Characterization of the Nonvolatile Compounds Formed During the Thermal Oxidation of Corn Oil. II. Phthalate Esters

E. G. PERKINS, Department of Food Science, The Burnsides Research Laboratory, University of Illinois, Urbana, Illinois

Abstract

Twenty compounds with molecular weights ranging from 390 to 970 have been isolated from thermally oxidized corn oil with the aid of countercurrent distribution, column, and thin-layer chromatography. The structure of one compound was shown to be a phthalic acid esterified with an eight carbon normal and branched chain alcohol in both carboxyl groups. A similar compound was found in the products resulting from the oxidation of 2-oleyl-1,3-distearin and 1-palmityl-2,3 diolein as well as in a fat obtained from a local drive-in restaurant.

Introduction

Nonvolatile decomposition products are formed in fats subjected to high temperature in the presence of oxygen (1). These materials have been shown to be nutritionally harmful to the growing rat (2,3). It is therefore important to ascertain the chemical nature of these compounds in order to understand the chemical processes taking place under these conditions. Although a considerable body of work has been published which describes the volatile compounds formed during thermal oxidation and deep fat frying (4,5), little has appeared which deals with the identification of specific nonvolatile components formed during thermal oxidation. In the present report, evidence is presented for the formation of phthalic acid esters during thermal oxidation.

Experimental

Fresh corn oil was thermally oxidized for 48 hr at 200C in the presence of air (0.15 ml/min/g) (6). The resulting oil was converted to its corresponding fatty acids (6). Countercurrent distribution (CCD) was carried out with an automatic 50-tube Craig-Post apparatus. Methyl esters were prepared by treatment of fatty acid with 2% sulfuric acid in methanol (6).

Partition column chromatography was carried out as follows: 60 g of 100 mesh silicic acid, activated for 2 hr at 100C was gently stirred in a mortar while 30% by weight of 80% aq ethanol was added. The mass was stirred until free flowing and then packed into a 25-mm diameter column. A general elution pattern using hexane, increasing proportions of diethyl ether in hexane and ethanol was employed. Adsorption chromatography was carried out using 100 mesh silicic acid activated for 2 hr at 150C.

Gas-liquid chromatography (GLC) was accomplished using a Barber-Colman Model 10 instrument equipped with a tritium diode detector. Components were separated on a 6 ft × 4 mm glass column packed with 16% ethylene glycol succinate coated on 60–80 mesh Chromasorb W (AW) obtained from Applied Science Laboratory, State College, Pa. The column was operated at 185C; detector 210C and injector at 225C. A column flow of 40 cc/min of Argon was employed.

Carbon hydrogen analyses were carried out by Clark Microanalytical Laboratory, Urbana, Ill. Mass spectra and NMR spectra were determined by the chemistry department of the University of Illinois, using an Atlas mass spectrometer and a Varian A 60 instrument, respectively. Molecular weight was determined by vapor pressure osmometry using a Mechrolab Osmometer.

Saponification and neutralization equivalents were determined as outlined by Smith and Shriner (7). Infrared and ultraviolet spectrophotometric measurements were carried out in a Beckman Model IR 7 and Cary Model 11 spectrophotometer. Thin-layer chromatography (TLC) was carried out according to the method of Stahl; (8) using hexane; diethyl ether (9:1) and isooctane: ethyl acetate (9:1) as developing solvents.

Isolation of Compound A

The fatty acids of thermally oxidized corn oil were subjected to countercurrent distribution in a 50 tube Post apparatus using equilibrated 80% ethanol and hexane as partitioning solvents. This process yielded a concentrate of oxidized material (36.6%) in the hyperphase. The concentrate was then converted to its methyl ester and redistributed in the same solvent system to achieve further fractionation. After 100 transfers the hyperphase was collected and the solvent removed. The material obtained in this way represented 12.4% of the total oxidized material. The material remaining in the hypophase represented more fully oxidized material of increased complexity. The hyperphase concentrate was subjected to partition chromatography on silicic acid containing 30% by weight of an 80% ethanol-water phase. This fraction was rechromatographed using another partition column as described above and then chromatographed once more on an adsorption column composed of activated silicic acid and again eluted with hexane. Purified material A, isolated in this manner, was a yellow viscous oil and was found in the original thermally oxidized corn oil at the 2.4% level.

Results and Discussion

The application of countercurrent distribution combined with column and thin-layer chromatography has resulted in the isolation of twenty compounds from thermally oxidized corn oil with molecular weights ranging from 390 to 970. The characterization of one of these compounds is outlined below:

The purity of compound A was determined by TLC and GLC. No impurities were detected when 50 μg of the compound were chromatographed in two different solvent systems composed of hexane/diethyl ether/glacial acetic acid (9:1) and isooctane/ethyl acetate (9/1). One peak was found in the gas chromatogram of "A." It had a relative retention time of 11.1 compared to methyl stearate on an ethylene glycol polyester column and an ester carbon number of 27.5. The molecular weight of A was determined by vapor pressure osmometry to be 440. The parent

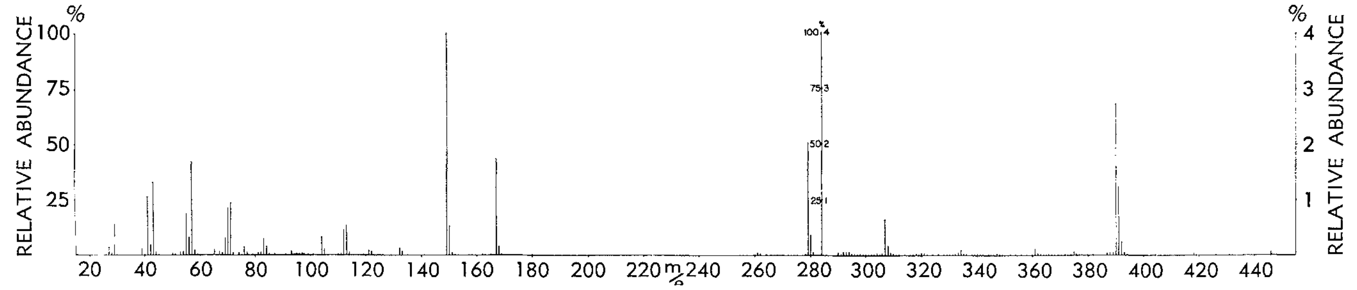

FIG. 1. Mass spectrum of compound A.

ion in the mass spectrum was at mass 390 (Fig. 1). Carbon hydrogen analysis indicated 75.13% C and 9.16% H. The analytical data were consistent with the empirical formula for A. $C_{24}H_{37}O_4$.

The NMR spectrum (Fig. 2) indicated the presence of an aromatic structure at 2.6 τ as well as a doublet at 5.7–5.8 τ which indicates that there is present an -O-CH$_2$-CH grouping in the alcohol moiety attached to the carbonyl group. The ultraviolet spectrum contained a main peak at λ max = 274 mμ (e = 1561) with a shoulder at 281 mμ (e = 1394) (Fig. 3). Supplementary data are as follows: no uptake of bromine from CCl_4 solution or uptake of hydrogen at atmosphere pressure and 25C with Adams catalyst. The compound did not form an adduct with urea (9). No reaction was observed when the Von Rudloff $HIO_4/KMnO_4$ oxidation was attempted (10).

The infrared spectrum of the compound contained bands at 1735, cm^{-1} indicative of ester; at 1580 and 1605 cm^{-1}, characteristic of phthalate esters, and at 745 cm^{-1}, indicating a 1,2-disubstituted benzene ring. The corresponding acid contained a carbonyl absorption at 1690 cm^{-1}. The mass spectrum of the compound (Fig. 2) is characteristic of the fragmentation patterns observed for phthalate esters (11). The base peak at m/e 149 represents the rearrangement ion from phthalic anhydride formed by loss of water from the diacid fragment at m/e 167. Fragments at m/e 279 and 167 represent loss of first one and then the second alcohol comprising the ester groups. Those wth m/e 361, 334, and 307 represent cleavage of hydrocarbon ions from the alcohol moieties and ions with m/e 112 to 28 represent the C_8 to C_2 hydrocarbon fragments, respectively.

Further evidence for the assigned structure was obtained as follows: The mass spectrum of the dimethyl ester of the acid from compound A prepared by reaction with diazomethane was identical with that of dimethyl phthalate. The UV spectra of the acid and phthalic acid were almost identical (Fig. 4). In addition, the acid, when heated above its melting point (173C) formed an anhydride (mp 128C) with UV (Fig. 4) and IR spectra identical to that of phthalic anhydride. Furthermore, reaction of the acid isolated from compound A with alkaline permanganate yielded the starting acid, thereby indicating that there were no oxidizable side chains other than the carboxyl groups.

The alcohol moiety was recovered from a saponification mixture. It had a carbon number of 7.7 on an ethylene glycol succinate column (EGS); on a 6 ft × 1/16 in. proapak Q (80–100 mesh) column, this coincided with the elution time of octyl alcohol and 2-ethyl hexyl alcohol. Both the UV and IR spectra of compound A, di-n-octyl phthalate, and the di-2-ethylhexyl ester were identical. The three compounds

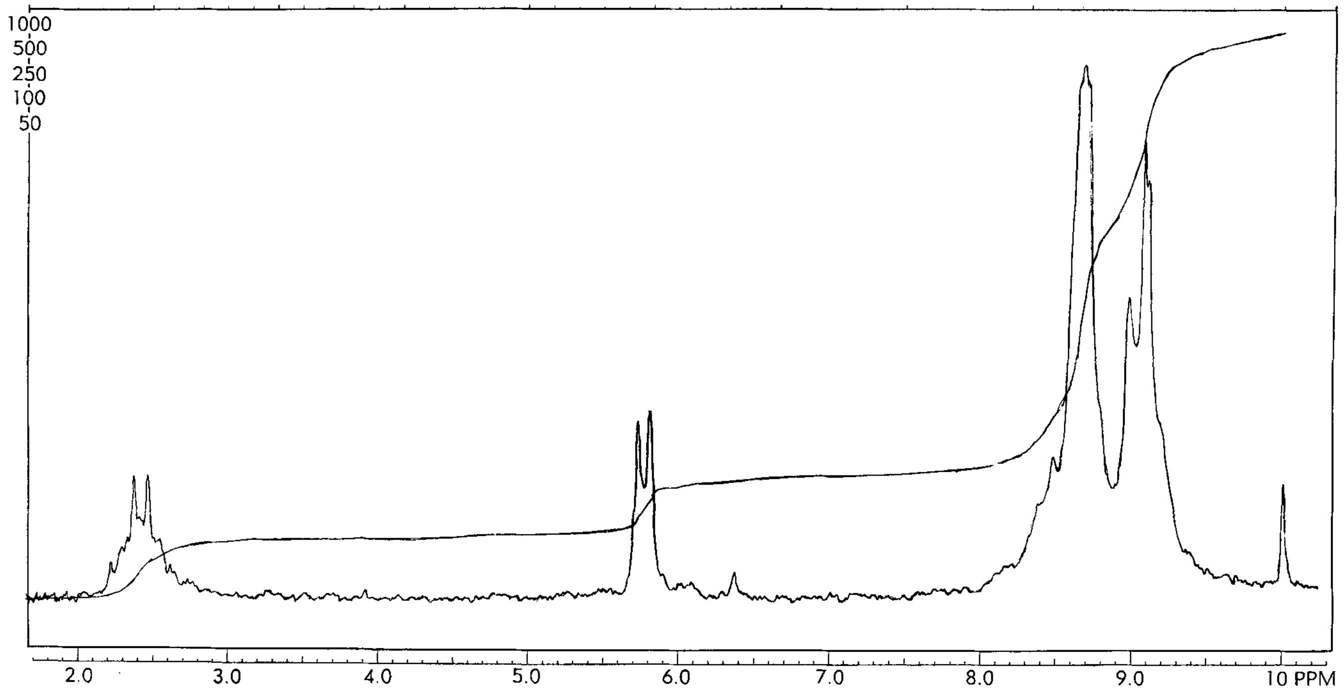

FIG. 2. Nuclear magnetic resonance spectrum of compound A.

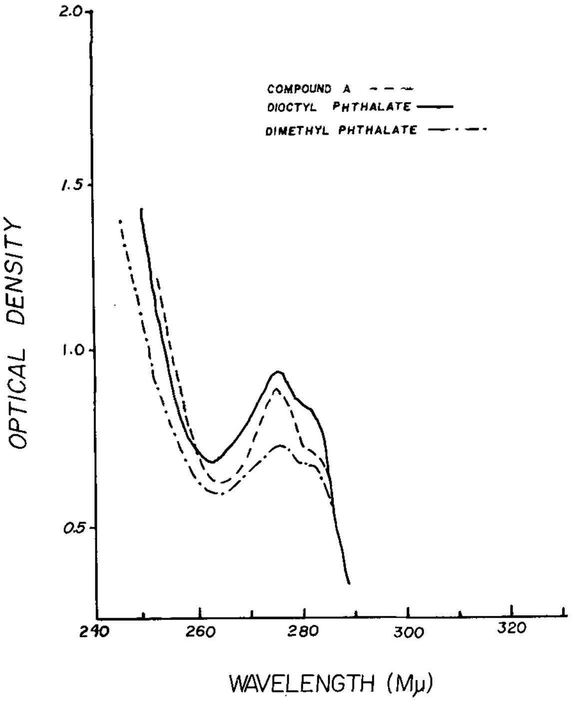

FIG. 3. Ultraviolet spectra of compound A compared with dioctyl phthalate and dimethyl phthalate.

could not be separated from one another by gas chromatography using an EGS column. These data indicate that the compound is phthalic acid esterified with an eight carbon alcohol which is probably a mixture of branched and straight chain moieties.

Thermal oxidation of 2-oleyl-1,3 distearin produced 3.3% of nonvolatile material. The mass spectrum and infrared spectrum was essentially identical to that obtained for the phthalate ester derivative isolated from oxidized corn oil. Thermal oxidation of 1-palmityl-2,3 diolein in a similar fashion yielded a material which has also been tentatively identified as a phthalate ester. More recent results indicate that this type of compound was also present in a fat obtained from the deep fryer of a local drive-in restaurant. Such compounds would be expected to possess biological activity since phthalic acid has a reported LD_{50} of 8 g/kg and octyl alcohol (2-ethyl hexyl) an LD_{50} of 3.2 g/kg in rats. A short chain derivative, dibutyl phthalate, has an LD_{50} of 4.14 g/kr determined on mice (12).

The formation of phthalic acid and esters is feasible since it could arise as a result of the oxidation and rearrangement of a cyclic monomer which had

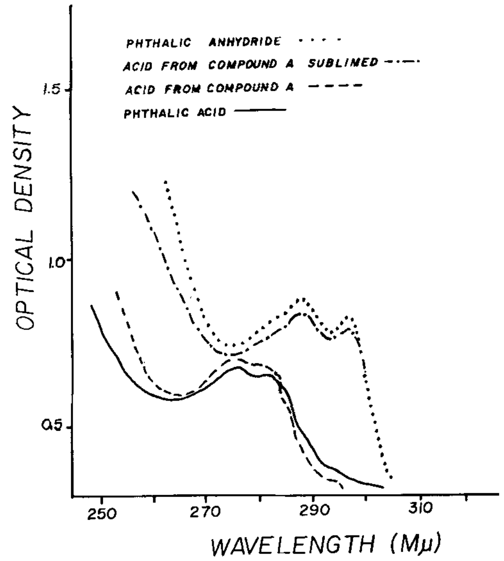

FIG. 4. Ultraviolet spectra of phthalic anhydride, acid from compound A sublimed, acid from compound A and phthalic acid.

aromatized. A mechanism for the formation of aromatic structures from linoleate has been proposed by Paschke and Wheeler (13). Further support of this is found in the isolation of benzoic acid from the volatile oxidation products of fats by Krishnamurthy and Chang (14). This compound also may have come about as a result of oxidation of an aromatized cyclic monomer.

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