# Factors Affecting the Stability of Highly Unsaturated Fatty Acids.[1] III. The Autoxidation of Methyl Eleostearate

R. R. ALLEN[2,3] and F. A. KUMMEROW,[4] Kansas Agricultural Experiment Station,[5] Manhattan, Kansas

PREVIOUS studies have shown that oxygen adds directly to the double bond of a conjugated system (1). The mechanism involved in the autoxidation reaction as yet has not been clearly defined. The hydroperoxide hypothesis proposed by Farmer (2) for the autoxidation of a nonconjugated fatty acid does not seem to apply to a conjugated system. Robertson, Hartwell, and Kornberg (3) have shown that the peroxides of conjugated acids may differ from those of the nonconjugated acids. Furthermore Triebs (4) observed differences in the reactions of hydroxylamine and lead tetraacetate with the autoxidation products of the two types of unsaturated fatty acids.

Brauer and Steadman (5) found that oxygen adds to eleostearic acid in dilute solution in the ratio of one-half mole of oxygen for every double bond destroyed. These workers were able to separate a dimer of the acid which was thought to have at least one new carbon to carbon bond between the molecules. Furthermore Triebs (6) was able to isolate a dihydroxy stearic acid after hydrogenation of partially oxidized eleostearic acid. By comparing this glycol with one obtained from oxidized ricinoleic acid, he concluded that oxygen added 1,4 to produce a peroxide which could polymerize through peroxide bridges or tautomerize to more stable forms. However Triebs effected the separation of the autoxidation product by distillation which may have caused some thermal decomposition of the oxidation product.

In the present study the properties of the main primary oxidation product of methyl eleostearate and the kinetics of the reaction were investigated.

## Experimental

*Preparation of Materials.* The eleostearic acid was separated from tung oil[6] by the method described by Brauer and Steadman (5). The pure acid was esterified with methanol, the ester extracted with petroleum ether and purified by allowing it to pass through a column of alumina. The ester was freed from the solvent under vacuum, subjected to distillation in a falling film type of molecular still, sealed under vacuum in small glass ampoules, and stored at −20°C. The specific absorption coefficient at 2700 Å in ethyl alcohol was 184.

*Analytical Procedures.* The hydroxyl oxygen was determined according to the method of Ogg, *et al.* (7) using one ml. of acetylating reagent. The $\alpha,\beta$ dihydroxy compounds were determined by the method of Ross, *et al.* (8). The ultraviolet absorption spectra of the oxidized samples were carried out in a solution of 15% ethyl ether in ethyl alcohol by means of a Beckman quartz spectrophotometer.

*Apparatus.* The rate of autoxidation was followed with the aid of an apparatus (Figure 1) similar to

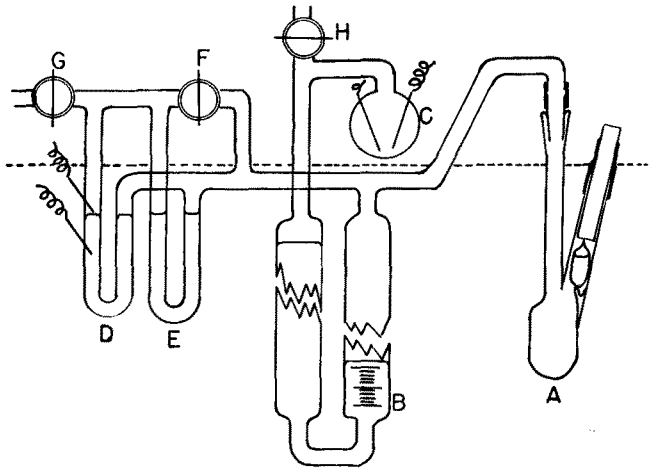


FIG. 1. Oxidation apparatus.

the one described by Kahman (9). A 15 ml. reaction flask *A* was fitted with a side arm of sufficient diameter to permit the introduction of a sealed ampoule of ester. A solid glass rod held by a short rubber tube served both to close the tube and to break the sealed ampoule of ester. The reaction flask was attached to a burette by means of a ground glass joint and a flexible heavy rubber tube. The latter permitted agitation of the reaction flask by a motor driven eccentric. The burette *B* of 30 ml. capacity was connected to a mercury reservoir and an electrolytic cell *C*. The latter contained a solution of oxalic acid, the electrolysis of which was controlled by the mercury manometer *D*. The manometer *E* was filled with butyl phthalate and served as a sensitive manometer to determine when the inside and outside pressures were equal. The entire apparatus was placed in a thermostatically controlled water bath which was fitted with both refrigeration coils and knife heaters.

The procedure used to measure the rate of autoxidation was as follows: The sealed ampoule of ester was introduced into the side arm of the reaction flask, the apparatus swept with dry oxygen, allowed to reach temperature equilibrium and the ampoule of ester broken. Stopcock *G* was opened and stopcock *F* closed, and the time, burette reading, and atmospheric pressure noted. The agitation of the reaction flask was started at two- to five-minute intervals, the mercury level in the burette was noted and the volume of oxygen absorbed calculated to moles of oxygen per mole of ester.

## Results

*Oxidative Changes in the Ultraviolet Absorption Spectra of Methyl Eleostearate.* The ultraviolet ab-

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[2] Portion of a thesis presented by Robert R. Allen as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry.

[3] Present address: Armour and Co., Chicago, Ill.

[4] Present address: University of Illinois, Urbana.

[5] Contribution No. 417, Department of Chemistry.

[6] Freshly pressed and sulfur isomerized tung oil were furnished through the courtesy of the Bagalusa Tung Oil Co., Bagalusa, La.

sorption spectra of the methyl eleostearate samples were determined during the autoxidation and found to show a decrease at 2700 Å while the band at 2340 Å increased as the amount of oxygen in the samples increased. The linear relationship of the changes in the absorption bands with the amount of added oxygen is shown in Figure 2.

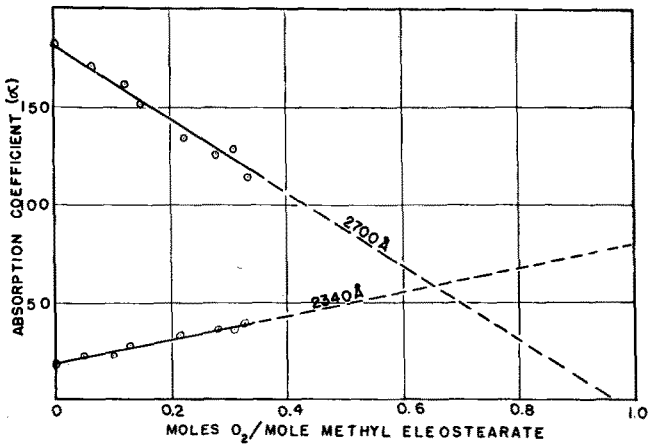



FIG. 2. Changes in absorption coefficient during oxidation.

*Isolation of Primary Oxidation Product.* The primary products obtained on autoxidation were separated by the fractional crystallization method described by Swift, *et al.* (10). One hundred twenty-one g. of methyl eleostearate was oxidized at 20°C. until 0.2 mole of oxygen per mole of ester had been absorbed. The resulting product was dissolved in 500 ml. of acetone and cooled to −80°C. in a dry ice-acetone bath. The liquid and solid phases were separated by filtration. The filtrate was reduced to 300 ml. of acetone in volume and again subjected to low temperature crystallization. The crystalline material was removed by filtration and the filtrate freed of acetone under vacuum at room temperature. The resulting viscous residue was poured into a separatory funnel and washed twice with 700 ml. of Skellysolve F. A flow sheet of the operations involved and the yields based on weights and ultraviolet absorption analysis of the fractions are given in Figure 3.

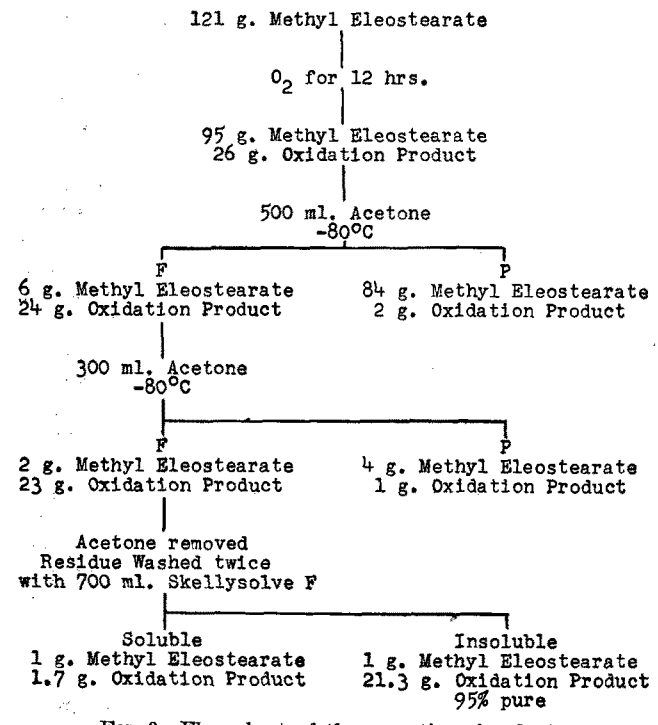



FIG. 3. Flow sheet of the operations involved.

*Properties of the Oxidation Product.* The products separated from both partially oxidized α and β methyl eleostearate were viscous colorless liquid which did not become solid at temperatures as low as −80°C. The characteristics of the products obtained from the autoxidation of the α and β forms of methyl eleostearate are shown in Table I with calculated values for methyl eleostearate + $O_2$. The ultraviolet spectra of typical preparations are shown in Figure 4.

The ultraviolet absorption spectra of the oxidation product in alcoholic potassium hydroxide solution was not altered in 1 and 5% alcoholic potassium hydroxide solution, but the absorption at the shorter wave lengths was decreased markedly in 10 and 15% solution as shown in Figure 4. Only slight increases were noted at the longer wave lengths in this solvent.

*Hydrogenation.* The hydrogenation of the oxidation product was accomplished by the use of Raney nickel catalyst and a hydrogen pressure of 60 p.s.i.

TABLE I

Characteristics of Oxidation Products From α and β Methyl Eleostearate

| | α | β | Calculated[d] |
|---|---|---|---|
| Peroxide value | 0 | 0 | ..... |
| Refractive index $N_D^{25}$ | 1.498 | 1.500 | ..... |
| Absorption coefficient 2340 Å | 62 | 60 | ..... |
| Absorption coefficient | 19[a] | 28[b] | 0 |
| % Carbon | 70.47 | 70.49 | 70.34 |
| % Hydrogen | 9.74 | 9.94 | 9.95 |
| % Oxygen (by difference) | 19.74 | 19.95 | 19.71 |
| Saponification equivalent | 320 | 321 | 324 |
| Molecular weight[c] | 660 | 632 | ..... |

[a] 2700 Å.
[b] 2680 Å.
[c] Determined by cryoscopic method using nitrobenzene as solvent.
[d] Methyl eleostearate + $O_2$.

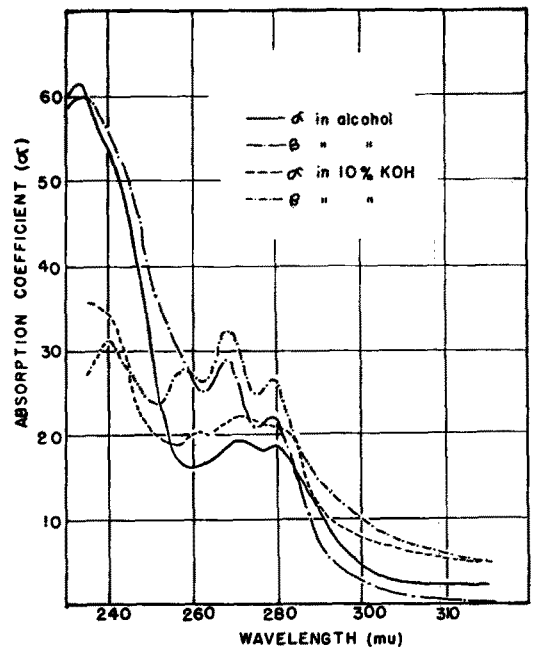



FIG. 4. Ultraviolet absorption spectra of autoxidation products.

The hydrogenated product was crystallized twice from 10% acetone in petroleum ether and resulted in a 70% yield of methyl dihydroxystearate. Analysis of this product indicated carbon, 68.96%; hydrogen, 11.9%; hydroxyl, 9.84%; saponification equivalent, 326; compared with the calculated values of carbon, 69.05%; hydrogen, 11.59%; and saponification equivalent, 330. The $\alpha,\beta$ hydroxyl groups varied from 4 to 12% in the different preparations.

The methyl dihydroxystearates were partially separated by chromatography, employing an alumina column and ether acetone as eluting agent. Fractions of the eluate were collected in tared beakers, the solvent evaporated, and the residue weighed. By combining the small residues, four main fractions were obtained. The first, a very small fraction, was identified as methyl stearate. The second and third, which had melting ranges of 61-63° and 56-57°, respectively, contained no $\alpha,\beta$ hydroxyl groups while the fourth had a melting range of 86-89° and contained 96% $\alpha,\beta$ dihydroxy groups. Analysis showed the latter three fractions were methyl dihydroxystearates.

*Oxidation With Potassium Permanganate.* Disruptive oxidation of the autoxidized product was carried out essentially by the method described by Armstrong and Hilditch (11). Characterization of the disrupted products showed that only valeric and azelaic acids were present.

*Kinetic Study.* The oxygen uptake of methyl eleostearate as a function of time at several different temperatures is given in Figure 5. The reaction started

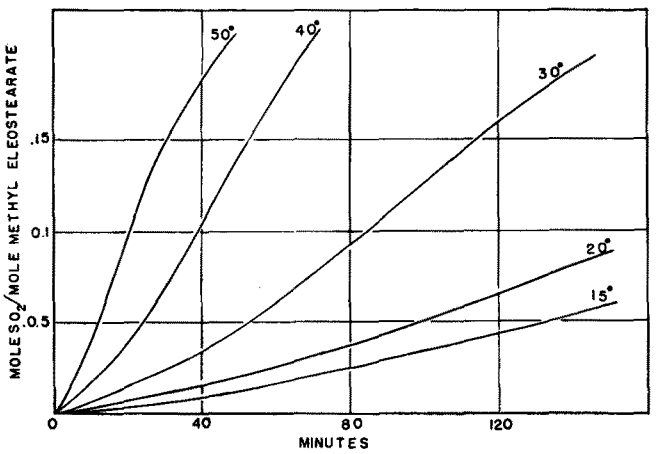



FIG. 5. Rate of autoxidation of methyl eleostearate.

immediately after the ester was allowed to contact the oxygen, but the rate of reaction was slow at first; it gradually increased to a maximum at about 0.2 mole oxygen uptake and then approached zero slowly. The rate of reaction (moles $O_2$/mole ester/minute) was not linear with the extent of oxidation so the reaction did not follow first order kinetics during the primary reaction. However the rate of reaction was found to show a linear relationship with the square root of the extent of oxidation as shown in Figure 6.

The effect of the initial concentration of methyl eleostearate on the rate of autoxidation is shown in Figure 7. Samples of methyl eleostearate were diluted with freshly distilled butyl stearate and the rates of reaction determined. The rate was found to

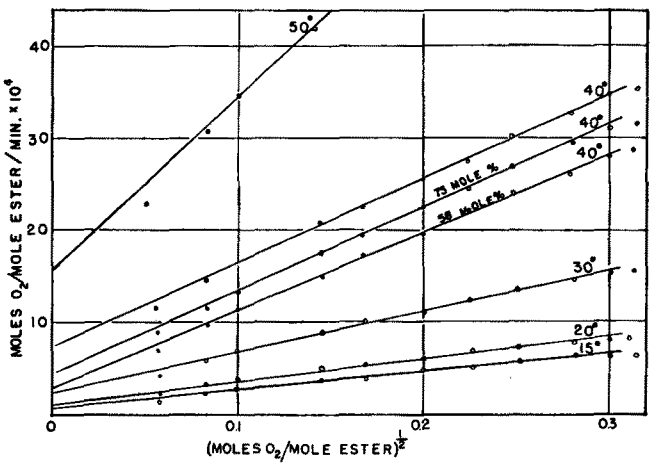



FIG. 6. Dependence of rate of autoxidation on the square root of product concentration.

be dependent on the initial concentration of methyl eleostearate.

The catalytic effect of benzoyl peroxide was investigated by the addition of benzoyl peroxide to methyl eleostearate. As shown in Figure 7, the rate of oxygen uptake was found to be proportional to the square root of the concentration of benzoyl peroxide.

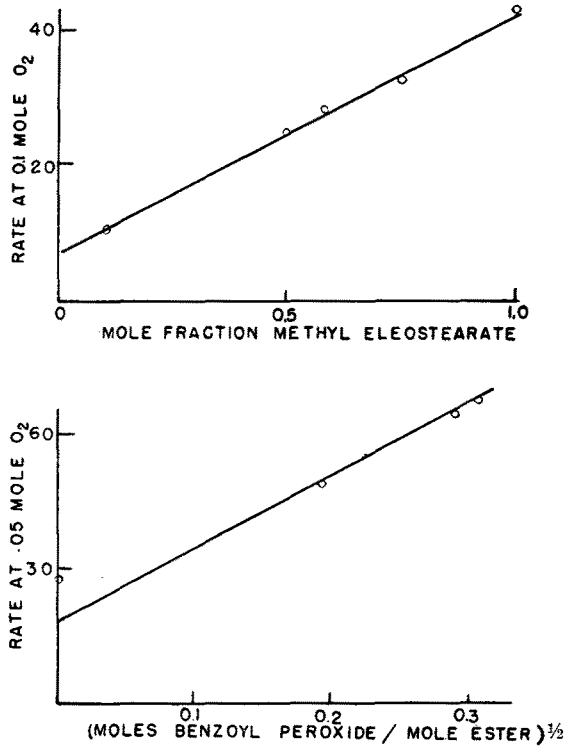



FIG. 7. Dependence of rate of autoxidation on initial concentration of methyl eleostearate and benzoyl peroxide concentration.

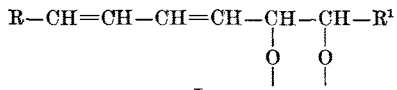
## Discussion

The results obtained from these studies are added evidence for the view (1, 3) that conjugated and isolated systems of double bonds give different initial products during autoxidation.

The changes in the ultraviolet absorption spectra of the fatty acid during the primary stages of autoxidation indicated that the triene system of double bonds was being destroyed from the outset of the reaction. Also the decrease in the absorption coefficient was linear with the oxygen added at low levels of oxidation and indicated that one mole of oxygen was necessary to destroy the triene system. This was in contrast to the conclusion reached by Brauer and Steadman (5) that one-half mole of oxygen will destroy the triene.

The chromaphore produced at 2320-2340 Å during the first stages of oxidation indicated the formation of a carbon diene system. Although the intensity of this band was reduced in strong alkaline solution, as would be expected from a carbonyl group, the absence of any new chromophore in basic solution indicated that the absorption band at 2340 Å was not due to an unsaturated carbonyl compound.

The data indicated that the position occupied by the oxygen in the oxidized ester was confined to the triene system and occurred at the 1,2, 1,4, and 1,6 positions, therefore the following structures were possible:



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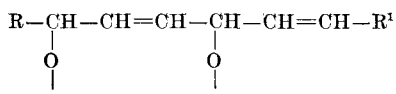

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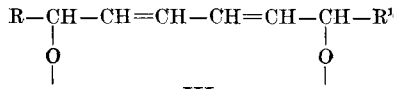


III

The experimental observations supporting such structures were as follows:

The production of only valeric and azealic acids by disruptive oxidation indicated that the reaction was confined to the triene system. The decrease of the triene and increase of the diene absorption bands were indicative of double bond reaction and also suggested the position of the oxygen. If the three structures were produced in equimolar amounts, the specific absorption coefficient at 2340 Å could be assumed to be two-thirds of 115, as only structures I and III would absorb at this wave length. The observed value of 62 of a product which was 90% pure agreed with the calculated value of 71.

Furthermore the formation by hydrogenation of at least three isomeric dihydroxy methyl stearates, only one of which contained an $\alpha,\beta$ dihydroxy group, added evidence that the three structures were produced.

From the kinetic study the rate of the reaction of oxygen with methyl eleostearate may be summarized by the expression:

$$do_2/dt = K\,(\text{Product})^{1/2}\,(\text{Ester})$$

From the observed kinetics of the reaction, the product of the reaction must be the sole catalyst and, in common with many different types of oxidation, be a chain reaction. The product was decomposed to form free radicals which initiated the chains, and termination of the chains occurred by reaction of two of the particular radicals formed. The generalized reaction scheme may be shown as follows, X and Y being designated as the chain carriers:

| | | |
|---|---|---|
| Initiation | | |
| | (1) Product → X or Y | $k_1$ |
| Propagation | | |
| | (2) X + $O_2$ → Y | $k_2$ |
| | (3) Y + ester → X + product | $k_3$ |
| Termination | | |
| | (4) X + X → | $k_4$ |
| | (5) X + Y → } end products | $k_5$ |
| | (6) Y + Y → | $k_6$ |

The reaction velocity constants for the various reactions are $k_1$ to $k_6$.

The concentration of Y at any time will be determined by the rate at which it is formed by initiation reaction 1 and propagation reaction 2, and by the rate at which it is destroyed by termination reactions 5 and 6. The high oxygen pressure employed in this study would adjust the concentration of X and Y so that termination would occur only by reaction 6. If $k_6$, is much larger than $k_1$, the "steady state" treatment may be applied. The concentration of the radical Y is soon reached so that the rates of initiation and cessation become equal and may be written:

$$k_1\,(\text{Product}) = k_6\,(Y)^2.$$

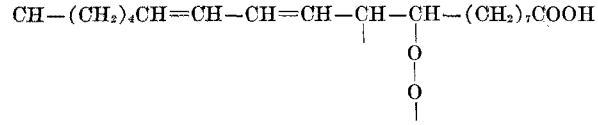
Solving this for Y we obtain: $(Y) = (k_1/k_6\,[\text{Product}])^{1/2}$

By using this expression to replace the concentration of Y in the propagation reaction 3, which is the reaction actually measured, the observed kinetics of the reaction, first order with respect to monomer and half order with respect to the product, are obtained.

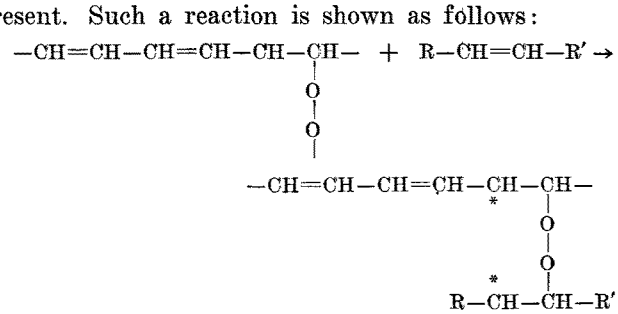
$$dO_2/dt = k_3\,(\text{Ester})\,(k_1/k_6\,[\text{Product}])^{1/2}$$

The benzoyl peroxide catalyzed reactions add proof to the above expression as it has been shown (12) that benzoyl peroxide decomposes by a first order chain reaction and the chains are terminated by mutual deactivation of two chain carriers.

In conformity with the foregoing general kinetic analysis X can be identified as an eleostearate molecule and Y as a diradical with the following structure:



This radical would be stabilized by resonance of the free radical center along the unsaturated carbon chain, thus producing free radical centers on any of the carbon atoms in the triene system. This radical could react with an olefinic double bond to form a dimer which would still have free radical centers present. Such a reaction is shown as follows:



The dimeric radical thus produced could again add oxygen to the free radical centers to produce per-

oxide radicals which could add olefin, thus building up a polymeric chain in which the repeating unit is —CHR'—CHROO—.

Although molecular weight measurement indicated only dimer formation, the solvent used possibly partially decomposed the polymers to the more stable perdioxane structure.

Treibs in a recent report (13) stated that only a 1,4 reaction occurred and produced polymers through peroxide bridges. The data presented here support this view but add the possibility of both 1,2 and 1,6 addition to the conjugated triene system.


### Summary

The autoxidation of methyl eleostearate was studied by observing the kinetics of the reaction and separating the primary oxidation products. The results indicate that an eleostearate free radical adds oxygen to the 1,2, 1,4, or 1,6 position of the triene system to produce polymers through peroxide bridges.



### Acknowledgment

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REFERENCES

1. Morrell, R. S., Bolam, T. R., Davis, W. R., Marks, S., Phillips, E. O., and Sim, W. S., Trans. Faraday Soc., *38*, 362-372 (1942).
2. Farmer, E. H., Trans. Faraday Soc., *38*, 356-361 (1942).
3. Robertson, W. V. B., Hartwell, J. L., and Kornberg, S., J. Am. Chem. Soc., *66*, 1894-1897 (1944).
4. Treibs, W., Chem. Ber., *75*, 1373-1378 (1942).
5. Brauer, R. W., and Steadman, L. T., J. Am. Chem. Soc., *66*, 563-567 (1944).
6. Treibs, W., Chem. Ber., *76*, 670-676 (1943).
7. Ogg, C. L., Porter, W. L., and Willits, C. O., Ind. and Eng. Chem., Anal. Ed., *17*, 394-396 (1945).
8. Ross, J., Gebhart, A. I., and Gerecht, J. F., J. Am. Chem. Soc., *67*, 1275-1278 (1945).
9. Kahman, G. T., J. Phys. Chem., *33*, 226-242 (1929).
10. Swift, C. E., Dollear, F. G., and O'Connor, R. T., Oil and Soap, *23*, 255-259 (1946).
11. Armstrong, E. F., and Hilditch, T. P., J. Soc. Chem. Ind., *44*, 180T-197T (1925).
12. Nayaki, K., and Bartlett, P. D., J. Am. Chem. Soc., *69*, 2299-2302 (1947).
13. Treibs, W., Chem. Ber., *80*, 423-426 (1947). C. A. *42*, 7707-7708 (1948).


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# Autoxidation of Methyl Oleate, Methyl Stearolate, and Methyl 9,10-Dideuteroöleate[1,2]


N. A. KHAN,[3] J. B. BROWN, and F. E. DEATHERAGE, Institute of Nutrition and Food Technology, Departments of Agricultural Biochemistry and Physiological Chemistry, Ohio State University, Ohio Agricultural Experiment Station, Columbus, Ohio


THE past decade has seen notable progress in understanding the nature of the autoxidation of fats and oils. Many of these advances have been made by studying the autoxidation of pure compounds which have led to postulations regarding the mechanisms involved in this complex phenomenon. Swern, Scanlan, and Knight (1) and Täufel and Rothe (2) have reviewed these theories. More recently a number of notable contributions have been made to the knowledge of the mechanism of the autoxidation of fats, and these will not be reviewed here.

It has been often reported that water is formed during the autoxidation process, and it was thought that perhaps some information of value might result when fat derivatives containing deuterium were studied and the resulting water characterized for deuterium content. Such studies might give information concerning the induction period, peroxidation, polymerization, and fragmentation of the molecule giving rise to rancidity. Accordingly this paper will deal with the autoxidation of methyl 9,10-dideuteroöleate, a mono-olefin where the olefinic hydrogens (on carbons 9,10) have been replaced by deuterium. In another paper there will be reported the autoxidation of 8,8,11,11-tetradeutero-cis-9-octadecene, a mono-olefin where the hydrogens, on the carbons 8 and 11, $\alpha$ to the double bond, have been replaced by deuterium. In both instances the corresponding compounds containing no deuterium are also to be reported.

Since the deuteroöleate was prepared from stearolic acid, the autoxidation of this compound and its methyl ester was also studied.

Some investigations have been carried out on the nature of the non-aqueous volatile matter from the autoxidized compounds which were reported by Deatherage and Mattill (3) to contain a high concentration of peroxidic oxygen.

### Materials

The substrates, "reduced" oleic acid, "reduced" methyl oleate, methyl 9,10-dideuteroöleate, stearolic acid, and methyl stearolate used for the autoxidation studies were prepared according to the methods described by Khan, Deatherage, and Brown (4) whereas those designated as "crystallized" oleic acid and "crystallized" methyl oleate were prepared according to the methods of Brown and Foreman (5). The characteristics of the different substrates were:

Reduced oleic acid: B.P., 183-184° at 1.8-2.0 mm.; I. N. (Iodine Number), 89.4; $n^{20} = 1.4600$; hydrogen uptake, 1.00 mole $H_2$ per mole.

Reduced methyl oleate: B.P., 171-172° at 1.9-2.0 mm.; I. N., 85.1; $n^{20} = 1.45215$; hydrogen uptake, 1.00 mole $H_2$ per mole.

Methyl 9,10-dideuteroöleate: B.P., 173-174° at 1.8-2.0 mm.; I. N. 84.7; $n^{20} = 1.45186$, $n^{26} = 1.4492$; deuterium content, 5.05 atoms per cent of hydrogen and deuterium (theory 5.55).

Stearolic acid: M.P. 46.0-46.5°; I. N., 89.5; $n^{54.5} = 1.4510$, $n^{61.5} = 1.4484$; hydrogen uptake, 1.98 moles $H_2$ per mole (theory, 2 moles). Freezing point curve, carbon and hydrogen analysis, and ozonolysis confirmed the purity of the material as 9-octadecinoic acid.

Methyl stearolate: B.P., 174-175° at 2.6-3 mm.; I. N., 86.2; hydrogen uptake, 2.00 moles $H_2$ per mole; $n^{20} = 1.4562$, $n^{52.5} = 1.4435$.

Crystallized oleic acid: M.P., 13.3-13.4°; I. N., 89.99; $n^{20} = 1.4600$.

Crystallized methyl oleate: B.P., 171-172° at 1.9-2.0 mm.; I. N., 84.97; $n^{20} = 1.4521$.

Hydroxy heptyl peroxide, 95%; methyl amyl ketone peroxide, 95%; and t-butyl hydroperoxide, 60%. These were donated by the Lucidol Division, Novadel-Agene Corporation.


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[2] Journal article No. J 31-50 of the Ohio Agricultural Experiment Station, published with the permission of the assistant director.

[3] A scholar of the government of Pakistan.