notes on methodology

New method for the reductive ozonolysis of double bonds in monoenoic fatty acid methyl esters

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SUMMARY Unsaturated methyl esters were cleaved to aldehydes and aldehydo-esters by ozonolysis followed by reduction with dimethyl sulfide after conversion to hydroperoxides. Cleavage products were identified by thin-layer chromatography and quantified by temperature programmed gas–liquid chromatography.

KEY WORDS ozonolysis–reduction · unsaturated methyl esters · ozonides · hydroperoxides · aldehydes · aldehydo-esters · dimethyl sulfide · dimethyl sulfoxide · thin-layer chromatography · gas–liquid chromatography

Several procedures have been used for the reductive ozonolysis of fatty acids. They include reduction with zinc and acetic acid (1), lithium aluminum hydride or sodium borohydride (2), triphenylphosphine (3), and 2,4-dinitrophenylhydrazine (4), and hydrogenation in the presence of palladium on charcoal (1, 5) or Lindlar catalyst (5, 6). Pappas, Keaveney, Gancher, and Berger (7) recently synthesized aldehydes from olefins by ozonization in the protic solvent methanol followed by reduction with dimethyl sulfide (DMS). Methanol converted the hydroperoxide dipolar ion derived from the ozonide to the α-methoxyhydroperoxide, which was reduced by the DMS (8). The DMS was in turn oxidized to dimethyl sulfoxide (DMSO). We have used this reaction for the synthesis of long-chain fatty aldehydes (9). In the present study, this reaction and a modification of this reaction which does not employ methanol have been applied to the reductive ozonolysis of the methyl esters of unsaturated fatty acids.

Materials. Methyl oleate, methyl petroselenate, methyl *cis*-vaccenate, and methyl palmitoleate were purchased from The Hormel Institute (Austin, Minn.). Normal pentane (spectroscopic grade) was purchased from the Phillips Petroleum Co. (Bartlesville, Okla.). Methanol (spectranalyzed) was purchased from the Fisher Scientific Company (Fair Lawn, N.J.). Methyl sulfide was purchased from Matheson, Coleman, & Bell (Cincinnati, Ohio).

Reductive Ozonolysis of Fatty Acid Methyl Esters. Ozone, generated in a Welsbach T-408 ozonator, was bubbled through 25 ml of pentane until a blue color developed. The pentane solution was maintained at −70°C with a dry ice–acetone bath. 10–25 mg of methyl esters, dissolved in 5 ml of pentane and cooled to 0°C, was added to the ozone solution. We flushed the flask with dry nitrogen in order to remove excess ozone, and the blue color disappeared. After 5 min, the pentane was evaporated in a stream of dry nitrogen. We facilitated the evaporation by removing the reaction flask from the dry ice–acetone bath for short periods of time. Flask temperature was not allowed to exceed 0°C. When the pentane had evaporated, 3 ml of methanol precooled to −70°C was added to the flask which was then removed from the bath for a short time to allow the ozonide precipitate to dissolve. The clear solution was cooled to −70°C, and 2 ml of DMS was added. We again removed the flask from the bath for a short time to allow any precipitate that had formed to dissolve completely; this was important if reduction was to be complete. We maintained the mixture at −70°C for 20 min and then warmed it slowly, while swirling, by alternately removing it from and immersing it in the bath. The mixture was brought to room temperature in 15 min by this process and allowed to stand for an additional 10 min at room temperature. Excess DMS was evaporated in a stream of dry nitrogen and methanol was evaporated with a water pump at 35°C. The reaction products were dissolved in ether and analyzed.

In small-scale reductive ozonolysis, 0.5–1 mg of the methyl ester mixture was dissolved in 1 ml of pentane, and this solution was added to 2 ml of pentane saturated with ozone and precooled to −70°C. The pentane was evaporated in a stream of nitrogen and 1 ml of DMS was added to the ozonides. After 30 min, excess DMS was evaporated in a stream of nitrogen. The product was dissolved in a small amount of ether and analyzed.

TLC. The reaction was monitored by TLC on Silica Gel G (Brinkmann Instruments Inc., Westbury, N.Y.). We developed plates with xylene or hexane–ether 90:10 in order to separate unreacted methyl esters and ozonides (10, 11). Aldehydes and ozonides were first made visible with a spray of fuchsin–sulfurous acid (9). All spots were then made visible with iodine vapor.

GLC. Simple aldehydes and aldehydo-esters were analyzed with an Aerograph 200 chromatograph equipped with a flame ionization detector. A 10 ft stainless steel column, $^{1}/_{8}$ inch o.d., containing 20% EGS on 60–80 mesh Gas-Chrom P (Applied Science Laboratories, Inc., State College, Pa.) was used for chromatographic separations. The column temperature was programmed

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Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; DMS, dimethyl sulfide; DMSO, dimethyl sulfoxide; EGS, ethylene glycol polyester.

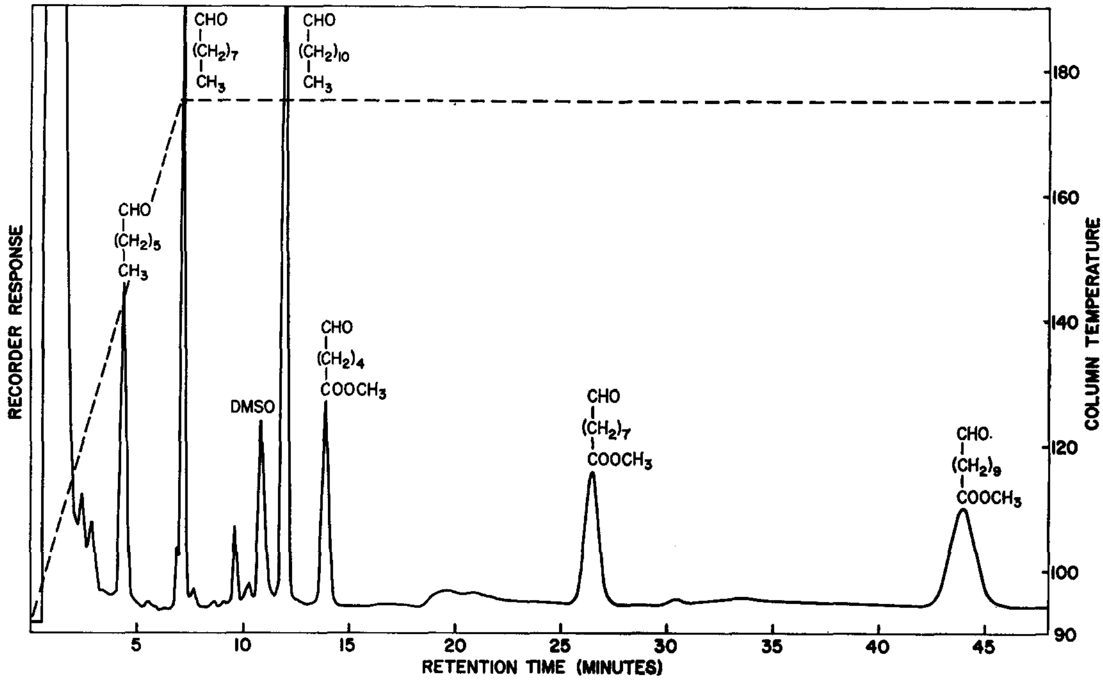

Fig. 1. GLC of the cleavage products obtained from a mixture of methyl oleate, methyl vaccenate, and methyl petroselenate (mixture F, Table 1). Compounds are identified on the figure. Column temperature is indicated by the dotted line. Operating conditions are described in the text.

to increase from 90 to 175°C per min and then maintained at 175°C for the duration of the analysis. The helium flow rate was 11 ml per min.

Results and Discussion. TLC of the reaction products showed that unsaturated fatty acid esters were cleaved quantitatively to their aldehyde and aldehydo-ester fragments by the ozonolysis–reduction reaction. The presence of aldehydes (R_f 0.46) and aldehydo-esters (R_f 0.06) and the absence of unreacted methyl esters (R_f 0.50) and ozonides (R_f 0.30) were demonstrated by chromatography in xylene. The presence of aldehydes (R_f 0.37) and aldehydo-esters (R_f 0.08) and the absence of methyl esters (R_f 0.41) and ozonides (R_f 0.30) were confirmed by chromatography in hexane–ether. DMSO remained at the origin in both solvent systems.

Specific peaks were identified by GLC of the products of reductive ozonolysis of known compounds. DMSO readily separated from the short-chain aldehydes. No unreacted methyl esters were detected. A typical separation is shown in Fig. 1.

The quantitative analysis of several mixtures is presented in Table 1. Peak areas were estimated by triangulation. The standard deviation for the GLC step in the analysis varied from ±0.2 to ±0.8. Composition was calculated by the method of Privett and Nickell (5), which employs the total area for the aldehyde and aldehydo-ester cleavage products of each compound rather than the area of a single class of fragments such as the aldehydes or aldehydo-esters. This calculation compensated for differences in molecular weight and therefore the detector response to each fragment.

The reductive cleavage of fatty acid ozonides with DMS is a simple quantitative reaction which is completed in 1 hr. The method does not require the preparation of a heterogeneous catalyst. DMS and DMSO do not interfere in the isolation and analysis of the cleavage products. Pappas et al. (7) performed the ozonization

TABLE 1 Analysis of Mixtures of Monounsaturated Methyl Esters by Ozonolysis Followed by Reduction with DMS

Mixture		Composition	
		Known	Found*
		wt %	
A	Palmitoleate	51.6	51.8
	Petroselenate	48.4	48.1
B	Oleate	72.1	72.0
	Petroselenate	27.9	28.0
C	Oleate	56.6	57.2
	Petroselenate	43.4	42.7
D	Oleate	51.5	50.8
	Vaccenate	48.5	49.1
E	Oleate	50.7	50.7
	Vaccenate	49.3	49.2
F	Oleate	30.7	30.6
	Petroselenate	35.3	35.5
	Vaccenate	34.0	33.7

* The total area of aldehyde and aldehydo-ester fragments was used in the calculation of the percentage composition of each component.

reaction in methanol. Fatty acid esters are more soluble in pentane than in methanol at −70°C. The yield is improved when pentane is used to prepare the ozonide and a small amount of methanol is then added to decompose the ozonide. The small volume of methanol is easily evaporated without the loss of short-chain monoaldehyde fragments such as heptyl aldehyde. Side-reactions are minimized by lowering the temperature of the methanol and reaction mixture. Methanol was not required for the reductive ozonolysis of 0.5–1 mg methyl ester mixtures. The elimination of methanol from the small-scale reaction prevented any losses which may be incurred by the evaporation of methanol at 35°C. Aldehyde and aldehydo-esters are readily formed by the reductive ozonolysis of diene and polyene esters; however, the volatile and reactive malonaldehyde fragment is not recovered. The quantitative recovery of malonaldehyde is under investigation.

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