A Study of the Volatile Compounds Produced by the Autoxidation of Methyl Oleate, Oleic Acid, and cis-9-Octadecene

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RANCIDITY is due to volatile, low molecular-weight compounds produced by the fragmentation of fatty acids or glycerides undergoing autoxidation. The nature of the carbon to carbon scission is complicated and only incompletely understood. Since only trace amounts of these volatile scission products are sufficient to produce rancidity, the characterization of these products is difficult. Volatile autoxidation products of fats and some of their derivatives can be condensed in dry ice traps (4), and this provides a method for the collection of larger quantities of the volatile scission products. The characterization of these volatile products might lead to a better understanding of the mechanism of rancidification.

Deatherage and Mattill (4) reported that the volatile products collected in the dry ice traps consisted of water and water-soluble substances and of a water-insoluble or oily fraction. Irrespective of the substrate oxidized, the oily material was colorless, had a typical rancid odor, and contained very active peroxide oxygen. On standing at room temperature, the acids in the volatile fraction increased while the peroxides decreased. The volatile material which escaped the dry ice traps were stopped by dehydrite, on which it formed a brownish-black resin. Khan, Brown, and Deatherage (7) observed that peroxide crystals could be isolated from the oily volatile fraction collected in dry ice traps during the autoxidation of methyl oleate. These peroxides could be reduced to carbonyl compounds. Polarographic reduction showed a similarity between the isolated solid peroxide and commercial preparations of hydroxyheptyl peroxide and methyl amyl ketone peroxide. These three peroxides showed the "A-wave" at 0.3 volts observed recently by Kalbag, Narayan, Chang, and Kummerow (6) in autoxidized soybean oil.

Experimental

Autoxidation Substrates. Methyl oleate was obtained from the mixed methyl esters of olive oil by fractional distillation and low temperature crystallization. This preparation had an iodine value of 84.5 (theory 85.7) and contained less than 0.01% of polyunsaturated esters (1).

The oleic acid, prepared by hydrolysis of the purified methyl oleate, had a melting point of 14–15°, a neutral equivalent of 281 (theory 282), and an iodine value of 89.1 (theory 89.9).

Cis-9-octadecene was synthesized from ethyl oleate, purified by low temperature crystallization, by a method similar to that of Deatherage and Olcott (5). Lithium aluminum hydride was used as reducing agent. The hydrocarbon had an iodine value of 100.2 (theory 100.5), n_D^{30} 1.4424, and a d^{30} 0.788.

Oxidation Procedure. Ten grams of substrate were oxidized at 80° by a stream of air (50 to 60 ml. per min.) which had been passed through scrubbing towers of concentrated sulfuric acid, calcium chloride, and sodium hydroxide. The volatile decomposition products were collected in dry ice traps and examined upon completion of each run.

Analytical Methods. For the determination of the C_1 to C_4 acids the chromatographic method of Bulen, Varner, and Burrell (3) was used. The solvent schedule was modified by using 50 ml. of 1%, 100 ml. of 5%, and 75 ml. of 15% n-butanol-chloroform mixtures. For the separation of the C_4 to C_{10} fatty acids the first fraction obtained from the above method was rechromatographed, using a modification of the method of Nijkamp (10).

The amount of formic acid in the aqueous volatile material was also determined by the gravimetric method of Auerbach and Zeglin (2).

The infrared spectra were taken on a Baird recording spectrophotometer with a NaCl prism. Crystalline samples were suspended in Nujol.

Bis-(Hydroxyheptyl) Peroxide. Pure heptanal was obtained by the preparation of the bisulfite addition compound from technical heptanal. The bisulfite addition compound was decomposed by steam distillation from a 10% sodium hydroxide solution. Twenty-eight grams (0.246 mole) of heptanal were mixed with 55 ml. of ethereal hydrogen peroxide containing 0.257 mole of active oxygen. The mixture was allowed to stand overnight at room temperature. The next day the mixture was cooled to −65°, and the precipitate was removed by filtration. The latter was recrystallized twice from petroleum ether at −20°. The crystals were then dried in a vacuum desiccator at 5-mm. pressure for 4 hrs. A yield of 8.7 g. of white crystals which had a melting point of 55–58° were obtained. Rieche (11) reported a melting point of 60° for bis-(hydroxyheptyl) peroxide. From the peroxide value a molecular weight of 271 (theory 262) was calculated. The infrared spectrum of this peroxide is shown in Figure 1.

Bis-(Hydroxycyclohexyl) Peroxide. This peroxide was prepared by the method of Milas, Harris, and

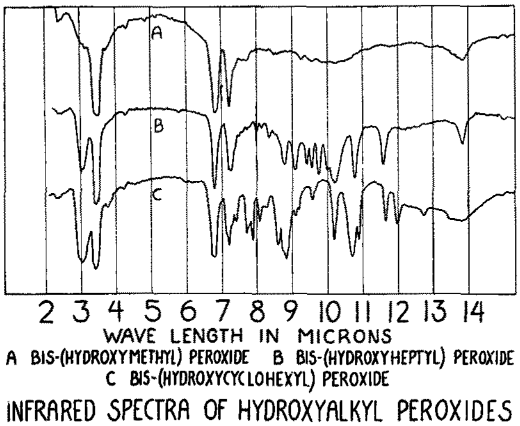


FIG. 1. Infrared spectra of bis-(hydroxymethyl) peroxide (A), bis-(hydroxyheptyl) peroxide (B), and bis-(hydroxycyclohexyl) peroxide (C).

TABLE I

Quantitative Estimation of Acids in the Volatile Aqueous Fractions

Substrate[a]	Methyl Oleate				Oleic Acid			9-Octadecene				
Days	6.8	10.0	14.1	18.9	7.9	13.9	21.7	10.8	13.8	13.8	19.0	22.7
Mol. C=C destroyed—Mol. sub.	......	.833	......	......	.865	.899	.922	.889	.881	.889	.917	.922
Mol. formic—Mol. sub.	.033	.134	.124	.138	.058	.069	.086	.118	.153	.163	.137	.211
Mol. formic—C=C destroyed	......	.161	......	......	.068	.078	.093	.133	.173	.183	.150	.229
Mol. acetic—Mol. sub.	.003	.015	.020	.021	.004	.007	.010	.008	.023	.016	.029	.042
Mol. acetic—C=C destroyed	......	.018	......	......	.005	.007	.011	.009	.032	.017	.032	.046

[a] Ten grams of each substrate was used.

Panagiotakos (9). The product had a melting point of 76–78° and a molecular weight of 243 (theory 230), as calculated from the peroxide value. The infrared spectrum of this peroxide is shown in Figure 1.

Bis-(Hydroxymethyl) Peroxide. Eighty-three ml. (1.0 mole) of formalin (A.C.S.) and 52 ml. of 30% hydrogen peroxide were added together and placed in the refrigerator for two days. The water was then removed by sublimation. The crystals remaining in the cups of the apparatus were carefully dissolved in 1.1 ether-alcohol and recovered by cooling the solution in dry ice. After filtration the peroxide was dried in a vacuum desiccator. A yield of 21.3 g. of white, salt-like crystals which had a melting point of 62–64° was obtained. The molecular weight calculated from the peroxide value was 109 (theory 94). The crystals had an ozone-like odor and liberated an average of 0.71 mole of hydrogen per mole of peroxide when decomposed in an alkaline solution at 26°. The infrared spectra of these crystals is shown in Figure 1.

Results and Discussion

The volatile decomposition products which were collected in the dry ice traps during the autoxidation of methyl oleate, oleic acid, and cis-9-octadecene exhibited the same properties as described by Deatherage and Mattill (4). The amounts and the peroxide values of the oily material were somewhat smaller. The amount of water was greater. A blank run showed that this was due to the incomplete removal of the moisture from the air used for oxidation. After the removal of the aqueous and oily fractions a crystalline product usually remained in the traps.

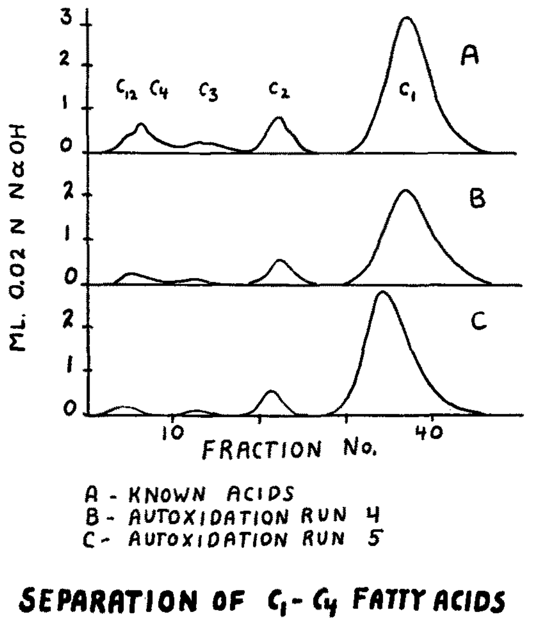


FIG. 2. Separation of acids from the volatile aqueous fractions. Chromatogram of known acids (A), of acids from autoxidation run No. 4 (B), and of acids from autoxidation run No. 5.

The aqueous fractions were highly acidic. A portion of this material of each run was neutralized, and after evaporation of the water the acids were separated by partition chromatography. By comparison with known chromatograms the acids were characterized as formic and acetic acid and occasionally traces of propionic and higher carboxylic acids. Some characteristic chromatograms of this separation are shown in Figure 2. A portion of the original aqueous fraction of the autoxidation of cis-9-octadecene was also chromatographed immediately after the removal from the traps without prior conversion into the sodium salts. The chromatogram showed the same characteristics as that obtained from the isolated sodium salts. The amount of formic and acetic acid based on this determination represented 91 and 95%, respectively, of the amounts calculated from the chromatographic determination after conversion into the sodium salts. This suggests that formic and acetic acid were present primarily as such in the aqueous volatile fraction. To confirm the chromatographic characterization of formic acid the amounts of sodium formate in the isolated sodium salts were also determined gravimetrically by the reduction of mercuric chloride. The percentages of sodium formate determined in this manner were in agreement with those calculated from the chromatographic analysis. After chromatographic separation a pH titration of the two major acid fractions (autoxidation of octadecene) was carried out. The inflection point of the acid fraction characterized as formic acid was 0.90×10^{-4} and that of a known formic acid solution 0.95×10^{-4}. For acetic acid a value of 1.10×10^{-5} was obtained for the unknown, and 1.26×10^{-5} was obtained for the known. The Duclaux values of the acetic acid fraction (autoxidation of oleic acid) were 6.2, 7.0, and 7.4; and the values of a known acetic acid solution determined in the same manner were 6.3, 6.95, and 7.6, respectively.

The total amounts of formic and acetic acid formed during the autoxidation of methyl oleate, oleic acid, and cis-9-octadecene (Table I) were calculated from the chromatographic determination of the acids found in the isolated sodium salts. When the moles of these acids produced per mole of substrate was plotted against time, a sigmoid type of curve was obtained. The maximum rate of formation of these acids occurred between 10 and 15 days of autoxidation. Oleic acid produced the least amount of these acids and cis-9-octadecene the most.

To investigate the rate of the volatile acid formation with respect to peroxide formation and double bond destruction, the substrates were oxidized in the usual manner, but the material in the dry ice traps was removed and titrated with standard sodium hydroxide once or twice each day. At the same time the iodine and peroxide values of the substrate were determined. The results for cis-9-octadecene are shown in Figure 3. The autoxidation of methyl oleate followed the same pattern. In each case a small amount of the volatile acids could be detected as soon as peroxide formation occurred, indicating that fragmentation of the substrate occurs at relatively early stages of autoxidation. Chromatographic analysis of the volatile material produced before maximum peroxide formation indicated that formic acid was the predominant acidic component.

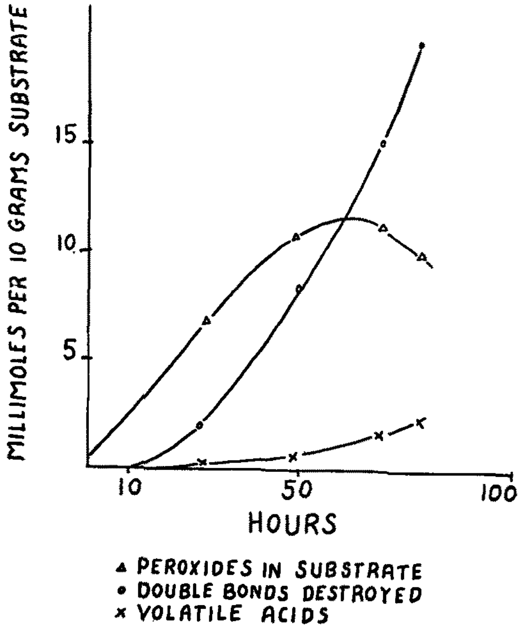


Fig. 3. Volatile acid formation during the autoxidation cis-9-octadecene.

To study the effect of moisture on the formation of formic and acetic acid, methyl oleate was oxidized with a stream of air from which the moisture was removed by condensation at dry ice temperature. The air was preheated before entering the reaction vessel. After 10 days of autoxidation 2.74 millimoles of formic acid and 0.43 millimole of acetic acid per 10 g. of methyl oleate were found. During the oxidation of an equal amount of methyl oleate over the same period of time with air passed only through the sulfuric acid, 4.36 millimoles of formic acid and 0.69 millimole of acetic acid were produced.

The crystals isolated from the volatile autoxidation products showed similar properties irrespective of the substrate oxidized. This material could be recrystallized from small amounts of alcohol (2 to 3 ml.) at —20°. Recrystallization from larger amounts of solvent could not be achieved. The melting point after the first crystallization ranged from 51 to 55°. After a second crystallization the melting point increased to about 58–60°. Further crystallization did not alter the melting point significantly. The purified crystals liberated iodine from potassium iodide in glacial acetic acid-chloroform at room temperature; however this reaction proceeded only slowly. From the peroxide value (1 hr. of reaction time) a mean molecular weight of 315 to 320 was calculated from samples of various autoxidation runs. The purified crystals were unstable when kept for an extended period at room temperature, were soluble in alcohol, ether, and petroleum ether but insoluble in water. They reacted with 2,4-dinitrophenylhydrazine reagent and did not discolor a weak bromine-carbontetrachloride solution. The crystals were neutral after crystallization but, when allowed to remain in solution, acids slowly formed. The acids which were produced during the decomposition of these crystals in a dilute alcohol solution neutralized each day over a period of two weeks were chromatographically analyzed. Caprylic, enanthic, and formic acid were the main acidic products (Figure 4). Traces of pelargonic, caproic, and acetic acid were also detected. The infrared spectrum of the crystals isolated from the autoxidation of oleic acid is shown in Figure 5.

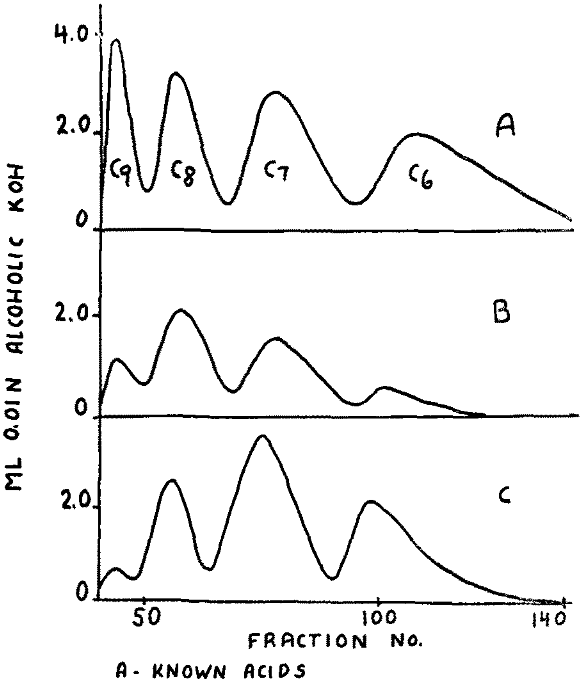


Fig. 4. Separation of C_9–C_6 fatty acids.

Since the isolated peroxides were alike irrespective of the substrate oxidized and contained no double bonds, it was concluded that they were scission products with nine or less carbons in the carbon chain. The fact that the crystals were neutral and could be partially converted into carbonyl derivatives indicated that they were not peracids or acyl peroxides. The absence of infrared adsorption at 5.8 μ (C=O) substantiates this conclusion. n-Alkyl hydroperoxides and peroxides with nine or less carbons in the carbon chain are liquids at room temperature (probably due to immediate decomposition) and do not decompose into acids in the manner observed for the unknown compounds. The properties of hydroxyalkyl hydroperoxides and peroxides described by Rieche (11, 12) resembled most nearly those of the isolated crystals. Bis-(hydroxyheptyl) peroxide was synthesized, and its properties were found to be very similar to the unknown material. The acidic products of the decomposition of bis-(hydroxyheptyl) peroxide in dilute alcohol solution at room temperature were primarily enanthic, formic, and acetic acid. The latter two acids must have resulted from the fragmentation of the original carbon chain of the known and unknown peroxide. It was concluded that the isolated peroxide was a mixture of hydroxyalkyl peroxides with predominantly 7 and 8 carbons in the carbon chain.

The infrared spectra of samples of the oily material obtained from the autoxidation of oleic acid and cis-9-octadecene are shown in Figure 5. The properties of these fractions were in many respects also similar to hydroxyalkyl peroxides. Upon standing at room temperature in a dilute alcohol solution, the acids increased. When a portion of the oily material of the autoxidation of octadecene was decomposed in the same manner as described above for the crystals caproic, enanthic, valeric, acetic, butyric, propionic, formic acid and pelargonic acid were found (Figure 4).

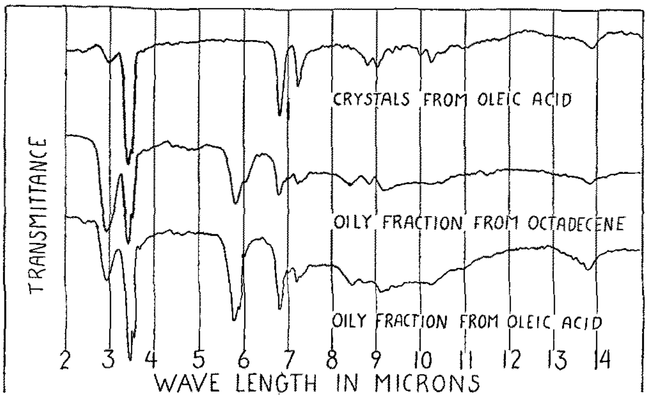
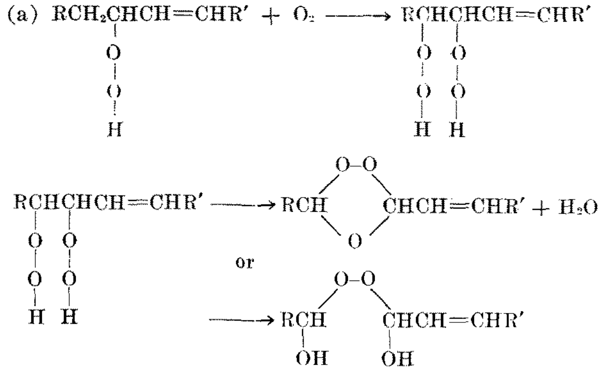


FIG. 5. Infrared spectra of volatile autoxidation products.

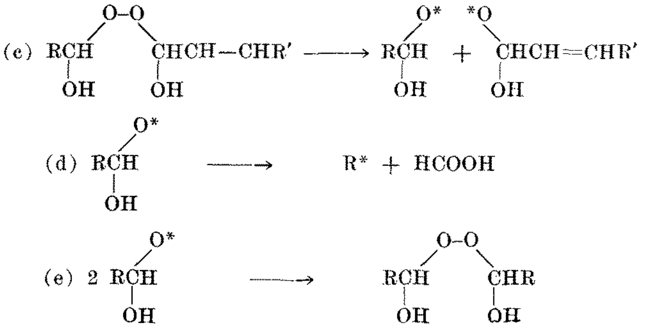
The observation that formic and acetic acids were produced during the decomposition of bis-(hydroxyheptyl) peroxide prompted a study of the acidic decomposition products of this peroxide at temperatures near that used for oxidation of the fatty materials. Bis-(hydroxyheptyl) peroxide was decomposed by heating a sample in a test tube at 100° for 1 hr. When the decomposition was carried out in the presence of air, 0.230 mole of formic acid, 0.08 mole of acetic acid, and 1.115 moles of enanthic acid per mole of peroxide were obtained; however in a nitrogen atmosphere only 0.016 mole of formic acid and 0.243 mole of enanthic acid were produced. Bis-(hydroxyheptyl) peroxide was also "autoxidized" at 80° by placing 5.0 g. of the peroxide in the autoxidation apparatus and bubbling air through the reaction mixture for 48 hrs. The volatile decomposition products which collected in the dry ice traps consisted of an aqueous fraction containing formic, acetic, and a trace of propionic acid, an oily fraction, and some crystals. The odor of the volatile materials resembled that obtained during the autoxidation of fatty materials. The oily material had a peroxide value of 2796 and was only slightly acidic. The oxidation residue had a peroxide value of 62.5 and consisted mainly of acids. All fractions were chromatographically analyzed for acids. A total of 0.188 mole of formic, 0.63 mole of acetic, 0.08 mole of propionic, and 1.361 moles of enanthic acid per mole of peroxide was found. The decomposition of bis-(hydroxycyclohexyl) peroxide at 100° in the presence of air occurred only slowly; however 0.027 mole of formic acid per mole of peroxide, among other acids, were found in the decomposition products.

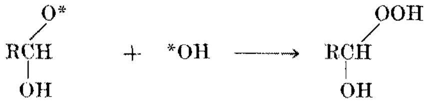
The monocarboxylic acids of the autoxidation residue of methyl oleate (14.1 days) and cis-9-octadecene (13.8 days) were removed by steam distillation and chromatographically analyzed. The acids present in the autoxidation residue from 10 g. of methyl oleate were 0.508 millimole of pelargonic, 0.481 millimole of caprylic, 0.081 millimole of enanthic, 0.012 millimole of caproic, 0.224 millimole of propionic, 0.494 millimole of acetic, and 0.205 millimole of formic acid. The acids found in the oxidation residue from 10 g. of cis-9-octadecene were 0.368 millimole of pelargonic, 1.064 millimoles of caprylic, 0.710 millimole of enanthic, 0.113 millimole of caproic, 0.018 millimole of propionic, 0.051 millimole of acetic, and 0.083 millimole of formic acid.

The isolation of formic acid and to a lesser extent acetic acid confirms the work of Skellon (14), who in 1931 reported that these acids are formed during the autoxidation of oleic acid. Recently King (8) showed that formic acid is also produced during the autoxidation of elaidic acid. The observation that formic and acetic acid are also produced during the decomposition of hydroxyalkyl peroxides suggests that this type of peroxide may be involved in the autoxidation process. Rieche (13) postulated that hydroxyalkyl peroxides are produced by the hydrolysis of ozonides. One may postulate that during autoxidation a secondary oxygen attack on an adjacent carbon of the initial hydroperoxide occurs. Such a dihydroperoxide could by the elimination of water and subsequent rearrangement form an ozonide or by a direct rearrangement form a hydroxyalkyl peroxide.

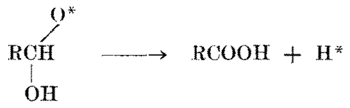


A fission of the O — O bond of the hydroxyalkyl peroxide produces a hydroxyalkoxy radical. The latter could then break another C — C bond producing formic acid, react with a second hydroxyalkoxy radical forming a bis-(hydroxyalkyl) peroxide, react with a hydroxy radical yielding a hydroxyalkyl hydroperoxide, or break a C — H bond to produce an acid.



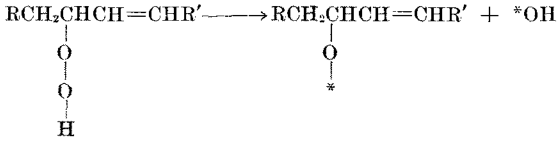
(f) 

$$R\overset{O^*}{\underset{OH}{C}}H + {}^*OH \longrightarrow R\overset{OOH}{\underset{OH}{C}}H$$

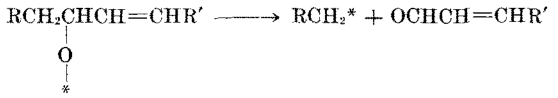
(g) 

$$R\overset{O^*}{\underset{OH}{C}}H \longrightarrow RCOOH + H^*$$

Since it is not possible to determine from the experimental evidence that the isolated hydroxyalkyl peroxides are intermediate products of the secondary reactions of autoxidation, the possibility that these peroxides represented end-products of side reactions induced by the experimental set-up must be considered. Should this be the case, the reactions postulated by Walsh (15) for the oxidation of hydrocarbons would serve for an explanation of the isolated oxidation products. Aldehydes and alkyl-free radicals are formed from the decomposition of the initial hydroperoxides. Hydroxyalkyl hydroperoxides and peroxides may then be produced in the traps by the reaction of the scission aldehydes with hydrogen peroxide in a manner analogous to the synthesis of these peroxides.

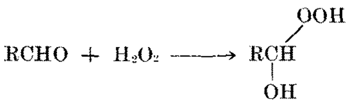
(h) 

$$RCH_2\underset{\substack{|\\O\\|\\O\\|\\H}}{C}HCH{=}CHR' \longrightarrow RCH_2\underset{\substack{|\\O\\|\\*}}{C}HCH{=}CHR' + {}^*OH$$

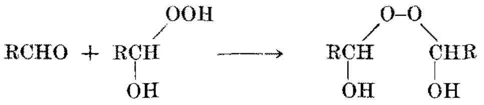
(i) 

$$RCH_2\underset{\substack{|\\O\\|\\*}}{C}HCH{=}CHR' \longrightarrow RCH_2{}^* + OCHCH{=}CHR'$$

(j) $2\ {}^*OH \longrightarrow H_2O_2$

(k) 

$$RCHO + H_2O_2 \longrightarrow R\overset{OOH}{\underset{OH}{C}}H$$

(l) 

$$RCHO + R\overset{OOH}{\underset{OH}{C}}H \longrightarrow \underset{OH}{RCH}\overset{O-O}{}\underset{OH}{CHR}$$

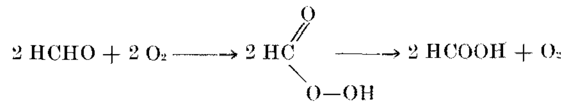
The alkyl-free radical could react with further oxygen and after acquisition of a proton yield a primary hydroperoxide. The latter would decompose into an alkoxy and a hydroxy radical. The alkoxy radical, which is of a highly unstable nature, could produce an aldehyde or formaldehyde. The latter would react with further oxygen to produce formic acid.

(m) $RCH_2{}^* + O_2 \longrightarrow RCH_2OO^*$

(n) $RCH_2OO^* + RCH_2R' \longrightarrow RCH_2OOH + R\underset{*}{C}HR'$

(o) $RCH_2OOH \longrightarrow RCH_2O^* + {}^*OH$

(p) $RCH_2O^* \longrightarrow RCHO + {}^*H$
or
$\longrightarrow R^* + HCHO$

(r) 

$$2\,HCHO + 2\,O_2 \longrightarrow 2\,HC\overset{O}{\underset{O-OH}{}} \longrightarrow 2\,HCOOH + O_2$$

Summary

Methyl oleate, oleic acid, and cis-9-octadecene were oxidized at 80° with a stream of air, and the volatile decomposition products were collected in dry ice traps. The volatile material consisted of an aqueous fraction and water-soluble substances, an oil fraction, and a crystalline product. The substances in the aqueous fractions were characterized as formic and acetic acid, and their formation was studied during the autoxidation of the various substrates up to 21 days. The crystalline material was purified, and its properties were examined. The purified crystals showed chemical and infrared properties very similar to a sample of synthesized bis-(hydroxyheptyl) peroxide. Formic and acetic acid were also shown to be produced during the decomposition of hydroxyalkyl peroxides.

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The Measurement of Lime-Soap Dispersion

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Numerous tests have been proposed to measure the lime-soap dispersion properties of synthetic surface-active agents. These tests generally fall into two classes: nephelometric (*i.e.*, tests designed to measure a decrease in turbidity of soap-dispersant solutions); and what may well be termed titrimetric (*i.e.*, tests which measure the quantity of undispersed lime soap by difference titration following removal of the undispersed material by filtration or centrifugation).

Examples of the former are methods of Dupin and Reutenauer (1) and the method reported by Baird, Brown, and Perdue (2) while titrimetric methods are typified by the technique recently described by Harris (3), a modification of one due to Ramachandran, Uppal, and Venkataraman (4).