Reductive Ozonolysis for Monoenoic Fatty Acid Structure Determination in the Microreactor Apparatus[1]

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ABSTRACT

An improved method of double bond location in unsaturated monoenoic fatty acids has been developed for the "integrated microreactor apparatus" (MRA) system. Solid triphenylphosphine (TPP), as contrasted with previously employed TPP solutions, effectively reduces ozonides to aldehydes and eliminates interferences of solvents with desired aldehyde peaks and the selective loss of volatile aldehydes. The need for separating homologous series of volatile aldehydes at subambient temperatures (beginning at -40 C) and of long chain aldehyde esters (up to 270 C) places stringent requirements upon column stability, polarity and resolution. An effective substrate system for gas liquid chromatography (GLC) has been developed that gives results amenable to digital computer analysis; also developed are techniques of utilizing temperature measurements from temperature programming and for eliminating detector poisoning by TPP. This improved MRA system has been applied to the analysis of isomeric fatty acids formed in hydrogenation of fats.

INTRODUCTION

During hydrogenation triglyceride oils are isomerized both in positional and geometric configuration of double bonds. A rubber column (1) will separate the monoenes from the other polyunsaturates. A rapid method to locate unsaturation is needed. Double bond rupture followed by gas chromatographic (GC) analysis of cleavage products presents a method of choice. Ozonization of the double bond to the ozonide, followed by pyrolytic or reduction cleavage of the ozonide and GC analysis, effectively meets these requirements. However, ozonization-pyrolysis of double bonds in methyl esters not only yields the desired aldehyde ester and aldehydes, but also methyl esters, hydrocarbon and acid-cleavage products. Alternately, reductive ozonolysis may be carried out; e.g., triphenylphosphine (TPP) reduction (2,3) of the ozonide. Reductive ozonolysis is preferred because the production of aldehydes is enhanced with resultant decrease of acids, esters and hydrocarbons.

One might correctly anticipate that volatile or low-boiling materials would be selectively lost from the conventional test tube TPP reduction of ozonides to aldehydes and aldehyde esters; this loss can be eliminated in a closed system such as the ozonization-pyrolysis procedure based on the integrated microreactor apparatus (MRA) (4,5). Because of the recognized disadvantages of the pyrolysis procedure (6) in forming artifacts, the possibility was explored of employing reductive ozonlysis in the closed integrated MRA system. In addition, partitioning substrates were developed, temperature measurements were effectively utilized and certain difficulties inherent with the use of TPP were avoided.

MATERIALS AND METHODS

The MRA system is based on the soldering gun heater described by Davison and Dutton (4). Later modifications that led to an integrated MRA system include such features as temperature control, ozone generation and monitoring and gas valving (5,7).

In the latest investigations, the 4 mm male Luer joint is equipped with a 23 gauge, 2 in. needle. A 2 cm packing of glass wool is inserted to such depth that a drop of sample on the end of a 1-μl microsyringe can be wiped onto the glass wool during the ozonization step (Fig. 1).

Since low-temperature ozonization held no advantages all operations are done at room temperature. Ozone in oxygen (3%) flow through the T arrangement to the Luer joint is adjusted to 10 cc/min. Ozone is generated and its concentration measured and recorded by the integrated MRA (5,7). The methyl ester sample, 0.1 to 0.7 μl depending on its complexity, is introduced through a septum to the glass wool carrier while the ozone flow is recorded. A decrease in concentration of ozone is apparent from the integrated MRA meter and a parallel strip chart recorder while the sample is being ozonized. After complete ozonization is indicated by the meter/recorder, flow is continued for an additional 4 min. Sample and chamber are then flushed with helium. While helium flow is continued the septum is removed and 3 mg of solid TPP is placed on the glass wool with a disposable capillary pipet. The pipet is inserted to the glass wool while that joint is in a horizontal position; the joint is tilted to a vertical position to empty contents of the pipet. After the glass wool is pushed down to the needle end with the pipet the septum is replaced and the MRA needle is injected into the heated inlet of the GC.

Helium is the carrier gas both for the GC and the integrated MRA system. The helium gas flow through MRA is adjusted while it is in an injection position to 40-50 cc/min, so that MRA pressure is greater than pressure in the GC injection inlet port. Temperature in the Luer joint of MRA is raised above the melting point (80-82 C) of TPP and held at this temperature by MRA controller for 3 min while TPP reduces the ozonide to yield the aldehydes and aldehydic ester cleavage products. Gas flow is maintained at 40-50 cc/min while MRA temperature is raised to 400 C for 2 min; this procedure transfers quantitatively all the aldehydes and aldehydic ester cleavage products to the GC for subsequent identification and quantitation. The MRA needle is then withdrawn from the GC septum.

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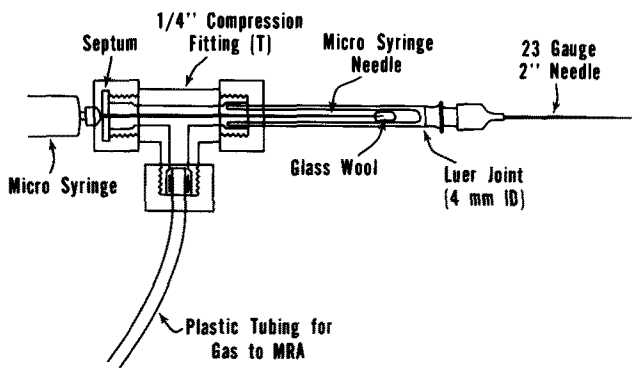

FIG. 1. Luer joint with fittings for microreactor apparatus (MRA).

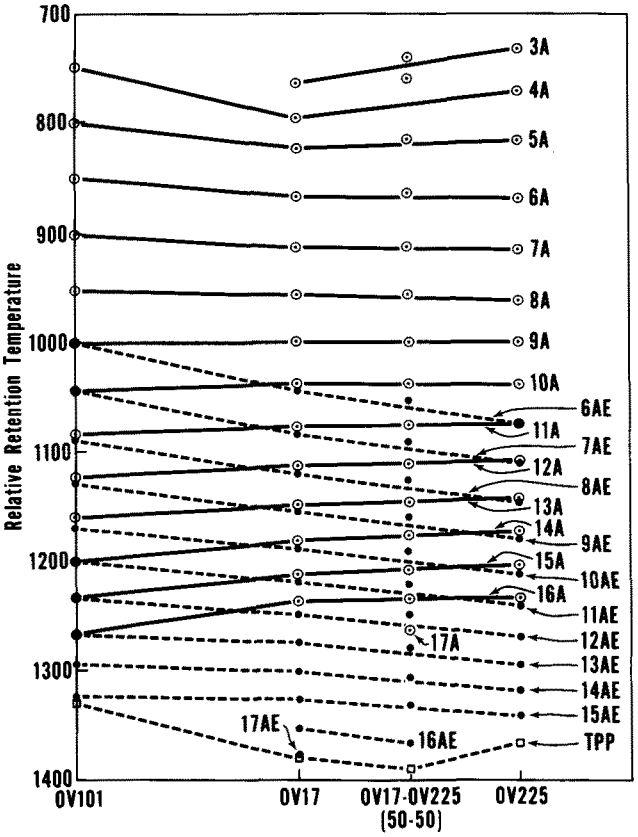

FIG. 2. Modified Rohrschneider representation of substrate (OV 101, OV 17 and OV 225) polarity. Relative retention temperatures of aldehydes (A), open circles; aldehydic esters (AE), solid circles; triphenylphosphine (TPP), open squares.

A Beckman GC-5 gas chromatograph with flame isonization detector was used with helium carrier gas flow at 38 cc/min, attenuation at 2×10^4, detector temperature at 350 C and temperature programmed from -40 C to 275 C. More specifically, temperature programmed from -40 C to 0 C in 1 min; then in the following 63 min, from 0 C to 275 C at 4.4 C/min. Four columns were used: (a) 4 ft x 1/8 in., 3% OV 101 (Supelco Inc., Bellefonte, Pa.) on 100/120 Chromosorb WHP; (b) 4 ft x 1/8 in., 10% OV 225 on 80/100 Chromosorb WHP; (c) 4 ft x 1/8 in., 10% OV 17 on 80/100 Chromosorb WHP; and (d) 2 ft x 1/8 in., 10% OV 17 on 80/100 Chromosorb WHP followed by 2 ft x 1/8 in. 10% OV 225 on 80/100 Chromosorb WHP. Each chromatographic column was followed by 2-1/2 in. x 1/8 in. zinc oxide on sand to remove traces of acids if present (4).

RESULTS AND DISCUSSION

Elimination of Solvents

Various solvents for TPP have been proposed (2,6,8,9). The more volatile solvents obscure low-molecular-weight aldehydes; for example propionaldehyde is lost when acetone, dichloromethane or pentane-hexane is the solvent for TPP. Methylformate offered the most promise because propionaldehyde appeared as a separate peak on the tailing edge of the large methylformate peak. Volatility of methylformate and methyl fluorides, however, made special handling necessary and presented some technical difficulties. Various so-called nonvolatile solvents for TPP were tried but all had certain undesirable qualities. Triglycerides as solvent for TPP had impurities, or fragmented and interfered at the temperatures required. Dow Corning 550 silicone oil and OV 101 itself were tried but were too viscous and either had to be heated when injected or lacked desired solubility characteristics.

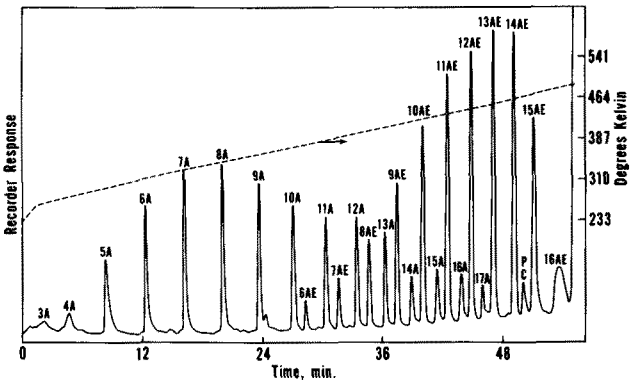

FIG. 3. Chromatogram of TPP-reduced ozonides of methyl esters of C_{18} monoenes with integrated MRA system. (A) aldehyde; (AE) aldehydic ester.

Solid TPP introduced into MRA obviates problems raised by a solvent. Masking of desired components is eliminated; also, the closed injection system prevents loss of volatile components.

Choice of Substrate

The multiplicity and range of volatility of ozonolysis products, which come from monounsaturated fats whose double bond varies in position from the second or third carbon atom to the 17th carbon atom, pose stringent requirements upon any liquid substrate used for quantitative resolution.

Stability. Since compounds produced vary in volatility from propionaldehyde to methyl-16-formylhexadecanoate, elution temperatures vary roughly from 0-250 C. In practice the temperature program of the GC runs from -40 C to 280 C. Not only should there be minimal column bleed at the highest temperature but the substrate should remain liquid to -40 C (10).

Resolution. Resolution problems divide themselves into the ability (a) to separate individual members of the homologous series 3-aldehyde to 15-aldehyde and 5-aldehyde esters to 17-aldehyde ester and (b) to resolve individual members of each homologous series from members of the second series. Although some aldehydes inevitably and unavoidably overlap certain aldehyde esters, it is desirable that the overlaps of significant components be at a minimum.

Retention of TPP. Because an excess of TPP is used it is necessary that this compound be eluted subsequent to the 16-aldehyde ester so as not to interfere with the analysis. To exemplify the nature of the substrate problem one might first observe that the polar polyester columns, EGSS-X and DEGS, perform successfully in the resolution of individual members of the homologous series from themselves, but they bleed excessively at the high temperatures and change phase at the lower temperature ranges. Further, they hold back the TPP so that it comes off in following chromatograms overlapping desired aldehyde members at unpredictable times.

After considerable exploratory work the series OV 101, OV 17 and OV 225 were studied in detail for polarity. All three have low bleed and adequate temperature stability. Figure 2 gives a modified Rohrschneider (11) representation of the relative retention temperatures (12) of these three substrates over the range of aldehydes and aldehydic esters encountered. Furthermore, OV 101 had the desirable characteristic of being liquid at temperatures below O C and hence gave a well-shaped propanal peak. As seen from Figure 2, individual members of a homologous series are adequately separated from one another. Further, TPP elutes at a temperature high enough not to interfere with aldehydic esters. However, there is overlap of important

members of the two classes of compounds; e.g., the 8-aldehyde ester overlaps the 11-aldehyde.

Of the three liquid phases, OV 17 is perhaps the best for it separates the members of aldehyde and aldehydic ester families. Moreover, overlap of the classes themselves is one carbon atom less than with OV 101; e.g., the 11-aldehyde overlaps the 7-aldehyde ester. For all practical purposes TPP is adequately held back.

OV 225 gives the greatest resolution of the two classes of homologous compounds; i.e., the 11-aldehyde overlaps the 6-aldehyde ester. However, overlap of important members of the aldehyde class with the aldehydic ester is totally unacceptable. The answer to this dilemma lies in optimal mixing of pairs of substrates, or of columns, to get the maximum resolutions of all components. An OV 17-OV 225 system was compared with an OV 101-OV 17 system because the former tends to resolve the two homologous classes the most. Mixing the two phases (13) was actually accomplished by using sequential columns; i.e., cutting the 4 ft OV 17 and the OV 225 columns in the middle and placing the 2 ft OV 225 tandem to the 2 ft OV 17 column. As predicted and as shown by Figure 2, for the 50% composition, resolution of the classes improved and overlap of important members was minimal. Shown in Figure 3 is the chromatogram for this mixture. The aldehyde and aldehydic esters seem equally spaced in position between one another. Undoubtedly the ratio between the two columns can be optimized further, but the present 50-50 mixture appears to be a practical compromise.

Temperature Programming. The temperature programming for this system as indicated in the broken line of Figure 3 presents as yet an unsolved problem. If the temperature is linearly programmed from -40 to 280 C, a propanal peak appears as its proper temperature-time location but as a very broad and sometimes indistinguishable peak. This peak may be sharpened by the expedient of raising the temperature rapidly from -40 up to 0 C and then programming at a constant rate. While this technique improves the shape of the aldehyde peak, it throws off the approximate linear relationship between carbon chain length and retention temperature and obviates the feasibility of a simple mathematical algorithm for subsequent computer identification of the peaks.

Detector Poisoning. In many installations where one sample is run each day, poisoning by TPP may be overlooked because the high-boiling TPP will gradually elute itself. When successive samples containing TPP are run, however, we have observed poisoning of the flame ionization detector, as evidenced by its excessive noise and going off scale many chart widths. Poisoning of the detector is corrected by its standing idle overnight or by dismantling and cleaning. A simple expedient to overcome this difficulty consists of inserting a tee in the tube just before the flame ionization detector. When the elution temperature for TPP is reached, as observed on recorder, the vacuum is connected to the normally closed tee so that air is pulled backward through the flame ionization detector and so that TPP is pulled from the column until all TPP has been eluted from the column. The flame is immediately extinguished as air at 2 l/min is pulled through the tee reducing hydrogen to ca. 1.5%, which is below the lower explosive limit of 4%.

Methods of computer processing of data shown in Figure 3 are described in a concurrent manuscript.

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