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Stereoisomer formation on the ozonization of esters of monounsaturated fatty acids*

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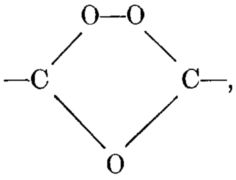
SUMMARY

The formation of stereoisomers of ozonides on the ozonization of methyl esters of monounsaturated fatty acids in pentane at −70° was demonstrated by thin-layer chromatography, infrared spectra, and other analyses. The *cis*- and *trans*-isomers of ozonides of *cis*- and *trans*-methyl 9-octadecenoates (methyl oleate and methyl elaidate) were isolated and characterized.

The "true ozonides" of the methyl esters of monounsaturated fatty acids give two spots on thin-layer chromatography (TLC). In contrast, the ozonides of polyunsaturated methyl esters give only one spot. The spots in the former represent stereoisomers.

"True ozonides" refer to compounds containing the well known structure, , established by Rieche et al. (1). These compounds are also referred to as monomeric ozonides, but this terminology appears to be a misnomer. Hence, the term ozonide is used for "true ozonides" in the present study.

During the course of our studies, Schröder (2) reported the formation of *cis*- and *trans*-isomeric ozonides on the ozonization of *cis*-di-*t*-butylethylene. Only the *trans*-isomer was detected on the ozonization of *trans*-di-*t*-butylethylene, however. Identification of the stereoisomers was made on the basis of gas-liquid chromatography (GLC), relative rates of reduction by $LiAlH_4$, and infrared spectral analysis.

In our study, the formation of *cis*- and *trans*-ozonides was observed on the ozonization of a number of methyl esters of monounsaturated fatty acids. *Cis*- and *trans*-ozonides were isolated from both *cis*- and *trans*-methyl 9-octadecenoates (methyl oleate and methyl elaidate, respectively) and their properties were determined.

EXPERIMENTAL METHODS

Materials. Highly purified (>99%) methyl oleate, petroselinate, linoleate, and elaidate were obtained from The Hormel Foundation.

Methyl 10-undecenoate was obtained from Eastman Organic Chemicals Division, Rochester, New York, and purified by fractional distillation.

Ozonization. This reaction was carried out as previously described by the authors (3). A pentane solution of approximately 0.03 M ozone was prepared by bubbling oxygen containing about 3% ozone through pentane at −70° for about 7 min at 100 ml/min. About 50 mg of sample was dissolved in 2–3 ml pentane and added to 10 ml of the ozone solution at −70°. The ozonization is instantaneous under these conditions. In order to avoid secondary reactions, the dissolved oxygen and excess ozone were removed from the solution as soon as possible by evaporation of part of the solvent, under reduced pressure, as it warmed up to room temperature.

TLC of Ozonides. The ozonides were analyzed by TLC on silicic acid-coated plates with various ratios of petroleum ether bp (35–60°) to diethyl ether. The spots were made visible by charring in an oven at 280° after spraying the plates with 50% aqueous sulfuric acid.

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TABLE 1. ELEMENTAL ANALYSIS OF OZONIDES

Source	Found C	Found H	Found O Difference	Found O Direct	Theory C	Theory H	Theory O	Formula
	%	%	%	%	%	%	%	
Methyl linoleate	58.7	8.63	32.67	31.9	58.5	8.8	32.8	$C_{19}H_{34}O_8$
Methyl petroselinate	66.15	10.30	23.55	23.9	66.4	10.5	23.2	$C_{19}H_{36}O_5$
Methyl oleate	66.51	10.34	23.15	23.6	66.4	10.5	23.2	$C_{19}H_{36}O_5$
Methyl elaidate	66.87	10.39	22.74	22.86	66.4	10.5	23.2	$C_{19}H_{36}O_5$
Methyl 10-undecenoate	58.51	9.0	32.49	32.00	58.5	9.0	32.5	$C_{12}H_{22}O_5$

Isolation of Ozonides. The isolation of *cis*- and *trans*-ozonides of methyl *cis*-9-octadecenoate (oleate) and methyl *trans*-9-octadecenoate (elaidate) was carried out by TLC using the same conditions as described above, except that the positions of the spots were located by spraying the plates with a solution of 0.1% 2,7-dichlorofluorescein in ethyl alcohol. In order to isolate sufficient material for analysis, about 50 chromatoplates (20 x 20 cm) were prepared in which the products of the reaction were spotted in rows of 10 spots of 50 μg each. The two spots into which the ozonides separated were scraped off each plate separately and recovered from the adsorbent by extraction with diethyl ether. The recovered products were then crystallized from petroleum ether (bp 35–60°).

Melting points were determined by a capillary tube method with a total immersion thermometer and a rise in temperature of 0.5°/min.

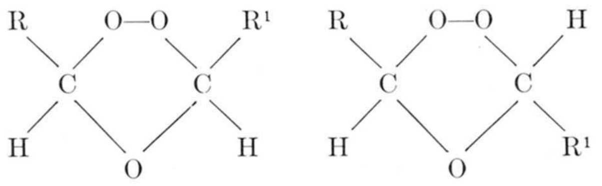
Infrared spectra were determined with a Perkin Elmer instrument (Model 21 Double Beam) in 10% solution of CS_2 (680–1400 cm^{-1}; 2400–4000 cm^{-1}) and in a 10% solution of C_2Cl_4 (1400–2400 cm^{-1}).

Elemental analyses were performed by the Clark Microanalytical Laboratory, Urbana, Illinois, and the Microanalysis Laboratories, Wilmington, Delaware.

RESULTS AND DISCUSSION

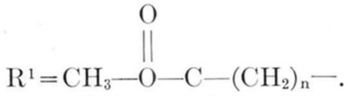
Thin-layer chromatography and elemental analyses of the products of the ozonization of several methyl esters are presented in Fig. 1 and Table 1, respectively.

These results, in accordance with previous studies (3), indicate that the major products of the reaction are ozonides. The spot for the ozonides of methyl linoleate (E, Fig. 1) has the lowest R_f value, as these compounds contain two ozonide groups per molecule. The ozonide of methyl undecenoate (A, Fig. 1) is less polar than those of methyl linoleate, but more polar than those of the octadecenoates because of its shorter chain. The ozonide of methyl petroselinate is more polar than those formed from methyl oleate and elaidate, because of the different position of the ozonide group in the chain. That the two components of the ozonides of methyl oleate and elaidate have the same R_f value is good evidence that their structures are identical. The separation of the ozonides of methyl oleate, elaidate, and petroselinate into two components (Fig. 1) is explained by the formation of stereoisomers as follows:



(*cis*) (*trans*)

where $R = CH_3—(CH_2)_n—$ and


$$R^1 = CH_3—O—\overset{\overset{\displaystyle O}{\|}}{C}—(CH_2)_n—.$$

Since the double bond in methyl 10-undecenoate is in the terminal position, the formation of stereoisomers

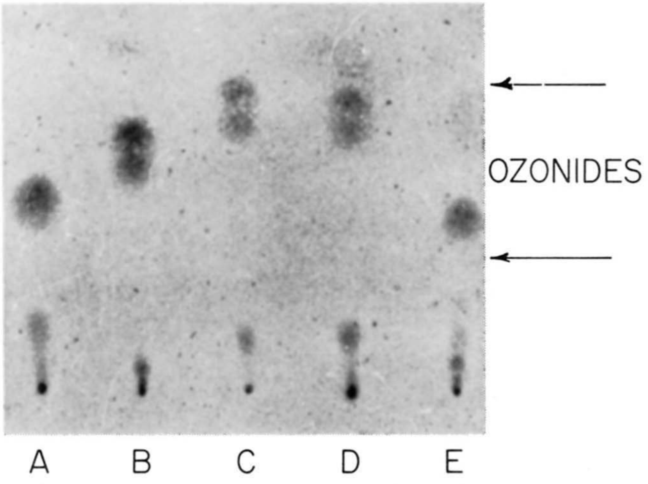


FIG. 1. Results of the TLC of the products of the ozonization of several methyl esters with 10% diethyl ether in petroleum ether (bp 35–60°). A, methyl undecenoate; B, methyl petroselinate; C, methyl oleate; D, methyl elaidate; E, methyl linoleate.

is not possible with this compound. Theoretically, a complex mixture of stereoisomers should be formed from polyunsaturated methyl esters because the number of possible isomers increases exponentially with the number of double bonds. Thus, one spot for the ozonides of methyl linolenate and arachidonate (not shown), as well as methyl linoleate, which may form four ozonides, is not unexpected, as the components would be most difficult to separate.

Since the basis of the separation of the component ozonides by TLC is a difference in polarity, the compound with the lower R_f value (greater polarity) would be expected to have the *cis* configuration. Evidence to this effect was obtained by the analysis of the two isomers isolated from methyl oleate (*cis*-9-octadecenoate) and methyl elaidate (*trans*-9-octadecenoate). Melting points of the compounds representing the two spots of the ozonides of these methyl esters are shown in Table 2. The compound separated as the lower spot in both cases had the lower melting point. The slight difference between the melting points of the corresponding high and low melting forms from these esters was due to the greater purity obtained with the ozonide prepared from methyl elaidate. That the two low and the two high melting forms had essentially the same melting points offered further proof for the formation of stereoisomers.

TABLE 2. MELTING POINTS OF OZONIDES

Source	*Cis* Form (Lower Spot)	*Trans* Form (Upper Spot)
Methyl oleate	4.5°	30.0°
Methyl elaidate	6.0°	31.8°

The infrared spectra of the two *cis* forms (low melting) and the two *trans* forms (high melting) were also identical. There were several differences in the infrared spectra of the *cis*- and *trans*-isomers as shown in Fig. 2. The *cis*-ozonide may be distinguished from the *trans* form by a band at 12.2 μ. The *trans*-ozonide has a band at 7.6 μ that is not present in the *cis*-isomer. Similar differences were observed between the spectra of the *cis*- and *trans*-ozonides of di-*t*-butylethylene by Schröder (2).

Cis-isomers are generally more reactive than *trans*-isomers because of their greater free energy. Hence, a difference in reactivity is often used as a means of distinguishing stereoisomers. In the present study, a

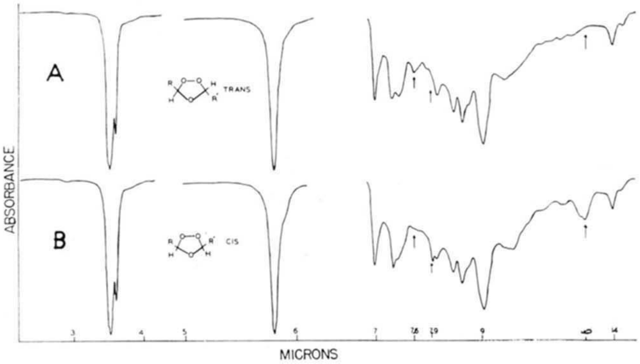


FIG. 2. Infrared spectra of the ozonides isolated from the products of the ozonization of methyl oleate. A, the upper compound of the "double spot," Fig. 1; B, the lower compound of the "double spot," Fig. 1.

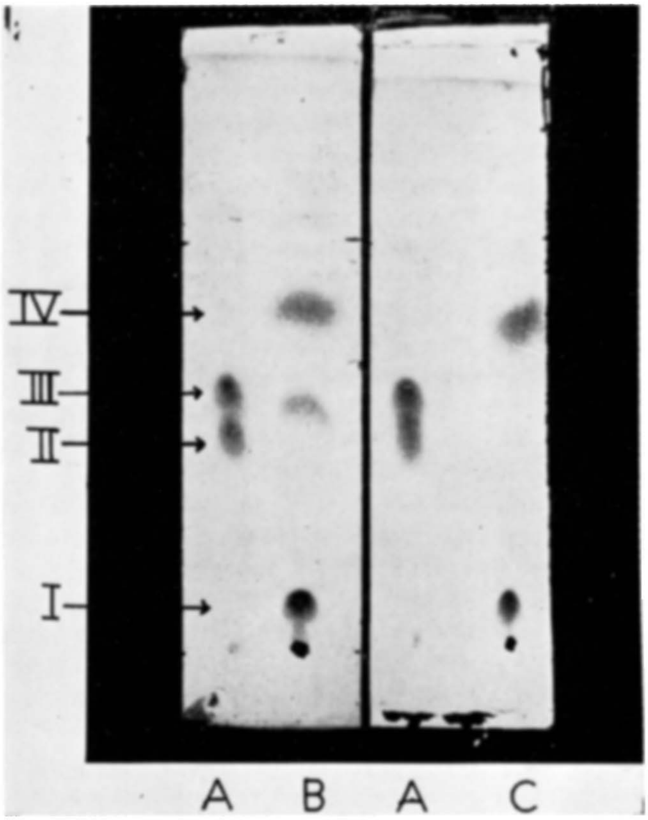
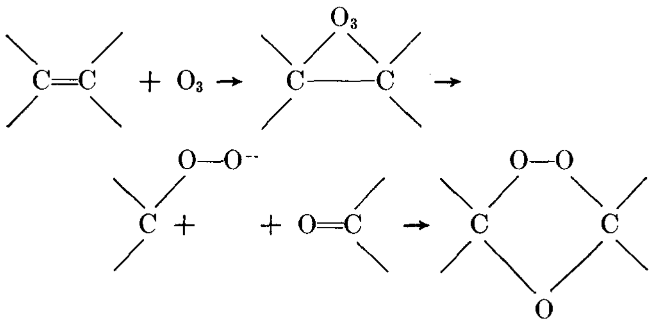


FIG. 3. Results of the TLC of the products of the reduction of methyl oleate ozonide with 10% diethyl ether in petroleum ether (bp 35–60°). A, at start of the reaction; B, after a reaction time of 3 min; C, at the completion of the reaction (15 min); I, methyl nononoate-9-al; II, *cis*-ozonide; III, *trans*-ozonide; IV, pelargonyl aldehyde.

further identification of the *cis*- and *trans*-ozonide isomers was made on the basis of their relative rates of catalytic reduction with the Lindlar catalyst (3). Analyses of the products of the reduction of methyl oleate ozonides at various intervals during the reaction by TLC (Fig. 3) showed that the ozonide represented by the lower spot (Figs. 1 and 3) was reduced at a much faster rate than the ozonide represented by the upper spot in accordance with the designation of these ozonides as *cis*- and *trans*-isomers, respectively.

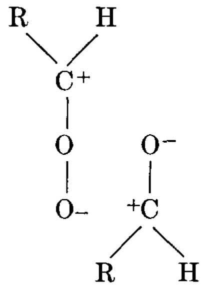
Quantitative analysis by densitometry of the charred spots showed that the two isomeric ozonides given by methyl oleate were formed in approximately equal amounts (48% *cis*, 52% *trans*). Roughly equal amounts of *cis*- and *trans*-isomers of the ozonides of methyl oleate and elaidate were isolated. Thus, it may be concluded that the *cis*- and *trans*-ozonides of monounsaturated fatty acid esters are formed in essentially equal amounts.

Present knowledge of ozonization reactions, recently reviewed by Bailey (4), indicates that the mechanism of ozonide formation is as follows.

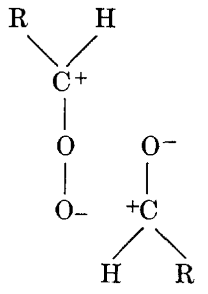


According to Criegee (5), *cis-trans*-isomeric olefins give the same and not stereoisomeric ozonides because "the intermediates are the same for both isomers and will combine to the same end products." Bailey (4) elaborates further: "the complete breakdown into the zwitterion and aldehyde followed by recombination of these fragments would lead to the more stable ozonide in each case."

Although the same intermediates are formed from *cis*- and *trans*-isomeric olefins by the above mechanism, it would appear that *cis*- and *trans*-ozonide isomers may be formed depending on the orientation of the intermediates at the time of their recombination, which may be visualized as follows:



(*cis*)



(*trans*)

Since approximately equal amounts of *cis*- and *trans*-ozonides are formed on the ozonization of monounsaturated methyl esters, steric factors do not appear to be involved in the formation of ozonides from these compounds. The report by Schröder (2) that the ozonides of *cis*-di-*t*-butylethylene consisted of a mixture of *cis*- and *trans*-isomers in contrast to *trans*-di-*t*-butylethylene, which gave only the *trans*-isomer, indicated that steric factors might be involved in the ozonization of some olefins. However, there was a considerable difference in the yield of ozonides from these compounds, 82% for the *cis* form vs 58% for the *trans* form; furthermore, the recoveries in the separation of the *cis*- and *trans*-ozonides by GLC were only of the order of 50%.

An alternative explanation for the formation of stereoisomers is a mechanism, such as that proposed by Milas (6), which does not involve a complete breakdown of the molecule. Although the products of the ozonization of many compounds can be explained on the basis of the zwitterion intermediate, the possibility that a complete breakdown of the molecule does not occur on the formation of some ozonides cannot be entirely precluded.

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