Volatile Cleavage Products of Autoxidized Methyl Linolenate[1]

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THE autoxidation of soybean oil results in the formation of volatile carbonyl compounds, one of which or a combination of which is responsible for the "reversion" or aged odor and flavor of soybean oil. These volatile aldehydes were fractionated upon a chromatographic column composed of silicic acid into what has been characterized as "painty" and "rancid" fractions (7). Certain component aldehydes of these fractions, namely hexanal, α-pentenal, propionaldehyde, and acetaldehyde, were identified as their 2,4-dinitrophenylhydrazone (DNPH) derivatives. Indirect evidence was obtained for the presence of crotonaldehyde. Under higher temperature conditions of autoxidation and with the use of steam deodorization at reduced pressure for removal of cleavage products, Daubert and coworkers have isolated acetaldehyde, α-heptenal, and 2,4-decadienal (8, 10).

Organoleptic identification studies of chemically modified glycerides show that the linolenic acid radical is an unstable precursor of reversion flavors (2). Consequently the isolation and identification of aldehydes from autoxidizing linolenate was undertaken. It was hoped that correlation with similar work on soybean oil might lead directly to the flavor principle or principles responsible for reversion.

Methods and Materials

The methyl linolenate was obtained from Hormel Institute[4] and was prepared by bromination, debromination, and distillation procedures.

Autoxidation of methyl linolenate (13 grams) was performed at room temperature by continuously bubbling a stream of oxygen through a 6-cm. layer for 7 days. Effluent oxygen conveying the constituents volatile at room temperature and at atmospheric presure was then passed through a cold trap immersed in an ethanol and solid carbon dioxide bath. The trap contained 15 ml. of pentane-hexane to facilitate the recovery and concentration of the volatiles.

Chromatographic procedures for separation of odor constituents on silicic acid were identical with those which have been previously described (7). Fractions (5 ml.) of the eluate were collected in the test tubes by means of a Technicon automatic fraction collector.[4] The chromatographic separation of the 2,4-dinitrophenylhydrazones was effected by combined techniques of elution and the cutting of the extruded column.

All solvents were purified as previously described and permitted ultraviolet absorptions to be determined upon solutions (7).

Results

Chromatographic adsorption of the cold trap contents fractionated the odor concentrate into rancid and painty components. Two major peaks are apparent in Figure 1, which shows the spectral absorption at 234 mμ and 268 mμ plotted against the tube number of the eluate. These wavelengths correspond to maxima in volatiles isolated from soybean oil (7). Despite the rough parallelism of these curves, the spectral absorptions of individual fractions in the two peaks differed greatly and confirmed the indications that separations of ultraviolet light absorbing substances had been effected (see also 7). The judgments of three experienced tasters associated paintiness with the first major peak and rancidity with the second. Based on this information, tubes 16-23 and 24-28 were combined to comprise the painty and rancid fractions. The 2,4-dinitrophenylhydrazones were then formed from each of the fractions.

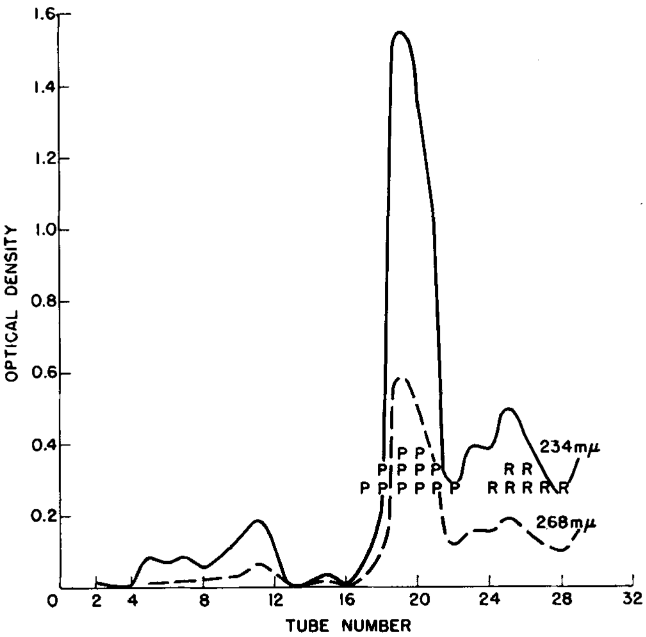

FIG. 1. Adsorption analysis of volatiles which absorb ultraviolet light. Optical densities and organoleptic evaluations are plotted against fraction number.

Chromatography of the hydrazones from the painty fraction resulted in the resolution of five bands:

Band	Color	Position	Weight
		cms.	*mg.*
P_A	Red	6.5- 7.0	38.4
P_B	Orange	12-14.3	164.7
P_C	Orange	16-19.0	123.9
P_D	Orange red	23-25.2	33.4
P_E	Diffuse yellow	28-eluate	64.7

Band P_B, after readsorption and recrystallization three times from 80% ethanol, yielded yellow plates

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[3] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

[4] The mention of products does not imply endorsement or recommendation by the Department of Agriculture over other products of a similar nature not mentioned.

melting 161°-162° C. [m.p. 168.5 for acetaldehyde DNPH, Bryant, J. Am. Chem. Soc. *60,* 2815 (1938)]. *Anal.* calcd. for $C_8H_8N_4O_4$: C, 42.86; H, 3.60; N, 24.99. Found: C, 42.95; H, 3.57; N, 25.00.

Band P_C, similarly purified, gave yellow crystals m.p. 151°-152° C. [m.p. 155° C. for propionaldehyde DNPH; Allen, J. Am. Chem. Soc. *52,* 2947 (1930)]. *Anal.* calcd. for $C_9H_{10}N_4O_4$: C, 45.38; H, 4.23; N, 23.52. Found: C, 45.65; H, 4.08; N, 23.9.

Band P_D gave fine red needles after further adsorption and crystallization steps, which melted at 157.5°-158.5° C. [m.p. 162°-163° C. for authentic α-pentenal DNPH described below]. *Anal.* calcd. for $C_{11}CH_{12}N_4O_4$: C, 50.3; H, 4.57; N, 21.19. Found: C, 50.00; H, 4.86; N, 21.3.

In connection with its isolation from soybean oil volatiles, α-pentenal had been synthesized by condensing ethyl hydrogen malonate with propionaldehyde to give ethyl 2-pentenoate. Reduction to the alcohol with lithium aluminum hydride and oxidation with dichromate to the aldehyde yielded α-pentenal. The semicarbazone derivative melted at 172°-173°C. and was compared to a value found in the literature [Delaby (1)] of 180°C. The DNPH of the synthetic pentenal melted at 162°-163°C. The mixed melting point of the synthetic and isolated DNPH was not depressed over that of the isolated pentenal DNPH.

Figure 2 gives the absorption spectrum of the isolated and authentic α-pentenal DNPH's and supports the identity of the pair.

The rancid component converted to the hydrazone derivative was subsequently adsorbed. The following bands became apparent:

Band	Color	Position	Weight
		cms.	*mg.*
$R_A{}^1$	Red	5.2- 5.4	93.0
$R_A{}^2$	Red	5.5- 5.6	
$R_A{}^3$	Yellow	5.6- 5.7	
R_B	Yellow	6.4- 6.7	33.1
R_C	Orange	8.6-13.0	206.8
R_D	Yellow	14.8-16.7	30.4
R_E	Light yellow	20.0-22.6	23.7

Band R_C was the most prominent, and after further chromatography and recrystallization, melted at 162°-163°C. [m.p. 168.5 for acetaldehyde DNPH: Bryant, J. Am. Chem. Soc. *60,* 2815 (1938)]. *Anal.* calcd. for $C_8H_8N_4O_4$: C, 42.86; H, 3.60; N, 24.99. Found: C, 43.00; H, 3.94; N, 24.8.

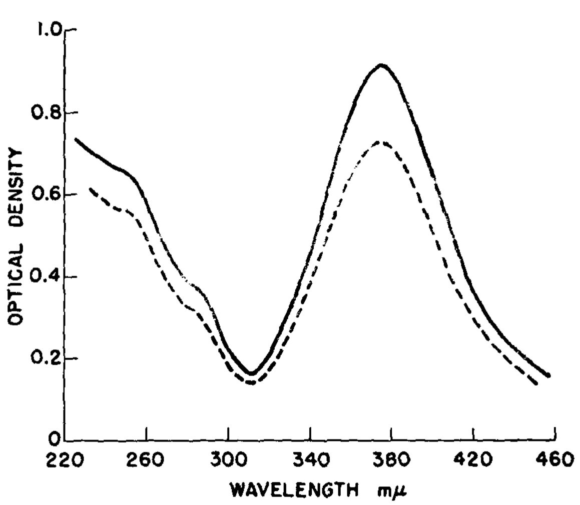

FIG. 2. Spectrophotometric curves for DNPH derivatives of a synthetic α-pentenal and of the isolated fraction.

Band R_A was studied by combining the upper portions of several columns. Excess reagent present in these bands was reacted with acetone. In order to dissolve these hydrazones and introduce them upon the chromatographic column, 5% acetone in benzene was used for solvent. This solvent was followed by pentane-hexane to stop the movement of the band. Development then took place in pentane-hexane containing 25% diethyl ether. Three bands were obtained, the lowest being the DNPH of acetone.

Band	Color	Position
		cms.
R_{Aa}	Orange-red	11.2-11.8
R_{Bb}	Yellow-orange	16.0-16.2
R_{Ac} (acetone DNPH)	Yellow	29.6-32.0

Band A_a was readsorbed twice in succession and yielded 42.3 mg. of deep brown-red crystals. Three recrystallizations from an ethyl acetate-ethanol mixture (1:5) gave crystals melting at 240°-248°C. and charring at 270°C. On crystallization from nitrobenzene and ethanol (1:1) 8.6 mg. of crystals which

TABLE I

Schematic Summary of Isolation and Identifications

Methyl Linolenate

Adsorption analysis

[Silicic acid + filter-cel (1 + 1),
5% ethanol in pentane-hexane]

Painty fractions

(Chromatography of DNPH's)

P_B, acetaldehyde DNPH
164.7 mg., m.p. 161°C.

	C	H	N
Calcd.	42.86	3.60	24.99
Found	42.95	3.57	25.00

P_C, propionaldehyde DNPH
123.9 mg., m.p. 151°C.

	C	H	N
Calcd.	45.38	4.23	23.52
Found	45.65	4.08	23.9

P_D, α-pentenal DNPH
33.4 mg., m.p. 158.5°C.

	C	H	N
Calcd.	50.3	4.57	21.19
Found	50.0	4.86	21.3

Rancid fractions

(Chromatography of DNPH's

R_{Aa}, dicarbonyl (Hexene-3-dial-1,6)
8.6 mg., m.p. 283°C.

	C	H	N
Calcd.	45.75	3.41	23.72
Found	45.0	3.48	23.4

R_C, acetaldehyde DNPH
81.5 mg., m.p. 165°C.

	C	H	N
Calcd.	42.86	3.60	24.99
Found	43.0	3.94	24.8

melted at 283°-284°C. were finally recovered. These crystals gave a deep blue color on treatment with 10% potassium hydroxide solution, which indicated the DNPH of a dicarbonyl (12). *Anal.* calcd. for di-DNPH of hexene-3-dial-1,6, $C_{18}H_{16}N_8O_8$: C, 45.75; H, 3.41; N, 23.72. Found: C, 45.0; H, 3.48; N, 23.4.

The absorption spectrum of the dihydrazones of dicarbonyls has been shown to differ significantly from that of the monohydrazone (9) and the absorption spectrum of the present preparation, as given in Figure 3, is characteristic of a dicarbonyl dihydrazone.

A schematic summary of the fractionation, isolation, and characterization data is given in Table I.

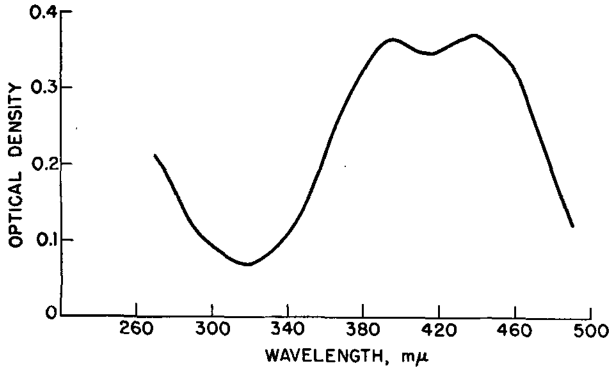

FIG. 3. Absorption spectrum of the dihydrazone of the dicarbonyl in dioxane.

Discussion

Volatile aldehydes isolated from autoxidizing methyl linolenate differ from those isolated from soybean oil autoxidized under the same conditions in the absence of hexanal and in the presence of appreciable amounts of a C_6 dial. It may therefore be assumed that acetaldehyde, propionaldehyde, and α-pentenal isolated from autoxidizing soybean oil could have arisen from linolenic acid.

The formation of acetaldehyde, propionaldehyde, crotonaldehyde, and α-pentenal can be rationalized according to present concepts of the mechanism of oxidation (3, 7). Rationalization of the occurrence of a C_6 dial can be made by postulating the decomposition of a 10,15-dihydroperoxide to give hexene-3-dial-1,6. Ultimate analysis, absorption spectrum, and color reactions are compatible with this postulate.

It seems surprising at the outset that the linolenate radical which comprises 6 to 9% of soybean oil should be the precursor of the principal aldehydes isolated. However studies on the isolated compounds have shown that linolenate oxidizes 1½ to 2 times faster than linoleate and 16 to 25 times faster than oleate (6, 11). Moreover recent studies have shown a marked difference in the course of linolenate oxidation compared to that of oleate or linoleate. Whereas monohydroperoxides of the latter two radicals are relatively stable, polymerization and scission occur immediately upon oxidation of linolenate (4, 5).

Hexanal was isolated from soybean oil but not from methyl linolenate. The presence of hexanal in reverted soybean oil can best be rationalized by considering oxidative reactions of linoleic acid. Hexanal has, in fact, been isolated from autoxidized cottonseed oil (13).

The relationship of the isolated aldehydes to the reversion flavors of soybean oil are the subject of present investigations.

Summary

Volatile cleavage products of autoxidizing methyl linolenate have chromatographically separated and the aldehydes have been isolated as 2,4-dinitrophenylhydrazones. Acetaldehyde, propionaldehyde, and α-pentenal have been identified. A six-carbon atom dialdehyde has been isolated as its hydrazone and is postulated to be hexene-3-dial-1,6.

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CORRECTION

In reference to the article (pp. 582-586) in the November 1952 issue of the Journal entitled "Detergency Evaluation" by Jay C. Harris, the Atlas Electric Devices Company of Chicago, Ill., has asked to have the correct spelling of Launder-Ometer published and some information provided on the size of the jars for the machine. The standard size handles one-pint glass jars or the new metal containers, which are 3½ in. by 8 in. with a capacity of 2½ pints. The company also manufactures a large Launder-Ometer which handles one pint, one quart, or two-quart glass jars or the new metal containers. A few larger metal containers have been made for this model.