Comparative Rates of Oxidation of Isomeric Linolenic Acids and Their Esters*

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NATURAL drying oils may be classified into two types, according to the sites of the unsaturation in their constituent glycerides. The first, of which linseed oil is the commonest example, dries only slowly in the absence of driers and has for its chief component the glycerides of linolenic acid, with its double bonds in the isolated 9-12-15 positions. The second, exemplified by tung oil, dries very rapidly and has for its main constituent the glyceride of *alpha* eleostearic acid, which is isomeric with linolenic acid and has its double linkages in the conjugated 9-11-13 configuration. These oils differ not only in their drying rates but in the character of the resulting films as well. Moreover, the drying characteristics of tung oil are profoundly altered by its conversion to "China wood butter," in which the *alpha*-eleostearic glyceride has been geometrically isomerized to the higher melting *beta* form. While practical application has long been made of these differences in the properties of the oils, fundamental data expressing quantitatively the effect of position and geometric isomerism within the series of the pure octadecatrienoic acids are lacking. This paper presents a study of the comparative rates of oxygen absorption by linolenic acid and ethyl linolenate, pseudo-eleostearic acid and its methyl ester, and *alpha* and *beta* eleostearic acids.

Previous investigators have already demonstrated the importance of structure in the oxidation reaction. Täufel (15) noted that elaidic acid becomes rancid much more slowly than oleic acid, which in turn oxidizes more rapidly than its methyl, glycol, and glycerine esters. In the presence of hemin (8) the rate of oxidation of oleic acid is fifty times that of elaidic acid, the two acids also differing in their course of oxidation (2). An exhaustive study on the effect of methylation of the carboxyl group of oleic acid and its reduction to an alcohol on the rates and products of oxidation has been made by Hamilton and Olcott (3). The isomeric maleic anhydride addition products of *alpha* and *beta* eleostearic acids have been shown by Morrell and his coworkers (12) to oxidize by distinctly different mechanisms. Again, linoleic acid, differing from oleic acid by the presence of only one additional double linkage, takes up oxygen thirty-five times as fast (4, 16).

Experimental

The procedure adopted in the present investigation was based on the use of the Warburg-Barcroft respirometer, which has been shown by many workers to be readily adaptable to studies of the oxygen absorption by oils and fatty acids (5, 8, 16). The apparatus employed was the standard small size, with reaction flasks of about 15 ml. capacity. Each determination was made at least in duplicate with the usual thermobarometric control. The flasks and manometers were calibrated by the mercury method described in detail by Dixon (1).

The actual determinations were made as follows: Small discs of filter paper, 1.60 cm.^{2} in area, were punched out from a sheet of Whatman No. 42 paper with a leaf punch. Each disc was then supported above the central cup of the reaction flask by a very thin glass rod pierced through the paper. One ml. of an ether solution of the fatty acid or ester, containing 1.6 to 2.0 mg./ml., was transferred by a calibrated hypodermic syringe to the paper disc at which a stream of pure tank nitrogen was directed. Sufficient nitrogen was passed through the flask to completely evaporate the ether. The flask was attached to the manometer and again swept out with nitrogen while 0.4 ml. of 40% potassium hydroxide was added to the side-arm of the flask. The manometer was next fastened in position with the reaction flask immersed in a water bath thermostated at 40° C. When temperature equilibrium had been established, the flask and manometer arm were swept out with tank oxygen for three minutes. Pressure readings were then taken at short intervals. The reaction was carried out in complete darkness, except for a weak electric light switched on for taking manometer readings.

It is assumed that under these conditions, change in pressure is entirely due to absorption of oxygen. The mols of oxygen absorbed per mol of acid for any reading is therefore given by:

$$\text{Mols } 0_2/\text{mol substrate} = \frac{h\text{mm.} \times \left(V\text{ml.} \times \frac{273}{T}\right)}{760 \times \frac{13.60}{D} \times 22{,}400 \times M}$$

where h represents the pressure decrease in mm. of manometric fluid of density D, M represents the mols of acid or ester used, and V is the gas volume of the flask and the closed portion of the manometer in ml.

Materials

The linolenic acid and its ethyl ester were prepared according to the procedure of Rollett (13), as described in detail in another communication from this laboratory (7). Briefly, it consists of the debromination with zinc and ethyl alcoholic HCl of hexabromstearic acid obtained by the bromination of linseed oil fatty acids and repeatedly crystallized from xylene to a m.p. of 178-178.3° C. After distillation in high vacuum, the resulting ester had an I. V. of 247.3, while the acid obtained from it by cold saponification had an I. V. of 271.0 (theory, 248.5 and 273.7 respectively). These, and the other substances described below, were preserved until needed for the determinations by sealing in highly evacuated tubes stored in the refrigerator.

The alpha eleostearic acid was obtained from fresh Florida tung oil.** The oil was saponified by boiling

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under nitrogen for several minutes with 20% alcoholic potassium hydroxide (100 gm. oil to a solution of 40 gm. KOH in 27 ml. water and 200 ml. 95% ethanol previously distilled from alkali). The cooled soap solution was diluted with water, covered with a layer of petroleum ether in a separatory funnel and carefully acidified with chilled 20% sulfuric acid. After thorough shaking, the ligroin layer was separated, washed well, dried over sodium sulfate and filtered. The solution was diluted with more low-boiling petroleum ether to about 10-15% and cooled to 0°. The precipitating solid was filtered with suction under nitrogen and the mother liquor chilled to about —20°. The crystals were filtered as before and the process of cooling to 0° and —20° repeated three times, the crystals appearing at the lower temperature being retained each time. The third recrystallization resulted in a pure white solid, m.p. 49°. While the filtrate in every case was perfectly clear, the solution of the solid acid in ligroin even without heating quickly produced a cloudiness due to slight oxidation and which could be removed only by the fractional crystallization described above. While this procedure entails considerable loss of eleostearic acid in the fraction dropping out at 0° and in the mother liquor, it quickly yields the pure acid, and the remainder is easily recovered in the less pure form. We have found crystallization from aqueous alcohol, as suggested by others (17), much less efficient and rapid, as well as inconvenient for the drying of the final product.

The beta eleostearic acid was prepared similarly from "tung butter" produced from the above tung oil by letting it stand for several days over a trace of flowers of sulphur in a tightly stoppered filled flask. Its m.p. was 71.5°.

The pseudo-eleostearic acid (10-12-14 octadecatrienoic acid) was made by isomerizing the linseed oil acids with 20% KOH in ethylene glycol according to the methods of Kass and Burr (6). Its m.p. was 79°, while its methyl ester, prepared in the usual manner by letting the acid stand over night with an excess of 4% sulfuric acid in methanol and then refluxing briefly under nitrogen, melted at 41°. As before, all crystallizations were made from ligroin under nitrogen.

Results

Curves showing the observed oxygen absorption with time are plotted as dotted lines in the accompanying figure. In all cases, maximum rates are attained which are approximately constant for some time. These rates are drawn on the figure as solid lines which are extrapolated for convenience in estimation. Their numerical values in mols O_2/mol substrate/100 minutes are indicated by the figures above and to the left of each curve. The rates obtained from these and from duplicate curves are listed in the following table.

Maximum Rates of Oxygen Absorption by Pure Octadecatrienoic Acids and Their Esters at 40° C.

Substance	Maximum Rate at 40°	
	Mols O_2/mol subst./100 min.	
A. *alpha*-Eleostearic acid	2.88 2.66	2.63 2.56
B. *beta*- " "	1.02	1.02
C. pseudo- " "	0.66	0.63
D. Methyl pseudo-eleostearate	0.43	0.41
E. Linolenic acid	0.53	0.52
F. Ethyl linolenate	0.23 0.24	0.24 —

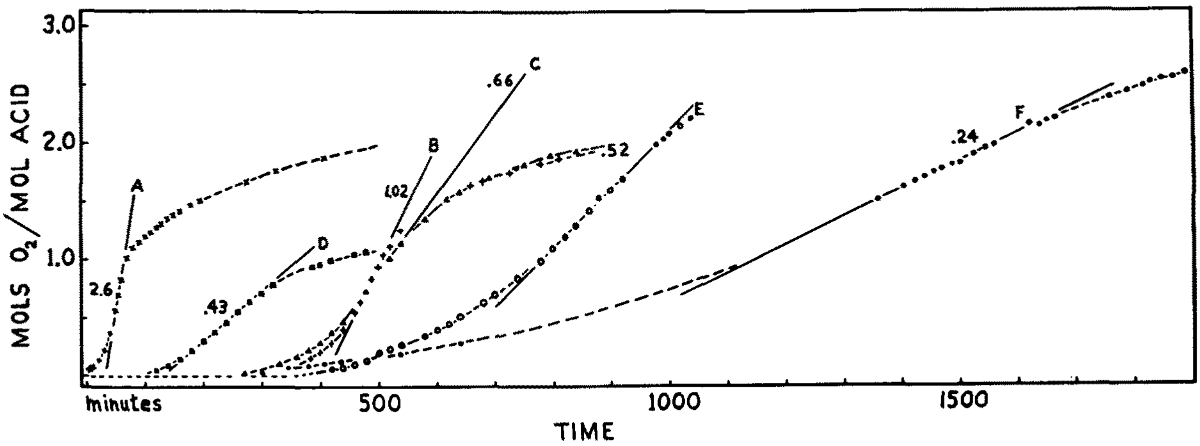
As expected, and in agreement with the data of Lederer (9), the conjugated compounds (A, B, C, and D) show considerably greater velocities of absorption than the compounds with isolated double linkages. Their curves are also more nearly S-shaped. However, the maximum rate attained by the conjugated compounds takes place approximately between the uptake of 0.5 and 1.0 mol of oxygen, while the maximum rate of the linolenic acid and its esters occurs between 1.0 and 2.0 mols of oxygen absorbed. These results may possibly be explained by the theory of partial valency.

Discussion

The influence of geometric configuration on the oxidation reaction is again strikingly borne out by the present data. The *alpha*- and *beta*-eleostearic acids have widely differing rates of oxygen absorption, though in both cases the unsaturation is located in the 9-11-13 positions. The contrast between the velocities of *beta*- and pseudo-eleostearic acids is less marked, although the sites of unsaturation are different in the two compounds. Pseudo-eleostearic acid probably resembles *beta*-eleostearic acid in its spatial construction, as further suggested by its melting point (10). Within either the conjugated or the isolated types of constitution, then, the maximum velocity of oxidation is affected more by geometric configuration than by the location of the double bonds. On the other hand, despite the marked differences in their maximum rates, the three conjugated acids required practically identical times for the uptake of from 0.5 to 2.0 mols of oxygen per mol of acid, i. e. 450 minutes.

In confirmation of the work mentioned in the introduction, the free acids oxidize much more rapidly than their esters. This may account for the accelerating effect of free acids on the rancidity of fats (4), since the free unsaturated acids can very quickly build up a sufficient amount of peroxides to destroy the antioxidants or to catalyze the oxidation of the neutral fat.

Considerable theoretical significance must be attached to the presence of the remarkably long induction periods preceding the attainment of maximum rates by these ostensibly pure compounds, also found by Täufel and Seuss (16) with purified oleic acid. It is very unlikely, especially in the case of linolenic acid and its ester, that any natural antioxidant could have come through into the purified compounds and thus account for the induction period. Stephens (14) states that pure compounds should have no induction periods. Accordingly, if the oxidation reaction is not autocatalytic, then this inhibitory effect must be due to some impurity resulting during the preparation of the materials. However, we have found that, while the maximum rates of a given compound is fairly reproducible with various samples, the induction period is not constant and represents merely the previous history of the particular sample. For example, a carelessly distilled preparation of a linolenate or a poorly protected sample of eleostearic acid will show no induction period at all, while the same compounds purified with utmost care exhibit prolonged induction periods. Although Morrell (11) found no induction period in the ethyl esters of drying oil acids, his results are not convincing in view of the work of Täufel and Seuss (16), who showed that oleic acid loses its induction period even after high vacuum distillation following exposure of the substance to air for some time. It is possible that the shortening of the induction period following re-



Rates of Oxygen Absorption by Octadecatrienoic Acids and their Esters at 40°

A—alpha Eleostearic acid
B—beta Eleostearic acid
C—pseudo Eleostearic acid
D—Methyl pseudo eleostearate
E—Linolenic acid
F—Ethyl linolenate

peated high-temperature distillations is not due to the removal of antioxidants but rather to the accumulation of catalyzing products of decomposition. The present data seem to indicate that pure fatty acids and their derivatives normally have induction periods not due to contaminating inhibitors and that the reaction is therefore autocatalytic. It would follow that in a series of pure compounds under the same physical conditions, the induction periods will fall in inverse order as their maximum velocities of oxygen absorption. This may be impossible to realize experimentally, since identical purities can never be assured. With any given compound, the induction period may obviously be explained on the one hand as the time required for the destruction of the inhibitor in the process of breaking chains in a chain reaction and by normal peroxide molecules, and on the other hand as the interval necessary for the accumulation of a sufficient amount of reaction product for autocatalysis. Solution of the problem whether pure substances normally have inductiton periods awaits the study of the mechanism of oxygen absorption by several samples of the same compound purified in various ways.

Conclusion

1. The maximum velocities of oxygen absorption as measured by the Warburg-Barcroft respirometer at 40° and expressed as mols 0_2/mol substance/100 minutes was found for a series of octadecatrienoic acids and their esters to be as follows: *alpha*-eleostearic acid, 2.68; *beta*-eleostearic acid, 1.02; pseudo-eleostearic acid, 0.64; methyl pseudo-eleostearate, 0.42; alpha-linolenic acid, 0.52; ethyl linolenate, 0.24.

2. The data showed the effect of spatial configuration as well as type of unsaturation and functional grouping on the oxidation reaction.

3. The apparently pure fatty compounds were found to have induction periods, pointing to the autocatalytic nature of the oxidation reaction.

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Methods of Measuring the Rate and Extent of Oxidation of Fats

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OUR interest in measuring the rate and extent of oxidation of edible fat is directed toward determining the relative susceptibility of fats to become rancid. Oxidative rancidity is not the only type of fat deterioration which is known but it is most common and the only type that is considered here.

A resumé of our knowledge of the mechanism of fat oxidation, the induction period, autoxidation, and antioxidants as directly related to the methods used to determine the rate and extent of fat oxidation have been presented in other articles in this symposium and this paper will therefore be confined to the methods that have been proposed to measure the oxidation of fats.

The literature on the oxidation of edible fats and oils is extensive and it cannot be completely reviewed