Methyl 2,3-di-O-linseed acyl-α-D-glucopyranoside (XX) was prepared from XIX (10 g) following the procedure given for XIII. The water-white oil (6.1 g, 68.1%) had the following properties: I.V. Calc.: 141.5; I.V. Found: 135.9; N_D^{25} 1.4831; $[\alpha]_D^{25}$ + 58.5° (c 1, $CHCl_3$). Anal. Calc. for $C_{43}H_{74}O_8$: C, 71.72; H, 10.30. Found: C, 71.69; H, 10.26.

Methyl 2-O-linseed acyl-4,6-O-benzylidene-α-D-glucopyranoside (XXI) was prepared by the dropwise addition of linseed acid chloride (17 g) to I (17 g) in pyridine (50 ml). After washing and drying 20 g of the crude product were passed through an alumina column (12 × 4.4 cm) and then through a silicic acid column (12 × 4.4 cm). The second column was eluted with 4–100 ml portions of hexane and with 4–100 ml portions of each of the following: 0.5, 1.0, 1.5, 2, and 10% ethyl acetate in hexane. After TLC analysis the fractions containing the product (second spot from the top, Fig. 1) were combined. Removal of the solvent gave 11.8 g of water-white oil. I.V. Calc.: 93.8. I.V. Found: 90.4. $[\alpha]_D^{25}$ + 102.3° (c 1, $CHCl_3$). N_D^{25} 1.5035. Anal. Calc. for $C_{32}H_{48}O_7$: C, 70.59; H, 8.82. Found: C, 70.17; H, 9.14.

Methyl 2-O-linseed acyl-α-D-glucopyranoside (XXII). Compound XXI (3.2 g) was dissolved in acetone (50 ml) containing 0.1 *N* hydrochloric acid (10 ml) and refluxed for 2 hr. The solvent was removed in vacuo and the residue extracted with ether. The ether solution was washed with water and dried. After removal of the ether, the residue was dissolved in hexane and passed through a silicic acid column (4 × 1.9 cm). The column was eluted with hexane (25 ml), 10% ethyl acetate in hexane (25 ml), 25% ethyl acetate in hexane (25 ml), and finally with ethyl acetate. Removal of the ethyl acetate gave a viscous yellow oil. I.V. Calc.: 110.5. I.V. Found: 109.7. $[\alpha]_D^{25}$ + 83.1° (c 1, $CHCl_3$). Anal. Calc. for $C_{25}H_{44}O_7$: C, 65.79; H, 9.65. Found: C, 65.62; H, 9.65.

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Further Observations on the Dicarbonyl Compounds Formed Via Autoxidation of Methyl Linoleate[1]

W. Y. COBB[2] and E. A. DAY

Department of Food Science and Technology, Oregon State University, Corvallis

Abstract

α-Dicarbonyls isolated from oxidized methyl linoleate and conclusively identified as DNP-osazones[3] were glyoxal, methyl glyoxal, α-keto hexanal, α-keto heptanal, and α-keto octanal.

Introduction

IN A PREVIOUS PAPER (1) tentative evidence was presented for the presence of a series of dicarbonyls occurring in oxidized methyl linoleate. Due to the small quantities of derivative isolated, conclusive identification of the compounds was not possible. The purpose of this paper is to present evidence for the identification of the dicarbonyls.

Experimental

The volume of packing used in the column chromatographic procedures (2–4) was increased and 2 in. diameter columns were employed to accommodate the rather large quantities of sample. Following complete resolution of the crude DNP-osazone mixture, pooled fractions were rechromatographed on partition columns (4); the column size was determined by the quantity of derivative isolated. Carbonyl-free solvents (5) were used throughout the experiment for column development and extraction purposes.

Methyl linoleate (19.62 g) was oxidized under a stream of oxygen in the manner described previously (1). The oxidized ester was passed over alumina 2,4 DNP-hydrazine reaction columns (2) containing about 60 g of packing. The DNP-hydrazones of monocarbonyls and the unaltered ester were eluted with 500 ml of benzene. The amount of 300 ml of acetic acid-chloroform (3:2) was then percolated through the columns to remove all remaining material. The eluate from 20 columns was pooled and the chloroform was removed by evaporation. Excess DNP-hydrazine reagent was added and the mixture was refluxed for 16 hr. Water was added, and the cooled mixture was extracted with chloroform. This extract was chromatographed on magnesia adsorption columns (3), and the DNP-osazone fraction recovered (1) was rechromatographed on Celite-ethanolamine columns (4). Columns for resolution of more polar derivatives (e.g., glyoxal, methyl glyoxal) consisted of 30 g Celite, 14 ml ethanolamine, and 4.5 g water. For the derivatives of longer chain dicarbonyls, columns consisting of 60 g Celite, 28 ml ethanolamine, and 2.0 g water were employed. All the columns were developed until adequate resolution of bands was obtained. The column packing was then extruded, and the individual

[1] Technical Paper No. 2012, Oregon Agricultural Experiment Station.
[2] Present address: Department of Food Science, North Carolina State of the University of North Carolina, Raleigh.
[3] DNP = 2,4,-dinitrophenyl.

TABLE I
Properties of 2,4 DNP-osazones from Oxidized Methyl Linoleate

Compound	Unknown M.P. (°C corr.)	Authentic M.P.	Mixed M.P.	UV λ Max. (mμ) in CHCl3	UV λ Max. (mμ) in alcoholic base	Concentration mM/M ester	Corroborative evidence
Glyoxal	321 (d.)	325.5 (d.)	–	393,440	567	8.07×10^{-2}	TLC(c)[1]
Methyl Glyoxal	291.5–295.5 (d.)	297–300.5 (d.)	296–300 (d.)	394,435	559	4.62×10^{-3}	TLC(c,h) IR
F1				395,435	554	1.33×10^{-3}	TLC(c,h)
α-Keto hexanal	220–222	220–223	218–221	395,435	557	2.2×10^{-2}	TLC(c,h) IR
α-Keto heptanal	177–179	181–182.5	176–181	395,435	557	1.05×10^{-1}	TLC(c,h) IR
α-Keto octanal	161–163	161–161.5	160–162	395,435	559	3.86×10^{-2}	TLC(c,h) IR
F4A-B2	124–129			395,435	555	5.34×10^{-3}	TLC(c,h) IR
α-Keto nonanal				395,435			TLC(c,h)
But-2-en-1,4-dial				400,448			TLC(c)

[1] TLC indicates thin-layer chromatography; c, class of dicarbonyl (12); h, homolog chain length (13).
[2] IR indicates infrared spectrum.

bands were cut out and extracted from the packing with chloroform. Each fraction was washed with distilled water to remove ethanolamine. Similar fractions were pooled and rechromatographed.

The DNP-osazones were characterized spectroscopically and chromatographically as described previously (1). The authentic dicarbonyls used as standards for comparison were synthesized by the procedure of Riley et al. (7), converted to osazones under reflux with 2,4 DNP-hydrazine (6), and purified over silicic acid (8). In order to obtain sharp melting points on the DNP-osazones of dicarbonyls from linoleate, it was imperative that these derivatives also be passed over silicic acid columns as one of the final steps in their purification. Very carefully prepared benzene, which yielded no residue upon evaporation, was used as the solvent in this procedure. The dicarbonyl fraction was collected and the osazones were recrystallized from either benzene-hexane or chloroform-hexane until sharp melting points were obtained.

Results and Discussion

Following oxidation, the ester had a TBA value (9) of 247 and a peroxide value (10) of 227 indicating that approximately 0.6 mole oxygen/mole of ester had been absorbed.

Some difficulty was experienced in the partition column procedure (4) when attempting to remove the ethanolamine from the column effluent. Passage of the effluent through properly charged cation exchange resin effectively removed the amine but a small amount of contaminant was leached from the resin which interfered with melting point analyses. Subsequently it was found that the amine could be removed completely from both benzene effluents and chloroform extracts of the extruded column by washing four times with equal volumes of distilled water.

The properties of the derivatives isolated from oxidized methyl linoleate are presented in Table I. Conclusively identified on the basis of melting points, mixed melting points, chromatographic and spectrometric evidence were glyoxal, methyl glyoxal, α-keto hexanal, α-keto heptanal, and α-keto octanal.

A weak carbonyl absorption band at 1725 cm^{-1}, indicating free carbonyl, was present in the infrared spectra of nearly all DNP-osazones that had been chromatographed on silicic acid columns. A small amount of $-CH_2$ stretch at 2925 cm^{-1} in the infrared spectrum of the glyoxal fraction from linoleate could not be explained. The spectrum of authentic glyoxal exhibited no absorption in this region.

The ultraviolet-visible absorption spectra of authentic DNP-osazones of dicarbonyls with carbon chains longer than C_2 exhibited strong maxima at 395 mμ and secondary maxima at 435 mμ; the ratio of the two was about 1.15. In contrast, the DNP-osazone of authentic glyoxal gave the strongest maximum at 440 mμ and a secondary maximum at 390 mμ. The glyoxal fraction isolated from linoleate exhibited the proper absorption maxima, but the ratio of the two was similar to the longer chain DNP-osazones. It was noted, however, that the ultraviolet spectra of all isolated dicarbonyls had been determined prior to silicic acid chromatography. Although a recheck of all compounds following silicic acid chromatography showed no changes in absorption maxima, the ratio of the maxima in the glyoxal derivative had fallen to 0.98. This phenomenon, as well as the presence of some free carbonyl absorption in the infrared spectrum of the osazones, suggests that silicic acid chromatography may, in some manner, alter the derivatives. Silicic acid has previously been found to cause some decomposition of the hydrazone derivatives of monocarbonyl compounds (11).

When the α-keto octanal fraction from oxidized linoleate was chromatographed on silicic acid, two intensely colored bands formed on the column. The first band to elute corresponded in melting point with authentic α-keto octanal, while the second derivative (F4A-B2) exhibited a melting range of 124C–29C after three recrystallizations. This latter derivative behaved similarly to authentic α-keto octanal on thin-layer class and homolog plates, gave lambda maxima of 395 and 435 mμ in the UV-visible region, and a maximum of 555 mμ in alcoholic base. The infrared spectrum of this compound indicated no unsaturation in the aliphatic chain, but it exhibited some free carbonyl absorption. The behavior of this compound on silver nitrate-impregnated thin-layer plates was similar to the other saturated dicarbonyls isolated from linoleate.

The fraction referred to previously (1) as "F1" was again present in insufficient quantities to obtain a melting point. However, further characterization via chromatographic and spectroscopic analyses suggests that this compound may be α-keto pentanal, contrary to what was previously postulated (1). The osazones with the properties of α-keto nonanal and but-2-en-1,4-dial were lost in a laboratory accident. Hence the proof of these compounds must remain tentative at present.

Relative to other dicarbonyls, α-keto heptanal and

glyoxal are present in much higher concentrations in the dicarbonyls isolated from linoleate. This suggests that these compounds are formed in oxidized lipids through favored reactions or possibly through more than one mechanism.

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Volatile Products from Mild Oxidation of Methyl Linoleate. Analysis by Combined Mass Spectrometry-Gas Chromatography

R. J. HORVAT, W. H. McFADDEN, HAWKINS NG, D. R. BLACK, W. G. LANE and R. M. TEETER [1]
Western Regional Research Laboratory, [2] **Albany, California**

Abstract

The volatile products from autoxidation of methyl linoleate have been analyzed by combined mass spectrometry-gas chromatography. The principal components were pentanal, hexanal, amyl formate, methyl octanoate, and substituted dioxolanes. Minor components included esters, alcohols, ketones, aldehydes, hydrocarbons, and acetals. Certain unsaturated carbonyl compounds, previously reported, were not detected.

Introduction

IN GENERAL CAPILLARY gas chromatographic analysis of the volatiles from oxidation of lipids indicates complex mixtures. To ascertain fully the nature of such mixtures, the combined technique of fast scan mass spectrometry-capillary gas chromatography (MS-Cap GC) has been applied (1,2). This method of analysis permits identification of many components from as little as 1 μl of a complex mixture (then split 1/100 in the injector of the chromatograph). In favorable cases, a component present in less than 1 part in 10^4 can be identified from this size sample. The method involves no chemical intermediates that might modify the unknowns.

Experimental

Preparation of Methyl Linoleate

Methyl linoleate was prepared from fresh safflower oil, a convenient source of this acid, by the method of Swern and Parker (3). The purity of the ester was estimated to be 97% by GLC analysis using a 75-ft, 0.01-in. capillary column coated with General Electric SF96-50 silicone oil and a 4-ft, 0.25-in. column containing 15% DEGS on firebrick. The peroxide value was negligible.

Oxidation of Methyl Linoleate

Methyl linoleate (19 g), on purified glass wool, was oxidized at room temperature (22C) and in diffuse daylight by purified oxygen passed at a rate of 10 ml/min. The oxygen was purified with Linde Type 5A molecular sieve. After 18 days the ester had a peroxide value of approximately 1000.

Volatile products of the oxidation were swept by the oxygen into a U-tube at –78C. Approximately 0.5 ml of condensate, primarily water, was obtained from the 19 g of the ester. The condensate was extracted with 50 μl of 2,2,4-trimethylpentane by slow rotation (overnight), and the 2,2,4-trimethylpentane extract was analyzed by combined gas chromatography and mass spectrometry.

A control run was performed simultaneously in a parallel apparatus without the ester. The control condensate (20–50 μl) was extracted and analyzed as described. Only solvent and solvent impurities were observed.

MS-Cap GC Analysis

Gas chromatographic separation of the volatile oxidation products was achieved with a 200-ft, 0.01-in. capillary column. The column was coated with General Electric SF96–50 silicone oil containing 1% Carbowax. A typical temperature-programmed chromatogram obtained with a hydrogen flame detector is shown in Figure 1.

For mass spectral analyses the column was removed from the flame detector and the column exit inserted directly into a vacuum manifold leading to the ion chamber of a Bendix Time-of-Flight mass spectrometer (Fig. 2). With the column exit pressure at vacuum, the inlet pressure must be correspondingly reduced in order to obtain the same average linear velocity in the column. This method has the advantages that 1) a greater fraction of the total eluate may enter the mass spectrometer and, 2) possible loss of chromatographic resolution due to eddy currents in corners, etc., is eliminated by the high linear velocities at the vacuum exit (4).

[1] California Research Corporation, Richmond, California.
[2] A laboratory of the W. Utiliz. Res. Devel. Div., ARS, USDA.