The Journal of the American Oil Chemists' Society
Volume 34 JULY, 1957 No. 7

Influence of High Energy Radiation on the Oxidation of Oleic Acid and Methyl Oleate. II. Sites of Oxygen Attack[1, 2, 3]

HAL T. SLOVER and L. R. DUGAN JR., American Meat Institute Foundation, The University of Chicago, Chicago, Illinois

AN EARLIER PAPER (1) reported some of the effects of γ-radiation on the rate and extent of oxidation of oleic acid and methyl oleate. Such radiation greatly increased the rate of autoxidation even at low temperatures; at higher temperatures the hydroperoxides were formed faster, but the breakdown to secondary products was also increased. No significant departure from non-irradiated autoxidations was found. There was a possibility however that the influence of these high energy γ-rays might cause the autoxidation to take a different course and that among the various types of compounds investigated—hydroperoxides, carbonyls, and acids—there might be some individual members peculiar to irradiated autoxidations. In the currently accepted, free-radical mechanism of autoxidation the chain-carrying step involves the activity of a methylene group α- to the double bond: a hydrogen atom is lost, and the resulting radical combines with oxygen to form a hydroperoxide radical. Loss of a hydrogen from carbon 11 results in a resonance hybrid that may combine with oxygen either at carbon 11 or carbon 9. Similarly loss of a hydrogen from carbon 8 may lead to union with oxygen at either carbon 8 or carbon 10. Evidence for the presence of all four of these hydroperoxides was found by Ross (5) in his work with methyl oleate autoxidized at 35°C. under ultraviolet light. The quantities varied in the order 10>11>8>9. It has also been shown (2) that the acidic secondary products resulting from such hydroperoxides include 8 and 9 carbon mono- and dibasic acids, and possibly some dibasic semialdehydes. In the presence of γ-radiation it was conceivable that other hydrogens along the chain might be activated, resulting in different hydroperoxides and secondary products. To test this possibility an attempt has been made to characterize the hydroperoxides and acids formed by the irradiated autoxidation of methyl oleate at two temperatures, 7.5°C. and 56°C. The acids formed in a cobalt-catalyzed, irradiated autoxidation have also been examined, though less extensively.

Experimental

The methyl oleate used in these experiments was prepared from commercial oleic acid and purified by solvent crystallization and fractional distillation. The material used for the three samples had the following characteristics: Sample I, IV = 80.8, $n_D^{20} = 1.4520$; Sample II, IV = 78.4, $n_D^{20} = 1.4520$; Sample III, IV = 79.0, $n_D^{20} = 1.4520$. During autoxidation, oxygen was introduced in a fine stream of bubbles from a fritted glass, gas dispersion tube while the sample was exposed to the radiation from a cobalt 60 source rated at 100 Rep./sec. When required, heat was provided by an internal, nichrome heating element. Using this procedure, three samples of methyl oleate were autoxidized for this study: one at 7.5°C., one at 56°C., and one, containing 0.5% cobalt stearate, at 73°C. These were tested for peroxide value, total carbonyl value, α,β-unsaturated carbonyls, and acidity.

Peroxide values were determined by a modification of the Wheeler (6) iodometric method. Total carbonyl values were determined colorimetrically by a modification of the Lappin and Clark (4) procedure. The $E^{1\%}_{1\,cm.}$ at 224 mμ was used as a measure of α,β-unsaturated carbonyls, as suggested by King (3). Mono- and dibasic acids were separated and identified chromatographically, using a reverse phase partition system described by Zbinovsky (7). An internal phase of methyl cellosolve/water supported on silicic acid was used to absorb the sample, which was then eluted by solvents of appropriate polarity. Skellysolve B was used for monobasic acids, and n-butyl ether for dibasic acids. Collecting small fractions and titrating them with standard base determined the distribution of the acids in the eluent. By comparing acid elution curves of the unknown samples with those of known mixtures, a tentative identi-

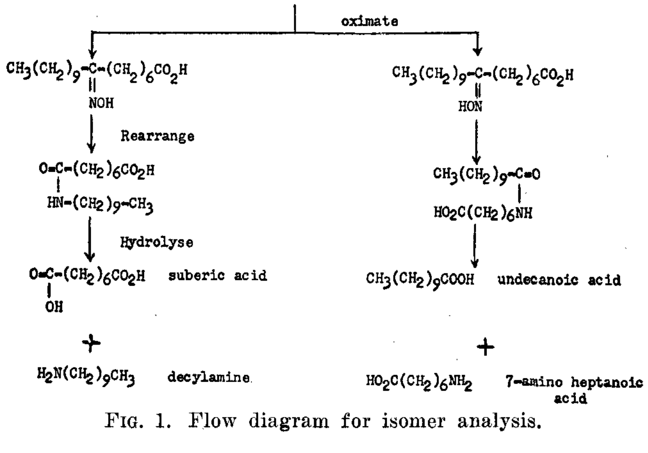

FIG. 1. Flow diagram for isomer analysis.

---

[1] Supported by a grant from the National Renderers' Association.

[2] American Meat Institute Foundation Journal Paper, Number 136.

[3] Presented at the fall meeting of the American Oil Chemists' Society, September 24–26, 1956, Chicago, Ill.

fication was possible. The recovering and rechromatographing of the tentatively identified acids, mixed with equivalent amounts of known acids, gave a more positive identification.

A method described by Ross (5) was used for characterizing the hydroperoxide isomers. This involved conversion into separable and identifiable fragments characteristic of the parent hydroperoxide. A flow diagram for one of the possible isomers, the 8-hydroperoxide, indicating the series of reactions involved, is shown in Figure 1. The first step is hydrogenation to the saturated hydroxy compound, using a platinum oxide Adams catalyst. Oxidation with chromic acid oxidizes the hydroxy compound to a keto acid, which is then converted to an oxime by reaction with hydroxylamine hydrochloride. In this reaction two stereoisomers are possible, each giving different end-products. A Beckmann rearrangement of the mixture of oximes, carried out by heating with concentrated sulfuric acid, converts them into amides. Hydrolyzing with alkali and acidifying gives, as final products, a dibasic acid and an amine from one isomer and a monobasic acid and an amino acid from the other. Table I lists the fragments to be expected from the 8-, 9-, 10-, and 11-hydroperoxides, which, if the γ-irradiated autoxidation follows the normal course, would be the only ones present. By separating and identifying one or more groups of these fragments, the original hydroperoxides may be characterized. Should there be other isomers present, they too could be reconstructed from a knowledge of their fragmentation products.

TABLE I

Products to be Expected from Degrading the Various Isomeric Methyl Hydroperoxidoöleates

Peroxide	Products			
	Monobasic acid	Amino acid	Dibasic acid	Amine
8-hydroperoxide	undecanoic	7-amino heptanoic	octandioic	10-amino decane
9-hydroperoxide	decanoic	8-amino octanoic	nonandioic	9-amino nonane
10-hydroperoxide	nonanoic	9-amino nonanoic	decandioic	8-amino octane
11-hydroperoxide	octanoic	10-amino decanoic	undecandioic	7-amino heptane

Results

The analytical data obtained for the autoxidized samples are given in Table II. At 7.5°C. there was little breakdown of peroxides; the principal secondary product was carbonyls. At 56°C., after 101 hrs., about 40% of the original methyl oleate was present as peroxide, calculated as the hydroperoxide. There was some breakdown to secondary products, as shown by the high carbonyl value and the amount of acid present. In the cobalt catalyzed sample the secondary products, principally acids, predominated, and very little peroxide was present.

TABLE II

Methyl Oleate Autoxidized Under Gamma Radiation

	Temp. (°C.)	Time (Hrs.)	PV[b] (Meq./Kg.)	CV[c] (Mol./Kg.)	$E^{1\%}_{1\,cm.}$[d]	Acidity (Meq./Kg.)
I	7.5	69	472	114.5	9.6	10.5
II	56	101	2453	618	39.5	299
III[a]	73	63	168	715	35.2	2030

[a] 0.5% cobalt stearate added.
[b] Peroxide value.
[c] Carbonyl value.
[d] Measured at 224 mμ.

The samples autoxidized at 7.5°C. and 56°C. were submitted to the procedure for isomer analysis. After acidifying the hydrolyzed amide, the monobasic acids were recovered by steam-distillation and chromatographed. Figure 2 shows the elution curves of the derived monobasic acids compared with that of a mixture of known octanoic, nonanoic, decanoic, and undecanoic acids. In each sample there are four peaks, corresponding approximately to the four known acids shown. The third peak in the 7.5° curve was rechromatographed with nonanoic acid. Both known and unknown came off in one peak, showing this acid to be nonanoic. The fourth peak was shown to be octanoic acid by the same procedure. By inference the other acids are assumed to be undecanoic and decanoic. In the 56°C. curves each of the four acids was rechromatographed with its nearest corresponding known acid, and the same proof was obtained. The relative amounts of each of the hydroperoxides may be roughly estimated from the amounts of acid found (Table III). In descending order the amounts were found to be 10 > 11 > 9 > 8 for both samples, with 10- and 11- predominating.

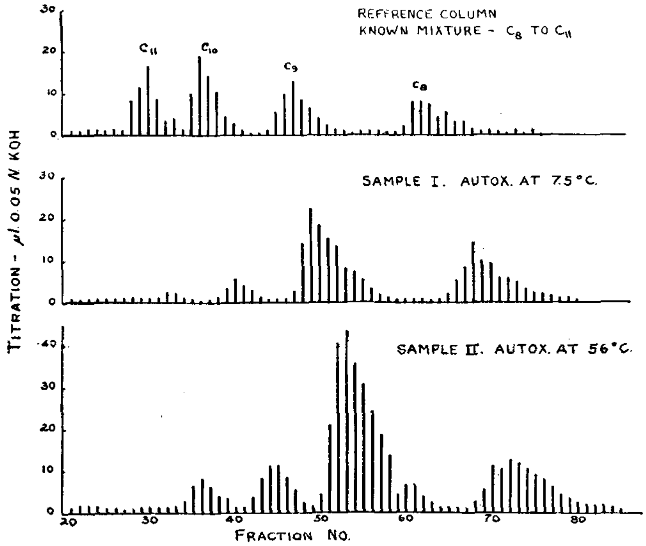

FIG. 2. Monobasic acids derived from hydroperoxides.

TABLE III

Percentage of Distribution of Hydroperoxide Isomers Formed from Methyl Oleate Under Gamma Radiation

Sample No.	Carbon No.			
	8	9	10	11
I–(7.5°C.)	1.9	7.8	56.0	34.0
II–(56°C.)	6.0	9.6	59.6	24.9

The sample autoxidized at 56°C. and the cobalt-catalyzed sample autoxidized at 73°C. were also examined for mono- and dibasic acids formed as secondary products of the autoxidation. They were first steam-distilled to separate volatile and non-volatile oils, then hydrolyzed and chromatographed on appropriate columns. The steam-distillate from the 56° autoxidation, when chromatographed on a methyl cellosolve/silicic acid column and eluted with Skellysolve B, gave a chromatogram (Figure 3) with two

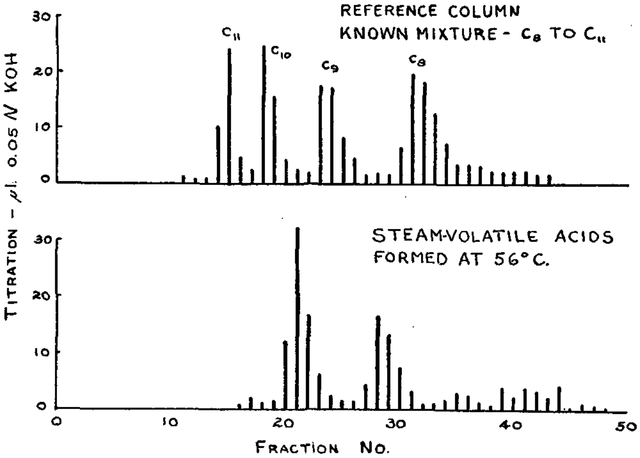

FIG. 3. Monobasic acids formed by autoxidation at 56°C.

peaks corresponding approximately to nonanoic and octanoic acids. The first of these peaks was shown to be nonanoic by recovery and rechromatographing mixed with known nonanoic acid; both known and unknown came off in one peak. By inference the second peak from the steam-distillation products was assumed to be octanoic acid. When the steam distillate was hydrolyzed and the resulting acids were chromatographed, the same two major peaks came off and, in addition, a much earlier large sharp peak, which could be a long-chain acid whose methyl ester was steam-distillable. Both before and after hydrolysis only 50% of the acid put on the column could be eluted. This could be caused by the presence of some acidic material that was steam-distillable but not eluted by Skellysolve B, perhaps the half-esters of dibasic acids or dibasic-semialdehydes. This will be established by further studies. Both the untreated steam-distillate and its hydrolysate were chromatographed on a column designed to separate dibasic acids. The unhydrolyzed material came off early with no separation. The hydrolysate however contained two minor components, chromatographing in approximately the same positions as nonandioic and octandioic acids, though most of the acids were not held in the column. This would indicate that some dibasic esters had distilled over. The non-volatile material, after hydrolysis, gave a complex chromatogram (Figure 4) when eluted with n-butyl ether. Most of the material was not held; the remainder was separated into a number of as yet unidentified minor components. Two of these correspond approximately to nonandioic and octandioic acids.

The steam-volatile acids from the cobalt-catalyzed autoxidation contained two components that have been tentatively identified as nonanoic and octanoic acids. Two lesser components were also present, in approximately the positions expected for heptanoic and hexanoic acids. The corresponding non-volatile fraction contained only minor and doubtful amounts of dibasic acid. There were several components that were rapidly eluted by n-butyl ether but not so rapidly as the simple mono-basic acids. These may possibly be dibasic semialdehydes, intermediate in polarity between mono- and dibasic acids.

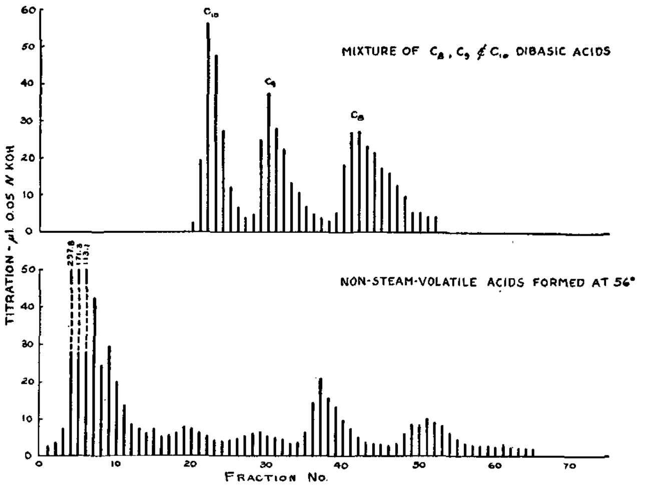

FIG. 4. Non-steam-volatile acids formed by autoxidation at 56°C.

Conclusion

The evidence indicates that γ-radiation at the level used has no specific effect on the products of autoxidation. The initial product, the hydroperoxides, consists of the same four isomers found in non-irradiated autoxidations. An examination of the mono- and dibasic acids, formed by chain scission and complete oxidation, also revealed no products not found in autoxidations energized in other ways.

Summary

1. The hydroperoxides formed by the γ-irradiated autoxidations of methyl oleate at 7.5°C. and 56°C. have been characterized. The mono- and dicarboxylic acids produced by γ-irradiated autoxidation at 56°C. and by cobalt-catalyzed γ-irradiated autoxidation at 73°C. have also been examined.

2. The hydroperoxides were found to be a mixture of the 8-, 9-, 10-, and 11-hydroperoxidoöleates in both samples. They were present in the order $10 > 11 > 9 > 8$.

3. Evidence is presented for the presence of octanoic, nonanoic, octandioic, and nonandioic acids in the 56°C. autoxidation. Octanoic and nonanoic acids were also present in the cobalt-catalyzed autoxidation.

4. No significant departure from non-irradiated autoxidations was found.

REFERENCES

1. Dugan, L. R., and Landis, P. W., J. Am. Oil Chemists' Soc., *33*, 152–154 (1956).
2. Ellis, G. W., Biochem. J., *46*, 129–141 (1950).
3. King, G., J. Chem. Soc., 2114 (1954).
4. Lappin, G. R., and Clark, L. C., Anal. Chem., *23*, 541 (1951).
5. Ross, J., Gebhart, A. I., and Gerecht, J. F., J. Am. Chem. Soc., *71*, 282–286 (1949).
6. Wheeler, D. H., Oil and Soap, *9*, 89 (1932).
7. Zbinovsky, V., Anal. Chem., *27*, 764 (1955).

[Received November 29, 1956]

ADDENDUM

The following reference: Wilson, Glenn R., *31*, 564–568 (1954) should have been cited at the end of the paper on "Fatty Alcohols," by Karl P. Schroeder, in the November 1956 issue of the Journal of the American Oil Chemists' Society (*33*, 565–568).