45 and 50° this soluble oil not only represents over 90 % of the products but may contain little else besides the unsaturated keto acids and these supposed dimerides. The latter are acids and contain the unsaturated ketone grouping. In this communication they are not included in the category of unsaturated keto acids which, for convenience, includes only the significant η - and κ -keto-elaidic (or oleic) acid products. They are more stable than the latter, resisting the alkaline hydrolysis which has led to the identification of these comparatively simple keto acids. This hydrolysis yields volatile and water-soluble products which can be removed and a *n*-hexane extract of the residues gives the supposed dimerides in their most concentrated form. This concentration is designated the 'colourless oil' obtained after operation 6 as shown in the separation chart (Fig. 2).

It may be thought that the experimental work so far carried out on this oily mixture of supposed dimerides (see below) does not justify the assignment of a formula at this stage. However, all observations so far made support the hypothesis that there is formed a substituted dihydrofuran ring, which may arise as a result of reactions indicated by the equations:

ketones obtained (in this case methyl hexyl ketone) has served as an indication of the concentrations of the autoxidation products. These unsaturated keto acids are contained, for the most part, in the light petroleum-soluble fraction of the oily products. This fraction is shown in the accompanying separation chart (Fig. 2) as the 'soluble oil' at the conclusion of operation 3. The oils insoluble in this solvent contain a smaller proportion of substances containing the unsaturated ketone grouping, but they also appear to contain β -ketone groups, as a result of further autoxidation. The yields of the unsaturated keto acids are set out in cols. 10 and 11, Table 1. These figures have been arrived at from the yields of methyl heptyl ketone and nonaldehyde given in col. 9 and obtained on hydrolysis of the 'soluble oil' as explained in the Experimental part.

The supposed isomeric dimerides of the η - and κ -keto-elaidic (or oleic) acids. It does not appear possible to avoid the formation of appreciable proportions of these substances in the autoxidation processes, whatever temperature is employed. They form one of a number of constituents in the 'soluble oil' referred to in the previous paragraph, providing the autoxidation has been conducted above 60°, but

The first reaction is regarded as initiated by polarized effects of the electromeric displacements in two molecules. The oxygen linkage is regarded as taking place simultaneously with a migration of the hydrogen (*) to the carbonyl carbon. The second reaction is the isomeric conversion of the $\beta\gamma$ - to the $\alpha\beta$ -form of the unsaturated-ketone grouping. Such a conversion would be liable to take place because of the more stable form resulting by entrance of the double bond into the ring (cf. Hugh & Kon, 1927).

Polymerized oils and their β -keto-acid content. The oily products insoluble in light petroleum are shown in Fig. 2 as resulting after the conclusion of operation 3. This fraction is almost absent in the products from the lower-temperature (45–50°) processes, but autoxidation at temperatures approaching 100° will give over 70 % of coloured, resinous substances. Boiling light petroleum will remove from these substances some nearly colourless oils which give definite enol reactions and which yield carbon dioxide on hydrolysis. These are regarded as β -keto-acid peroxidation products. This fraction of the oily products also contains most of the monoacyl esters from the epoxide referred to above. It therefore yields on saponification the nearly insoluble di-

hydroxystearic acid with water-soluble and volatile acids, which are the hydrolytic products of these esters and which can be separated from the remaining oily or resinous material. The higher-temperature processes yield some 50 % of these resins (Table 1, in Part 2, Ellis, 1936). They have been fractionated into substances of increasing hardness and decreasing solubility. They disappear from the products of autoxidation as the temperature employed falls, and as the yields of the unsaturated keto acids increase. It is well known that unsaturated ketones readily polymerize, and it is shown for those obtained from ricinoleic acid (Ellis, 1950) that this polymerization takes place rapidly at the higher temperatures employed in these autoxidations.

The presence of peroxides. No oily fraction of these autoxidation products has failed to give a small peroxide value as indicated by liberation of iodine from potassium iodide and acetic acid. In all cases the simple method of peroxide estimation proposed by Lea (1938) has been adopted. The results were calculated as a peroxide of oleic acid (giving mol. wt. 314) with one available oxygen atom. The average concentration found in oily products of sixteen different experiments was 1.7 % (range 0.3–3.4 %). No significance can be placed on the variations obtained because of the number of disturbing factors both in the autoxidation process itself, and in the isolation of the products examined. Some of the higher results were obtained from oils extracted from the large bulk of the dispersing medium with a considerable volume of ether. The use of this peroxide-forming solvent has been replaced, in recent experiments, by *n*-hexane, in which the whole products of the lower-temperature autoxidations, including the peroxide-containing substances, are soluble. Eleven more recent experiments have given lower peroxide figures (average 1.2 %). Nearly all these results were from catalysed experiments, but a recent preparation of carefully purified oleic acid after an oxygen uptake of 21 % by weight gave total products with a peroxide content of 1.4 %. It has been observed on a number of occasions that, on removing the source of heat from an autoxidation process, there continues a slow absorption of oxygen, and it has been ascertained that this uptake is accompanied by an increase in the peroxide content of the products, particularly after one of the lower-temperature autoxidations. It was observed that the total products from elaidic acid after an oxygen uptake of 15 % by weight at 45–55°, gave 0.9 % peroxide, but these products, on further exposure to oxygen at room temperatures for 3 days, gave a peroxide value of 1.7 %. For an experiment at 65–70° with an oxygen uptake of 20 % the products gave 1.0 % peroxide and on the 3-day exposure to oxygen at room temperatures only a small increase to 1.2 % was obtained. It is only in the first of these two

experiments that an appreciable unsaturated ketone formation would be observed. The light petroleum-soluble oil, obtained after saponification of the products of autoxidation and referred to above as supposed dimerides, contains the unsaturated ketone grouping, and the peroxide-free oil will acquire a value of over 2 % on exposure to oxygen during some days at room temperatures. There is little doubt that the peroxide-forming properties of these substances must be attributed to their containing this unsaturated-ketone grouping. As mentioned above, pure unsaturated keto acids have been prepared and they give much higher peroxide values than have been obtained in any of these autoxidation products.

On the whole, the peroxide contents of the autoxidation products must be regarded as of a low value with little variation. If these peroxides are formed by an unstable addition of molecular oxygen to the unsaturated ketone grouping, in a manner such as is suggested below, they may donate *both* oxygen atoms to the substrate. Hence the average value of 1.2 % given above must be halved. It seems probable that a rise of temperature may increase their formation, but apparently owing to a mass action effect and their increased reactivity at the higher temperatures, their concentration may not materially change.

Carbon dioxide evolved during autoxidation. The more recent methods used in the autoxidation processes have lent themselves to simple and accurate means of determining the volume ratio of carbon dioxide formed to oxygen uptake. The results are given in col. 8, Table 1, and for the nine experiments in which the autoxidation of elaidic acid was carried out at temperatures below 60° the average ratio for CO_2/O_2 is 0.20, while the results recorded in cols. 5 and 6, Table 1, Part 2 (Ellis, 1936) for nine experiments, in which the temperatures were 60° and over, gave an average of 0.17. This difference is not a great one. The extreme interest in these observations is that the amount of carbon dioxide formed in the autoxidation process is not less, but actually more, at the lower temperatures employed. Elaidin (Exp. 21, Table 1) has given a higher ratio, and oleic acid of doubtful purity (Exp. 4) has given a low ratio, otherwise there is a fairly constant ratio of about 0.2 representing 20 % of the oxygen uptake being evolved as carbon dioxide. In the earlier work it was shown that appreciable quantities of water were also formed and assuming these two products to represent chain degradation ($\text{CH}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O}$) the oxygen so evolved in the form of water and carbon dioxide must be about 30 % of the uptake.

When the autoxidation is prolonged for some period after the maximum velocity has been attained, this ratio increases from about 0.2 to 0.4. If water be included, as indicated above, the ratio must reach 0.6 and must represent 60 % of the oxygen uptake being evolved as carbon dioxide and water (Table 2).

Table 1. *Autoxidation of elaidic and oleic acids. Oxygen uptake and yields of some resulting products*

(Bracketed figures are approximate.)									
No. of exp.* and method	Wt. of acid taken† (g.)	Cobalt wt. as metal† (g.)	Temp. (°)	Duration of exp. (5)	O ₂ uptake (% by wt.) (6)	O ₂ evolved as CO ₂ (% by wt.) (7)	Methyl ketone‡ (9)	η-Keto-elaidic acid (%) (10)	γ- and κ-keto-elaidic acids (%) (11)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1a	20.9	0.10	78	8 hr.	20.8	(2.11)	(0.10)	2.3	8.3
2a	5.22	0.03	100	8 hr.	25.5	6.21	0.24	Nil	Nil
3b	4.6	0.07	50	4 days	7.5	—	—	3.3	11.9
4b	4.4	0.02	37	6 days	11.8	1.86	0.16	3.1	11.2
5b	3.0	Nil	63	3 days	17.8	—	—	2.1	7.6
6c	4.45	0.02	53	7 hr.	12.8	2.67	0.20	3.1	11.2
7b	10.9	0.1	48	2 days	15.3	3.20	0.21	6.8	24.6
8a	47.6	0.20	48	24 hr.	13.9	—	—	6.0	21.7
9a	105	Nil	68	2 days	13.2	2.29	0.17	6.0	21.7
10a	20.5	0.05	63	24 hr.	18.2	2.96	0.16	8.0	28.9
11a	22.1	0.05	60	20 hr.	13.6	—	—	5.5	19.9
12a	20.2	0.02	60	24 hr.	17.8	—	—	10.0	36.0
13b	29.7	0.3	46	19 days	23.6	4.90	0.21	7.2	44.2
14e	20.0	0.02	55	5 days	23.9	4.24	0.18	12.3	26.0
15b	10.1	0.03	45	4 days	21.2	—	—	(12.0)	(21.5)
16e	4.2	0.02	50	2 days	13.6	—	—	8.4	15.1
17b	4.5	0.05	48	2 days	12.6	2.60	0.20	15.1	27.1
18b	3.2	0.02	48	2 days	9.2	—	—	15.1	27.1
19b	24.1	0.1	48	3 days	17.4	3.37	0.19	12.2	21.9
20b	47.8	0.2	50	—	17.3	3.60	0.21	25.4	45.5
21b	20.4	0.5	50	7 days	11.7	4.77	0.41	4.3	7.8
22c	4.56	0.02	50	3.5 hr.	6.8	1.25	0.20	11.4	20.5
23c	2.68	0.02	55	20 hr.	10.7	1.91	0.18	8.4	15.1
24d	3.13	0.02	(50)	6 hr.	12.3	2.68	0.22	(6.0)	(10.8)
25d	1.07	0.01	58	28 hr.	22.7	3.89	0.17	11.9	21.3
26a	41.8	0.2	68	14 hr.	18.4	—	—	14.1	50.6

* Letters a, b, c, d and e refer to the methods of autoxidation (p. 133).

† Weights in g. of elaidic acid unless oleic acid is referred to.

‡ Present as cobaltous elaidate or oleate.

§ Percentages refer to weights in col. 2 irrespective of the presence of any unchanged acid in the products.

|| Ratio of carbon dioxide formed to oxygen uptake, or CO₂/O₂.

¶ Methyl heptyl ketone, nonaldehyde and its acid. Products of hydrolysis of fractions noted under 'Remarks' column.

** 'Petrol' signifies the use of light petroleum (b.p. 40–60°) or of n-hexane. See separation chart, Fig. 2.

Remarks on different fractions obtained from total oily products or from petrol** soluble fraction of same

(12)

Fraction soluble in petrol**

Different fractions examined

From oleic acid

Similar to 3. See text

Unchanged acid present

A similar fraction

Unchanged acid removed

Whole product

Not further fractionated

Fractionation of Mg salts

From oleic acid

Ether-soluble Pb salt

Unchanged acid present

Autoxidation of an emulsion

Water soluble Mg salts removed

Autoxidation of an emulsion

Chromatographic separation

A similar process

Solids deposited below 0°

Girdard's reagent separation

Elaidin, partial autoxidation

Autoxidized at 2 atm. pressure

A similar experiment

Heat from an electric lamp

A similar experiment

Volatile acids removed

Petrol** soluble fractions of oils, being nearly total oils for experiments at lower temperatures

Oxygen uptake in the autoxidation process. Ample evidence was obtained in Parts 1 and 2 (Ellis, 1932, 1936) for regarding at least 20 % by weight as the oxygen required to bring about the disappearance of elaidic acid in this process. In col. 6, Table 1, are set out further records of oxygen uptake. In most of these experiments the autoxidation has been stopped to avoid possible destruction of the initial products sought. In those cases unchanged elaidic acid was observed and often separated by the processes shown in Fig. 2. When an uptake of a little over 20 % had been obtained the autoxidation was always stopped, because it was realized that only traces of unchanged acid would be present, moreover, there was at this stage a marked falling off of the rate of uptake (cf. Ellis, 1932). In Exp. 21 the uptake was 12.6 % and the recovered acid was 50.4 % with possible traces of the epoxy acid. This result indicates an uptake of over 24 % for complete autoxidation. If the molecular uptake were 4 atoms of oxygen a 23.7 % addition by weight would be required.

EXPERIMENTAL

Apparatus employed

The large bulk of sand used in the earlier work to disperse the acids required a continuous current of O_2 to displace the CO_2 formed in the autoxidation. It was thought that this displacement was uneven owing to pockets of resistance to the current being set up in the sand. An unsteady O_2 uptake would thereby result. It was therefore decided to devise a method which eliminated the use of sand. However, some experiments, marked (a) in col. 1, Table 1, were made with the earlier method.

Autoxidation is a surface reaction taking place very slowly when O_2 is passed into solutions of elaidic or oleic acids in organic solvents, with or without a catalyst, nor when O_2 is passed through aqueous colloidal solutions of the soaps of these acids. In order to obtain the initial products of autoxidation as free as possible from secondary products, fine dispersion is of paramount importance. A fine emulsion of the acid should, therefore, be most suitable. Such experiments with fine emulsions have been used and are marked (e) in col. 1, Table 1, but the difficulty of handling sufficiently large quantities and of separating the products from the emulsifier has not warranted extensive use of this method.

Most of the experimental results recorded in Table 1 have been obtained by use of a simple form of apparatus consisting of the common type of desiccator of about 16 cm. diam. In the bottom half is placed NaOH to absorb the CO_2 formed. The lid is provided with clamps to make it gas-tight. Its central opening is fitted with a rubber bung through which passes a thermometer and two tubes, one of which reaches nearly to the bottom of the desiccator. The tubes are for displacing air and for supplying O_2 . Thin sheets of filter paper are reduced to a third of their length by folds of about 5 mm. depth and are cut into strips of a width equal to the depth of the upper part of the desiccator. These cuts are made just off right-angles with the folds so that by reversing alternate layers of the strips they are prevented

from falling into each other's folds. A drum is thereby formed of considerable surface area and of a size to fit loosely into the desiccator over a wide-meshed gauze. A dispersion of about 1 mg. of the acid to be autoxidized to 1 cm. surface was generally allowed for. In dispersing elaidic acid over the paper the catalyst (cobalt elaidate) is at first dissolved to a clear solution in the melted acid and this solution is diluted with sufficient light petroleum to cause dispersion over the entire paper of the drum. This dispersion is carried out in a suitable beaker, and immediately the solution has been absorbed the drum must be taken out and the paper strips spread out for evaporation of the solvent. Evaporation from the beaker draws most of the acid to the upper edges of the paper strips. The drum is finally re-assembled and placed in position in the desiccator, which is supplied with O_2 from a graduated gas holder and brought to the required temperature (48–55°) in an oven or incubator. Experiments with this apparatus are marked (b) in col. 1, Table 1.

Fig. 1. Apparatus for autoxidation. *A*, folded filter paper; *B*, NaOH solution; *C*, dropping funnel containing NaOH; *D*, tap; *E*, oxygen-supply tube.

A modified form of this apparatus (Fig. 1) has been used in the smaller-scale experiments. It was designed especially to enable periodic determinations of the CO_2 formed during a prolonged autoxidation as described below. The desiccator is replaced by a glass cylinder drawn in and fused with a capillary tube for ready removal of the alkali without dismantling the apparatus. It is heated in a beaker of water and contains the drum of fluted paper strips supported by a glass grid over the alkali.

A third, slightly modified, form (c) of the last described makes use of a wide-mouthed bottle. The stopper is of metal, well clamped to the neck so as to carry out an autoxidation at increased pressures. The results obtained are recorded in Exps. 22–23 of the table. Yet another slight modification (d)

was made to enable an autoxidation to be carried out in the presence of artificial light, which also provided the source of heat. Little work has been done with this form. The wide bottle contains in its centre a low-powered electric lamp and the drum of fluted papers is replaced by a single, deeply fluted paper lining the inner side of the bottle. The most convenient form of this modification has been to use a bright tin canister of 12.5 cm. diameter. A 15 W. lamp gives the required temperature near the heavy flutings of the paper. An 'opal' lamp seems to give a more even distribution of heat than does one of clear glass. The alkali is placed in a Petri dish, the sides of which support a metal ring of 1 cm. width which, in its turn, supports the paper lining fluted to similar dimensions. The results of Exps. 24 and 25 given in Table 1 were obtained by use of this form of apparatus.

Monocyl derivatives from elaidic acid epoxide. Experiments relating to these esters were conducted to ascertain the proportion they formed of the oily products. The unfractionated oils obtained by autoxidations carried out at over 60° yielded about 12% of *th*-dihydroxystearic acid, m.p. 133°. Some oily fractions after saponification with aqueous NaOH and acidification gave precipitates containing little solid matter. This was nearly always the case when oils obtained at the lower temperatures (45–55°) were saponified. Other fractions of oils obtained at over 60° and so treated gave precipitates that contained so much of the solid dihydroxystearic acid as to be readily filterable through a small paper disk. After washing such a precipitate with water, drying and removing any oil with a minimum volume of ether, the dihydroxystearic acid was nearly pure and could be weighed for its estimations. One crystallization from methanol yielded the product with its correct m.p. None of its isomer of m.p. 95° has been identified.

The esters giving rise to dihydroxystearic acid on hydrolysis are therefore only formed at the higher-temperature autoxidations, under which condition the free elaidic acid epoxide is often present in the products to the extent of over 16% (Table 1, Part 2, Ellis, 1936), but in the lower-temperature autoxidations its concentration is much less, and in Exp. 19 (Table 1), carried out at about 48° for 3 days, only 4.1% of the epoxide was isolated.

At the higher temperatures, and for the duration of autoxidations so conducted, it has been possible to demonstrate that ester formation readily occurs between the epoxide and certain of the free acids found in the products of those autoxidations. Molar proportions of the epoxide and octane-1-carboxylic acid were heated at 65–68° for 5 days and gave about 60% of an oily product. It was purified by crystallizing out the unchanged epoxide from cold light petroleum and removing the unchanged acid by steam distillation. The product was soluble in light petroleum and gave an equiv. wt. of 509 (theory 465 for a mixture of the two isomeric esters). Saponification yielded 66% of dihydroxystearic acid, m.p. 133° (theory requires 69%). The epoxide esterifies by intermolecular reaction when heated alone under these conditions. This accounts for the high equiv. wt. A specimen of equiv. wt. 299 heated to 68° for 12 hr. gave an equiv. wt. 321. Heated at 68° for 16 days it gave a thick oil of equiv. wt. 564. This substance gave evidence of some polymerization. Similarly, the epoxide was found to combine readily with azelaic acid, another of the scission products of autoxidation. Thick oils, insoluble in light petroleum, were obtained but they were not

further investigated. Deatherage & Mattill (1939) have shown that monoacyl derivatives are formed by heating autoxidation products of different monenes with acetic acid.

Oils from one of the higher-temperature autoxidations were dissolved in ethanol and water was added until most of these oils were thrown out. They were thereby freed from the dibasic scission products azelaic and suberic acids, for these acids are very soluble in dilute ethanol. This process was followed by washing with warm water. On saponification they yielded a further amount of both acids which were separated and identified by the methods described in Part 2 (Ellis, 1936) for the same acids present in the free state. These acids are the strongest present and are the more liable to esterify with the epoxide present. The insolubility of these esters in light petroleum, as compared with the solubility of others that are formed with octane-1-carboxylic acid, accounts for the much higher yields of dihydroxystearic acid when the light petroleum-insoluble fractions of the oils were saponified than are obtained from the soluble fractions. In Exp. 12, Table 1, Part 2 (Ellis, 1936) an autoxidation carried out at 68° was shown to give 15.1% of the free elaidic acid epoxide. Subsequently, it was found that the oils, which were given as 30.5% soluble in light petroleum and 36.7% insoluble, yielded on saponification 11.0 and 23.6%, respectively, of dihydroxystearic acid. These figures represent a formation of about 26% of elaidic acid epoxide, free and esterified.

From the above results it is evident that for the higher-temperature autoxidations not only must the epoxy acid formation be much higher than reported in Part 2 but so also that of the acid scission products. The latter were reported as totalling a 16–20% yield and suberic acid occurred in largest amount; possibly heptane-1-carboxylic acid predominated as the monobasic acid product. The total formation may be regarded as reaching about 30%.

Concentration and identification of the unsaturated keto acids. The accompanying separation chart (Fig. 2) summarizes the preliminary processes of fractionation by which the products of autoxidation were generally resolved into their components. In operation 1 the products are dissolved in 2–3 times their bulk of 96% methanol or in 3–4 times their bulk of 80% ethanol. The separation of the crystalline deposit through a Büchner funnel presents no difficulty. The second operation gives a fine emulsion, but when the mother liquors are not required the addition of a little anhydrous Na₂SO₄ assists the complete deposition of the oily matter. In the third operation the mutual solvent action of the oils becomes apparent. They are completely soluble in a small proportion of light petroleum, but precipitation of some of these oils on the addition of more solvent seems to proceed indefinitely. The solution has generally been obtained by the addition of at least 10 vol. of the solvent to 1 vol. of the oils. This solution becomes clear on standing for some time. On evaporation, using reduced pressure during the last stages so as to avoid unnecessary heat, there remains a clear light-yellow oil which readily solidifies below 0°. It may form 90% of the autoxidation products of the lower-temperature experiments of short duration, while in the higher-temperature experiments it may represent as little as 10%, being replaced by the formation of a large proportion of the coloured, resinous oils, by a large proportion of the oily monoacyl esters derived from the epoxide formed, and by an appreciable proportion of the volatile scission products already referred to. For the

majority of experiments of recent work, found the most convenient for operation, yields of 60–70% of this fraction have been obtained.

under cool conditions, in order to obtain the soluble oil, tends to leave the β -keto acids amongst the coloured insoluble oils.

Fig. 2. SEPARATION CHART

Showing preliminary separation of autoxidation products

(Abbreviations: h.t. exps. refer to those conducted above 60°; l.t. exps. refer to those conducted below 60°; Op.=operation; M.L.=mother liquor.)

Dispersing medium extracted with ether, or (for l.t. exps.) with *n*-hexane, yielding:

Notes. (a) Odour strongly reminiscent of that of the gorse flower.

(b) I.e. 2:10-diketoelaidic acid. (See text, p. 138.)

(c) Or treated with a view to further concentration of its unsaturated ketone content.

(d) In h.t. exps. esters of the epoxy acids may yield 8:9-dihydroxystearic acid.

This main product is shown in Table 1 and Fig. 2 as the 'soluble oil'. It contains nearly the whole of the unsaturated keto acids. It may contain some of these acids which have undergone β -oxidation. Such unsaturated diketo acids are less soluble in light petroleum than are the unsaturated keto acids themselves. Using a large volume of the solvent

The identification of the unsaturated keto acids and the determination of their concentration in the soluble oil have been effected by saponification of this oil and by subsequent isolation of the different hydrolysis products. An outline of the processes involved is shown in the remaining operations given in Fig. 2. By means of the apparatus described below

it has been possible to experiment on a much smaller scale than might have been necessary. The methyl heptyl ketone with some nonaldehyde and octane-1-carboxylic acid formed therefrom, is isolated during saponification by use of the still-head shown in Fig. 3. A flask (*B*) of about 400 ml. capacity is connected by a ground joint to the reflux tube (*C*) which has a length of about 30 cm. and a bore of about 11 mm. The U-tube (*E*) has a bore of 5 mm. (not less) and the arm connecting it with the reflux tube should be of 6-7 mm. bore. It will be observed that the upper portion of the fused connexion made between this arm and the reflux tube has been rounded off. This shaping is essential in

Fig. 3. Diagram of still-head for isolating small quantities of steam-distillation products. *A*, bunsen flame protected from draught; *B*, saponification flask with ground-glass fitting; *C*, air-cooled reflux condenser; *D*, condenser; *E*, inner arm containing distilled oil over water; *F*, beaker to collect water distillate.

directing the condensed methyl ketone products from the reflux tube to the U-tube. The slope of the reflux should be about 27° to the vertical. The ebullition of the reaction mixture in the flask must be slow and steady. The addition of pumice stone, or the like, is essential. So, too, is an easily adjustable flame protected from draughts. In this manner the steam line in the reflux tube can be regulated to come a few cm. above the U-tube outlet. The reflux tube is not, of course, hermetically sealed, but the small tube containing cold water serves to limit convection currents in the reflux and provides an emergency condenser in case of over-heat applied to the flask. The whole condensing and collecting parts of the still-head must be thoroughly clean and free from grease before a saponification is started. If this precaution has been taken the condensed steam will run back almost entirely as a continuous film, while the condensed volatile products will run as droplets over the water film into the U-tube to form an upper layer over the water with which the tube has been filled. Any water entering the U-tube finds its way through the oil layer to the beaker (*F*). This water is used from time to time for flushing down the reflux tube. A long Pasteur pipette is used for this purpose and a shorter one is used to convey the oily layer to a weighing bottle. The U-tube must be fused to the reflux tube so as to allow of the approach of this pipette. The efficiency of this

simple apparatus has rendered it almost indispensable throughout the course of these investigations on the ketonic products of autoxidation, and the technique may prove equally effective in other spheres of work. The accuracy of the method is over 90% for the small quantities examined: 0.0143 g. of methyl heptyl ketone distilled with 0.05 *N*-alkali (the excess used in the saponifications) yielded, in the above described apparatus, 0.0133 g. or 93%. Nonanol (0.1191 g.) gave 0.1090 g., or 91.6%. Nonaldehyde (0.1722 g.) gave from water distillation 0.1550 g., or 90.0%. Distilled from alkali, aldehydes gave low yields as will be considered below.

The other factor upon which this method of computing the concentration of an unsaturated ketone depends is the quantitative nature of the actual formation of these volatile products on alkaline hydrolysis. It has been possible to prepare heptadec-9-en-11-one-1-carboxylic, which should bear close analogy with those in these autoxidation products. It gave 32% methyl hexyl ketone (Ellis, 1950). The question also arose as to the proportion of the methyl heptyl ketone from (I) and of nonaldehyde, or its acid, from (II). The aldehyde imparted a Schiff reaction to the volatile saponification products, but it was seldom very pronounced. The slow hydrolysis by the 0.05 *N*-alkali used, and the consequent long exposure of small quantities of the aldehyde to air in the condenser of the still-head described, brought about nearly complete autoxidation to octanecarboxylic acid. This acid is appreciably soluble in water, but as the aqueous distillate was repeatedly used during saponification to flush down the volatile products so as to bring them into the U-tube for collection, most of the acid appeared as part of the volatile oil and was eventually removed and weighed. The octanecarboxylic acid content of these oils was determined by titration of a weighing-bottle collection from a number of experiments: 0.3649 g. required 7.7 ml. of 0.1 *N*-sodium hydroxide, corresponding to 33% of octanecarboxylic acid. Also 0.3729 g. required 9.15 ml., equal to 39%. A third collected lot gave 36% the average of the first two. The recovered acid gave equiv. wt. 162; calc. for octanecarboxylic acid, 158. A Zn salt gave the characteristic long plates of m.p. and mixed m.p. 131° . It was evident that nonaldehyde was one of the saponification products of the soluble oil, but the action of the alkali on it and its susceptibility to oxidation allow of only a very approximate estimate of its yield. Experiments showed that the 0.05 *N*-alkali used in the saponification processes brought about considerable decomposition of pure nonaldehyde when this was distilled in the apparatus described: 0.0689 g. gave 0.0350 g., a recovery of 51%; 0.0988 g. gave 0.0564 g., a recovery of 57% and from 0.0348 g. a trace only was recovered. Titration of the distillate from the last gave 11% octanecarboxylic acid and, on acidification of the flask contents and distilling, acid equivalent to a further 75% of the aldehyde was obtained. Had larger amounts of material been used in these estimations the losses of the aldehyde would have been of less significance, but it has been the practice to use about 0.25 g. as more suitable for the particular apparatus described, and in some cases less material was available.

The 36% of octanecarboxylic acid in the saponification distillates could have had no other source than that of the nonaldehyde. Octanecarboxylic acid was often in the oils examined because it was often one of the scission products of an autoxidation. In an experiment with this acid in the presence of 0.05 *N* excess alkali no hydrolysis of its sodium

salt on 2 hr. distillation was found since no acid appeared in the distillate. It is evident from the experiments with non-aldehyde described that the 36% of its acid in the distillates, with traces of the aldehyde itself present, must, owing to loss, represent appreciably more nonaldehyde as having been formed by hydrolysis of the η -ketoelaidic (or oleic) acid in the sample examined. It is probable that this aldehyde is actually liberated in larger amounts than is the methyl heptyl ketone from the κ -keto acid. This ketone represents the remaining 64% of the distillate. Suberic acid, which has been referred to as the most abundant of the scission products of an autoxidation (Part 2), provides further evidence for the predominance of the η -keto acid, since this dibasic acid suggests cleavage at C₍₇₎.

Methyl heptyl ketone is readily purified from the remaining 64% of the distillate, which sets to a crystalline mass on the addition of sodium hydrogen sulphite. Filtered, pressed and washed with a little ether, this mass has been distilled with sodium carbonate in the above-described apparatus which conveniently isolates the pure product. (Found: C, 75.9; H, 12.7. Calc. for C₉H₁₈O: C, 76.0; H, 12.8%.) The semicarbazone crystallized from ethanol gave well-defined plates, identical in microscopic appearance with those of an authentic specimen. It gave m.p. and mixed m.p. 119°.

The total distillation products obtained during a saponification are set out for a number of experiments in col. 9, Table 1. They are expressed as a percentage of the oil examined. The deduced concentrations of the κ -ketoelaidic (or oleic) acid are given in col. 10. These figures have been arrived at as follows. From evidence given above the total distillate reduced by 64/100 gives the percentage of methyl heptyl ketone yields. As mentioned above it was shown that heptadec-9-en-11-one-1-carboxylic acid gave 32% methyl hexyl ketone, theory requiring 43%. Hence, from close analogy, the above percentage of 64/100 may be increased to 64/100 $\times$ 43/32. Since the κ -keto acid (mol. wt. 296) is hydrolysed to 1 mol. of the methyl heptyl ketone (mol. wt. 142) the percentage concentration of the oil under examination becomes 64/100 $\times$ 43/32 $\times$ 296/142. The figures given in col. 10 are therefore obtained by multiplying those of col. 9 by this factor. Assuming that the unsaturated η -keto acids are formed in equal amounts the total of these acids as given in col. 11 are double those of col. 10.

Further concentration of the unsaturated keto acids. No attempts have been made to concentrate the small yields of these acids in the products obtained by autoxidations carried out at over 70° (see Exps. 1 and 2, Table 1). Below 70° the 'soluble oil' (see Fig. 2) may be a complex mixture. The mutual solubility of its constituents is very high. The removal of any one set such as the volatile scission acids (heptane- and octane-carboxylic) by steam distillation or by the greater solubility of their Mg salts in water, makes possible further effective fractionation in light petroleum. Exps. 15 and 26 give results illustrating this concentration. The Ba salts, and to a less extent, the Pb salts of the unsaturated keto acids are more soluble in ether than those of the other constituents of the oil, but the manipulations involved in this separation bring about too much decomposition of the required acids (Exps. 10 and 12). The very high solubilities of the unsaturated keto acids in apparently all the common organic solvents led to their concentration in the mother liquors of cooled solutions, even when the solutions were cooled below -50°. The supposed dimerides seem, however, to possess a similar solubility.

As would be expected, the soluble oil from the lower-temperature processes, containing, for the most part, only the unsaturated keto acids and their supposed dimerides, has afforded the highest concentrations of these acids so far obtained, and it is probable that chromatographic methods will eventually effect a complete separation of these constituents. In an experiment the oil (1 g.) was dissolved in *n*-hexane and passed through a column (15 cm. $\times$ 10 mm.) of silica, on the top of which was anhydrous Na₂SO₄ (2 cm.). The filtration and elution was conducted at 0° and after some hours four bands could be distinguished. These were separated. The top one contained a trace of coloured oil, the second was an oil giving 10.5% of the volatile products (methyl heptyl ketone and nonaldehyde), the third gave an oil yielding 15% of the volatile products, answering to about 54% of the unsaturated keto acids (Exp. 17). Girard's reagent does not react smoothly with the unsaturated ketones. It reacts with nearly the whole of this soluble oil, but to give substances which are not easily hydrolysed. The most advantageous conditions of employing this reagent for separation of these ketones may not have been arrived at. The oil (4.4 g.) warmed for a few minutes with the reagent (1.7 g.) in dry ethanol (10 ml.) and acetic acid (5 ml.) yielded only 0.54 g. of an ether extract after dilution and partial, ice-cold, neutralization. This extract gave 25% of the methyl ketone products, corresponding to a 91.7% concentration of the unsaturated keto acids. Other slightly lower concentrations have been effected by these means.

The yields shown by the different fractions given in Table 1 are not closely comparable. There are too many factors involved, not only in the autoxidation process but in the isolation of the different fractions examined. The comparatively low results obtained for oleic acid (Exps. 3, 4 and 7) are due to the difficulty in removing the unchanged acid from the products.

Separation and examination of the supposed dimerides of the unsaturated keto acids. This fraction is shown in Fig. 2 as the colourless oil obtained after operation 6. It is slightly coloured when isolated from the products of the higher-temperature autoxidations. It is not easily freed from unchanged acid, from traces of heptane- and octane-1-carboxylic acids and other impurities. Since the supposed dimerides are regarded as being formed by combination of any two of the two unsaturated keto acids forming the main products of autoxidation, there may be present in the mixture six dimerides, which are not likely to lend themselves to separation. This mixture of supposed dimerides retains its unsaturated ketone groupings after being subjected to the alkaline hydrolysis which destroys η - and κ -keto elaidic or oleic acids. How far it will remain unaffected by more concentrated alkali than the 0.05N-solutions employed has not been ascertained.

Taking into consideration the impurities present, analyses agree fairly well with the proposed structure. (Found: mol. wt. 424. Calc. 593. Equiv. wts. have varied between 240 and 302 (calc. 296).) The value 302 was obtained by subjecting the oil to prolonged steam distillation which volatilized most of the impurities. Iodine values for a number of different preparations varied from 36 to 50 with an average of 45%. This points to a normal addition (calc. 43%) which is not always the case with the unsaturated ketone grouping. A carbonyl determination by the author's method (Ellis, 1927) was less affected by the impurities, as might be expected. It gave 4.8% CO (calc. 4.7%). An

approximate determination of hydroxyl by Grignard reagent in ether gave 4.3%. It points to an absence of an alcohol group since the carboxyl groups alone require 5.7% OH.

When recently prepared from a saponification process this oil has no peroxide value. Left in open beakers it soon acquires one. This O_2 uptake has been studied quantitatively. A specimen (0.1772 g.) was dispersed on filter paper and placed in a tube filled with O_2 and fitted with a calibrated Hg manometer. At the bottom of the tube was placed a saturated solution of $Ba(OH)_2$. The tube was placed in diffused light and the temperature during about 8 weeks was near 17°. The O_2 uptake was about 12 ml. (nearly 10% by wt.). The $Ba(OH)_2$ solution contained a ppt. which dissolved with effervescence on adding HCl. The filter paper was extracted with peroxide-free ether. The extract was no longer completely soluble in *n*-hexane, and contained some crystalline matter. The *n*-hexane-soluble fraction (0.1317 g.) gave a peroxide value of 2.3%, calc. as that of a peroxide of the supposed dimerides. If both oxygens were available this value must be halved. The insoluble fraction gave a value of 3.4%.

This property of peroxide formation and a consequent self-peroxidation has been demonstrated for a pure unsaturated keto acid (Ellis, 1950). The intense pyrazoline colour reaction, which is given by the unsaturated keto acid fractions is also given by these supposed dimerides. The bluish purple of the former is replaced by a reddish purple of the latter.

The presence of β -keto acids. Definite enol reactions have been obtained with some of the fractions of the oily products. They have given intense red or bluish red colours in ethanol solution with $FeCl_3$ and an immediate deep blue with the Folin-Ciocalteu reagent. These are fractions soluble in hot light petroleum. They may be obtained as a deposit from a solution of oils in this solvent, to which more of the solvent has been added.

All of the oily fractions obtained in this work have given a saponification value regarded as due to three causes: (1) The presence of the monoacyl esters derived from the epoxy acid. Their concentration may give a value nearly equal to that of the free acid. (2) A Cannizzaro reaction on the aldehydes resulting from the hydrolysis of the unsaturated keto acids and also the formation of acids by some oxidation of these aldehydes. (3) The presence of enol-reacting substances, regarded as the β -keto acid products.

For confirmation of (3) it has been necessary to demonstrate the liberation of CO_2 during a saponification process. This work has involved the carrying out of some 100 such processes and in many cases the final titration has resulted in small liberations of CO_2 . Excess of the H_2SO_4 could be added and a permanent colour (phenolphthalein) restored by heating the solution. The gas could be detected in the flask after such treatment. A few quantitative experiments have been conducted: a fraction deposited from a light-petroleum solution by further addition of the solvent

(1.8513 g.) was heated with excess of H_2SO_4 after its saponification. CO_2 -free air was drawn through the apparatus fitted to a condenser to arrest any heptane- or octane-1-carboxylic acids. It was then passed into 0.1 *N*-alkali. The excess was determined (3.0 ml.). A blank experiment, with the reagents only, gave 0.3 ml. The difference, calc. as β -ketoalaidic acid, gave 4.3% in the oil. From another autoxidation a similar fraction (0.5142 g.) gave $BaCO_3$ (0.2220 g.) by passing the CO_2 through a solution of $Ba(OH)_2$. A blank experiment, with the reagents only, gave 0.0070 g. The difference calc. to the keto acid gave a concentration of 4.7%. The carbonate precipitates gave clear solutions in a few drops of HCl. Two other fractions of oils, not giving definite enol reactions, gave only 2 and 1% of the keto acid. All the concentrations are low. The fractions examined came from autoxidations of less than 20% O_2 uptake. It is reasonable to suppose that further autoxidation would give higher formations indicating this β -oxidation, just as prolonged autoxidation has been shown above to give larger yields of CO_2 itself.

Ratio of oxygen uptake to carbon dioxide evolved. The results obtained for the R.Q. of autoxidizing elaidic acid are given in column 8, Table 1. The O_2 uptake and CO_2 formed, from which the ratios CO_2/O_2 were obtained, are shown in the previous columns of the Table. The CO_2 figures have been arrived at by titration of the excess alkali in the autoxidation chamber after the O_2 uptake, or by liberating the gas from conc. alkali so placed. Both alternatives give almost identical results, since there are apparently no other volatile acids absorbed by the alkali. The CO_2/O_2 ratio has been followed during the autoxidation of elaidic acid for a period of 39 days. The apparatus used has been described above, and is shown in Fig. 1. The alkali (5–10 ml. *N*) was introduced by way of the dropping funnel (*C*) and washed down. After a period the O_2 uptake was read and the alkali forced out of *B* by way of the exit *D*, rinsing with water through *C*. The excess alkali was determined and the amount of CO_2 deduced. The results are shown in Table 2. The method has few sources of error, but movement of the apparatus has caused the alkali to contact the dispersion media (pleated filter papers) and give a slightly higher result for the CO_2 , as is shown for the 5-day period in the Table.

These results show clearly that the R.Q. of autoxidation is comparatively low during the initial stages, as would be expected, and that beyond these stages the ratio is fairly constant at about 0.41. Since water is also given off freely, also from the early stages (Part 1, Ellis, 1932), these two chain degradation products must account for over 60% of the O_2 uptake. It will be noticed, however, that the rate per day of O_2 uptake as shown in Table 2, line 5, are those of a die-away curve similar to the actual curve given in the graph shown in Part 1. The nearly complete cessation of O_2 uptake is accompanied by the disappearance of the unsaturated keto acids. The autoxidation products contain about 17.7% of the soluble oil, which on saponification gives 3.6% volatile products with very little odour of the

Table 2. *Oxygen uptake and carbon dioxide formed by 2 g. elaidic acid during 39 days*

Successive periods (days) ...	1	1	2	2	2	3	5	7	11	5	Totals
O_2 (ml.)	75	95	71	42	44	64	58	51	72	22	594, 42%*
CO_2 (ml.)	12	20	19	16	16	27	27	21	28	96	196, 14%*
Ratio CO_2/O_2	0.14	0.21	0.27	0.38	0.36	0.42	0.47	0.41	0.39	0.44	Average
O_2 uptake/day	75	95	36	21	22	21	12	7	7	4	0.33

* Percentage by weight of oxygen.

methyl heptyl ketone. These products are, however, of an aldehyde or ketone nature. They represent only 0.6 % yield of the acid autoxidized.

DISCUSSION

It has been shown that the $\alpha\beta$ -unsaturated keto acids are initial products of autoxidation. Whether these are formed from a hydroperoxide by rearrangement of its oxygen atoms and elimination of water or whether they are slowly formed in an autoxidation process by a reaction which is greatly accelerated by the catalytic effects of certain metals will be considered below. Formed in appreciable amounts at temperatures of about 50°, these acids decrease in concentration as the temperature of the process is raised, to be replaced by their coloured, resinous polymers. At all temperatures these acids give rise to an appreciable proportion of their apparent dimerides. Together they constitute over 90 % of the products of autoxidation for the lower-temperature processes. It has been shown also that the molar oxygen uptake by elaidic (or oleic) acid is about 4 atoms at that stage when unchanged acid disappears. Even at lower temperatures it has been shown that about 30 % of this uptake is evolved as carbon dioxide and water and that on continuing the autoxidation beyond this stage the oxygen evolved as these products may be 60 % of any further uptake. It has been shown, further, that these unsaturated keto acids are peroxide-forming. So also are their supposed dimerides which apparently contain the unsaturated ketone grouping, but in a more stable form than that found in the initial products; a comparative stability which has been explained to be due to the presence of the double bond in a ring formation. These probable dimerides resist alkaline hydrolysis, but when recovered after saponification of the products of an autoxidation they show an oxygen uptake and then give a peroxide reaction.

After the source of heat of an autoxidation process is removed and the reaction chamber has assumed room temperature, there continues a slow oxygen uptake, and the peroxide value of the products shows an increase as though these peroxides were more stable at the lower temperature while continuing to be formed. The author (Ellis, 1950) has shown that a pure unsaturated keto acid exposed to air at room temperatures is distinctly peroxide-forming. Investigating substances of lower molecular weight, Eccott & Linstead (1930) observed that one of the butylideneacetones exploded after keeping some time at ordinary temperatures. In addition to the formation of a peroxide the author's pure product referred to gave an epoxy acid and, in addition to apparent β -ketonic acid formation, an evolution of carbon dioxide was observed. The unsaturated keto acids therefore give peroxides and peroxidation products. Elaidic acid epoxide has been prepared by

Figulevski & Petrova (1926), and by subsequent workers, as a product of peroxide reagents on the acid. So also, from the work of Dakin (1908), β -ketonic acid and carbon dioxide formation can be attributed to peroxide action.

In seeking for the nature of the peroxide responsible for these peroxidation products formed in the autoxidations studied, it would appear that it results from oxygen addition to the unsaturated keto acids. It does not seem reasonable to offer any peroxide structure as forming part of an autoxidation process unless the reduced form is clearly shown to accumulate in the autoxidation products or unless that form is able to resume the peroxide structure in the presence of oxygen gas. The peroxide formed must be very unstable, even at room temperatures, at any rate when in the presence of the oxidizable substrate. It is as though little more than an adsorption of the oxygen takes place. Such an adsorption might be due to electrostatic attraction, but a reaction involving movement of the double bond of the unsaturated ketone system and addition of the oxygen molecule to the β -carbon and to the carbonyl oxygen is suggested in accordance with the equation:

Such a structure would afford a peroxide with two available oxygen atoms and its reduced form would be the original unsaturated keto acid. It has been shown that the disappearance of any appreciable concentration of the unsaturated keto acids as a consequence of the prolonged autoxidation is contemporary with a nearly complete cessation of the reaction.

The above salient findings of this work seem to suggest a simple explanation for the autoxidation process as applied to the mon-ene fatty acids. It would appear that nearly the whole, if not the whole, of the unsaturated acid forms a mixture of the η - and κ -keto-oleic or elaidic acids. These form unstable and therefore active peroxides which give rise, by peroxidation, to the epoxy derivatives and their monoacyl esters, to the scission acids, to β -oxidation and to the other signs of chain oxidation and degradation such as the evolution of carbon dioxide and water. The supposed dimerides would represent a secondary reaction of the unsaturated keto acids, as would the formation of the coloured polymers caused by the action of heat.

In recent years, however, other workers have demonstrated the formation of hydroperoxides in autoxidation processes. It is in the same η - and κ -positions of the carbonyl groups found in this work that Farmer & Sutton (1943) have found hydro-

peroxide groups as a result of the autoxidation of the methyl esters of oleic and of elaidic acids under the influence of ultraviolet light. The same hydroperoxide groups in the same positions have been observed by Atherton & Hilditch (1944), who showed that by oxidation with potassium permanganate their preparations yielded suberic and heptane-1-carboxylic acids, indicating the η - and κ -positions respectively.

The substitution of a hydroperoxide group for one of the hydrogens in a methylene group adjacent to a double bond was previously noted as a result of a similar autoxidation of *cyclohexene* by Criegee, Pilz & Flygare (1939). The same product was also prepared by Farmer & Sundralingam (1942) by autoxidation in the presence of ultraviolet light at a temperature not exceeding 40°. No recognizable amount of a cyclic ketone was formed, but Dupont (1936*a, b*) had identified a 6-keto derivative of 1-methyl*cyclohexene* by autoxidation at 95° in the absence of a catalyst, and at 70° from 1:2-dimethyl*cyclohexene* a 3-keto product was obtained. Also, Cook (1938) obtained the 1-methyl*cyclohexen*-1-one at 70° in the presence of the catalyst ferrous phthalocyanine. Farmer & Sundralingam (1942) drew attention to these ketone formations at 70° or over, as compared with their own observations that no ketone groups, but those of a hydroperoxide, were formed in the same positions at temperatures below 40°. They observed that the catalyst employed by Cook produced active decomposition of their hydroperoxides and that ketones could be recognized in the products formed. It should be noted, however, that catalysts are not necessary to the autoxidation process, although they accelerate the initial reaction.

The hydroperoxide groups as introduced into the unsaturated fatty acids by Farmer & Sutton (1943) were found to be remarkably heat-stable. No ketones accompanied their formation, nor could ketones be obtained from them by the action of heat. They did not oxidize their substrate. The methyl esters of the fatty acids containing these hydroperoxides, in excess of the unchanged esters, could be concentrated by distillation of the latter at 65°. This is a temperature within the higher range employed in the autoxidation processes of this work.

It is difficult, therefore, to regard the hydroperoxides as precursors of the unsaturated keto acids found in this work. It is still more difficult to regard these hydroperoxides as the oxygen donors to the oxidizable substrate giving rise to the peroxidation products.

There is, on the other hand, little difficulty in fitting all the observations made in this work into a sequence of reactions which go to form the autoxidation processes as a whole. In the first place there are slow formations of the η - and κ -keto groups.

These formations are greatly accelerated by the presence of the well-known polyvalent metallic catalysts, but in the absence of these they are so slow as to constitute the so-called 'induction period'. In the second place the $\alpha\beta$ -unsaturated ketone systems resulting, take up molecular oxygen to form unstable, and therefore active, peroxides which accelerate the formation of further unsaturated keto acids and so account for the recognized autocatalytic action of a non-catalysed drying oil. At the lower temperatures employed these unsaturated keto acids accumulate and give rise to dimerides, these together constituting perhaps 90 % of the oily products of autoxidation. At the higher temperatures they polymerize giving coloured oils and resins which may then form the greater proportion of the autoxidation products. In the third place the peroxides of the unsaturated ketone groupings, at all temperatures employed, give rise to the peroxidation products, which include the epoxides, the acid scission products (suberic, heptane-1-carboxylic, etc., formed more at the higher temperatures), together with β -ketonic acids, carbon dioxide and water, constituting chain degradation products and representing over 60 % of the reaction of the later stages of autoxidation.

SUMMARY

1. Further evidence has been obtained for the conclusion arrived at in the earlier parts of this work that an oxygen uptake of over 20 % is required in the autoxidation process before there is a marked falling off of the initial rate. A near observed percentage is 22.7, corresponding to an uptake of 4 atoms/mol. of the acids (elaidic or oleic).

2. *Autoxidation at about 100°* yields the following products. (i) A small proportion of the epoxy acid and an appreciable proportion of the monoacyl esters derived from it, the scission products suberic, azelaic, heptane- and octane-1-carboxylic acids. (ii) A predominating proportion of the coloured, resinous polymers of the $\alpha\beta$ -unsaturated keto acids, which latter are, as a consequence, reduced to a small yield. There is also present an appreciable proportion of an apparent mixture of isomeric dimerides of the $\alpha\beta$ -unsaturated keto acids.

3. *Autoxidation at about 65°* yields the same products, but the coloured resinous polymers are less and the supposed dimerides are more. There is now present nearly 20 % of the epoxy acid (Part 2, Ellis, 1936) and about another 15 % of this acid as monoacyl esters. There are now present significant amounts of the $\alpha\beta$ -unsaturated keto acids.

4. *Autoxidation at about 50°* yields the $\alpha\beta$ -unsaturated keto acids (*cis*- or *trans*-heptadec-8-en-7-one-1-carboxylic and *cis*- or *trans*-heptadec-8-en-10-one-1-carboxylic acids). Their supposed dimerides will be present in a larger or smaller proportion, but

together they may represent over 90 % of the products. There is present about 5 % of the epoxy acid, but little or no monoacyl esters and little or no coloured, resinous oils and the acids forming the scission products also appear to be absent or nearly so.

5. *At all the above temperatures* the products give evidence for the presence of β -ketonic acids and considerable volumes of carbon dioxide and water vapour are evolved. This apparent chain oxidation and degradation is regarded as being brought about

by peroxides present in small amounts and formed from the unsaturated keto acids which are the initial products whose formation is probably catalysed by certain metals.

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Studies on the more Soluble Complexes of Soil Organic Matter

2. THE COMPOSITION OF THE SOLUBLE POLYSACCHARIDE FRACTION

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In the first paper of this series (Forsyth, 1947a) a method of fractionation of the soluble complexes of soil organic matter was presented and the observation made that polysaccharide fractions containing uronic acid groups could be readily isolated in reasonable yield from diverse types of soil. Because of the important effect that such polysaccharides might well have on the physical properties (Martin, 1946) and the biochemical properties (Forsyth, 1948a) of the soil, their composition has been studied and to some extent elucidated.

EXPERIMENTAL

Purification of the polysaccharide complex

The polysaccharide fraction was isolated by the selective adsorption procedure previously described (Forsyth, 1947a).

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It was first purified by precipitation into 4 vol. of ethanol containing 2 % (v/v) acetic acid followed by further purification by the addition of excess CuSO_4 to an alkaline solution of the gum. The precipitated copper complex after washing with water was decomposed with ethanol containing HCl and washed free from Cu^{++} and Cl^- with ethanol. The polysaccharide remained as a white powder which, after a further ethanol precipitation, could be dried by washing with ethanol followed by ether to give an ash-free powder readily soluble in water. Polysaccharide complexes were prepared in this manner from the heath, pine-forest, and agricultural soils previously described (Forsyth, 1947b), and also from deciduous woodland and permanent pasture soils, all from north-east Scotland. A similar fraction was prepared from a garden soil in Trinidad, British West Indies. All the preparations from the diverse sources had a slight negative rotation $[\alpha]_D^{20} - 2^\circ$ to -10° in water (c, 1.0, as Na salt) and a nitrogen content of 0.1-0.5%. However, on titration with 0.02N-NaOH equivalents were obtained ranging from 1000 to 1300.