Volatile Decomposition Products of the Oxidative Polymers of Ethyl Linolenate[1]

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IN recent studies involving the flavor reversion of soybean oil (4, 6, 7, 8, 10, 12) carbonyl compounds have been isolated from reverted soybean oil. It was suggested in these studies that the initial oxidation products of the di- and tri-unsaturated fatty acids in soybean oil were precursors for the carbonyl compounds. However it has been shown that soybean oil, hydrogenated to a degree where linoleic and linolenic acid are undetectable, will still develop a reverted flavor (11). Therefore if the unsaturated fatty acids are precursors of reversion compounds, they must be present in an undetectable form and cannot be readily removed by catalytic hydrogenation.

Recent work on the oxidation of ethyl linoleate in our laboratory has indicated that the polymers formed during oxidation may be a precursor of some of the reversion compounds (1, 2). In the work to be described in this paper ethyl linolenate was oxidized and the oxidative polymers separated by solvent extraction. The polymeric fractions were oxidatively decomposed in a closed system, and the volatile decomposition products trapped in cold traps. The carbonyl compounds in the volatile material were isolated and identified as their 2,4-dinitrophenylhydrazones. These compounds were compared to those isolated from soybean oil by other workers.

Experimental and Results

The ethyl linolenate was prepared in this laboratory from linseed oil by a bromination-debromination method (9). It had a Wijs iodine value of 248.2 (theoretical 248.5). The ethyl linolenate was autoxidized by bubbling oxygen through it for 170 hours at 30°C.

The autoxidized ethyl linolenate was separated into polymeric fractions by a solvent-extraction process, using Skellysolve F and diethyl ether as a solvent pair. In this extraction procedure the oxidized ethyl linolenate was weighed into a round bottom flask, and twice its volume of the appropriate solvent mixture added. The mixture was agitated with a mechanical stirrer for 30 minutes and then allowed to stand until all of the undissolved portion had settled to the bottom of the flask. The top layer was drawn off into a clean flask with the aid of a fritted glass filter stick, and the vacuum created with a water pump. The solvent was removed under vacuum, the fraction weighed, and representative constants determined (Table I). These polymer fractions were stored at —20°C. The Skellysolve F used in the extraction was distilled before use. Anhydrous diethyl ether was used without treatment.

TABLE I

Physical Constants of Polymeric Fractions Separated From 150 g. of Oxidized Ethyl Linolenate

Property	Fraction			
	8	12	16	17
Solvent				acetone
% Ether	24	44	76	....
% Skellysolve F	76	56	24	....
Ref. index n_D 25°C	1.4864	1.4911	1.4983	....
Molecular weight	536	1268	....	gel
Weight in grams	6.625	15.356	10.034	3.701
Peroxide value	1450.0	1023.2	934.3	832.0

In order to follow the oxidation of the polymers and to insure trapping of the volatile products, a closed oxidation system was devised (Figure 1). The reaction tube *A* was placed in the constant temperature bath *I*. Oxygen was passed into the reaction tube through a fritted glass filter stick *B* sealed into the bottom of the tube. The reaction tube was connected to two cold traps, *C* and *D*, which were cooled to —70°C. by a mixture of dry ice and acetone. The traps were connected to a Sigmamotor *H* which, in turn, was connected through glass tubing to the bottom of the reaction tube. The direction of the flow of oxygen through the system could be controlled by means of three-way stopcocks, *E* and *G*.

At the start of the oxidation, oxygen was introduced into the system through stopcock *E*. This oxygen was freed of carbon dioxide, water, and carbon monoxide by means of purification train *N*. The latter consisted of an absorption tube of magnesium perchlorate to remove water, an absorption tube of ascarite to remove carbon dioxide, a Vycor tube packed with copper oxide heated to red heat in order to convert carbon monoxide to carbon dioxide and hydrogen to water, and absorption tubes of perchlorate and ascarite to absorb the carbon dioxide and water, respectively. The second train *M* was set up in the same manner and all of the tubes in train *M* were accurately weighed. With this system it was possible to calculate the amounts of carbon monoxide, carbon dioxide, hydrogen, and water formed during

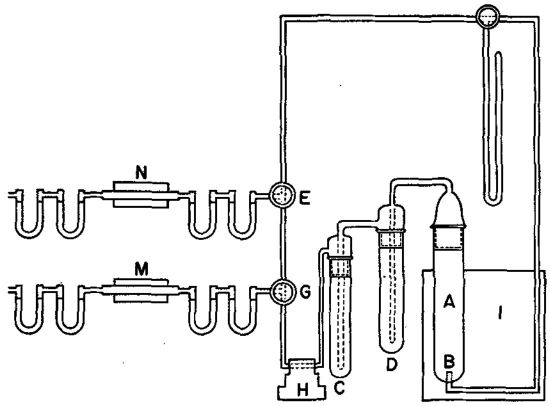

FIG. 1. Diagram of apparatus used for autoxidation of the ester in a closed system.

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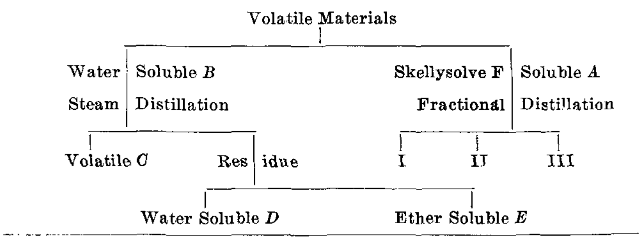
[2] With the armed forces.

TABLE II

Fractionation of the Volatile Material Collected in the Cold Traps



the oxidation. Except for a short piece of Tygon tubing in the Sigmamotor, all of the connections and tubing used in the system were glass; ground glass ball joints were used for all necessary connections.

The following procedure was used in oxidizing the polymeric fractions. The reaction vessel and the cold traps were tared, and the entire system was flushed out with purified oxygen until the absorption tubes had reached a constant weight. The polymer sample was then added to the reaction tube, the stopcocks properly arranged, and the Sigmamotor started. At 48-hour intervals the Sigmamotor was stopped, the system was flushed out with purified oxygen and the various absorption tubes were weighed. As the polymeric fractions were very viscous, it was necessary to disperse each fraction on glass wool and introduce the polymer into the reaction tube *A* in this form. In actual operation approximately 25 g. of glass wool were added to the reaction tube, an ether solution of the polymer was poured over the glass wool, and the solvent was removed under vacuum.

The molecular weights were determined by the cryoscopic method, using purified benzene as the solvent. The peroxide value was determined by the method outlined by Fugger *et al.* (3).

The volatile material collected in the cold trap was diluted with distilled water and subjected to a preliminary separation by three extractions with equal volumes of Skellysolve F (Table II). The Skellysolve F soluble material was fractionally distilled at atmospheric pressure. Three fractions were obtained: one, *(A-I)* distilled below 50°C. (this fraction also contained the Skellysolve F); two, *(A-II)* distilled between 50-70°C.; and three, *(A-III)* the residue fraction which did not distill. The water-soluble fraction *(B)*, which was left after extraction of the volatile material with Skellysolve F was steam-distilled; a steam-distillable fraction *(C)* was obtained. The non-steam distillable residue was extracted with three 10-ml. portions of ether, giving fraction *(E)*. The non-ether soluble residue was designated as fraction *(D)*. All of the fractions were converted into 2,4-dinitrophenylhydrazones by the method of Gordon *et al.* (5). Twenty ml. of an alcoholic solution of the various fractions were added to 100 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid and the mixture digested at 0°C. for one hour. The precipitate was filtered on a Buchner funnel, washed with 100 ml. of water, and dried under high vacuum for 3 hours.

The method of Gordon *et al.* was used for the chromatographic separation of the 2,4-dinitrophenylhydrazones. A column 20 × 4 cm. was packed with 2:1 mixture of silica gel,[3] and Celite filter aid.[4] The adsorption media was activated separately by heating to 190°C. for 2 hours. The mixture was packed into the column with the aid of a plunger. The top of the column was then covered by an aluminum cap which had been perforated with many small holes. A 100- to 150-mg. sample of the 2,4-dinitrophenylhydrazones was dissolved in 20 ml. of chloroform, poured carefully into the top of the column, and allowed to pass through it. The chloroform solution was followed by 500 ml. of Skellysolve F, which was forced through the column under a pressure of two to five pounds of ntirogen. The column was developed with 2, 4, and 6% ethyl ether in Skellysolve F. After the column had been sufficiently developed, the bands were eluted with 8 to 12% ethyl ether solutions. The 2,4-dinitrophenylhydrazone in each band was collected as it was eluted, and the solvent removed under vacuum. Each 2,4-dinitrophenylhydrazone was then taken up in chloroform, rechromatographed, and recrystallized from alcohol. It was identified by melting point, mixed melting point, ultraviolet absorption, and ultimate analysis.

One 2,4-dinitrophenylhydrazone was found in fraction *(A-I)*. This compound melted at 113-114°C. and had an analysis and absorption peaks similar to that of the 2,4-dinitrophenylhydrazone of methyl ethyl ke-

TABLE III

Comparison of the 2,4-Dinitrophenylhydrazones of the Isolated Carbonyls With Those of Known Carbonyls

Compound	Color	Melting point	Mixed melting point	Maximum absorption	Carbon	Hydrogen	Nitrogen
		°C.	°C.		%	%	%
Unknown A-1	Orange	113-114	119-116	360-362	47.88	4.63	21.62
Methyl ethyl ketone	Orange	115-117		362	47.60	4.79	22.21
Unknown C-1	Yellow	161-163	164-165	356	42.12	3.84	24.76
Acetaldehyde	Yellow	168		356	42.86	3.60	24.99
Unknown C-2	Orange	148-150	152-154	354	45.65	4.35	23.24
Propionaldehyde	Orange	155		355	45.38	4.23	23.52
Unknown D-1	R. O.	160-169	......	369-370	48.04	4.63	22.86
Crotonaldehyde	R. O.	190		375	47.95	4.03	22.81
Unknown E-2	Red	160-161	161-163	374	50.53	4.88	21.38
Alpha-pentenal	Red	163		(375)	50.30	4.57	21.19

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[3] Obtained from Mallinckrodt Chemical Company, St. Louis, Mo., analytical grade 100-mesh.

[4] Johns-Manville and Company, Manville, N. J., analytical grade.

tone (Table III). Several other bands were present on the column, but the small amount of material in these bands made further study of the 2,4-dinitrophenylhydrazones impossible. The second fraction *(A-II)* gave no 2,4-dinitrophenylhydrazones. The 2,4-dinitrophenylhydrazone of the final fraction *(A-III)* separated into a wide diffused band, which yielded a single compound. After repeated recrystallizations, fine yellow-orange needles were obtained, melting point 85-86° C. The ultimate analysis of this 2,4-dinitrophenylhydrazone indicated that the derivative contained a five-carbon-atom carbonyl. However comparison with known five-carbon-carbonyl compounds did not reveal its identity. Further work on this compound and others which were present in minor bands, remains to be completed.

The 2,4-dinitrophenylhydrazone mixture from the steam volatile fraction *(C)* yielded two compounds when chromatographed. The first *(C-1)* was rechromatographed and recrystallized twice from 75% ethanol yielding yellow needles, melting point 161-163°C. An admixture of compound *(C-1)* with the 2,4-dinitrophenylhydrazone of acetaldehyde gave a mixed melting point of 164-165°C. Comparison of ultraviolet absorption peaks gave further proof that compound *(C-1)* was acetaldehyde. The second compound *(C-2)* was rechromatographed and recrystallized several times from ethanol yielding orange needles, melting point 148-150°C. The ultimate analysis, mixed melting point, and absorption peaks indicated that compound *(C-2)* was the 2,4-dinitrophenylhydrazone of propionaldehyde.

The 2,4-dinitrophenylhydrazone of the non-ether soluble fraction *(D)* gave one major band and several very minor bands on the chromatograph column. The minor bands did not contain enough 2,4-dinitrophenylhydrazones for positive identification. The major band was resolved and the compound *(D-1)* recrystallized several times from 80% ethanol yielding a red-orange powder, melting point 160-169°C. The melting point could not be improved by further recrystallization. As the compound *(D-1)* gave a positive test for the presence of an alpha-beta unsaturated carbonyl, it may have been crotonaldehyde. It has been pointed out by other workers in this field that crotonaldehyde and propionaldehyde cannot be separated because their movement on a silica column is almost identical (6).

The 2,4-dinitrophenylhydrazones in fraction *(E)* gave two major bands on the column, plus a wide diffused band at the bottom of the column. Further separation of the latter proved unsuccessful. After removal from the column, the material in the top band *(E-1)* was recrystallized twice from ethanol, yielding yellow-orange crystals, melting point 160-162°C. The constants determined on this compound indicated that it was the 2,4-dinitrophenylhydrazone of acetaldehyde.

The compound in the second band *(E-2)* was rechromatographed, and then recrystallized twice from ethanol. The fine red needles, which were obtained, melted at 160-161°C.; the constants indicated that they were the 2,4-dinitrophenylhydrazone of alpha-pentenal.

Gaseous products were continuously evolved. However the rate of evolution of carbon monoxide, carbon dioxide, hydrogen, and water vapor varied during the oxidation, with a marked decrease toward the end of the oxidation (Figure 2). The rate of carbon dioxide evolution gave an indication of the decomposition of the polymer fraction. The amount of carbon dioxide evolved increased rapidly during the early part of the oxidation and then gradually decreased until it became relatively constant at the end of the oxidation (Table IV). The polymer remaining in the reaction tube had changed in color from a light yellow to dark red, and its molecular weight, refractive index, and peroxide number had decreased (Table V).

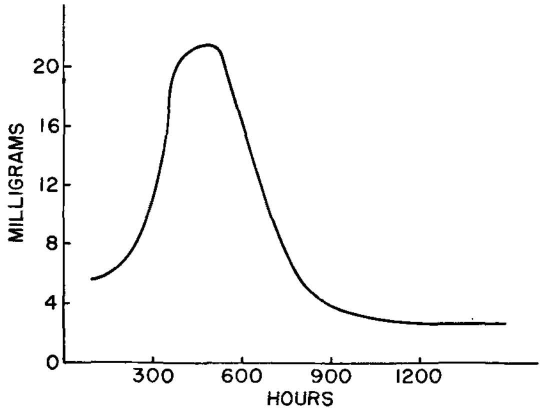

FIG. 2. The rate of carbon dioxide evolution during oxidation of 5 g. of the polymer.

TABLE IV

Volatile Products Formed During Oxidation of Polymer Fraction (Fraction 616)

	Time (hours)			
	0	487	754	1,228
Polymer weight—gm.	5.12	......	......	4.69
Materials in traps—gm.	......	1.01	2.21	2.49
Weight of gaseous—mg.				
H_2O (total wt.)	......	21.8	44.4	65.0
CO_2 (total wt.)	......	62.2	80.8	100.4
H_2 (total wt.)	......	1.6	2.5	3.0
CO (total wt.)	......	8.4	11.5	15.4

TABLE V

Comparison of Polymer Fraction After Oxidation

	Hours	
	0	843
Color	Yellow	Red
Refractive index	1.4910	1.4764
Peroxide value	946.0	273.0
Molecular weight	832.0	572.0
Weight	12.01	11.52

Discussion

The present work indicated that the oxidative polymers of ethyl linolenate can be autoxidized to carbonyl and gaseous products. Acetaldehyde, propionaldehyde, and alpha pentenal have been identified, and possibly crotonaldehyde was also present. All of these compounds have been isolated from oxidized soybean oil (4, 6, 7, 8, 10, 12). In addition, evidence for the presence of methyl ethyl ketone was obtained. The lack of knowledge of the structure of the oxidative polymers and the mechanisms of polymer decomposition does not allow one to speculate to any great extent on the mechanism of formation of these carbonyl compounds. The mechanisms which have been put forth by other workers (6, 7, 10) suggest that hydroperoxides break down with simultaneous cleavage of the adjacent carbon to carbon bond to form an aldehyde and some unidentified fragment. The specific aldehyde which is formed depends upon the position of the hydroperoxide group on the chain, and the length of the carbon chain which is cleaved from the parent molecule. The formation of acetaldehyde, propionaldehyde, alpha pentenal, and crotonaldehyde can be postulated from the mechanism by appropriate shifts in double bonds. The formation of methyl ethyl ketone cannot be due to a simple decomposition of a hydroperoxide and cleavage. However the initial cleavage product could be a beta-keto acid, this acid then losing carbon dioxide to form the methyl ketone. The gaseous products are mainly carbon dioxide and water vapor with traces of carbon monoxide and hydrogen. The mechanism whereby these gaseous products are formed is not known.

Three of the compounds which were isolated in the present work, acetaldehyde, propionaldehyde, and alpha pentenal, have been included among the compounds that have been isolated from reverted soybean oil. The presence of these compounds in the volatile decomposition products of the oxidative polymers of ethyl linolenate indicated that oxidative polymers may serve as precursors of flavor reversion compounds in soybean oil.

Summary

Oxidative polymers which had been isolated from autoxidized ethyl linolenate were oxidatively decomposed in a closed system at room temperature. The decomposition products were collected in appropriate traps and separated into various fractions on the basis of their volatility and solubility. The aldehydes and ketones thus obtained were converted into 2,4-dinitrophenylhydrazones, chromatographed on silica gel, and the isolated 2,4-dinitrophenylhydrazones identified by standard procedures. Methyl ethyl ketone, propionaldehyde, acetaldehyde, and alpha-pentenal were positively identified, and evidence for the presence of crotonaldehyde and an unknown five-carbon-carbonyl compound was obtained.

Acetaldehyde, propionaldehyde, and alpha-pentenal have been isolated from the decomposition products of oxidatively decomposed reverted soybean oil by previous workers. If oxidative polymers of linolenate are present in reverted soybean oil, they could serve as precursors of the aldehydes and ketones which have been associated with flavor reversion.

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Modification of Vegetable Oils. XIV. Properties of Aceto-Oleins[1]

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IN the industrial utilization of fats and oils the need often arises for oils which remain liquid at low temperatures and yet are relatively resistant to oxidative polymerization and other forms of deterioration. In the manufacture of mayonnaise and salad oils, which require oils remaining liquid at the temperature of the domestic refrigerator (4° to 10°C.), these needs can be fulfilled by winterized cottonseed oil, corn oil, and dewaxed soybean oil. However the latter possesses some objectionable characteristics because of its high degree of unsaturation. When oils which remain liquid well below 0°C. and simultaneously possess good stability are needed, few naturally occurring triglycerides can be employed.

The simple triglycerides of saturated fatty acids occurring most profusely in nature, those having 12 to 18 carbon atoms, are solid at temperatures below 46.5°C. Tricaprin and tricaprylin, whose fatty acids occur as glycerides in amounts up to 8% in a few commercially produced oils, are solid below 8.3°C. Tricaproin and tributyrin solidify at temperatures of −25°C. and approximately −60°C., respectively; but their fatty acids occur in natural fats to a very limited degree. Butterfat, the best natural source of caproic and butyric acids, contains from 2 to 4% of each.

Triglycerides of oleic, linoleic, and linolenic acids are, of course, plentiful in nature and are employed where an oil melting at a relatively low temperature is needed. However triolein solidifies at 5.5°C. Trilinolein and trilinolenin solidify at lower temperatures but are more unsaturated, having iodine values of 173.21 and 261.61, respectively, compared to 86.01 for triolein.

In Figure 1 are shown the melting points of triglycerides of naturally occurring fatty acids as a function of chain length and degree of unsaturation.

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[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.