method, the effects of linoleate hydroperoxide, hemoglobin concentrations, and temperature on the rate of hydroperoxide decomposition was studied. In addition, the catalytic activities of several hemoproteins, metallo-protoporphyrins, and metal chelate compounds were determined.

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Products of Unsaturated Fatty Acid Oxidation Catalyzed by Hematin Compounds

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THE IMPORTANCE of further knowledge of the mechanism of unsaturated fatty acid oxidation catalyzed by hematin compounds is given in previous papers (8, 15, 16). The critical part of this mechanism is the reaction between the hematin compounds and peroxides of the fatty acid in which free radicals are produced.

However very little is known concerning this interaction of hematin compounds and peroxides. The purpose of this research was to identify the products of the hematin-hydroperoxide reaction in an unsaturated fatty acid system.

The first section of this paper describes the identification of the initial products of hematin-hydroperoxide interaction, using both α-cumyl hydroperoxide and a methyl linoleate hydroperoxide. Alpha-cumyl hydroperoxide was chosen because of its well-studied reactions with ferrous ion and methyl linoleate hydroperoxide because of its similarity to hydroperoxides present in oxidizing fats. Since alkoxy free-radicals were the expected initial products, hydroquinone was used as a radical trap in the reaction system to convert the alkoxy radicals into their corresponding alcohols. The alcohols were then identified by standard chemical and physical methods.

The second section of this paper describes the identification of the secondary reaction products of a hematin-linoleate hydroperoxide-potassium linoleate system. Because of the extreme complexity of this reaction the identification of reaction products was limited at this time to separation into solubility classes and quantitative determination of functional groups.

Experimental

Reactants. Commercial 69% α-cumyl hydroperoxide (Monomer-Polymer Inc.) was used without further purification. A methyl linoleate hydroperoxide concentrate containing 1.05 meq./g. (33.2%) was prepared by the lipoxidase catalyzed oxidation of an emulsion of pure methyl linoleate (Hormel Foundation). Potassium linoleate hydroperoxide (0.068 meq./liter) was prepared from pure linoleic acid (Hormel Foundation) by the lipoxidase catalyzed oxidation of a 0 11 M potassium linoleate solution (8).

Reaction Systems for Identification of Initial Products. The α-cumyl hydroperoxide system contained 23.6 millimoles (5.0 g.) of α-cumyl hydroperoxide, 47.2 millimoles of hydroquinone, and 0.085 millimoles of hematin in 33 ml. of water with sufficient methanol added to produce a homogeneous solution.

The methyl linoleate hydroperoxide system contained 7.1 millimoles (6.76 g.) of the hydroperoxide, 14.3 millimoles of hydroquinone, and 0.036 millimoles of hematin in 20 ml. of 95% aqueous ethanol.

After 30 min. at 25°C. in a nitrogen atmosphere each system was filtered to remove the crystalline quinhydrone, and the reaction products were taken up in diethyl ether. The ether solutions were washed with 0.01 M NaOH and dried, and the ether was removed by vacuum distillation.

Products of the Cumyl Hydroperoxide System. Removal of the ether left 4.13 g. of crude product (82.6% recovery), which gave a negative peroxide test. Vacuum distillation of this material resulted in 3.30 g. (80.0%) of distillate (105°/25 mm.) and 0.11 g. of residue. The distillate formed a 2,4-dinitrophenylhydrazone, which melted at 250°C. and was thus identified as acetophenone. However quantitative gravimetric analysis of the distillate by the formation of this derivative showed that only 4.0% (wt./wt.) of the distillate was acetophenone.

The distillate, after removal of the acetophenone by washing with sodium bisulfite, had a refractive index of n_D^{25} 1.5199. It formed a derivative with 3,5-dinitrobenzoyl chloride, m.p. 105–106°C. A mixed melting point determination with the 3,5-dinitrobenzoate of pure α-cumyl alcohol gave m.p. 106°C. Thus the distillate was principally α-cumyl alcohol. The pure α-cumyl alcohol was prepared by reduction of the α-cumyl hydroperoxide with sodium sulfite (3). The reduced product, 8.01 g., yielded 6.95 g. (86.6%) of distillate (105°/25 mm.), which gave n_D^{25} 1.5207, 3,5-dinitrobenzoate m.p. 106–107°C., and a negative carbonyl test. Analysis showed that the original α-cumyl hydroperoxide contained about 17.4% α-cumyl alcohol.

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TABLE I

Analysis of Linoleate Hydroperoxide and Products Resulting from Hematin Catalyzed Decomposition

Analysis	Initial linoleate hydroperoxide	Products of the reaction			
		Nonacid ether-soluble fraction	Acidic, ether-soluble fraction		
			Unreduced	Reduced with $SnCl_2$	Reduced with $NaHSO_3$
Material recovered (g.)	24.20	1.07	22.78	.....	.....
Diene conjugation (meq./g.)	1.91	0.660	0.703	.....	.....
Peroxide (meq./g.)	2.06	.....	0.420	0.356	0.104
Carboxyl (meq./g.)	3.235	0.0	3.230	.....	.....
Carbonyl saturated (meq./g.)	.....	0.185	.....	0.076	0.115
Carbonyl unsaturated (meq./g.)	.....	1.04	.....	0.448	0.525
Carbonyl total (meq./g.)	.....	1.22	.....	0.524	0.640
Oxirane (meq./g.)	.....	0.0	0.923	.....	.....
Hydroxyl (meq./g.) total, includes chlorohydroxy	.....	0.0	1.89	.....	.....
Hydroxyl (meq./g.) corrected for chlorohydroxy	.....	.....	0.962	.....	.....
Density (g./ml. at 24°C.)	0.9731	0.9250	0.9871	.....	.....
Refractive index (n_D^{25})	1.4838	1.4714	1.4840	.....	.....
Specific extinction coefficient, 280 mμ (gm./l., 1 cm.)	1030	15200	8200	.....	.....
Viscosity (centipoises)	1380	1400	10000	.....	.....
Solubility in 60% (vol./vol.) aqueous ethanol	sol.	insol.	insol.	.....	.....

Products of the Methyl Linoleate Hydroperoxide System. Removal of the ether left 5.32 g. (78.7% recovery) of residue, which contained 9.1×10^{-3} millimoles/g. of peroxide (99% peroxide destruction). Quantitative determination of the hydroxyl group concentration by the acetic anhydride method (10) showed that 0.985 millimole/g. (93.8%) of the methyl linoleate hydroperoxide had been converted into the corresponding alcohol. Attempts to obtain a crystalline 3,5-dinitrobenzoate derivative of the alcohol were unsuccessful.

Reaction System for Identification of Secondary Products. Hematin, 0.272 millimole, was added to 800 ml. of the potassium linoleate hydroperoxide solution, in the absence of oxygen. The system was allowed to react for 90 min. at 25°C. before extraction. A total of 165 moles of peroxide were decomposed per mole of initial hematin.

Extraction Procedure. The reaction solution was adjusted to pH 8 and extracted with diethyl ether to remove the nonacid, ether-soluble decomposition products. Removal of the ether left 1.07 g. (4.4% of the starting material) of a light yellow, oily material which had an extremely rancid odor. The alkaline aqueous phase was acidified to pH 3 and extracted with ether to remove the acidic products. Most of the hematin and its decomposition products coagulated and were removed by filtration. Removal of the ether left 22.78 g. (93.2% of the starting material) of an oily, brownish, highly viscous residue. The brown color was later shown to be residual hematin.

Analytical Methods. Total peroxides were determined by the method of Lundberg and Chipault (7). A modification of the method of Henick, Benca, and Mitchell (6) was used to determine saturated and unsaturated carbonyl oxygen. The method of Swern *et al.* (13) was employed to determine oxirane oxygen. The acetic anhydride method of Ogg, Porter, and Willits (10) was used to determine hydroxyl oxygen. The method is known to give high results in the presence of oxirane groups. However, if the oxirane groups are first converted to chlorohydroxy groups, total hydroxy oxygen can be accurately determined (6). The hydroxyl oxygen content of the original sample can then be calculated from the difference between total hydroxyl (hydroxyl plus chlorohydroxy) and oxirane oxygen. For the determination of conjugated diene concentration the sample was dissolved in 60% aqueous ethanol, and the conjugated diene concentration was calculated from the absorbance at 232.5 millimicrons by using a molar extinction coefficient of 27,400 (14). For reduction of peroxides the stannous chloride method of Holman and Greenberg (4) and the sodium bisulfite method of Knight and Swern (6) were employed. For isolated *trans* double bond concentration the method developed by Shreve *et al.* (12), using the absorbance at 10.36 microns, was applied. Infrared spectra were obtained with a Beckman IR2 instrument, using sodium chloride cells and a sample thickness of 0.03 mm.

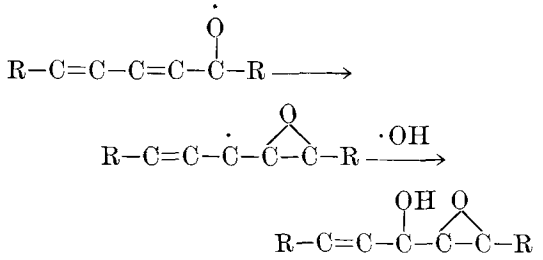
Results and Discussion

Initial Products of the Cumyl Hydroperoxide System. The yield of α-cumyl alcohol from the hematin catalyzed decomposition of α-cumyl hydroperoxide, 77.5% of the recovered product, compared well with that obtained by sodium sulfite reduction of α-cumyl hydroperoxide, 86.6% of the recovered product. The small amount of acetophenone produced during hematin catalysis indicates that the hydroquinone was not completely effective in stopping the reaction at the alkoxy radical stage. Thus, in the presence of a hydrogen-donor, the hematin catalyzed decomposition of α-cumyl hydroperoxide produces α-cumyl alcohol almost exclusively. This shows that hematin catalyzes the homolytic cleavage of the –O–OH bond of α-cumyl hydroperoxide and produces α-cumyl alkoxy radical as the initial product. Kharasch, Fono, and Nudenberg (5) reported that ferrous ion, at a much higher concentration than the hematin used here, catalyzed this same reaction.

Methyl Linoleate Hydroperoxide System. The hematin catalyzed decomposition of methyl linoleate hydroperoxide, in the presence of hydroquinone, produced a methyl linoleate alcohol which accounted for 94% of the initial hydroperoxide. Thus, just as with α-cumyl hydroperoxide, hematin catalyzed the homolytic cleavage of the –O–OH bond of methyl linoleatehydroperoxide and yielded methyl linoleate alkoxy radical as the initial product. It can be concluded therefore that the catalytic, homolytic cleavage of the –O–OH bond of hydroperoxides is a general property of hematin, and most probably of the other hematin compounds as well.

Analysis of Secondary Products for Oxygen-Containing Functional Groups. Table I lists the analytical data obtained. Considerable amounts of oxirane (0.923 meq./g.) and hydroxyl (0.962 meq./g.) groups were found in the acidic, ether-soluble fraction. Whether or not these two functional groups are present in the

same molecule is not known. The close similarity of their concentrations however would seem to indicate some relationship. Possibly a hydroxy-oxirane compound is formed by the intramolecular attack of an alkoxy radical on an adjacent double bond, followed by radical combination between the alkyl radical produced and a hydroxyl radical:



This type of reaction could help account for the excess of oxygen-containing functional groups (2.19 meq./g.) over that predicted (1.66 meq./g.) from the amount of hydroperoxide decomposed [assuming the hydroxyl radical goes into regenerating the catalyst (15)]. The loss in conjugated double bonds would also fit this hypothesis.

The carbonyl content of this material is not known exactly because the presence of peroxide results in high carbonyl values. Removal of the peroxide by reduction was not successful since stannous chloride proved to be inefficient in reducing peroxides and sodium bisulfite produced carbonyl as a side reaction. Judging from the results obtained with stannous chloride, the carbonyl content of this material is in the range of 0.2 to 0.5 meq./g. with a mean value of 0.35 meq./g.

Approximately 4.4% by weight of the starting material is found in the nonacid, ether-soluble fraction. This material must have been produced as a result of cleavage of the linoleate molecule. About 0.35 meq./g. of carbonyl oxygen was found in this material, but hydroxyl or oxirane oxygen was not present in detectable amounts. Apparently a considerable portion of this fraction is hydrocarbon in nature. Sufficient material was not available to pursue its characterization farther.

The carboxyl group concentration of the linoleate hydroperoxide and the linoleate hydroperoxide decomposition products is identical, indicating that no carboxyl groups are produced by the decomposition reaction.

The polymeric nature of the acidic, ether-soluble fraction is shown by the great increase in viscosity and loss of solubility in 60% ethanol.

Interpretation of Infrared and Ultraviolet Spectra. The most important evidence obtained from the infrared spectra is that concerning the configuration about the double bonds. The linoleate hydroperoxide (Figure 1) contains mainly conjugated *cis, trans* double bonds. This is shown by the double absorption at 10.18 and 10.53 microns (9). The greater intensity of the 10.53 micron band is probably due to the absorption of residual linoleic acid at 10.5–10.6 microns. The weak band at 6.03 microns is due to *cis* bonds.

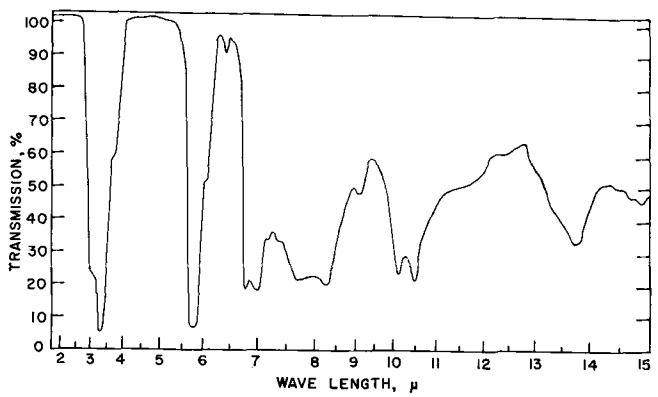


FIG. 1. Infrared spectrum of the linoleate hydroperoxide concentrate.

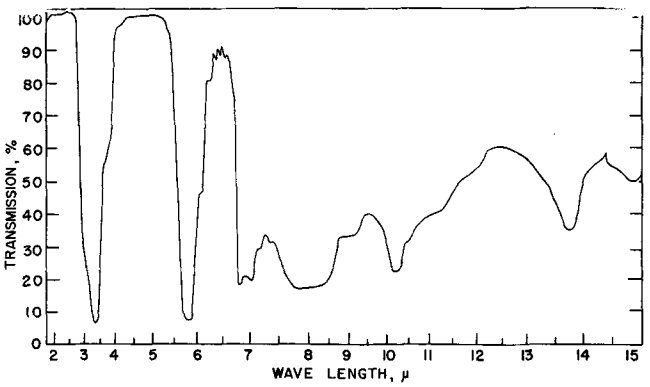


FIG. 2. Infrared spectrum of the acidic, ether-soluble products resulting from the hematin catalyzed decomposition of linoleate hydroperoxide.

The linoleate hydroperoxide decomposition products (Figure 2) contain mainly isolated *trans* double bonds as shown by the strong band at 10.34 microns. There is also an indication of some conjugated *cis, trans* double bonds, probably from unreacted linoleate hydroperoxide. This loss of conjugated double bonds is substantiated by the absorbance at 232.5 millimicrons, which shows a 61.8% loss in diene conjugation after hematin catalyzed decomposition of the linoleate hydroperoxide. The concentration of isolated *trans* double bonds can be estimated, using the method of Shreve *et al.* (12), who give the molar extinction of elaidic acid. Paschke, Jackson, and Wheeler (11) have found that the absorptivities of the isolated *trans* band at 10.3 microns are additive in nonconjugated compounds, thus allowing the method of Shreve *et al.* to be extended to the analysis of polyunsaturated, nonconjugated olefinic acids. Using this method, the acidic, ether-soluble fraction is found to have a concentration of 1.2 moles/liter of isolated *trans* double bonds. If all of the double bonds of the linoleate hydroperoxide had been converted into *trans, trans* isolated double bonds, the concentration of *trans* double bonds would be 3.3 moles/liter. These results indicate that approximately half of the original double bonds were destroyed when the linoleate hydroperoxide was catalytically decomposed by hematin.

The presence of aldehydic or ketonic carbonyl in the decomposed fraction is shown by the broadening of the band at 5.8–5.9 microns. The carboxyl carbonyl also absorbs strongly in this region, thus masking the other carbonyls to some extent. Ultraviolet absorption at 280 millimicrons also indicates the presence of carbonyl groups.

The presence of hydroperoxyl and hydroxyl groups is shown by the band at 2.8–3.0 microns in the linoleate

hydroperoxide and linoleate hydroperoxide decomposition products, respectively.

The infrared spectrum of the nonacid, ether-soluble fraction shows in Figure 3 an intense band at 5.75–5.85 microns, indicating the presence of considerable amounts of carbonyl compounds. This agrees with the chemical evidence for the presence of carbonyl compounds and with the ultraviolet absorption at 280 millimicrons. The broad band at 10.07–10.28 microns and the shoulder at 10.45–10.55 microns indicates the presence of conjugated *cis, trans* double bonds (peak at 10.17 and 10.55 microns) and conjugated *trans, trans* double bonds (peak at 10.12 microns). This is in agreement with the ultraviolet absorption at 232.5 millimicrons, which shows the presence of conjugated dienes (0.66 mmoles/g.). The peak at 6.1 microns is characteristic of double bonds in general.

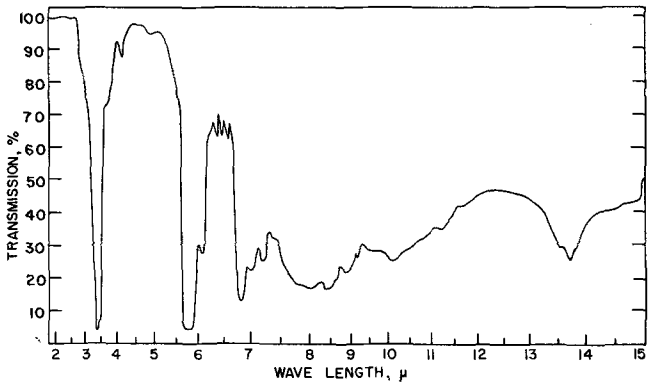


FIG. 3. Infrared spectrum of the nonacid, ether-soluble products resulting from the hematin catalyzed decomposition of linoleate hydroperoxide.

Pathway of the Reaction. The first step in the decomposition of hydroperoxides by the hematin compounds involves the catalytic, homolytic cleavage of the O–OH bond of the hydroperoxide.

The alkoxy radical then reacts in a variety of ways to produce oxirane, hydroxyl, and carbonyl compounds along with cleavage of the carbon chain, loss of conjugated double bonds, and polymer formation.

A reaction which may account for the production of oxirane groups has been given in this paper. This reaction would also account for the loss of double bonds and formation of hydroxyl groups, all of which fit the experimental data. However it is possible that only the oxirane group is formed in this reaction since the intermediate alkyl radical could abstract hydrogen from another linoleate molecule.

Hydroxyl groups could be formed from the alkoxy radical by hydrogen abstraction from another linoleate molecule.

The process of polymerization in a system of this type would most probably occur by radical combination or radical addition to double bonds.

Ketonic carbonyl could be formed by one electron oxidation of the alkoxy radical. In addition, the presence of carbonyl in the nonacid fraction shows that carbon chain cleavage has occurred, possibly in the formation of carbonyl compounds.

All of the data found in this study suggest the following mechanism for the hematin compound catalyzed decomposition of hydroperoxides in the absence of oxygen:

$$1)\ \overset{H}{Fe}OH+ROOH \rightleftharpoons \overset{H}{Fe}OOR+HOH$$

$$2)\ \overset{H}{Fe}OOR \longrightarrow \overset{H}{Fe}O\cdot+\cdot OR$$

$$3)\ \overset{H}{Fe}O\cdot+RH \longrightarrow \overset{H}{Fe}OH+R\cdot$$

$$4)\ \overset{H}{Fe}O\cdot+R\cdot \rightleftharpoons \overset{H}{Fe}OR \xrightarrow{HOH} \overset{H}{Fe}OH+ROH$$

$$5)\ \overset{H}{Fe}O\cdot+RH \longrightarrow \overset{H}{Fe}OH+R\cdot$$

This mechanism is an expansion of the one proposed previously (15, 16). Reaction 4 has been added to account for the incorporation of hydroxyl radical oxygen into the products. Evidence for the existence of the hematin-radical (FeOH) has been reported by George (1) in hemoglobin-H_2O_2 systems.

Summary

A study has been made of the initial and secondary products resulting from the hematin catalyzed decomposition of hydroperoxides. The initial product was found to be the alkoxy free-radical produced by the homolytic cleavage of the O–OH bond of the hydroperoxide. The system was arranged so that the alkoxy radicals reacted with a hydrogen donor as they were formed to produce the corresponding alcohol. The alcohol was then identified by standard physical and chemical procedures.

The secondary products resulting from the hematin catalyzed decomposition of linoleate hydroperoxide in the absence of oxygen were found to be composed principally of oxirane, hydroxyl, and carbonyl compounds. Cleavage of the carbon chain, loss of conjugated double bonds, and polymerization also took place. Reactions to account for these products were proposed, along with a general mechanism of hematin catalysis.

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