Mass spectrometry of derivatives of cyclopropene fatty acids

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ABSTRACT Diketo fatty acids prepared by ozonization of cyclopropene fatty acids have been separated and purified by chromatographic techniques. Mass spectra of esters of these compounds and of methanethiol adducts of cyclopropene acid esters are reported and interpreted. Location of the ring from examination of mass spectra of these derivatives appears to be a straightforward matter.

KEY WORDS cyclopropene fatty acids · diketo fatty acids · thiol adducts · mass spectrometry

BECAUSE OF THE HIGH REACTIVITY of the cyclopropene ring, purification and manipulation of fatty acids that contain this functional group are difficult. If polymerization and addition reactions, which readily take place, are to be minimized, low temperatures and mild conditions are necessary. In studies of the structure and biosynthesis of these fatty acids, use has been made of derived compounds which have greater stability and other useful characteristics. Thus, conversion to the diketo acid by ozonolysis (1, 2), addition of thiols (3, 4), and catalytic reduction to the saturated cyclopropane compounds (5, 6) have proved to be useful procedures. These reactions are summarized in Fig. 1.

The 1,3-diketone system produced by oxidative cleavage of the ring double bond provides increased stability and polarity and can be identified by a color reaction resulting from treatment with ferric ion (1). The diketone can be separated readily from other ozonolysis products by silicic acid chromatography. The diketo acids are crystalline solids with intense absorption at 276 mμ. The esters of various chain lengths can be separated by gas–liquid or reversed-phase chromatography, and mass spectra of the esters provides an excellent method for locating the position of the ring (2). For biosynthetic studies, the 1,3-diketone group provides a point of attack for degradation of the chain (7).

The addition of methanethiol across the ring double bond has been employed by Raju and Reiser (4) for the preparation of distinctive derivatives useful in gas chromatographic separations. The technique is simple and quantitative. The derivatives appear to have high stability and can be recovered from gas chromatographic effluents. The thiol is added randomly to the double bond to give an unresolved mixture of isomers in which the sulfur atom is attached at one or the other end of the original double bond. We have found that mass spectrometric examination of the mixture of thiol addition derivatives makes a definitive assignment of the ring position possible.

Cyclopropene acids can also be reduced to the much less reactive cyclopropane acids by catalytic hydrogenation in the presence of palladium catalyst in alcohol. Further reduction to the ring-opened compounds allows assignment of ring location by mass spectrometry (5, 6), but this process is more complicated than the methods described in this paper for the conversion of cyclopropene acids to diketones and thiol addition products. Furthermore, the natural sources of cyclopropene acids generally yield the analogous cyclopropane acids as well, and separation of these prior to catalytic reduction of the cyclopropenes was, until recently, difficult (8).

The present paper describes mass spectrometric studies on diketo and thiol derivatives of two naturally-occurring cyclopropene acids, sterculic acid and malvalic acid. The data obtained indicate that locating the ring position in other cyclopropene acids would be a straightforward task.

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

* U. S. Public Health Service Predoctoral Fellow.

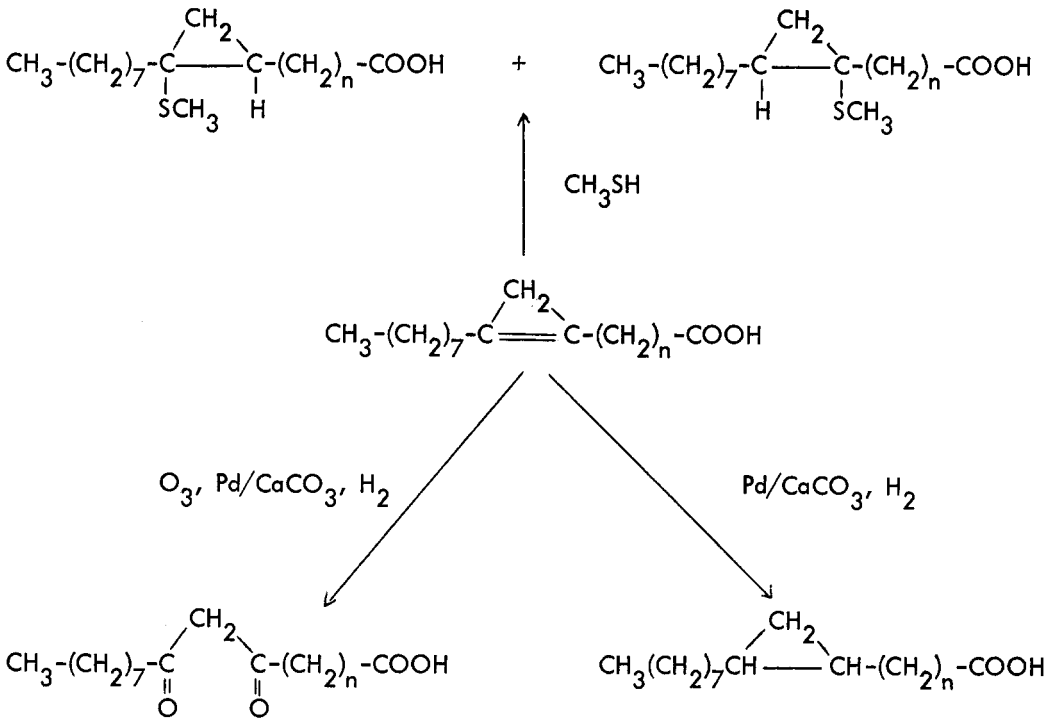


FIG. 1. Derivatives found useful in the characterization of cyclopropene acids. If n = 6, the central formula is malvalic acid; if n = 7, sterculic acid.

MATERIALS AND METHODS

Sterculia foetida seeds were obtained from the Superintendent, Royal Botanic Gardens, Peradenya, Ceylon. *Hibiscus syriacus* seeds were purchased from Harry Saier, Diamondale, Mich. Methanethiol was purchased from Eastman Organic Chemicals, Rochester, N. Y.

Isolation of Fatty Acids

Seeds were ground in a water-cooled Micromill (Chemical Rubber Company, Cleveland, Ohio) and the oily meal was extracted with chloroform–methanol 2:1, 1.5 ml/g. The suspension was filtered and the filter cake was reextracted twice with the same mixture, 1 ml/g. The filtrate was reduced to dryness in vacuo. The yield of crude lipids was about 25% of the weight of the seed meal. Crude lipids were suspended in 10 volumes of 20% KOH in water–methanol 1:1 and the mixture was stirred for 12 hr at 0°C. After the extraction of a nonsaponifiable fraction with pentane, the mixture was acidified at 0°C with HCl and a crude fatty acid fraction was extracted with ether. The ether phase was washed with 1% NaCl and reduced to dryness in vacuo.

Formation of Diketo Acids

Seed fatty acids were subjected to urea fractionation in methanol, a procedure modified from Nunn (1). Fatty acids were freed from the urea complexes by washing with water and extracting the aqueous mixture with ether. Recovery of cyclopropene acids in the filtrate was approximately 60%. Cyclopropene acid content of the enriched fraction was about 80% for *Sterculia* acids and 30% for *Hibiscus* acids as determined by a quantitative Halphen test (9).

Fractions rich in cyclopropene acids were ozonized to a potassium iodide end point in 40 volumes of ethyl acetate at −60°C in a Welsbach ozonizer with a flow rate of 0.5 ft³/hr of 2% O_3 in oxygen. Palladium on carbon (10%) catalyst was added immediately (100 mg/g fatty acid) and the solution was hydrogenated overnight at 0°C. The catalyst was filtered off and the solution was reduced to dryness in vacuo. The oil was extracted three times with 10 volumes of ethyl ether and reduced to dryness. This fraction gave a deep red color with saturated $FeCl_3$–$CHCl_3$ solution, which indicated the presence of β-diketo compounds.

Column Chromatography

Reduced ozonolysis products were put on 3.7 cm × 40 cm columns packed with a 3:1 mixture (w/w) of silicic acid (Mallinckrodt 100 mesh; washed with ethyl ether and hexane) and Celite (Johns-Manville Corp., New York; washed with diethyl ether and hexane) to give a total packing volume of 450 ml. Samples were applied in 2% diethyl ether in petroleum ether (bp 50–60°C); the columns were eluted with successive 4-liter portions of 2, 3, 4, 5, 6, 7, 8, 9, and 10% diethyl ether in petroleum

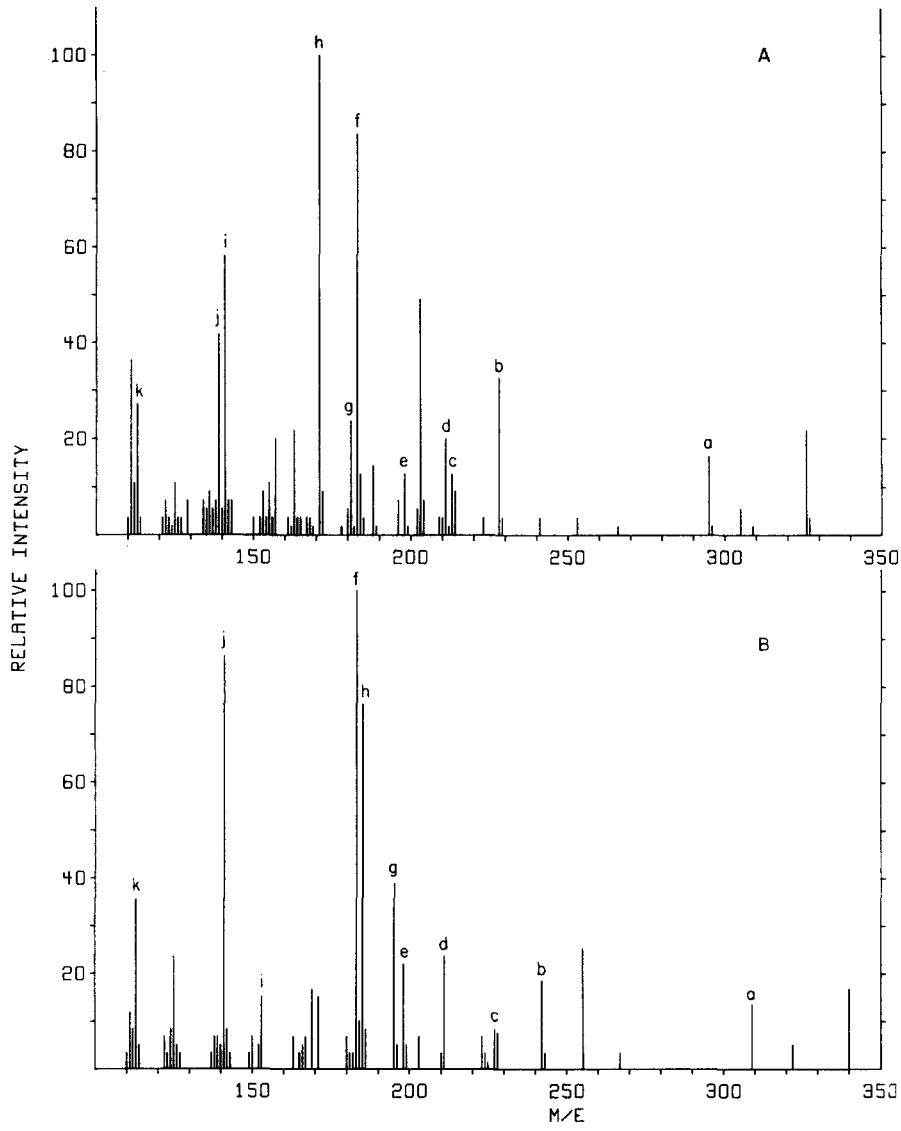


FIG. 2. Portions of mass spectra of diketo fatty acids: A, 18-carbon, and B, 19-carbon compounds.

ether and 500-ml fractions were collected. The $FeCl_3$ test showed that diketo acids were eluted with 8% ether in petroleum ether.

Column fractions were also monitored by a reversed-phase TLC method based on that of Kaufman and Ko (10). One part Reversil (Applied Science Laboratories Inc., State College, Pa.) was slurried with two parts of decane–hexane 15:85 and the mixture was applied to plates. The moving phase (acetic acid–water 7:3) was equilibrated overnight with decane–heptane 15:85 prior to development of plates. This solvent system resolved the C_{18} and C_{19} diketo acids which we detected by spraying the plates with saturated $FeCl_3$–$CHCl_3$ and heating them on a hot plate for 10 sec. This TLC system revealed that column chromatography had partially separated the 8,-10-diketooctadecanoic acid from the 9,11-diketononadecanoic acid. The separations were not complete and the two acids were finally resolved by fractional crystallization from redistilled hexane.

Samples of the diketo acids were converted to their methyl esters by treatment with diazomethane. The esters could be easily separated by GLC on columns of 3.8% SE-30 on Diatoport S at 210°C in an F & M model 402 gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.).

UV spectra of diketo acids in methanol were obtained with a Cary model 15 recording spectrophotometer (Cary Instruments, Monrovia, Calif.). Melting points were taken in capillaries or on a hot stage, and are uncorrected.

Preparation of Thiol Adducts

Fatty acid methyl esters were treated with thiol compounds as indicated by Raju and Reiser (4), and GLC

(see above) was used to prepare pure adducts for mass spectrometry. Mass spectra were obtained with an AEI MS9 instrument. Samples were introduced directly into the ionization chamber by means of a ceramic probe.

RESULTS

Properties of the Diketo Acids

Identical diketo acids were obtained from both *Sterculia* and *Hibiscus* seed acids; the 19-carbon compound predominated in *Sterculia* and 18-carbon acid in *Hibiscus*. The 18-carbon compound had a melting point of 56.0–56.2°C, while the 19-carbon compound melted at 58.0–58.2°C. A mixture of the two compounds melted at 46–54°C. Both compounds had strong absorbance at 276 mμ with molar extinction coefficients of approximately 10^4.

Mass Spectra of Diketo Esters

Parent ions were clearly discernible in the mass spectra of the diketo fatty acids. Portions of the spectra of the 18- and 19-carbon compounds are shown in Fig. 2 A and B. Origins of the most abundant ions in these spectra are summarized in Table 1.

Properties of the Thiol Adducts

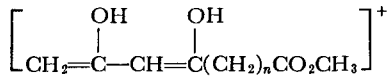
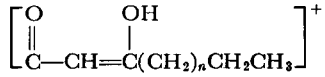
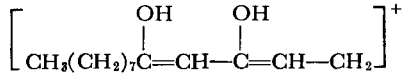
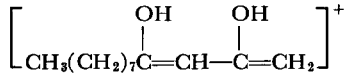
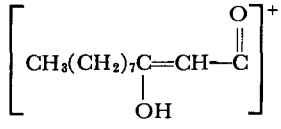
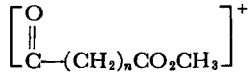
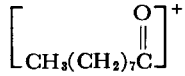
Portions of mass spectra of methanethiol adducts are shown in Fig. 3 A and B. Table 2 summarizes the major peaks of these spectra.

DISCUSSION

Although only malvalic and sterculic acid were available for study, the results of mass spectrometry on derivatives of these compounds indicate the usefulness of this technique for ring location. Quantities of less than 1 mg suffice for conversion to either diketo compounds or thiol adducts. Purification by GLC is convenient and simple, since the derivatives are well separated from normal contaminating acid esters.

The assignments of probable structures for major ions in the spectra of the diketo esters are in accord with the fragmentation behavior of monoketo fatty acid esters

TABLE 1 PROBABLE STRUCTURE AND ORIGIN OF IONS IN DIKETO FATTY ACIDS (FIG. 2)

m/e	Designation in Fig. 2	Probable Structure	Probable Origin
M − 31	a		Loss of CH_3O from parent ion
M − 98	b		Rearrangement ion from loss of last 7 carbons from methyl end of chain*
M − 113	c		Cleavage at carbonyl*
211	d		*
198	e		Rearrangement ion from loss of carbons at carboxyl end*
183	f		Cleavage at carbonyl*
M − 145	g		Loss of CH_3OH from M − 113
M − 155	h		Cleavage at carbonyl
M − 187	i		Loss of CH_3OH from M − 155
141			Cleavage at carbonyl
113	k	$[CH_3(CH_2)_6CH_2]^+$	Cleavage at carbonyl

* Enol structures are written only to indicate groups contained in ions. The spectra give no evidence as to whether keto or enol structures are present.

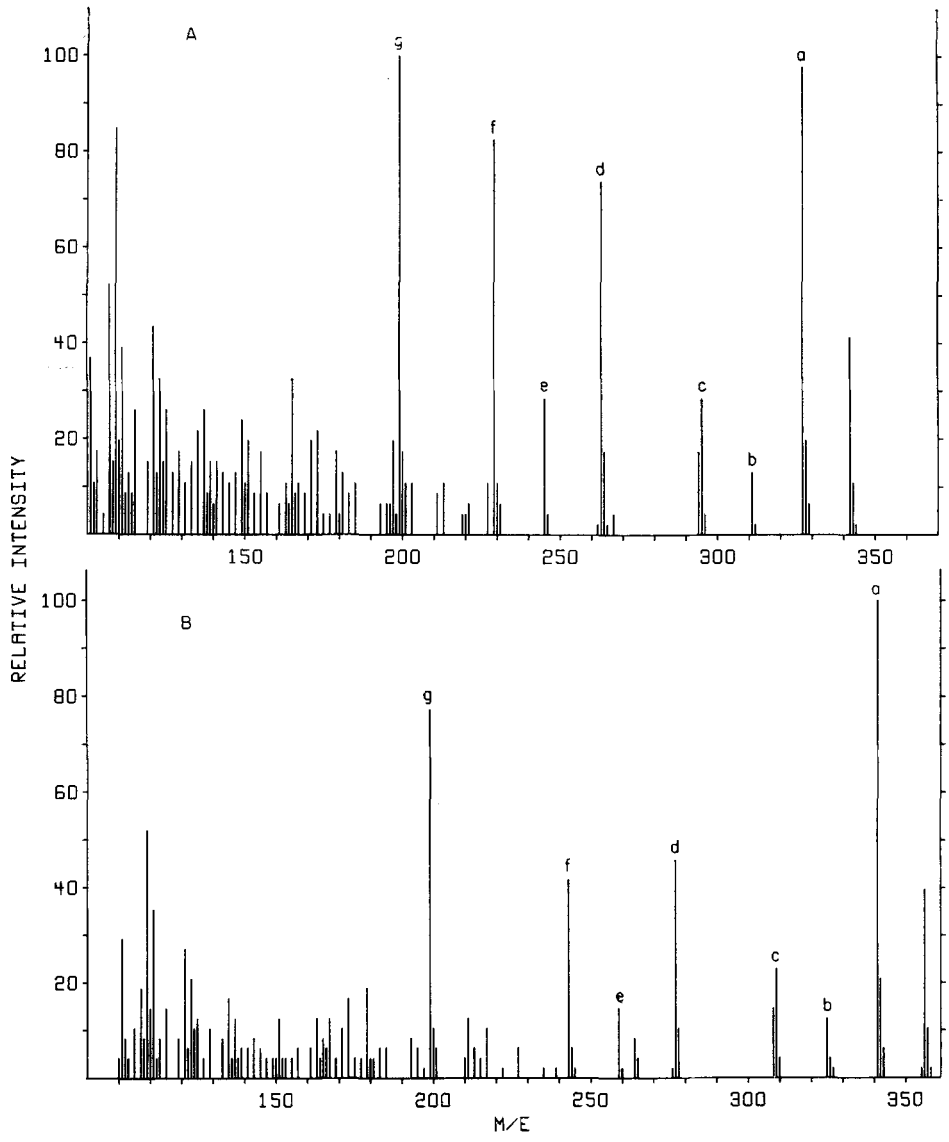


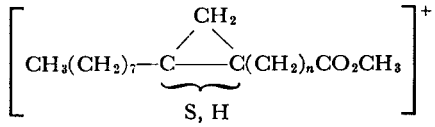
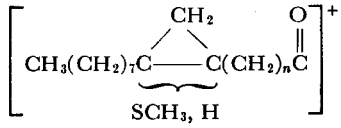
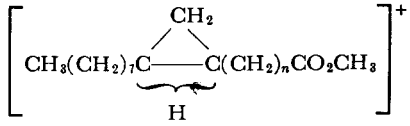
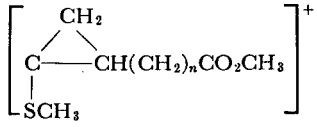
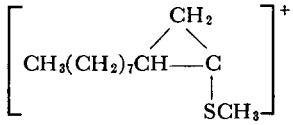
FIG. 3. Portions of mass spectra of methanethiol adducts. A, 18-carbon, and B, 19-carbon compounds.

(11, 12). Thus simple cleavage at the carbonyl group gives ions c, f, h, j, and k, while rearrangements of the McLafferty type (13) give ions b and e. Loss of methanol from the parent ion and ions c and h give a, g, and i, respectively (12).

Assignment of the major peaks in the thiol compounds is based upon the behavior of ordinary fatty acid esters (11, 12) and of thiol ethers (14). The indication in Table 2 that the cyclopropane ring is still intact should not be taken too seriously, for the ring has probably opened in these ions. The proposed sequence, ion c → ion d → ion e, is strengthened by metastable peaks in each spectrum (in Fig. 3 A, m/e 295 → m/e 263, calcd 234.5, found 234.3; m/e 263 → m/e 245, calcd 228.2, found 228.4; in Fig. 3 B, m/e 309 → m/e 277, calcd 248.3, found 248.4; m/e 277 → m/e 259, calcd 242.2, found 242.3). In addition, accurate mass measurements on the parent ion and ions a, b, f, and g (Table 2) were in accord with the proposed empirical formulas.

In addition to the methanethiol derivatives, we have prepared and examined the ethanethiol, methyl 2-thiopropanoate, N-acetylamino-2-thioethane, and N-acetyl cysteine methyl ester adducts of sterculic acid. All of these can be purified as the methyl esters by GLC. The mass spectra are more complicated than those of methanethiol, but the major ions are completely in accord with the interpretations of the spectrum of the methanethiol adduct. Here also, accurate mass measurements on major ions and the predicted location of metastable peaks help to confirm structure assignments for these compounds. The cysteine derivative may prove useful in experiments to test the hypothesis that sterculic acid

TABLE 2 PROBABLE STRUCTURE AND ORIGIN OF IONS IN METHANETHIOL ADDUCTS (FIG. 3)

m/e	Designation in Fig. 3	Probable Structure	Probable Origin
M − 15	a		Loss of CH_3
M − 31	b		Loss of methoxyl
M − 47	c		Loss of SCH_3
M − 79	d		Loss of CH_3OH from M − 47
M − 97	e		Loss of H_2O from M − 79
M − 113	f		Cleavage at ring with loss of 8 carbons from the methyl end
199	g		Cleavage at ring with loss of the carbonyl end of the chain

readily attaches itself to an essential thiol group in the fatty acid desaturase (3, 15). The mass spectra of all of these compounds lend considerable support to the structures of thiol derivatives proposed by Kircher (3) and by Raju and Reiser (4, 15). We will be pleased to supply these spectra to interested workers who request them.

The spectra were prepared in their present form by a computer program and plotter by courtesy of Dr. George Waller.

This work has been supported by a grant from the National Institute of General Medical Sciences, GM 13863.

Manuscript received 31 August 1967; accepted 1 November 1967.

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