The Aldehydes Produced During the Autoxidation of Cottonseed Oil[1]

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RECENTLY Δ^2-undecenal was shown to be a decomposition product of methyl hydroperoxido oleate (1). Assuming that methyl hydroperoxido oleate is a prototype of the hydroperoxides formed when fats oxidize, Δ^2-unsaturated aldehydes, such as Δ^2-undecenal and its analogs and homologs, should be among the constituents of oxidizing fats systems. The present report represents an extension of previously reported work and is concerned especially with the validity of the above-mentioned premise and the identity of the aldehydes produced during the autoxidation of cottonseed oil. The results reported here indicate that during the oxidation of this oil the unsaturated and saturated aldehydes, $\Delta^{2:4}$-decadienal, Δ^2-octenal, and hexanal, are produced.

EXPERIMENTAL

Preparation and Fractionation of Semicarbazones of Carbonyl Compounds Produced During the Oxidation of Cottonseed Oil

Ten kg. of refined cottonseed oil was heated at *ca.* 70°C., and aerated by bubbling air through it until the oil attained a peroxide value of approximately 250, after which it was heated to 100°C. and steam distilled to decompose the peroxides and to separate volatile carbonyl compounds. The peroxide value was reduced to *ca.* 120 during the steam distillation. About 3 l. of steam distillate was collected in a receiver containing 5 g. of semicarbazide hydrochloride and 7.5 g. of sodium acetate. The mixture in the receiver was allowed to stand overnight, whereupon 1.2 g. of semicarbazones crystallized and was separated by filtration. Another fraction consisting of 1.0 g. of semicarbazones was extracted from the filtrate with chloroform.

A 10-kg. batch of cottonseed oil was alternately oxidized and steam distilled from two to five times. Yields of the corresponding crystallized and extracted fractions (Fractions 1 and 2, respectively) were combined. The characteristics of these fractions are shown in Table I. The above-described method was modified during the preparation of Fraction 1Ay, which will be discussed later. During the preparation of the latter fraction an atmosphere of carbon dioxide was maintained in the steam distillation apparatus.

Fraction 1 was separated into several further fractions by crystallization from ethanol as follows: 6.5 g. of the mixture was dissolved in 130 ml. of ethanol. After the solution had been allowed to stand for 30 minutes, it was filtered and a fraction consisting of 1.6 g. of crystalline semicarbazones (Fraction 1A) was separated. The filtrate was allowed to stand for several hours at room temperature and a second fraction, consisting of 2.1 g. of semicarbazones, was separated. Water (100 ml.) was added to the filtrate, and the solution was maintained overnight at 0°C., whereupon 2.0 g. of semicarbazones (Fraction 1B) separated. The melting points, spectral absorption, and composition of Fractions 1A and 1B are shown in Table I.

Identification of Aldehydes from the Fractionated Semicarbazones

Fractionation of the semicarbazones produced appreciable separation as indicated by the absorption spectra and compositional data for Fractions 1A, 1B, and 2 (Table 1). The differences in the absorption spectra and composition reflect the differences in structures and chain lengths, respectively, of the components of these fractions. As will be shown later the structure of the principal aldehydic component of each fraction of semicarbazones was determined on the basis of its absorption spectrum and that of its semicarbazone, and chain length was determined by conversion to and identification of the corresponding *n*-aliphatic-2,4-dinitrophenylhydrazone.

$\Delta^{2:4}$-*Decadienal, Component of Fraction 1A:* Two crystallizations of Fraction 1A (1.5 g.) from ethanol yielded 0.2 g. of a product (Fraction 1Ax) which had the properties shown in Table I. To supplement

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TABLE I

Characteristics of Fractions of Semicarbazones

Fraction No.	M.P. °C.	Spectral absorption, extinction coefficients, $E^{g./l.}_{1cm.}$, at maxima				Composition					
						Found			Calculated		
		Maximum mμ	$E^{g./l.}_{1cm.}$	Maximum mμ	$E^{g./l.}_{1cm.}$	Carbon %	Hydrogen %	Nitrogen %	Carbon %	Hydrogen %	Nitrogen %
1	148-152	265	117.9	290-291	49.4[1]	60.4	9.2	21.6	......	......	......
1A	160-162	266	119.1	293-294	86.1	61.3	9.1	20.6	......	......	......
1Ax	178-179	....	....	293-294	162.7	61.9	9.1	20.4	......	......	......
1Ay	181-183	....	....	294-295	198.5	62.6	9.0	20.3	63.1[2]	9.1	20.1
1B	156-160	264-265	147.0	....	....	59.1	9.0	22.5	59.0[3]	9.3	23.0
2	85-100	229-230	71.0	....	....	54.1	9.4	26.0	54.1[4]	9.6	26.8

[1] Shoulder of absorption maximum.
[2] Calculated for semicarbazone of $\Delta^{2:4}$-decadienal.
[3] Calculated for semicarbazone of Δ^2-octenal.
[4] Calculated for semicarbazone of hexanal.

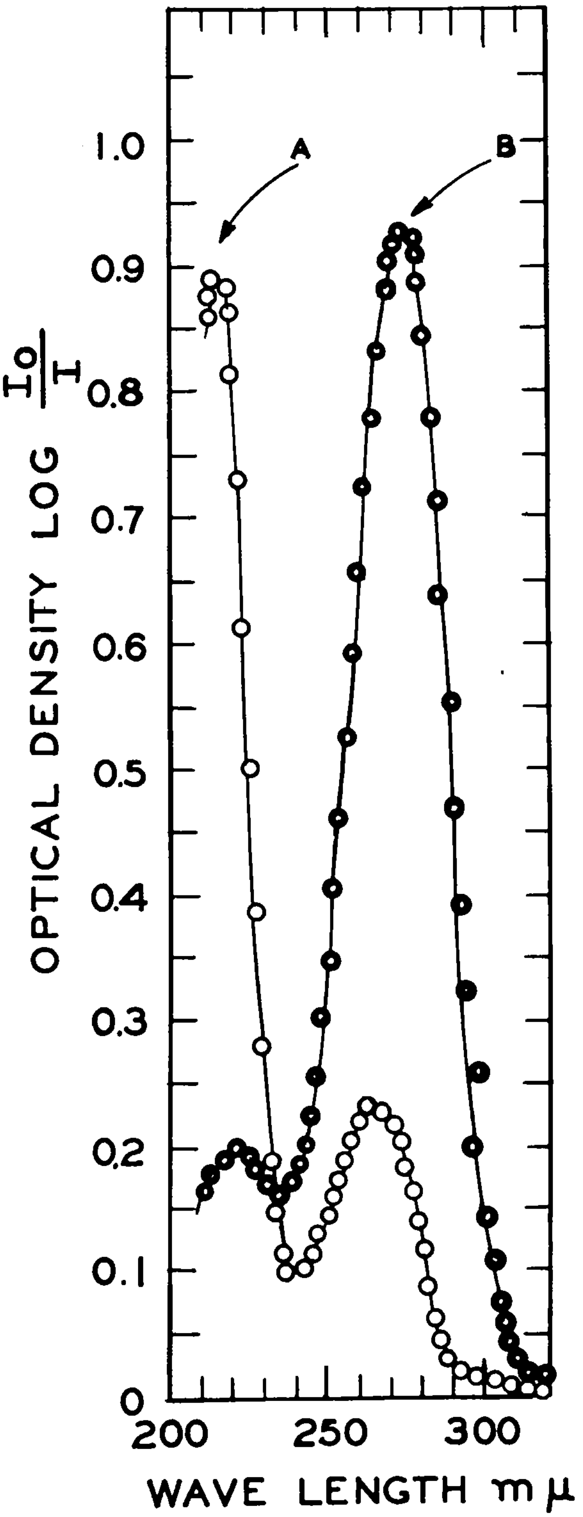


FIG. 1. Ultraviolet spectral absorption of aldehydes. Curves A and B represent hydrolysis products of Fractions 1B (Δ^2-octenal) and 1Ay ($\Delta^{2:4}$-decadienal).

the small amount of Fraction 1Ax which was available, an additional, corresponding fraction (Fraction 1Ay) was prepared. During the preparation of Fraction 1Ay an atmosphere of carbon dioxide was used to protect the carbonyl compounds, especially during the initial steam distillation.

As shown in Table I, the absorption spectra of Fractions 1Ax and 1Ay exhibited maxima at 293-294 mμ and 294-295 mμ and extinction coefficients, $E^{g./l.}_{1cm.}$ at these maxima of 162.7 and 198.5, respectively. Apparently, the preparation of Fraction 1Ay under an atmosphere of carbon dioxide yielded a product of higher purity. As indicated in Table II, Fraction 1Ay had an absorption spectrum corresponding to that of a semicarbazone of a $\Delta^{2:4}$-dienal (or a dienone with an equivalent triene structure), based on the data of Evans and Gillam (2) whose results indicate that these compounds exhibit absorption at 301 ± 8 mμ.

A portion (0.1 g.) of Fraction 1Ay was hydrolyzed in 4% hydrochloric acid. The spectrum of the regenerated aldehyde (Figure 1) exhibited a maximum at 273-274 mμ and an extinction coefficient, $E^{g./l.}_{1cm.}$ (273-274 mμ) = 170.6. As shown in Table II, the absorption of the regenerated aldehyde corresponds to the absorption of a $\Delta^{2:4}$-dienal, likewise based on the data of Evans and Gillam (2) whose results indicate that absorption at *ca.* 270 mμ is characteristic of $\Delta^{2:4}$-dienals (such as $\Delta^{2:4}$-hexadienal).

A portion (0.15 g.) of Fraction 1Ay was hydrogenated in *ca.* 20 ml. of ethanol using palladium (0.1 g.) as catalyst. The catalyst was separated by filtration, and the filtrate was heated to boiling with sufficient 2,4-dinitrophenylhydrazine to produce a saturated solution. Two drops of concentrated hydrochloric acid was added, and the solution was boiled for *ca.* 2 minutes. The solution was allowed to cool whereupon a yellow precipitate of 2,4-dinitrophenylhydrazone separated. After two crystallizations from ethanol the precipitate was analyzed. As shown in Table III the composition corresponds to the 2,4-dinitrophenylhydrazone of decanal, $C_{16}H_{24}N_4O_4$. It melted at 101.0-101.5°C. and a mixture of the product with an authentic specimen of the 2,4-dinitrophenylhydrazone of decanal melted at 101.5-104.0°C.

Δ^2-Octenal, a Component of Fraction 1B: As shown in Table I and Figure 2, the absorption spectrum of Fraction 1B exhibited a maximum at 264-265 mμ and an extinction coefficient, $E^{g./l.}_{1cm.}$ (264-265 mμ)= 147.0. The absorption of Fraction 1B at 264-265 mμ, as indicated in Table II, corresponds to the absorption of the semicarbazones of Δ^2-enals or -enones having an equivalent diene structure which according to the data of Evans and Gillam (4) exhibit characteristic absorption at 267 ± 7.5 mμ.

A portion of Fraction 1B was hydrolyzed in 4% hydrochloric acid and the regenerated aldehyde was recovered. The absorption spectrum of the aldehyde exhibited a maximum at 216-217 mμ (Figure 1) which is in the region, 220 ± 5 mμ, of the absorption of Δ^2-enals (4). A comparison of the positions of the maxima of these aldehydes is shown in Table II.

A portion (0.3 g.) of Fraction 1B was dissolved in 20 ml. of ethanol and hydrogenated with palladium (0.2 g.) as catalyst. The catalyst was removed, and the hydrogenated semicarbazone was treated with 2,4-dinitrophenylhydrazine as described in the case of Fraction 1Ay. After several recrystallizations the derived product melted at 99°-101°C. It was identified as the 2,4-dinitrophenylhydrazone of octanal on the basis of its composition which corresponded to $C_{14}H_{20}N_4O_4$. When mixed with an authentic specimen of the 2,4-dinitrophenylhydrazone

TABLE II

Spectral Absorption of Semicarbazones and Regenerated Aldehydes

Product	Spectral absorption at position of maximum		Type of aldehyde based on spectral absorption		
	Maximum mμ	$E^{g./l.}_{1cm.}$	Compound	Position of maximum, mμ	Reference
Fraction 1Ay	294-295	198.5	Semicarbazone of $\Delta^{2:4}$-dienal	301 ± 8	(2)
Aldehyde regenerated from Fraction 1Ay	273-274	170.6	$\Delta^{2:4}$-Dienal	*ca.* 270	(2)
Fraction 1B	264-265	147.0	Semicarbazone of Δ^{2}-enal	267 ± 7.5	(4)
Aldehyde regenerated from Fraction 1B	216-217	......	Δ^{2}-Enal	220 ± 5	(4)
Fraction 2	229-230	71.0	Semicarbazone of hexanal[1]	229-230	......

[1] Spectrum of an authentic specimen of hexanal semicarbazone.

of octanal (m.p. 106°C.) the mixture melted at 104°-106°C. These data are shown in Table III.

Hexanal, a Component of Fraction 2: The data shown in Table I indicates that Fraction 2 had a composition which corresponded to $C_7H_{15}ON_3$ and an absorption spectrum (Figure 2) which exhibited a maximum at 229-230 mμ and an extinction coefficient, $E^{g./l.}_{1cm.}$ (229-230 mμ) = 71.0. This spectrum, as indicated in Table II, corresponded to the spectrum of an authentic specimen of the semicarbazone of hexanal which exhibited a maximum at 229 mμ and an extinction coefficient, $E^{g./l.}_{1cm.}$ (229 mμ) = 78.0.

A solution of 0.5 g. of Fraction 2 in ethanol was treated with 2,4-dinitrophenylhydrazine as previously described in the case of Fraction 1Ay. After several recrystallizations the reaction product melted at 102°-103°C. and was identified as the 2,4-dinitrophenylhydrazone of hexanal on the basis of the correspondence of its composition with $C_{12}H_{16}N_4O_4$. Mixed with an authentic specimen of the 2,4-dinitrophenylhydrazone of hexanal it melted at 102°-104°C. These data are shown in Table III.

Discussion

In relating present and previous results reported in this series of communications to the oxidation of fats, it is recognized that the nature of the oxidation products which are formed depends to some extent on the method of oxidation. Previous investigators (5) have identified a series of oxidation products from oxidized fats consisting of hexanal and a number of other aliphatic aldehydes to which the odors and flavors of rancid fats have often been attributed. In the present investigation several unsaturated, but only one saturated aldehyde, namely, hexanal, appeared to be present in oxidized cottonseed oil. The fact that a relatively simple mixture of aldehydes was obtained may be attributed to the fact that they were isolated before they had undergone fission, further oxidation, polymerization, or condensation which might occur under other experimental conditions or during long periods of storage.

Origin of $\Delta^{2:4}$-Decadienal, Δ^{2}-Octenal, and Hexanal: Linoleic acid is the principal fatty acid constituent of cottonseed oil as well as the most readily oxidized component of the oil. Oxidized linoleic acid, or specifically its hydroperoxides, represent the probable precursors of the three aldehydes which have been identified. Bolland (6) and Bergström (7) have reported that the hydroperoxides of methyl linoleate comprise a mixture containing a large proportion of the methyl esters of $\Delta^{10:12}$-9-hydroperoxido (I) and $\Delta^{9:11}$-13-hydroperoxido (II) octadecadienoic acids. In addition, Bolland has proposed that the methyl ester of $\Delta^{9:12}$-11-hydroperoxido octadecadienoic acid (III) is one of the hydroperoxides of methyl linoleate, but this was not confirmed in Bergström's investigation.

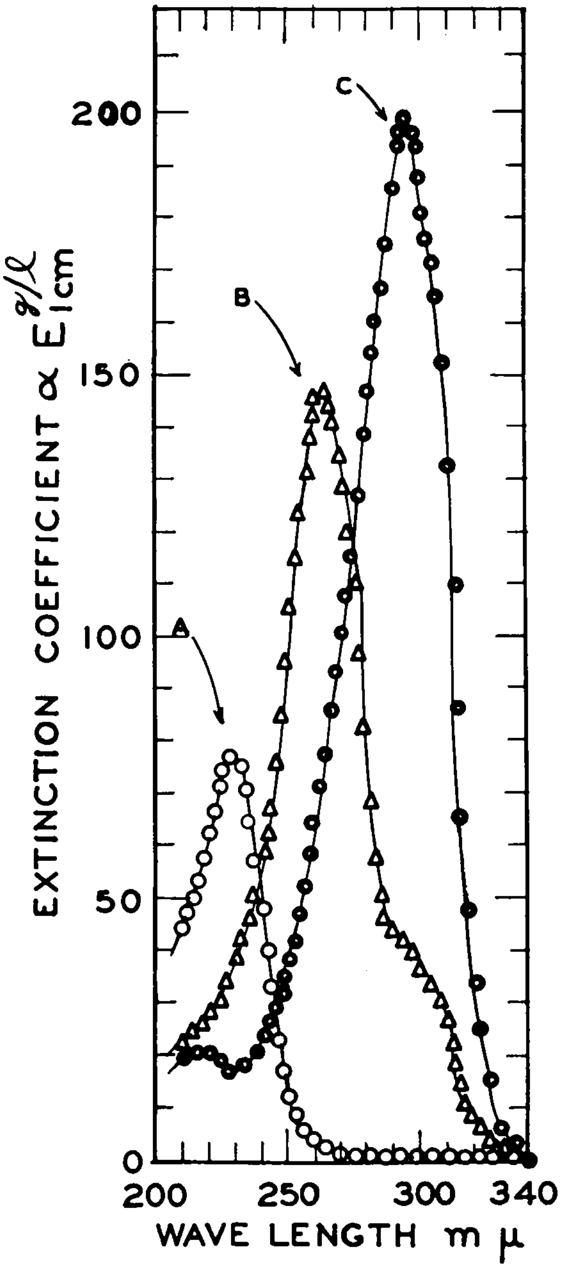


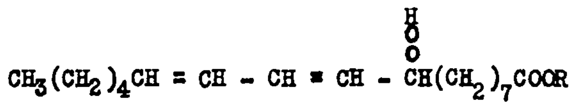
Fig. 2. Ultraviolet spectral absorption of semicarbazone fractions. Curves A, B, and C represent Fractions No. 2 (hexanal), 1B (Δ^{2}-octenal), and 1Ay ($\Delta^{2:4}$-decadienal).

TABLE III

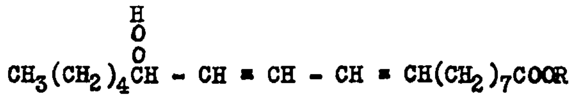
Identification of 2,4-Dinitrophenylhydrazones Prepared from Semicarbazones

Semicarbazone		Procedure of identification	Characteristics of 2,4-dinitrophenylhydrazones of saturated aldehydes								
			M. P. °C.	M. P.[1] authentic specimen	Mixed m. p. °C.	Composition					
						Found			Calculated		
Fraction No.	Aldehyde component identified					Carbon %	Hydrogen %	Nitrogen %	Carbon %	Hydrogen %	Nitrogen %
1 Ay	$\Delta^{2:4}$-Decadienal	Semicarbazone hydrogenated, converted to 2,4-dinitrophenylhydrazone of decanal	101.0-101.5	103.5-104.0	101.5-104.0	57.0	7.1	16.6	57.1	7.0	16.7
1B	Δ^{2}-Octenal	Semicarbazone hydrogenated, converted to 2,4-dinitrophenylhydrazone of octanal	99-101	106	104-106	54.9	6.5	18.0	54.6	6.6	18.2
2	Hexanal	Semicarbazone converted to 2,4-dinitrophenylhydrazone of hexanal	102-103	104	102-104	51.8	5.7	20.1	51.4	5.7	20.0

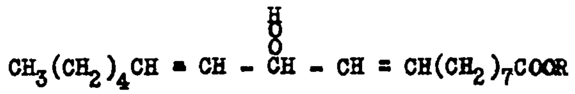
[1] Melting points of 2,4-dinitrophenylhydrazones of decanal, octanal, and hexanal reported to be 104°C., 106°C., and 104°C., respectively (3).

I. 

$\mathrm{CH_3(CH_2)_4CH=CH-CH=CH-\overset{\overset{\overset{H}{O}}{O}}{C}H(CH_2)_7COOR}$

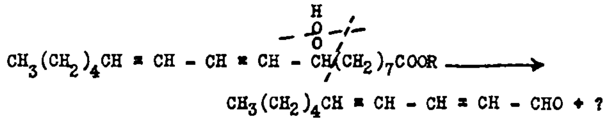
II. 

$\mathrm{CH_3(CH_2)_4\overset{\overset{\overset{H}{O}}{O}}{C}H-CH=CH-CH=CH(CH_2)_7COOR}$

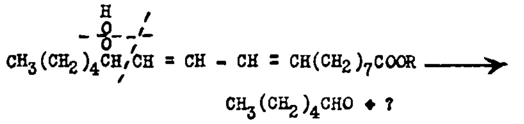
III. 

$\mathrm{CH_3(CH_2)_4CH=CH-\overset{\overset{\overset{H}{O}}{O}}{C}H-CH=CH(CH_2)_7COOR}$

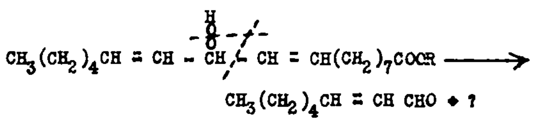
Assuming fission of hydroperoxide isomers I, II, and III occurred as shown in equations A, B, and C, respectively, $\Delta^{2:4}$-decadienal, hexanal, and Δ^{2}-octenal would be produced.

A. 

$\mathrm{CH_3(CH_2)_4CH=CH-CH=CH-\overset{\overset{\overset{H}{O}}{O}}{C}H(CH_2)_7COOR \longrightarrow CH_3(CH_2)_4CH=CH-CH=CH-CHO + ?}$

B. 

$\mathrm{CH_3(CH_2)_4\overset{\overset{\overset{H}{O}}{O}}{C}H\,CH=CH-CH=CH(CH_2)_7COOR \longrightarrow CH_3(CH_2)_4CHO + ?}$

C. 

$\mathrm{CH_3(CH_2)_4CH=CH-\overset{\overset{\overset{H}{O}}{O}}{C}H\,CH=CH(CH_2)_7COOR \longrightarrow CH_3(CH_2)_4CH=CH\,CHO + ?}$

While the above reactions cannot be exactly represented stoichiometrically, they appear to offer a reasonable explanation of the present results. A similar decomposition reaction was postulated by Swift *et al.* (1) to explain the production of Δ^{2}-undecenal during the decomposition of methyl hydroperoxido oleate. Martin *et al.* (8) recently advanced evidence indicating that Δ^{2}-heptenal is a constituent of reverted soybean oil. The peroxide decomposition reactions postulated afford an explanation of the origin of this aldehyde as a decomposition product of the hydroperoxides of linolenic acid, only if extreme mobility of double bonds is assumed.

Absorption Spectra of Oxidized Fats: A number of investigators (6-11) have reported considerable data relative to the development of absorption bands in the ultraviolet spectral region in autoxidizing fat systems. In several instances absorption in the region *ca.* 275 mμ has been reported and Holman *et al.* have examined the spectra of a number of compounds which might be considered as being responsible for this absorption band. However, none of the compounds examined corresponded in absorption characteristics to the compounds in autoxidizing fat systems (9, 12, 13). Bolland (6) and Bergström (7) attributed the observed absorption at 275 mμ to the presence of a conjugated diene ketone. In view of the isolation from autoxidized cottonseed oil of $\Delta^{2:4}$-decadienal which exhibits absorption at 273-274 mμ it appears that this compound may account for the absorption maxima observed by various investigators.

Summary

Spectrophotometric evidence was obtained that Δ^{2}- and $\Delta^{2:4}$-unsaturated aldehydes are produced during the autoxidation of cottonseed oil.

The unsaturated carbonyls were converted into semicarbazones and 2,4-dinitrophenylhydrazones whose characteristics indicated that the oxidation of cottonseed oil produces $\Delta^{2:4}$-decadienal, Δ^{2}-octenal, and hexanal.

Although the exact mechanism of their formation has not been established, these aldehydes are believed to result from the decomposition of the isomeric hydroperoxides of linoleic acid, the principal fatty acid constituent of cottonseed oil.

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