on the weight of cocoa fat, do not affect mouthing quality significantly, yet they impart marked rigidity to the chocolate mass at temperatures at which cocoa fat softens and melts. Because the hard fat is practically insoluble in cocoa fat at the melting point of the latter, the hard fat, under the conditions adopted, does not alter the melting characteristics of the cocoa fat, and the short softening range of the cocoa fat is retained.

The addition of the completely hydrogenated oil greatly retards the rate of fat leakage from chocolate at temperatures of about 36°C.(97°F.).

The addition of small amounts of very hard fats to chocolate should be useful in improving the performance of molded bars. However if the modified chocolate is to be used for coating confections, the increase in viscosity which results on the addition of the hard fat presents a serious disadvantage.

REFERENCES

1. Chapman, D., Crossley, A., and Davies, A. C., J. Chem. Soc., *1957*, 1502–1509.
2. Lovegren, N. V., Guice, Wilma A., and Feuge, R. O., J. Am. Oil Chemists' Soc., *35*, 327–331 (1958).
3. Meara, M. L., J. Chem. Soc., *1949*, 2154–2157.
4. Ward, T. L., Gros, Audrey T., and Feuge, R. O., J. Am. Oil Chemists' Soc., *32*, 316–318 (1955).
5. Working, E. B., U. S. Patent 1,781,672 (November 11, 1930).

[Received May 5, 1958]

Rate Studies of Unsaturated Fatty Acid Oxidation Catalyzed by Hematin Compounds[1]

VINCENT P. MAIER[2] and A. L. TAPPEL, Department of Food Technology, University of California, Davis, California

HEMATIN catalyzed unsaturated fatty acid oxidation is a basic mechanism of *in vivo* pathological reactions found in vitamin E-deficient animals (7, 14) and in oxidative fat rancidity of meat, poultry, and fish (11, 12, 16). In contrast to these deleterious reactions, hematin catalysis might be used beneficially. Hematin catalyzed peroxide decomposition might be used in processes requiring initiation of free-radical propagated polymerization reactions.

Many important aspects of this catalytic oxidation are still unknown. The catalytic activity of the hematin compounds depends on the presence of peroxide in the system (13), but little is known concerning the interdependence of catalyst, unsaturated fat, and peroxide concentrations and their effect on the rate of the oxidative reaction. The catalysis has been reported to occur only in heterogeneous systems (5, 11), but no systematic study of this phenomenon has been conducted. Long, unexplained induction periods have also been observed (1, 13). In order to help clarify these problems and to establish a firmer basis for further work, a comprehensive study was carried out in which the effects of homogeneous and heterogeneous linoleate, hydroperoxide concentration, catalyst concentration, and temperature on the rate of oxygen absorption were determined.

The rate of oxygen absorption is a good measure of the rate of oxidation of unsaturated fatty acid and thus of the over-all reaction, but it yields very little information concerning the initiation reactions. The kinetics of this important reaction between hematin compound and fatty acid hydroperoxide have never been studied, nor have the peroxidative activities of the various hemoproteins and related compounds been directly determined. In order to study this reaction a spectrophotometric method was developed, based on the decrease in diene conjugation of linoleate hydroperoxide when catalytically decomposed by hemoglobin. Using this technique, a study was made of the effects of linoleate hydroperoxide concentration, hemoglobin concentration, and temperature on the rate of the reaction. In addition, the catalytic activities of several hemoproteins, metallo-protoporphyrins, and other interesting metal-chelate compounds were determined.

[1] Supported in part by grants from Wm. J. Stange Company, Chicago, and General Foods Corporation, New York.

[2] General Foods Fellow 1955–57. Present address: U.S.D.A. Fruit and Vegetable Laboratory, Pasadena, Calif.

Experimental

Reactants. Homogeneous (soluble) linoleate was prepared from potassium linoleate by dilution with 0.1 M ammonium buffer, pH 9, and heterogeneous (colloidal) linoleate by dilution with 0.1 M phosphate buffer, pH 7 (11, 13). Linoleate hydroperoxide was prepared by the lipoxidase catalyzed oxidation of potassium linoleate (9). Purified hemoglobin was prepared from fresh cattle blood.

Measurement of Oxygen Absorption. Standard manometric techniques, employing a Warburg respirometer, were used to follow the rate of oxygen absorption. With the exception of the study to determine the effect of temperature on the rate of the reaction, in which the flasks were gassed with oxygen, air was used as the gas in all experiments. The rate of oxidation was obtained from the slope of the initial straight line portion of the oxygen absorption *versus* time plots. When induction periods were encountered the rates were those after the induction period.

Reaction Systems for Unsaturated Fatty Acid Oxidation. A comparison of the rates of oxidation of homogeneous and heterogeneous linoleate in the presence of 7.5×10^{-5} M hemoglobin was made at various linoleate concentrations. The ability of hematin to catalyze the oxidation of homogeneous linoleate was also tested, and similar results were obtained.

To determine which of the components were responsible for the occurrence of induction periods in the more dilute solutions, the following series of experiments were carried out: a) total linoleate and linoleate hydroperoxide varied, hemoglobin constant; b) linoleate hydroperoxide varied, total linoleate and hemoglobin constant; c) hemoglobin varied, total linoleate and linoleate hydroperoxide constant.

The effect of temperature on the hemoglobin catalyzed oxidation of 0.022 M homogeneous linoleate was determined over the temperature range 2° to 50°C.

Diene Conjugation and Total Peroxide. The conjugated double bonds in linoleate hydroperoxide provide a convenient method of quantitatively determining this compound since they have strong absorption at 232.5 mμ. In an effort to determine whether this property could be used as the basis of a method for following the decomposition of linoleate hydroperoxide by hemoglobin, the relationship between diene conjugation and total peroxide at various stages of the hemoglobin catalyzed reaction was investigated. Linoleate hydroperoxide was catalytically decomposed by hemoglobin in the absence of oxygen at 25°C. Three different levels of initial linoleate hydroperoxide concentration were used. The extent of hydroperoxide decomposition was governed by the amount of hemoglobin employed. Total peroxide was determined by the method of Lundberg and Chipault (8). Absorption measurements at 232.5 mμ were made in ammonium buffer solution by using a Beckman DU spectrophotometer. The results of this study are given in Figure 1 and show that the concentration of conjugated dienes is linearly related to total peroxide concentration. The fact that this linear plot does not pass through the origin indicates that some of the diene conjugation present in the reacting system is not due to the linoleate hydroperoxides.

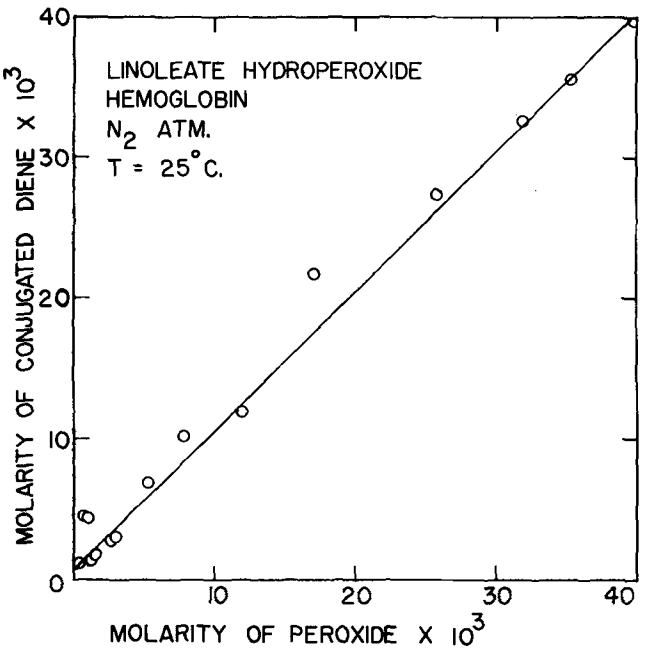

FIG. 1. Correlation of diene conjugation and peroxide in the hemoglobin catalyzed decomposition of linoleate hydroperoxide.

Spectrophotometric Rate Measurements. Based on the foregoing results, the rate of decrease in absorbance at 232.5 mμ in a nitrogen atmosphere was used as a measure of the rate of decrease in linoleate hydroperoxide concentration. In a typical rate measurement the cuvette was filled with 3 ml. of homogeneous linoleate hydroperoxide solution and loosely fitted with a cork stopper. The cuvette was placed in a vacuum chamber, and the chamber was evacuated and gassed five times with pure nitrogen. The chamber was then opened, and the stopper was immediately pressed down to give a tight seal. The reaction was initiated in the spectrophotometer by rapid introduction of 0.5 ml. of oxygen-free hemoglobin solution with the aid of a hypodermic syringe, the needle of which pierced the stopper. Sufficient turbulence was created by the hemoglobin solution to provide complete mixing of the system. Absorbance readings were then taken at 10- to 15-second intervals for several minutes. Since the reaction does not result in the complete disappearance of absorption at 232.5 mμ (30% of the initial absorbance remaining at infinite time), the data were treated so that the effective concentration of conjugated dienes was used rather than the total concentration. The initial effective conjugated diene concentration is is given by $D_0 - D_\infty$, where D_0 represents the absorbance at 232.5 mμ at zero time and D_∞ represents the absorbance at infinite time. The effective concentration of conjugated dienes remaining at any time during the reaction is given by $D - D_\infty$, where D is the absorbance at that time. When $1/D - D_\infty$ was plotted against time, a linear relationship was obtained.

Reaction Systems for Hydroperoxide Decomposition. Using the above method, the effect on the rate of varying the initial linoleate hydroperoxide concentration, 0.54×10^{-5} M to 3.26×10^{-5} M, and the initial hemoglobin concentration, 1.61×10^{-7}, 3.22×10^{-7}, and 6.44×10^{-7} M was determined. The influence of temperature, 4° to 39°C., on the rate of the reaction was determined in a system which contained 2.16×10^{-5} M linoleate hydroperoxide and 3.22×10^{-7} M hemoglobin.

Activities of Other Catalysts. A survey was conducted to determine the catalytic activity of several hemoproteins, of various metallo-protoporphyrins, and of other interesting related compounds. Linoleate hydroperoxide, 2.16×10^{-5} M, was used in all of these reactions.

Crude beef-liver catalase, crude horseradish peroxidase, and crystalline horse-heart cytochrome were obtained from commercial sources. The hemoprotein content of each was determined by spectral analysis of their iron-protoporphyrin contents.

Manganic-, zinc-, cupric-, and cobaltous-protoporphyrins were prepared by the methods of Hill (6). Manganic-protoporphyrin, 1.48×10^{-5} M, gave rate constants of 1.60 and 1.70×10^3 $(D - D_\infty)^{-1}$ sec.$^{-1}$/mole in duplicate determinations. Zinc-, 8.30×10^{-7} M, cupric-, 1.40×10^{-6} M, and cobaltous-, 1.35×10^{-6} M, protoporphyrins were inactive.

Ferric-triethylenetetramine was prepared by the method of Wang (15). Copper-chlorophyllin and crystalline vitamin B_{12} were obtained from commercial sources. Ferric-triethylenetetramine, 6.3×10^{-7} M, was catalytically active and had a rate constant of 4.5×10^3 $(D - D_\infty)^{-1}$ sec.$^{-1}$/mole. This was about 10 times less active than hematin. Copper-chlorophyllin, 2.86×10^{-6} M, and vitamin B_{12}, 3.8×10^{-6} M, were inactive.

Results and Discussion

The data presented in Table I show that hemoglobin catalyzes the oxidation of both homogeneous and heterogeneous linoleate at comparable rates. Hematin was also found to oxidize homogeneous and heterogeneous linoleate catalytically. The rate of oxidation decreases with linoleate concentration, and long induction periods occur at linoleate concentrations of 7.1×10^{-3} M and below.

TABLE I

Rate of the Hemoglobin Catalyzed Oxidation of Homogeneous and Heterogeneous Linoleate as a Function of the Linoleate Concentration[a]

Linoleate concentration, $M \times 10^2$	Hydroperoxide concentration, $M \times 10^4$	Homogeneous linoleate		Heterogeneous linoleate	
		Induction period, hr.	Rate, μl. O_2/hr.	Induction period, hr.	Rate, μl. O_2/hr.
8.8	59	0	1120	0	1200
4.3	30	0	725	0	1210
2.4	16	2.2	875	0.7	780
2.0	13	2.0	650	2.5	870
1.8	12	4.5	105	5.0	835
0.71	5	>26	.....	>26	.....
0.09	0.6	>22	.....	>22	.....
0.01	0.06	>22	.....	>22	.....

[a] Hemoglobin concentration 7.5×10^{-5} M, temperature 20.0°C.

Table II shows that the total linoleate concentration is the most important factor in determining whether oxygen absorption will occur, providing some peroxide is present. Long induction periods begin to occur at total linoleate concentrations below 2.2×10^{-2} M. Boyd and Adams (1) reported long induction periods, using 1.6×10^{-2} M sodium linoleate.

TABLE II

Rate of Hemoglobin Catalyzed Oxidation of Heterogeneous Linoleate as a Function of Total Linoleate and Linoleate Hydroperoxide Concentrations[a]

Linoleate, $M \times 10^2$	Linoleate hydroperoxide, $M \times 10^2$	Rate of oxygen absorption, μl. O_2/hr.	Induction period, hr.
8.8	4.3	1080	0
4.4	2.1	1130	0
2.2	1.1	1070	0
10.0	1.1	1030	0
0.44	0.21	0	~24
10.0	0.26	1150	0
0.22	0.11	0	>29
10.0	0.11	1040	0
0.02	0.01	0	>29

[a] Hemoglobin concentration 7.5×10^{-5} M, temperature 20.0°C.

The absence of oxygen absorption in systems having a low linoleate concentration is not caused by the lack of the initiation reaction between hemoglobin and hydroperoxide since Table II shows this reaction to occur at a hydroperoxide concentration of 1.1×10^{-3} M. and Table III shows it to occur at a concentration of as little as 5.4×10^{-6} M. Also, in connection with another study, hematin was shown catalytically to decompose linoleate hydroperoxide and di-t-butyl peroxide in glacial acetic acid-chloroform (2:1) solution. In both cases 40 milliequivalents of peroxide were decomposed per milliequivalent of initial hematin.

TABLE III

Rate Constants for Hemoglobin Decomposition of Linoleate Hydroperoxide[a]

Linoleate hydroperoxide, $M \times 10^5$	Second order rate constant, $(D - D_x)^{-1}$ sec.$^{-1}$
0.538	0.065
1.11	0.083
2.16	0.078
3.26	0.13

[a] Hemoglobin concentration 3.22×10^{-7} M, temperature 25.0°C.

However at low total linoleate concentrations (Table II) the propagation phase of the chain reaction is apparently nonfunctional since no oxygen is absorbed. Higher linoleate concentrations are necessary to insure the chain character of this reaction. The closely similar rates of the systems without induction periods (Table II) are caused by the limiting rate of oxygen diffusion into the liquid phase.

The rate of oxidation of linoleate increases with increased hemoglobin concentration (Table IV) to a maximum rate at 7.5×10^{-6} M. The slower rate at 7.5×10^{-5} M hemoglobin is unexplained. For this wide range of hemoglobin concentration there was no simple relationship between hemoglobin concentration and rate. The induction periods encountered at the lower hemoglobin concentrations are probably caused by the small amount of peroxide decomposition taking place, which results in a lack of sufficient free radicals to initiate the chain reaction.

TABLE IV

Rate of Hemoglobin Catalyzed Oxidation of Linoleate as a Function of the Hemoglobin Concentration[a]

Hemoglobin concentration, M	Rate of oxygen absorption, μl. O_2/hr.	Induction period, hr.
0	22.0	20
7.5×10^{-9}	29.0	12
7.5×10^{-8}	43.5	3
7.5×10^{-7}	516	0
7.5×10^{-6}	1000	0
7.5×10^{-5}	756	0

[a] Total linoleate concentration 0.022 M, containing 0.011 M linoleate hydroperoxide; temperature 20.0°C.

These foregoing results show that the ability of hemoglobin and hematin catalytically to oxidize linoleate is not limited to heterogeneous systems as previous workers have reported. The most critical factor involved in this oxidative process is the concentration of unsaturated fat or fatty acid. If the system contains 7.1×10^{-3} M or less, long induction periods result. The hydroperoxide concentration is not critical, provided there is a small amount present. Catalyst concentration becomes critical only at low concentrations (below 7.5×10^{-7} M).

The activation energy of 5 ± 1 kilocalories per mole for the hemoglobin catalyzed oxidation of homogeneous linoleate (Figure 2) compares favorably with that reported previously by Tappel (12) for heterogeneous linoleate, 3.3 kilocalories per mole. It should be added however that this is the minimum activation energy of this reaction since there is a three-fold decrease in oxygen solubility caused by the temperature increase from 0° to 50°C.

The second-order rate constants (Table III) for the

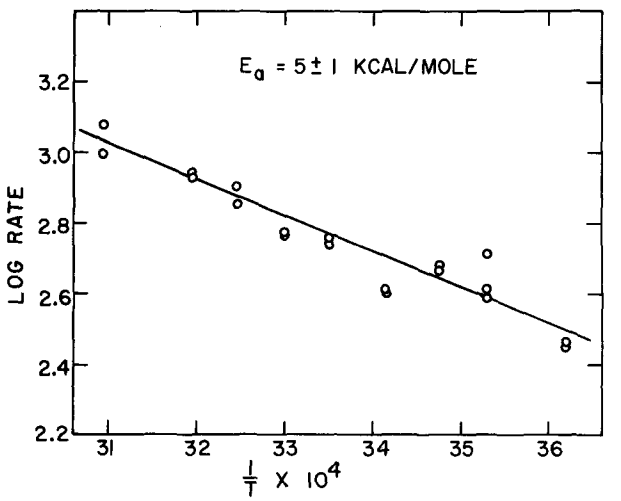

FIG. 2. The effect of temperature on the rate of hemoglobin catalyzed oxidation of linoleate.

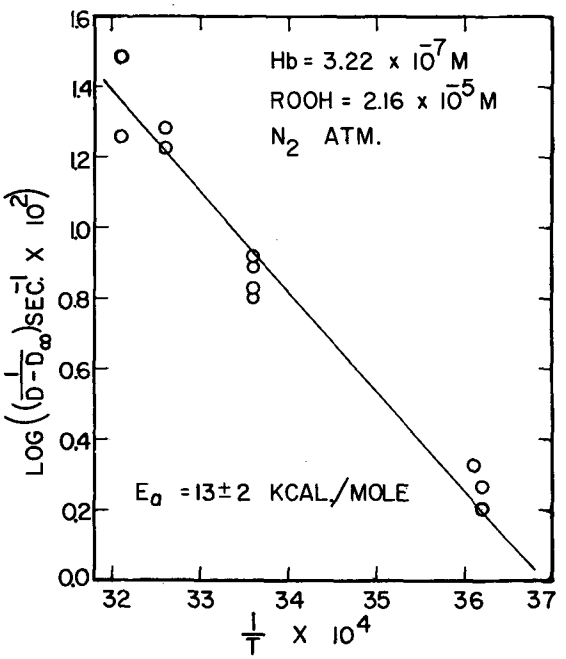

FIG. 3. Effect of temperature on the rate of hemoglobin (Hb) catalyzed decomposition of linoleate hydroperoxide (ROOH).

hemoglobin catalyzed decomposition of linoleate hydroperoxide in the absence of oxygen increased with increased hydroperoxide concentration. In addition, a two-fold increase and decrease in the hemoglobin concentration caused a marked deviation from the linear second-order plot. Both these facts show that this is not a true second-order reaction. Further work is necessary to determine the rate equation of this complex reaction. However the fact that this reaction does fit the second-order plot under special conditions allows this method to be used in comparing the activities of similar catalysts and for determining the effect of various factors on the reaction.

The activation energy of 13 ± 2 kilocalories per mole (Figure 3) for the hemoglobin catalyzed decomposition of linoleate hydroperoxide compares favorably with that obtained by Fordham and Williams (3) of 12.0 kilocalories per mole for the ferrous ion catalyzed decomposition of α-cumyl hydroperoxide. The activation energy for the spontaneous decomposition of α-cumyl hydroperoxide is 30.4 kilocalories per mole (2). Both these activation energies were obtained in the absence of radical induced hydroperoxide decomposition whereas with linoleate hydroperoxide this possibility was not eliminated.

The activation energy of 5 ± 1 kilocalories per mole, given previously for the over-all chain reaction (measured by oxygen absorption), is considerably smaller than that of the initiation reaction (measured by hydroperoxide decomposition). It has been shown by Frost and Pearson (4) however that this situation can arise when the activation energy of the chain-breaking reaction is zero or, more likely, when a second-order chain-breaking process occurs.

All of the hemoproteins studied were found to be catalysts for the decomposition of linoleate hydroperoxide (Table V). Previously for linoleate oxidation Simon *et al.* (10) found catalase to be inactive while Tappel (13) reported very low activity. In view of this the ability of catalase catalytically to oxidize heterogeneous linoleate was tested and found to be comparable to hematin on a hematin-equivalent basis. The activity of these hemoproteins and hematin strongly indicates that the peroxidative activity of these compounds is a function of their iron-protoporphyrin moiety. The differing activities of these compounds probably results from some effect of the protein on the activity of the iron-protoporphyrin portion of the molecule. In the case of catalase and hemoglobin the protein may cause an activation of the iron-porphyrin while in the case of cytochrome c and peroxidase a deactivation results. Present knowledge of the protein-iron-porphyrin linkage and structure of these compounds and their surface-active effects in this reaction system is not sufficiently detailed to allow definite conclusions to be drawn.

TABLE V

Comparative Activities of Hematin Compounds as Catalysts for Linoleate Hydroperoxide Decomposition[a]

Hematin compound	Rate constant $(D - D_\infty)^{-1}$ sec.$^{-1}$/hematin equivalent $\times 10^{-4}$
Catalase	36.0
Hemoglobin	29.4
Hematin	4.75
Cytochrome c	1.65
Peroxidase	0.22

[a] Linoleate hydroperoxide concentration 2.16×10^{-5} M, temperature 25.0°C., N_2 atmosphere.

Of the metallo-protoporphyrins studied, only mangani-protoporphyrin catalyzed the decomposition of linoleate hydroperoxide. This compound was about 25 times less active than hematin. This shows that the metal ion specificity of the catalyst is very strict. The inactivity of the zinc- and cupric-protoporphyrins is readily explained by their lack of open coordinating orbitals for bond formation with the hydroperoxide. Iron-, mangani-, and cobalt-protoporphyrins each have two open coordinating orbitals available for bond formation. The inactivity of the cobalt-protoporphyrin however appears to be an exception to this criterion for catalytic activvity and indicates that additional factors are involved.

Ferric-triethylenetetramine was only 10 times less active than hematin in catalyzing the decomposition of linoleate hydroperoxide. This chelate compound also has two open coordinating orbitals but differs from the metallo-protoporphyrins in that these orbital positions are adjacent rather than separated. It is of interest that of the many metallo-triethylenetetramines tested by Wang (15) for their ability catalytically to decompose hydrogen peroxide, including Fe^{+3}, Zn^{+2}, Mn^{+2}, and Co^{+3}, only the ferric- and manganous-triethylenetetramines were active.

Neither copper-chlorophyllin nor vitamin B_{12} were catalytically active. Since both of these compounds are similar to metallo-porphyrins, their inactivities are in agreement with those of the copper- and cobalt-protoporphyrins mentioned previously.

Summary

Hemoglobin and hematin were found to catalyze the oxidation of homogeneous as well as heterogeneous linoleate systems. The interrelationship of several factors affecting the rates of this reaction were studied.

A spectrophotometric method was developed for studying the rate of decomposition of linoleate hydroperoxide by hematin compounds. By using this

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.

REFERENCES

1. Boyd, D. H. J., and Adams, G. A., Can. J. Biochem. Physiol., *33*, 191 (1955).
2. Fordham, J. W. L., and Williams, H. L., Can. J. Research, *B27*, 943 (1949).
3. Fordham, J. W. L., and Williams, H. L., J. Am. Chem. Soc., *72*, 4465 (1950).
4. Frost, A. H., and Pearson, R. G., "Kinetics and Mechanism," p. 232, John Wiley and Sons, New York (1953).
5. Haurowitz, F., and Schwerin, P., Enzymologia, *9*, 193 (1940).
6. Hill, H., Biochem. J., *19*, 341 (1925).
7. Hove, E. L., Am. J. Clin. Nutr., *3*, 328 (1955).
8. Lundberg, W. O., and Chipault, J. R., J. Am. Chem. Soc., *69*, 833 (1947).
9. Privett, O. S., Nickell, C., Lundberg, W. O., and Boyer, P. D., J. Am. Oil Chemists' Soc., *32*, 505 (1955).
10. Simon, F. P., Horwitt, M. K., and Gerard, R. W., J. Biol. Chem., *154*, 421 (1944).
11. Tappel, A. L., Food Research, *18*, 104 (1953).
12. Tappel, A. L., Food Research, *18*, 560 (1953).
13. Tappel, A. L., Arch. Biochem. and Biophys., *44*, 378 (1953).
14. Tappel, A. L., Arch. Biochem. and Biophys., *54*, 266 (1955).
15. Wang, J. H., J. Am. Chem. Soc., *77*, 4715 (1955).
16. Watts, B. M., Adv. Food Research, *5*, 1 (1954).

[Received May 12, 1958]

Products of Unsaturated Fatty Acid Oxidation Catalyzed by Hematin Compounds

VINCENT P. MAIER [1] **and A. L. TAPPEL, Department of Food Technology, University of California, Davis, California**

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.

[1] General Foods Fellow 1955–57. Present address: U.S.D.A. Fruit and Vegetable Laboratory, Pasadena, Calif.