Antioxidants and the Autoxidation of Fats

Autoxidation of Oleic Acid, Methyl Oleate, Oleyl Alcohol, and *cis*-9-Octadecene¹

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An apparatus for the study of the autoxidation of fats and related materials has been designed to permit the collection and analysis of the various volatile products formed in the reaction, the measurement of the oxygen consumption, and analysis of the oxidation residue.

Oleic acid, oleyl alcohol, methyl oleate, butyl oleate, and *cis*-9-octadecene appear to be autoxidized in a similar manner to yield the same types of products—among others, peroxides, peracids, aldehydes, substituted ethylene oxides, acids, alcohols, combinations of these, and water.

After the addition of oxygen to form peroxides at the ethylene linkage, these peroxides may cleave to give aldehydes; they may react with another double bond to give two moles of ethylene oxide; or they may aid in the further oxidation of the carbon chain. The aldehydes formed also autoxidize to give peracids and acids.

The oxygen consumption per mole of double bond destroyed is least for oleic acid, most for oleyl alcohol. The amount of oxygen consumed is about the same for methyl oleate and *cis*-9-octadecene. In each case, about one-fourth of the oxygen taken up appears as water.

Oxido derivatives are among the main products of the autoxidation process. When oleic acid is oxidized, oxidoöleic acid does not appear as such but is apparently converted to half esters of dihydroxy stearic acid. The oxido derivatives are all of the same geometrical configuration and correspond in each case to the high-melting dihydroxy isomeric derivative of the original substrate.

THE reactions which occur during the autoxidation of fats and oils are complicated and only incompletely understood. Among the ultimate products are the short-chain aldehydes and monocarboxylic acids (16), 9,10dihydroxystearic acid, 10-hydroxystearic acid (18), oxidoöleic acid (16), and epihydrinaldehyde (13). In order to formulate the reactions responsible for such a variety of compounds, a knowledge of the behavior of highly purified fats or fat acids during autoxidation is essential; there have been few such investigations. It is generally agreed that the first event is the formation of a peroxide at the double bond. Although the kinetics of this reaction are of the utmost importance, the conceptions are still speculative (7). None of the theories so far offered (4) can suitably explain the observations which follow.

In continuation of the experiments described by Hamilton and Olcott (\mathcal{P}) , comparative autoxidation studies have been made of oleic acid, its esters, oleyl alcohol, and *cis*-9-octadecene. *cis*-9-Octadecene was a valuable addition to the substrates studied by them, since it contains no functional group other than the autoxidizable double bond. An improved apparatus permitted the measurement of oxygen consumption and also trapped out the volatile products as they were formed.

Apparatus

The apparatus (Figure 1) was a closed system consisting of a reaction vessel, V, in series with several traps and a circulating pump, P. In water-jacketed gas burets B the amount of oxygen used was measured from time to time during the course of a run. The system operated at atmospheric pressure; as oxygen was absorbed, the falling pressure shifted the level of the mercury in manometer M and thus broke the platinum-mercury contact, C-W. The relay then started motor A which lifted mercury reservoir R on elevator E, and mercury flowed into B, replacing the oxygen absorbed. When atmospheric pressure was restored, contact at C was again made and the motor stopped. Ahead of the reaction vessel in a constant-temperature bath

Ahead of the reaction vessel in a constant-temperature bath was a glass coil fitted with a ground-glass joint which served to bring the incoming oxygen to the same temperature as V (75° C.). The oxygen inlet extended to the bottom of V and ended in a bellshaped enlargement, with several small holes opening below the surface of the substrate. Continuous circulation of the oxygen served for stirring. The oxygen outlet of V was connected to the first of six traps through another ground-glass joint. All such joints were provided with mercury seals; the other connections were of pure gum rubber wired to prevent leakage. The first three traps were W-tubes immersed in a mixture of ether and solid carbon dioxide, the rest were U-tubes; traps IV and VI were filled with dehydrite, and V was filled with ascarite. The apparatus was easily dismantled for weighing the reaction vessel and all of the traps.

A uniform circulation of oxygen was maintained by pump P; it consisted of a round metal case, one side of which was a rubber diaphragm, D, of small displacement, attached to an eccentric driven with a small variable-speed motor. The magnitude of the displacement of D was insignificant compared with the total volume of the apparatus; hence it did not interfere appreciably with the buret readings. Undirectional gas flow was achieved by small glass valves, Y. At its most satisfactory speed, the pump circulated the gas at a rate of about 1 cc. per second.

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FIGURE 1. APPARATUS FOR MEASURING OXYGEN CONSUMPTION

MANIPULATION. Before a run was started, V, T, and each of the traps were weighed. These parts were then assembled, but the joints were not sealed with mercury. A supply of oxygen, free of water and carbon dioxide, was introduced at the three-The of water and carbon dioxide, was introduced at the three-way stopcock, 1; before the train was joined at X, V and traps I, II, and III were thoroughly flushed out with oxygen. Con-nection was then made at X, and the three-way stopcock 2 was opened so that both sides led to the exterior; the oxygen flowed in at 1 and out at 2. The ether-carbon dioxide mixture was placed around traps I, II, and III; all of the stopcocks in the system except 3 were then opened; and circulating pump P was started. In this way the remainder of the system was flushed out with oxygen. Bursts R were then filled by lowering the reservoir with oxygen. Burets B were then filled by lowering the reservoir. Next the pump was stopped, but the flow of oxygen was main-tained and the train was broken between V and T. The open-ing to T was capped, and oxygen continued to escape from V. A weighed amount of substrate was introduced into V with a pipet, the cap on T was removed, and the joint was again seated. All mercury seals were then made, stopcock 1 was turned to connect the burets, stopcock 2 was adjusted to exclude air and leave the system open from P to the coil and V, and the pump was then started.

started. To read the burets, the pump was stopped, stopcock 3 was opened to equalize the pressure, R was lowered until the relay started the motor, and the elevator was stopped when atmospheric pressure within the system was re-established; the burets were then read. This was repeated twice, and the three readings usually agreed within 0.1 cc. of one another on each buret. Care was to buret the Darwar factor surrounding trans I. II. and III. was taken that the Dewar flasks surrounding traps I, II, and III were well filled with the cold mixture.

When more oxygen was needed, the burets were easily filled by opening stopcock 1 to the oxygen supply and stopcock 2 to the outside, with the pump stopped. Suitable corrections of the oxygen consumption were made for the time required for filling the burets.

The runs continued for 100-150 hours; on completion the residue in V was analyzed for double bonds, free and combined carboxyl, free hydroxyl, and peroxide. In the trap contents, free acid, peroxide, water, and carbon dioxide were determined.

Methods

WATER. Expressed in milligrams or millimoles, water was determined volumetrically by the calibrated capillary tubes at the bottom of traps I, II, and III after the contents were allowed to melt. Since the ratio of water to nonaqueous material in trap III was the same as in trap II, it was obvious that some water was also taken out in trap IV (dehydrite) where it could not be determined separately. An estimate of this amount (5 to 10 per cent of the total) was made by applying the above-mentioned ratio to the increase in weight of trap IV. The weight of the aqueous portion was then added to the water in the first three traps. No allowance was made for the mutual solubility of water and the other sub-

stances in the traps. CARBON DIOXIDE. The increase in weight of the ascarite and dehydrite tubes V and VI was taken as carbon dioxide and other volatile acids.

FREE CARBOXYL. Samples (0.4 to 0.5 gram) were dissolved in 95 per cent (for octadecene, 100 per cent) ethanol, previously adjusted to the cresol-red end point with sodium hy-droxide, and were titrated with 0.1 N sodium hydroxide. Triplicates checked within 0.5 per cent.

ESTERIFIED CARBOXYL. Samples weighing 0.3 to 0.4 gram were dis-solved in 25 cc. of 0.3 N alcoholic weighing out to be the matrix the data solved in 25 cc. of 0.3 N alcoholic sodium hydroxide and warmed (in a glass-stoppered flask) on the steam bath for 45 minutes. Twenty-five cubic centimeters of approximately 0.31 N hydrochloric acid and 100 cc. of 95 per cent ethanol (100 per cent in the case of octadecene) were added, and the contents titrated to the cresol-red end point with 0.1 N sodium hydroxide. Corrected by blanks, the results represented total carboxyl; esterified carboxyl was the difference between total and free carboxyl. As Hamilton and Olcott (9) pointed out, heating a rancid substrate may out, heating a rancid substrate may

increase the free carboxyl content; possibly the peroxides de-compose to form acids. During saponification the solutions turned dark, obscuring the cresol-red end point. Agreement within one per cent was usually possible in triplicate determinations; better agreement was obtained when pure compounds were analyzed.

FREE HYDROXYL. Hydroxyl groups were determined by the method of West, Hoagland, and Curtis (19) on 0.2-gram samples. The increase in free acid on heating may influence these determini nations so that the results are too low. Oxido compounds may esterify to some extent (9). Solutions of oxidized material became dark and thus obscured the end point, and triplicate determina-tions varied as much as 5 or 10 per cent in some cases. With pure compounds triplicate determinations checked within 0.5 per cent or less.

DOUBLE BOND. The pyridine sulfate dibromide (Rosenmund-Kuhnhenn) reagent was used according to the directions of Yasuda (20). Triplicate determinations agreed within 0.5 per cent or less and the results, indicated by -CH=CH-, are

recorded as millimoles per gram. PEROXIDE. The method of French, Olcott, and Mattill (8) was followed. Depending upon the peroxide content of the materials to be analyzed, samples of 0.07 to 0.3 gram were used. The strength of the sodium thiosulfate was also varied from 0.05 The strength of the sodium thiosuffate was also varied from 0.05 to 0.005 N, depending on the amount of iodine liberated by the sample. Peroxide oxygen, $-O_{-}$, is recorded as millimoles per gram. Triplicate determinations usually agreed within 1 to 2 per cent, although occasionally greater variance was encountered. All glassware was washed with hot soap solution, rinsed with distilled water, washed with 20 per cent hot alkali, again rinsed the period by dividing on the source of the period by the distilled water.

thoroughly with distilled water, and finally dried in an oven at 120° C.

Materials

OLEIC ACID was prepared in a manner similar to that previously reported (ϑ) , by a combination of the methods of Skellon (17) and Raymond (14). The extraction of the unsaponifiable matter of Raymond (14). The extraction of the unsaponinable matter of olive oil was omitted, since this fraction was lost in the process of recrystallizing the lead and lithium soaps of oleic acid. The acid was peroxide-free and had the following constants:—COOH, 3.56 millimoles per gram; —CH==CH--, 3.50 (theoretical for both, 3.54). The preparation was thus 98.8 per cent pure oleic acid, the remainder probably consisting chiefly of palmitic acid.

METHYL OLEATE. Oleic acid was esterified with methyl alcohol and sulfuric acid. The crude ester was purified by distillation in vacuo. The constants were: —COOC—, 3.37 millimoles per gram; —CH—CH—, 3.36 (theoretical for both groups, 3.38). A methyl oleate concentration of 99.3 per cent was thus indicated indicated.

BUTYL OLEATE. Olive oil was esterified with butyl alcohol according to the procedure recorded in *Organic Syntheses* (11). Analyses indicated that the preparation contained 91.6 per cent butyl oleate: —COOC—, 2.92 millimoles per gram; —CH= CH—, 2.71 (theoretical for both, 2.96). A trace of free acid but

Note: 2.11 (theoretical to both, 2.50). A trace of free action but no peroxide was present. OLEVL ALCOHOL was also prepared by the method in *Organic Syntheses* (11). After two fractional distillations at reduced pressure, the alcohol assayed 96 per cent. The constants were 3.58 millimoles of --CH=-CH-- per gram, and 3.73 of --OH (theoretical for both, 3.73). As in the other preparations, the impurities appeared to be saturated compounds

impurities appeared to be saturated compounds. 1,9,10-OCTADECANETRIOL. André and Francois (1) prepared two isomeric triols, one from oleyl alcohol melting at 126° C., the other from elaidyl alcohol melting at 98° C. For this work only the former isomer was synthesized.

I-OCTADECANOL. Oleyl alcohol in absolute ethyl alcohol was hydrogenated in the presence of platinum. The catalyst was removed by filtration, and octadecyl alcohol was crystallized from the solvent by cooling. After recrystallization, the crystals melted at 58° C. The reported melting point for octadecyl alcohol is 58.5° C.

cis-9,10-OCTADECENE. In a recent communication, Deatherage and Olcott (5) described the synthesis of this compound from oleyl alcohol. The preparation contained 3.89 millimoles of --CH=-CH- per gram (theoretical, 3.97); hence it was about 97.5 per cent octadecene.

9,10-OCTADECANEDIOL. The synthesis of this compound from nonyloin was also described by Deatherage and Olcott (5). The diol melted at 127° C. and gave the theoretical value for free —OH groups

9,10-DIHYDROXYSTEARIC ACID, melting at 132° C., was pre-pared by the permanganate oxidation of sodium oleate at room temperature (10).

Oxygen Consumption and Changes in Functional Groups

The data in Table I and Figure 2 on oxygen uptake at 75° C. show that, although the fundamental processes may be the same, some of the reactions concerned are more prominent in one substrate than in another.



		TABLE	I. Oxyg	en Con	SUMPTION	r	
Olei Time, br. 6.3 16.5 20.3 25.3 30.5 41.0 48 8 64.5 69.1 73.5 78.9 97.0 97.0 99.3 102.4 112.2	ic Acid ^a Moles O ₁ / mole 0.022 0.212 0.352 0.422 0.546 0.685 0.775 0.816 0.853 0.896 0.985 1.025 1.025 1.025 1.064 1.1060	ABLE Oleyl Time, 3 11.8 22.5 22.5 31.2 31.2 31.2 31.2 35.5 59.8 70.8 78.2 84.0 101.3 108.2 119.3 3	Alcohola Alcohola Moles O ₂ / mole 0.013 0.084 0.423 0.660 0.764 0.984 1.220 1.410 1.520 1.800 2.000	Methy Time, hr. 6.8 17.1 23.3 31.1 40.8 49.8 49.8 54.2 65.1 70.7 78.3 8 54.2 65.1 70.7 78.3 8 54.2 65.1 103.4 103.0 108.4 113.5 124.5	$ \begin{array}{c} \text{SUMPTION}\\ \text{d} \ \text{Oleate}^a \\ \text{Moles O}_{\text{f}} \\ \text{mole} \\ 0.024 \\ 0.114 \\ 0.220 \\ 0.383 \\ 0.565 \\ 0.715 \\ 0.784 \\ 0.955 \\ 1.038 \\ 1.149 \\ 1.238 \\ 1.275 \\ 1.325 \\ 1.325 \\ 1.440 \\ 1.495 \\ 1.540 \\ 1.540 \\ 1.630 \\ \end{array} $	Octas Time, hr. 8.8 18.7 25.1 32.7 43.8 50.2 57.9 66.6 73.6 73.6 98.0 91.7 98.0 91.7 114.1	decene ⁴ Moles O ₂ mole 0.027 0.162 0.268 0.358 0.650 0.761 0.965 1.043 1.170 1.222 1.287 1.360
126.3	1.185						

^a Quantities of substances used: oleic acid, 7.133 grams; oleyl alcohol, 7.718; methyl oleate, 8.026; octadecene, 7.246. ^b Following this reading the power went off on the constant-temperature bath for about 16 hours. After coming back to temperature, the next reading was taken using an extrapolation of the previous points for the time (Figure 2) reading wa (Figure 2).

Oleic acid took up less and oleyl alcohol consumed more oxygen than did the esters and octadecene. Apparently octadecene autoxidizes more nearly like methyl oleate than like the acid or alcohol. This is shown more clearly in Table II where, for each of the substrates, the oxygen necessary to destroy one mole of double bond is indicated. Oleic acid required less than the esters and the hydrocarbon; oleyl alcohol required more.

TABLE II. OXYGEN CONSUMPTION PER MOLE OF DOUBLE BOND DESTROYED

		Millimoles of Double Bond						
Run No.	Substrate	Millimoles O2 Consumed	Start of run	End of run	De- stroyed	Moles O ₂ / Mole —CH=CH— Destroyed		
1 3 5 6 7 9 10	Oleic acid Oleic acid Butyl oleate Methyl oleate Oleyl alcohol Octadecene Octadecene	$\begin{array}{c} 30.87\\ 30.05\\ 31.04\\ 43.97\\ 49.47\\ 38.59\\ 38.33 \end{array}$	25.0 24.8 21.5 26.9 24.8 28.2 28.2 28.2	5.7 5.4 5.0 5.8 7.3 8.2 7.9	$19.3 \\ 19.4 \\ 16.5 \\ 21.1 \\ 17.5 \\ 20.0 \\ 20.3$	1.60 1.55 1.88 2.08 2.83 1.93 1.89		

All of the substrates have comparable induction periods; furthermore, all of the curves are of the sigmoid type characteristic of this class of autoxidation reactions.

Data concerning the appearance and disappearance of the various functional groups in and from the substrate are out-lined in Table III. The figures from two runs on the same substrate (1, 3, and 9, 10) show fair agreement throughout. The composition of the residue varied more than the oxygen consumption.

The values for ---COOC--- may be high and those for ---OH may be low, owing to the presence of peroxides which may interfere with the determinations; the data are at least qualitatively significant.

Combined carboxyl increased in all the substrates but most in oleic acid. Correspondingly, a definite increase in free carboxyl groups occurred in every case except in oleic acid. No significant comparisons can be made between the peroxide figures; less peroxide was found in oleic acid, but these runs were longer and there was more time for the peroxides to disappear. Hamilton and Olcott (9) showed a decrease in peroxide content in the later stages of autoxidation. All substrates showed a general increase in -OH with the exception of oleyl alcohol; in this case the terminal hydroxyl may have been oxidized. No significant differences appeared in the amount of double bond remaining.

		TABLE I	II. Oxid	ation R	ESIDUES			
Run No.	Substrate	Total Time, Hr.	Moles O ₂ / Mole Substrate	-соон	Residue, -COOC	Millimoles, CH = CH-	/Gram 0H	-0-
1 2 3 4 5 6 7 8 9 10	Oleic acid ^a Oleic acid Oleic acid ^b Butyl oleate Methyl oleate ^a Oleyl alcohol ^a Octadecene ^a Octadecene	136.626.8135.0126.9122.9119.3144.0118.7114.1	1.235 1.21 1.44 1.63 2.00 1.37 1.36	3.05° 3.38° 3.04° 2.80 0.839 1.06 1.05 0.718 0.765 0.799	2.04 0.45 2.11 2.61 3.73/ 4.28¢ 1.89 1.21 1.29 1.26	$\begin{array}{c} 0.82\\ 2.70\\ 0.81\\ 0.67\\ 0.67\\ 0.73\\ 0.98\\ 0.68\\ 1.25\\ 1.25\\ 1.25\\ \end{array}$	$1.52 \\ 0.59 \\ 1.45 \\ 1.93 \\ \\ 1.07 \\ 2.94h \\ 1.04 \\ 1.37 \\ 1.30 \\ 1.30 \\ 1.30 \\ 1.01 \\ $	$\begin{array}{c} 0.174\\ 0.272\\ 0.150\\ 0.142\\ 0.349\\ 0.51\\ 0.365\\ 0.192\\ 0.428\\ 0.410\\ \end{array}$
 a See curve on Figure 2. b Petroleum-ether-insoluble residue. c Crude sample. d Temperature varied somewhat during run. At start -COOH = 3.56. f At start -COOC = 2.92. f At start -COOC = 3.37. h At start -OH = 3.73. 								



Each of the residues was fluid and, with the exception of that from octadecene, somewhat insoluble in light petroleum ether. That from oleic acid was the least soluble and very viscous; a sample was extracted twice with petroleum ether, dissolved in ethyl ether, and thrown out with an excess of petroleum ether. This process was repeated three times, and the oil was extracted once more with petroleum ether. The yield varied from 50 to 70 per cent of the residue. The constants for this preparation are given in entry 4 of Table III. Since the material was practically odorless, most of the lower molecular weight compounds must have been removed; oleic

acid is miscible with petroleum ether, so that most of the unoxidized acid was also removed. Compared with the total residue, this portion contained less free carboxyl groups, much more combined carboxyl and hydroxyl groups, and only slightly less double bonds and peroxides. The manner of obtaining this material suggests that these double bonds were not those of free oleic acid; whether they were in combined oleic acid or had been created during the process of autoxidation is uncertain.

According to Hamilton and Olcott (9) the aldehyde content of an autoxidizing mixture is never great; once formed, aldehydes either react with other autoxidation products or are themselves autoxidized. A small amount of freshly redistilled pelargonic aldehyde was added to inert paraffin and autoxidized in the usual way. The rate was exceedingly fast, as Figure 3 shows. The point of origin of the oxygen consumption curve was obtained by extrapolation, a procedure probably justified by the oxygen balance of the run. The peroxide content calculated as perpelargonic acid was about 10 per cent of the nonparaffin residue; most of the remainder was probably pelargonic acid. The aldehyde may thus have been oxidized to the acid via the peracid in the usually accepted manner. The residue and trap contents were negative to the Kreis test, and no water was detected. A small amount of aldehyde escaped oxidation and was found in the first ether-solid carbon dioxide trap. Aldehydes formed during autoxidation also undergo autoxidation.

Products of Autoxidation

The larger fraction of volatile end products was condensed in the ether-solid carbon dioxide traps; most of the remainder was obtained in the first dehydrite trap. These fractions consisted (Table IV) of water and water-soluble substances (the latter in very small amount) and of the water-insoluble or oily-products which made up the main part. Irrespective of the substrate oxidized, the volatile materials were alike in several respects.

The oily substance was water-white, had the typical stinging odor of rancidity, and was moderately volatile so that weighing was difficult. That which escaped the first three traps was stopped quantitatively by dehydrite on which it formed a brownish black resin; ascarite could stop the material but not so well as dehydrite; calcium chloride entirely failed to stop it. It gave an intense Kreis test. This is contrary to the idea of Powick (13) that the substance responsible for the Kreis test is nonvolatile. (The residue in the reaction vessel also gave the Kreis test but much less intensely.) The oil contained very active peroxide oxygen which attacked rubber almost as vigorously as does ozone. These observations do not entirely agree with those of Roschen and Newton (15) who steam-distilled rancid lard.

In the cases where enough material was available for analysis (Table IV), the molar content of peroxide oxygen in trap I was found to be larger than that of acid. On standing at room temperature, the acid increased while the peroxide decreased; this again suggests that the peroxides oxidized aldehydes to acids or themselves spontaneously decomposed to acids. The volatile products of run 6 were allowed to stand several hours before titration; hence the difference between acid and peroxide was not great. Runs 9, 10, and 11 were analyzed immediately following the dismantling of the apparatus.

	Т	'ABLE IV.	Volati	le Oxii	ATION	PRODUCT	s	
Run	Substrate	←Moles I Mole	H ₂ O per:- Mole double bond	CO2 ^a ,	Other Pro	Volatile oducts Mg./ millimole of substrate	Contents of Millimole	of Trap I, ss/Gram
INO.	Bubstrate	substrate	uestroyeu	1V1 g.	IVI B.	Bubstrate	00011	
1	Oleic acid	0.68	0.89	20	972	38.8		2.21
3	Oleic acid	0.78	1.02	1	1182	47.5		1.80
5	Butyl oleate	0.73	0.97	112	1216	56.5	1.465	2.27
6	Methyl oleate	0.79	1.01	32	1141	42.5	2.62	2.94
7	Olevi alcohol	1 13	1.58	89	1235	49.9		
ġ	Octadecene	0.62	0.91	18	1695	60.2	1.70	2.48
10	Octadecene	0.56	0.83	19	1873	66.5	1.53	2.97
îĭ	Oleic acid						1.42	3.20
a,	And any other v	olatile acid	s.					

Contrary to many reports, carbon dioxide was found in insignificant amounts, especially with oleic acid, methyl oleate, and octadecene. Since calcium chloride is often used instead of dehydrite to stop water, and since the volatile oil passes calcium chloride and is absorbed by ascarite, some of this oil would be weighed as carbon dioxide. The reason for the exceptional amounts of carbon dioxide found in the autoxidation of oleyl alcohol and butyl oleate is not known. Comparable amounts of water were formed in the autoxidation of oleic acid and its esters; that formed from octadecene was low, possibly owing to greater volatility of the autoxidation products. The large amount of water from oleyl alcohol suggests the possibility that the alcohol group is attacked by peroxides to form water in addition to that formed in the principal autoxidation process.

Notwithstanding the similarity in kind and amount of the volatile products from the different substrates, there are differences in the oxygen consumption and changes in the various functional groups (Tables II, III, and IV and Figure 2) which may now be considered and analyzed more carefully.

In the hands of several investigators the saponification of autoxidized oleic acid has yielded dihydroxystearic acid (melting point, 132° C.). We were also able to isolate this compound from the products of oleic acid oxidation but from the other substrates, only traces or none at all of the corresponding hydroxy compounds. Furthermore, oleic acid was the only compound of which the free carboxyl groups decreased during the course of autoxidation. If the carboxyl group reacted with some other group formed during oxidation, this could not well be a free hydroxyl because more water should be formed from oleic acid than from the other compounds, contrary to our observations. A functional group which reacts readily with a carboxyl group to form an ester, but which will not form water in the process, is the ethylene oxide group. Confirming the work of Ellis (6), we were unable to find oxidoöleic acid in the uncatalyzed autoxidation of oleic acid. Perhaps when oxidooleic acid is formed, it quickly reacts with a carboxyl group to form a half ester of dihydroxystearic acid and thus prevents appreciable accumulation of oxidoöleic acid. If this assumption is justified and if all the other substrates are autoxidized in the same fundamental manner, their oxido derivatives should be found in the respective residues.

The presence of these compounds in considerable quantity was demonstrated by heating the residues with glacial acetic acid for several hours and subsequently saponifying the resulting esters to form the corresponding dihydroxy derivatives. In each case, as in oleic acid, the high-melting dihydroxy isomer was found. The following experimental procedure proved satisfactory: The residue was dissolved in four or five volumes of glacial acetic acid and heated on the steam bath overnight; ten volumes of water were added, the esters were extracted with ether, and the extract was washed free of most of the acetic acid. The ether was evaporated, and the residual esters were saponified with alcoholic potassium hydroxide. After being heated on the steam bath for an hour. the alcohol solution was acidified and the dihydroxy compounds were extracted with ether. The ether was washed until neutral and then evaporated, and the residue was crystallized two or more times from benzene or alcohol.

The compound formed from oleyl alcohol was isolated by precipitation from benzene by petroleum ether instead of crystallization, to ensure a sufficient yield for analysis. The product melted at $116-118^{\circ}$ C.; the melting point of the highmelting isomer of 1,9,10-octadecanetriol is 126° C. This discrepancy suggested that the product might be another compound, but determination of the number of hydroxyl groups (10.0 millimoles of --OH per gram, theoretical 9.9) left little doubt that the substance was a slightly impure sample of the high-melting triol. By the same procedure the high-melting octadecanediol was obtained from autoxidized octadecene. Its melting point (127° C.) was exactly that of the known diol, and the mixed melting point showed no depression. From methyl oleate, dihydroxystearic acid (melting point 132° C.) was obtained but only after the acetic acid treatment. On the basis of this evidence ethylene oxide derivatives play an important role in the autoxidation of unsaturated fat acids and related substances as illustrated by Equation 1:



Compound o was found in autoxidized octadecene, esters of oleic acid, and oleyl alcohol, but not in oleic acid; c was found in appreciable amounts only in autoxidized oleic acid, and appears to have had its origin in oxidoöleic acid and a molecule of oleic acid.

The analogous reaction for the other substrates is not prominent because no original —COOH is available. For this reason also the oxido derivatives of the other materials are present in relatively large amounts during the course of autoxidation, and a reaction similar to Equation 1 occurs when their autoxidation residues are esterified with glacial acetic acid.

Since in all cases the *cis* double bond was attacked by oxygen so as to give only the high-melting dihydroxy compounds, these compounds are derived from only one of the two possible oxides; whether this is the cis or trans form has not yet been determined. Raymond (14) reported that, when oleic acid and benzaldehyde are autoxidized together, the oxidoöleic acid is formed which gives rise to the low-melting dihydroxystearic acid. Since the aldehyde, according to Raymond, is oxidized to perbenzoic acid and this oxidizes the ethylene linkage of oleic acid to the ethylene oxide group, the peracid oxygen is responsible for the formation of the ethylene oxide isomer that yields the low-melting dihydroxy compounds. Pigulevskii and Petrov (12) showed in 1926 that perbenzoic acid oxidizes oleic acid in the cold to the oxidoöleic acid which yields the low-melting dihydroxystearic acid. Hilditch (10) found that concentrated hydrogen peroxide in acetic acid or acetone converted both oleic acid and methyl oleate to the low-melting dihydroxy derivatives. Böeseken (2, 3) showed that peracetic acid would react with oleic acid to give a monoacetate of dihydroxystearic acid (melting point 95° C.), a compound similar to c (Equation 1). It is therefore fairly well established that peracid oxygen reacts with cis double bonds to form the oxido derivatives which yield the low-melting dihydroxy compounds. These appear only in negligible amounts in autoxidized oleic acid, its esters, oleyl alcohol, and octadecene. In other words, the oxido compounds encountered in this work have not been formed through the reaction of peracids with the ethylene linkage.

Half esters of dihydroxystearic acid could be formed by the reaction of oxidoöleic acid with any free carboxyl group as in oleic acid, oxidoöleic acid, or short-chain acids. The viscous residue of autoxidized oleic acid (Table III, entry 4) contained considerable amounts of material not soluble in light petroleum ether. The double bond in compound c also probably undergoes characteristic autoxidation reactions.

The oxygen consumption curves (Figure 2) for the various substrates are all S-shaped; the point of inflection represents the most rapid autoxidation when the oxidizable material is at its highest concentration. With oleic acid this point is reached more rapidly and at a lower level of oxygen uptake than with the other substrates. An explanation for this more rapid removal of autoxidizable material when oleic acid serves as the substrate is suggested by the formation of half esters of dihydroxystearic acid; each molecule that is so converted is changed to the status of a saturated aliphatic compound, in so far as the autoxidation is concerned, and as the oxidation proceeds the amount of oxidizable substrate decreases more rapidly than with the other substrates.

Oleyl alcohol takes up more oxygen than would be expected by comparison with the other substrates. Hamilton and Olcott (9) suggested a possible dehydration at the terminal hydroxyl with creation of a new double bond which would autoxidize. With 1-octadecanol this reaction did not occur to any extent, nor was there any appreciable oxygen uptake (0.072 mole oxygen per mole in 90 hours). In the case of oleyl alcohol the increased oxygen uptake is more probably the result of the oxidation of the terminal hydroxyl to aldehydes in the presence of peroxides. These are then oxidized to peracids and acids by means of atmospheric oxygen. The additional water formed, the increased total carboxyl, and the decreased hydroxyl (Table III) all substantiate this interpretation. For oleyl alcohol, 1.58 moles of water were formed per mole of double bond destroyed; all of the other substrates yielded 1 mole or less.

Octadecene is autoxidized like the oleic acid esters, except that it consumes somewhat less oxygen than do the esters, and more volatile products are formed (Tables I and IV). Octadecene peroxide cleaves to two molecules of pelargonic aldehyde; similar cleavage of methyl oleate peroxide yields only one molecule of pelargonic aldehyde and one molecule of the less volatile methyl ester of azelaic half aldehyde. The latter compound would tend to stay in the reaction vessel longer and would have more opportunity to autoxidize to the peracid and acid. Differences in volatility of the cleavage products may be small but sufficient to account for the difference in oxygen consumption as between octadecene and the esters of oleic acid.

Water is an important autoxidation product of all of the compounds studied. Octadecene produced a somewhat smaller amount than the other substrates (Table IV). The volatile materials formed in autoxidation, largely peroxides and peracids, are probably responsible for the oxidation of the carbon chain with the formation of water and the creation of new double bonds; they in turn undergo autoxidation reactions. The escape of volatile peroxides from the reaction mixture thus delays oxidation of the chain, and less water is formed. The volatility of the autoxidation products of octadecene thus influences not only the total oxygen consumption but also the amount of water formed.

Comparison of the oxygen consumed (Table II) with the moles of water formed (Table IV) per mole of double bond destroyed reveals that about 25 per cent of the oxygen taken up appears as water. Since approximately one atom of oxygen out of every four absorbed is thus converted to water, the formation of the peroxides and ethylene oxide compounds takes place early. The following series of hypothetical equations may account for the formation of water, the oxidation of the products formed, the high content of peroxide found, and the intense Kreis test given by the volatile oil caught in the traps:

The peroxide aldehyde, *a*, is typical of a number of volatile autoxidizable aldehydes that could be formed and that may polymerize in a way similar to the observed polymerization of volatile products on dehydrite in trap IV.

There is good evidence that such a type of compound, though unstable, is actually formed. Analysis of the waterinsoluble contents of the first freezing trap showed a greater molar concentration of peroxide oxygen than of carboxyl group (Table IV). Much of the active oxygen is therefore contained in a volatile peroxide which is not an acid and which may be made up of aldehydes; indeed, the volatile oil from this trap gave a strong test for aldehydes with 2,4-dinitrophenylhydrazine.

The volatile materials in each of the cold traps from every substrate gave a stronger Kreis test than the nonvolatile residue, and with hydrochloric acid and ethereal phloroglucinol, gave a precipitate similar to the one obtained by Powick (13) upon treating acrolein and hydrogen peroxide with the same reagents. Powick demonstrated that the Kreis test is due to epihydrinaldehyde, CH₂CHCHO, either present

as such, or produced by the reaction of hydrochloric acid with a fat acid chain that had been oxidized at several places. The scheme which he proposed, involving compounds similar to those just suggested, requires the formation of carbon dioxide or carbon monoxide; little or none of the former is produced and none of the latter (9). Furthermore, the large amount of volatile material giving the Kreis test and the small amount of oxygen consumed are not compatible with such a process. Hence it must be concluded that a positive Kreis test may be produced by substances other than epihydrinaldehyde, possibly by its higher homologs. Such compounds could be formed by the reduction of products of Equation 3:

$$-CH-CH-CHO \rightarrow -CH-CH-CHO + -O-$$

or by the partial oxidation and cleavage of the products of Equation 2:

No all-inclusive theory of autoxidation can yet be formulated, but it is hoped that these data and the suggestions for their interpretation may uncover new approaches to the study of the mechanism of the autoxidation of unsaturated fat acids and related substances.

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H. S. Olcott was instrumental in the inauguration of the study of pure substrates and made suggestions for the improvement of this manuscript. Our thanks also go to Lever Brothers Company of Cambridge, Mass., for a grant in aid of this work.

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Pentachlorophenol for Wood Preservation

Pentachlorophenol, which is now available commercially, has been intensively studied and found to be a valuable woodpreserving chemical. Its physical and chemical characteristics make it especially suited for this purpose, and its fungicidal potency gives it a high rank as a toxic against wood-rotting and wood-staining organisms. It has also been demonstrated to be effective in preventing termite attack, and its use for preventing marine borer and powder post beetle attack is strongly indicated. Because of its lack of color and objectionable odor, it is useful where a "clean" treatment for wood and fibers is desired. The fact that it can be formulated so that treatment with the material does not impare puttyability, paintability, and further finishing is of utmost importance in connection with its use as a wood preservative.

LTHOUGH the use of chemicals for the preservation of wood and wood products is not new, modern conditions of use have created a wider demand for preservatives as well as a need for improved properties over those possessed by chemical treatments formerly available. Marked differences exist between virgin growth wood formerly used in construction and in second growth wood more commonly used today. Greater demands are continually being placed on wood by changes in architectural design, by the trend especially in the North toward heated and finished basements, and by the advent of modern air conditioning. All of these changes create a more favorable atmosphere for decay or termites.

Much of the future for wood in certain industrial applications is dependent upon the development of effective and permanent "clean" treatments which, while protecting the wood against degradation, will not alter its characteristic

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feel or appearance nor interfere with subsequent fabrication or application of surface coatings. Failure on the part of wood producers and fabricators to recognize the value of proper preservative treatments can result only in the ultimate displacement of wood in favor of substitutes of greater permanency, even though such substitution may involve the sacrifice of the esthetic value man has always placed upon wood.

Obviously creosote and many of the older treating materials cannot meet the requirements imposed by these new demands. This does not mean that creosote will not continue to be a favorite for railway ties, for piling, and for other such uses. But for millwork and much of our structural building materials, most of the older treatments are inadequate either because of their color, odor, or lack of permanence, or because of adverse effects upon the appearance of the treated products. Hence, a definite demand has developed within the woodusing industries for a satisfactory toxic chemical which will enable them to supply the public with properly preserved wood products.

The possible value of chlorinated phenolic compounds as wood preservatives has been suggested many times over a number of years. Several factors have prevented their extensive use. In general, the lower chlorinated phenols possess objectionable odors and are not sufficiently stable. The higher members of the series have not been commercially available until recently. Only since 1936 has pentachlorophenol been studied with sufficient intensity to permit its true usefulness as a wood preservative to be evaluated.

In 1932 Iwanowski and Turski (13) patented "polychloro deriva-tives of phenol" as wood preservatives, but they limited their claims to "chlorinated phenol compounds containing at least two and not more than three chlorine atoms". Because of the high vapor pressure, high water solubility, objectionable odor, and comparatively low degree of toxicity to wood-rotting and stain-ing fungi of some of the members, the lower chlorinated phenols are not generally satisfactory for use in wood preservation

are not generally satisfactory for use in wood preservation. Although pentachlorophenol was prepared as early as 1841, it was not produced commercially until 1936. In the intervening years, little actual experimental work was done upon it. In 1914 Aylsworth (1) patented it and other chlorinated benzene derivatives as flame-extinguishing materials. In his patent specification he said: "Fabrics or materials coated or impreg-nated with the improved composition in addition to having flame-extinguishing properties may likewise be given water-repellent qualities and rendered aseptic against the ravaging of