Characterization of Some Dicarbonyls from Autoxidized Methyl Linoleate[1]

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Abstract

The 2, 4-dinitrophenylosazones of dicarbonyl compounds isolated from oxidized methyl linoleate were resolved by means of adsorption and partition chromatography. Individual components were characterized by means of ultraviolet absorption, thin layer chromatographic techniques for class determination and for separation of homologous series, column co-chromatography of authentics and the unknowns, IR spectroscopy and melting point analysis. Evidence was obtained for the presence of glyoxal, methyl glyoxal, but-2-en-1, 4-dial, α-keto hexanal, α-keto heptanal, α-keto octanal, and α-keto nonanal.

Introduction

EVEN THOUGH dicarbonyl compounds long have been recognized as lipid oxidation products, information on the types present in lipid systems is very limited. The dicarbonyl most often discussed is malonaldehyde, which is reportedly responsible for the red pigment in the 2-thiobarbituric acid-oxidized lipid reaction (1,2). Evidence for other dicarbonyls has appeared in the literature: α-ketooctanal (3), but-2-en-1,4-dial (4), and hex-3-en-1, 6-dial (5). Recently Lillard and Day (6) reported a series of mono- and dicarbonyls resulting from the oxidation of individual unsaturated monocarbonyl compounds.

Lack of suitable procedures has hindered progress in the elucidation of the composition of dicarbonyl mixtures. However, recent developments in column (7,9) and thin-layer chromatography (TLC) (10,11) enable at least a partial separation of the 2,4-dinitrophenylsazones and provide new insight into the types of compounds produced in oxidized lipid systems. The purpose of this investigation was to employ these methods to partially characterize the dicarbonyl products of oxidized methyl linoleate.

Experimental

Methyl linoleate was obtained from the Hormel Institute, Austin, Minnesota, and was used without further purification. Gas-liquid chromatography (GLC) indicated that the ester contained a trace of linoleate. The ester, 3.72 g, was oxidized as described previously (6) and oxidation was terminated at an oxygen uptake of 0.45 mole/mole of ester. The peroxide value (12), 2-thiobarbituric acid number (TBA) (13) and free monocarbonyls (14) were immediately determined on the oxidized ester, and the values were calculated as mmoles per mole of ester.

In an effort to obtain a more complete picture of the dicarbonyl products, both the volatile and nonvolatile fractions were studied. The schematic diagram illustrating manipulation of these two fractions is shown in Figure 1. The fractions are denoted as distillate and pot residue. The distillate fraction was obtained by vacuum steam stripping the ester with the apparatus described by Day and Lillard (15), except the size of containers was reduced to accommodate the sample. Approximately 100 ml of distillate was collected. The pot residue remaining after distillation was analyzed for peroxides and TBA number.

The distillate was reacted 12 hr with 2,4-dinitrophenylhydrazine, DNPH (16), and the mixture was then refluxed 6 hr to facilitate more complete conversion of the mono-DNP-hydrazones to the *bis* derivatives. Water was added to the cooled mixture and the DNP-osazones were isolated by chloroform extraction. Following removal of the chloroform in vacuo, the remaining residue was dissolved in benzene-methanol (1:1) and passed over a column of Dowex 50 cation exchange resin for removal of excess DNP-hydrazine (17).

The benzene-methanol solvent was removed under reduced pressure and the residue was dissolved in ethylene chloride and subsequently chromatographed on magnesia-Celite columns (18). The DNP-hydrazones passed through these columns with the ethylene chloride solvent, while the DNP-osazones were adsorbed to the top of the packing. The DNP-osazones were recovered by extruding the column packing, cutting out the blue band, treating it with cold 3N HCl and extracting with chloroform. After the chloroform extract was dried over calcium chloride, the chloroform was removed in vacuo to yield a dry DNP-osazone residue.

The pot residue, remaining after distillation, was reacted with DNPH via the alumina reaction column of Keith and Day (14). In their procedure the DNP-hydrazones of monocarbonyls, as well as nonpolar material, are eluted with benzene, leaving the DNP-hydrazones of dicarbonyls adsorbed to the column. In this study, these latter compounds were subsequently eluted with acetic acid-chloroform (3:2). The acetic acid-chloroform effluent was refluxed with an excess of DNPH to convert dicarbonyl hydrazones to *bis* derivatives. Thereafter, water was added to the flask contents and the osazones were extracted into chloroform.

The DNP-osazone mixtures from both the distillate and pot residue were fractionated according to the column partition method of Schwartz (8). The water content of the stationary phase was varied to facilitate derivative movement. In initial separations the DNP-osazones were chromatographed on columns consisting of Celite-ethanolamine-water in a ratio of 15:7:3. The longer chain derivatives rapidly moved down these columns as a yellow band, while the osazones of glyoxal, methyl glyoxal, and but-2-en-1, 4-dial formed blue bands with measurable retention volumes. The longer chain dicarbonyls contained in the yellow band of the initial separation were then fractionated on columns made up of a ratio of 15:7:0.5, respectively, of the aforementioned constituents. Since several columns were necessary for resolution of the total quantity of derivatives, individual fractions were pooled and later rechromatographed to insure purity.

Following purification by column partition chromatography, each fraction was made to a known volume in chloroform and the absorbance at the lambda maximum was taken for quantitative calculations. Thereafter, several procedures were employed in conjunction with one another to charac-

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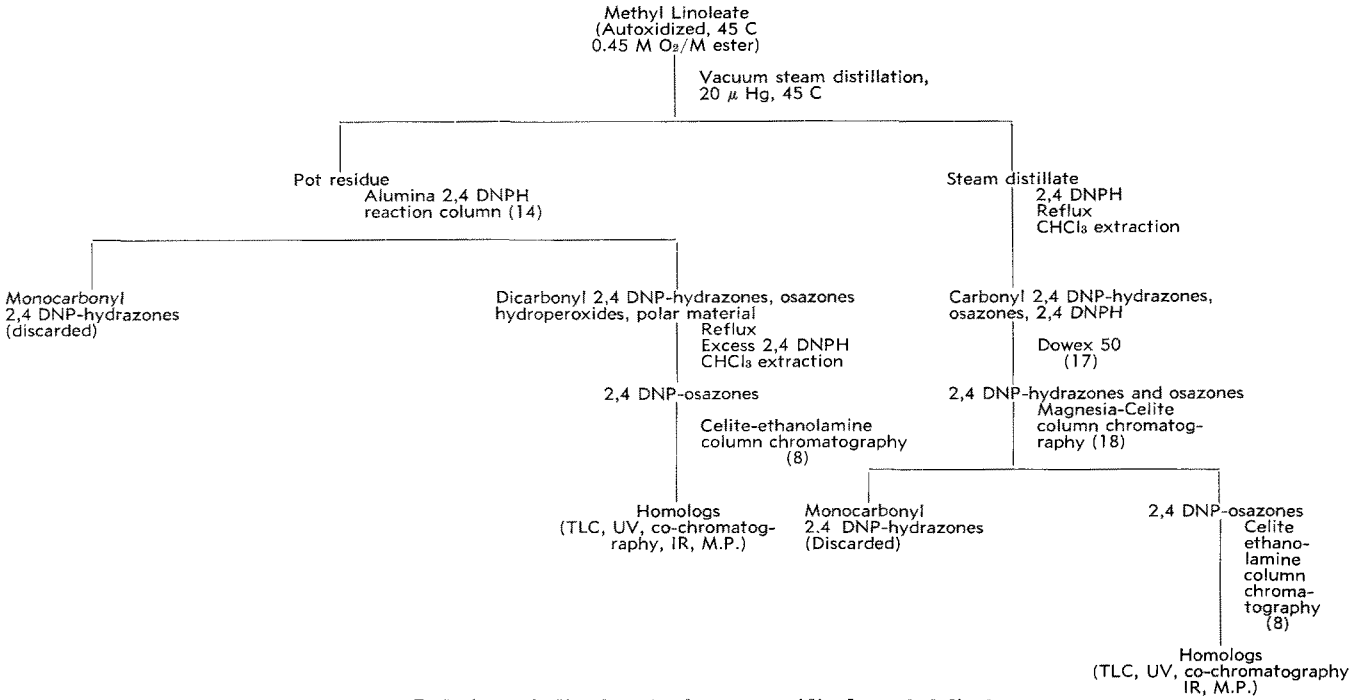

FIG. 1. Isolation of dicarbonyls from autoxidized methyl linoleate.

terize the derivatives. These included thin-layer chromatography (TLC) for determining the dicarbonyl class (10) and the carbon chain length of individual homologs (11), co-chromatography of authentic derivatives with unknowns on partition columns (8), determination of the ultraviolet absorption maxima of alcoholic-KOH solutions of the derivative (19), and, when quantities permitted, IR spectra and melting points.

Results and Discussion

The data in Table I give some indication of the oxidative state of methyl linoleate when the oxidation was terminated. For all practical purposes, hydroperoxide decomposition effected by the distillation treatment was insignificant. Hence, the dicarbonyls obtained in the distillate existed as free compounds in the oxidized ester.

Qualitative and quantitative data for the dicarbonyls in the two fractions are presented in Table II. Inadequate quantities of certain DNP-osazones limited the qualitative analysis to those listed in Table II. The DNP-osazones designated as F_1 and F_2 were among the more abundant fractions and their behavior in several chromatographic systems was studied. According to ultraviolet absorption spectra and behavior on liquid-liquid partition chromatography columns, fraction F_1 resembled α-ketopentanal and F_2 behaved like α-ketononanal. These data could not be confirmed by the TLC method for class separation (10) nor by the TLC method for separation of an homologous series (11). It would appear that these two derivatives represent unconjugated unsaturated vicinal dicarbonyls. Compounds of this type with isolated double bonds would behave like their saturated analogs under certain conditions. This phenomenon also has been observed in work with monocarbonyls (20).

The qualitative differences between the distillate and pot residue fractions may be partially attributed to the inefficiency of the distillation procedure. However, the additional compounds observed in the pot residue may result from decomposition of more stable hydroperoxides. The quantitative data are considered approximate and reflect the relative amounts of the various compounds present.

Mechanisms for dicarbonyl formation in oxidizing lipid systems are speculative at this point. Lillard and Day (6) have shown that they can result from the oxidation of alk-2-enals and alk-2, 4-dienals. Theo-

TABLE I
Properties of Autoxidized Methyl Linoleate [a]

	Oxidized ester	Oxidized ester minus distillate
Peroxide value	209	205
Malonaldehyde [b]	1.72	1.39
Monocarbonyls		
Alkanals	13.9	
Alk-2-enals	0.339	
Alk-2,4-dienals	1.87	

[a] Values expressed as mmoles/mole ester.
[b] Calculated from the TBA value.

TABLE II
Characterization of 2,4-Dinitrophenylosazones from Oxidized Methyl Linoleate

Compound	Ultraviolet maxima: Authentic	Ultraviolet maxima: Unknown	Methods of characterization	Concentration mmoles/mole ester
Distillate Fraction				
Glyoxal	390,435	389,435	A,C,D	4.76×10^{-4}
F_1		395,435	A,C,D	3.87×10^{-3}
α-keto C_6	395,438	393,433	A,C,D	1.23×10^{-2}
α-keto C_7	395,438	393,433	A,C,D,G	.335
α-keto C_8	395,438	393,433	A,C,D,F	.101
Pot Residue				
Glyoxal	390,435	390,435	A,B,C,D,F	[a]
Methyl Glyoxal	390,435	388,433	A,C,D	2.54×10^{-4}
But-2-en-1,4 dial	405,446	403,445	A,B,C,D	4.28×10^{-4}
F_1		395,435	A,C,D,E	6.34×10^{-3}
α-keto C_6	395,438	395,437	A,C,D,E,F	4.60×10^{-2}
α-keto C_7	395,438	395,437	A,C,D,E,F,G	0.278
α-keto C_8	395,438	395,437	A,C,D,E	3.72×10^{-2}
F_2		396,436	A,C,D,E,F	0.264
α-keto C_9	395,438	395,437	A,D,E	6.15×10^{-3} [b]

A–Ultraviolet maximum, $CHCl_3$
B–Ultraviolet maximum, Ethanolic KOH (19)
C–Schwartz column co-chromatography (8)
D–Thin layer class chromatography (10)
E–Thin layer homolog chromatography (11)
F–IR spectrometry
G–Melting point
[a] Glyoxal not quantitated.
[b] Mixture of α-keto nonanal and another ketoalkanal which did not separate with column chromatography (8,9), but was resolved with TLC homolog chromatography (11).

retically, the unsaturated monocarbonyls resulting as secondary degradation products from linoleate hydroperoxides; i.e., deca-2,4-dienal and oct-2-enal, could upon subsequent oxidation account for α-keto heptanal and α-keto octanal. However, according to the literature the presence of the 11-hydroperoxide of methyl linoleate is questionable; hence, the origin of α-keto heptanal and/or α-keto octanal from the further oxidation of oct-2-enal is doubtful. α-Keto heptanal, which was one of the more abundant dicarbonyls present, might also be produced by direct attack at the 12, 13 double bond of the linoleate molecule.

At the present time none of the observed dicarbonyls have been directly associated with flavor defects in oxidizing lipid systems. It is evident, however, that these compounds could assume a significant role in food systems. Dicarbonyls can serve as key reactants in the Strecker degradation of amino acids as well as in nonenzymatic browning reactions, both of which are important deterioration mechanisms in food materials.

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Methods for the Determination of Cyclopropenoid Fatty Acids V. A Spectrophotometric Method for Cottonseed Oils Based Upon the Halphen-Test Reaction

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Abstract

A spectrophotometric method of analysis for the quantitative estimation of cyclopropenoid fatty acids in cottonseed oil based upon the Halphen-test reaction has been described. Various parameters involved in the reaction have been investigated and two pigment fractions responsible for the characteristic Halphen-test cherry-red color have been isolated. The method is applicable to relatively small amts of sample material. The average deviation from the actual cyclopropenoid acid contents as determined by the stepwise HBr titration method was less than ± 0.02% in both the refined and crude oil series.

Introduction

In 1897 Halphen (1,2) found that cottonseed oil contains a minor constituent which produces a cherry-red color when the oil is heated with a mixture of amyl alcohol, carbon disulfide, and sulfur. This color test has recently been attributed to the presence of cyclopropenoid constituents, presumably malvalic and sterculic acid moieties (3–5).

Previous attempts (5–7) to develop an accurate quantitative method based upon this colorimetric test have not been entirely successful. In general, these methods have the advantage of requiring only a small amt of sample material. However, they lack precision since the color developed is a composite of several colored components, the relative proportions of which are dependent upon a number of hard-to-control parameters. This results in a variability in color response from test to test on the same sample. In addition, no authentically pure standard substance has been available which is sufficiently stable for calibration purposes, i.e., to establish a reliable, accurate relationship between the cyclopropenoid acid concn and the color intensity. A recently developed method of analysis (8) based upon a stepwise HBr titration now makes it possible to determine the cyclopropenoid acid content of cottonseed oils to within 0.01%. This titration method affords a means of setting up reliable calibration standards.

There is still a need for a method of analysis applicable to very small samples. Such a method would be advantageous, for example, in the development of a procedure for the cyclopropenoid analysis of cottonseed meals since very large amts of meal would be necessary for the isolation of relatively small amts of the fatty acid constituents.

The purpose of the present investigation was to study the various parameters involved in the Halphen-test reaction in order (*a*) to establish a set of reaction conditions under which the intensity of the color obtained for cottonseed oils by the Halphen reaction could be reproduced to within reasonable limits and (*b*) to relate the intensity of the color, as determined spectrophotometrically, to the known cyclopropenoid acid content of a series of cottonseed oils.

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