Mass spectral discrimination between monoenoic and cyclopropanoid, and between normal, iso, and anteiso fatty acid methyl esters

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ABSTRACT Measurement of the ratio of the intensity of the parent molecular ion to that of the parent molecular ion minus 32 (the mass of methanol) can distinguish between monoenoic and cyclopropanoid fatty acid methyl esters with the same carbon number. A similar technique can be used to distinguish between isomeric normal, iso, and anteiso fatty acid methyl esters.

SUPPLEMENTARY KEY WORDS intensity ratios · GLC–mass spectrometry · isomers

THE FULL VALUE of combined gas–liquid chromatography–mass spectrometry in studying complex biological mixtures will probably be achieved only when it is possible to characterize completely each component in a given mixture on the basis of the information obtained from a single injection into the GLC–MS unit. At present there are several impediments to the realization of such an ideal state of affairs. A major one is the frequently encountered difficulty of choosing between alternative structural isomeric solutions to the mass spectral data.

The purpose of the current investigation was to gain greater knowledge of the fragmentation characteristics of isomeric long-chain fatty acids. A method has been found for discriminating between monoenoic and cyclopropanoid fatty acid methyl esters. The same method serves for distinguishing normal, iso, and anteiso fatty acid methyl esters.

Abbreviations: GLC, gas–liquid chromatography; GLC–MS, combined GLC–mass spectrometry.

METHODS

Gas–liquid chromatography was performed on an F & M model 402 gas chromatograph equipped with a hydrogen flame detector. The 6 ft (2 m) glass column was packed with 3% OV-1 (methyl silicone polymer) on acid-washed and silanized Gas-Chrom S, 100–120 mesh. GLC–mass spectrometric data were obtained from an LKB 9000 combined GLC–mass spectrometer working at the indicated electron voltages with an electron current of 60 μA, an accelerating voltage of 3.5 kv, a molecule separator temperature of 250°C, and an ion source temperature of 270°C. The 2 m glass column was packed with 3% OV-1 on silanized Gas-Chrom Q, 60–80 mesh.

Samples of the methyl esters of palmitoleic acid (16:1), oleic acid (18:1), 11-eicosenoic acid (20:1), erucic acid (22:1), and nervonic acid (24:1) were obtained from Supelco, Inc., Bellefonte, Pa. The corresponding cyclopropanoid derivatives were synthesized from these monoenoic esters by the method of Christie and Holman (1). Samples of methyl heptadecenoate (17:1) and methyl nonadecenoate (19:1) were obtained from the lipids of *Mycobacterium phlei* (2). Methyl *cis*-9,10-methylene-octadecanoate was the gift of Dr. A. Chung.

We obtained the fatty acid methyl esters of *Escherichia coli* by saponifying the cellular paste with methanolic KOH extracting the acidic materials formed, and methylating them with ethereal diazomethane.

Samples of normal, iso, and anteiso fatty acid methyl esters were obtained from Supelco, Inc. and Applied Science Laboratories, Inc., State College, Pa. Samples of fatty acid methyl esters with methyl branches at

TABLE 1 P/(P–32) Intensity Ratios for Methyl Oleate and Methyl *cis*-9,10-Methylene-octadecanoate at Various Electron Energies

	Inlet by	Electron Energies				
		14	15	17	20	70
Methyl oleate	Probe	53/100	41/100	31/100	29/100	22/100
	GLC	35/100	30/100	24/100	19/100	15/100
Methyl *cis*-9,10-methylene-octadecanoate	Probe	11/100	10/100	9/100	8/100	8/100
	GLC	8/100	7/100	7/100	5/100	5/100

TABLE 2 Intensity Ratios $P/(P-32)_{17ev}$* for Monoenoic and Cyclopropanoid Fatty Acid Methyl Esters of Carbon Number 16–25

Carbon Number of Fatty Acid and Position of *cis* Double Bond or Ring	Monoene	Cyclopropane
16 (9, 10)	32/100	—
17 (9, 10)	36/100†	9/100
18 (9, 10)	25/100	—
19 (9, 10)	18/100†	8/100
20 (11, 12)	18/100	—
21 (11, 12)	—	8/100
22 (13, 14)	15/100	—
23 (13, 14)	—	7/100
24 (15, 16)	12/100	—
25 (15, 16)	—	7/100

* Designates that the ratio was measured with an electron energy of 17 ev.

† The two materials were obtained from lipids of *M. phlei*. The position and stereochemistry of the double bond are uncertain.

intermediate points in the carbon chain were obtained from the lipids of *M. phlei* (2).

RESULTS

Monoenoic and Cyclopropanoid Fatty Acid Esters

In their paper on the mass spectrometry of cyclopropanoid fatty acid esters, Christie and Holman (1) confirmed the observation of Wood and Reiser (3) that the spectra of the methyl esters of monoenoic and cyclopropanoid fatty acids were "similar." Published bargraphs corroborated this statement; the 80 ev spectra of methyl oleate and methyl *cis*-9,10-methylene-octadecanoate showed the same general fragmentation pattern. Notwithstanding this "similarity," there were some apparent differences in the relative ion intensities. In particular, it could be seen that, whereas in methyl oleate the parent ion (P) had 30% of the intensity of the P–32 ion, in the cyclopropanoid derivative the corresponding value was 15%. The obvious questions now arose—were such differences in the P/(P–32) intensity ratio reproducible, and if so, could they be made the basis of a method of discriminating between monoenoic and cyclopropanoid fatty acid methyl esters?

We first confirmed the findings deduced from the results of the work of Christie and Holman. The values of the intensity ratio, P/(P–32), for methyl oleate and methyl *cis*-9,10-methylene-ocadecanoate were determined with electron energies in the range 14–70 ev; the value for the oleate was always significantly higher than that for the 9,10-methylene-octadecanoate (Table 1). This difference was independent of whether samples were introduced into the mass spectrometer by direct probe or via the GLC inlet system. The values obtained by the former method, however, were invariably higher by a few per cent—a fact attributable to variation in the ion source temperature.[1]

To determine whether this difference in intensity ratio was a general phenomenon or solely a characteristic of the two compounds studied, we measured the intensity ratio, P/(P–32), for the methyl esters of the monoenoic fatty acids, 16:1, 18:1, 20:1, 22:1, and 24:1, and compared them with the ratios for the methyl esters of the corresponding cyclopropanoid fatty acids C_{17}, C_{19}, C_{21}, C_{23}, and C_{25}. Spectra were obtained via the GLC inlet system and were recorded at 17 ev since this voltage gave the best agreement between GLC and direct probe measurements (Table 1).

The comparison of intensity ratios is shown in Table 2. Values for a methyl heptadecenoate and a methyl nonadecenoate are also included. From these and other measurements, we can conclude that, in the range C_{16}–C_{25}, monoenoic esters invariably have a higher $P/(P-32)_{17ev}$ intensity ratio [(10–40)/100] than the cyclopropanoid isomers [(5–11)/100]. This difference tends to decrease with increasing carbon number.

To test the utility of the P/(P–32) intensity ratio method in solving structural problems in a natural mixture, we studied the fatty acid methyl esters of *E. coli*. This organism is known to produce C_{16} and C_{18} monoenoic fatty acids together with C_{17} and C_{19} cyclo-

[1] As will be fully considered in a future paper, P/(P–32) intensity ratios vary with ion source temperature, but not with molecule separator temperature. Thus, with a constant separator temperature of 250°C, methyl oleate has $P/(P-32)_{17ev}$ ratios of 48/100, 41/100, 39/100, 31/100, 28/100, and 24/100 at source temperatures of 170, 190, 210, 230, 250, and 270°C, respectively. When the source temperature is held constant at 270°C and the separator temperature is varied from 150 to 250°C, methyl oleate has a constant $P/(P-32)_{17ev}$ ratio of 24/100. We appreciate the suggestion of a reviewer to include these data in this paper.

propanoid fatty acids (4). The GLC trace of the methylated fatty acids of *E. coli* is shown in Fig. 1. GLC-MS indicated that components 1–4 had the retention time, molecular weight, and gross fragmentation pattern of 16:1, 17:1, 18:1, and 19:1 methyl esters, respectively. However, the $P/(P-32)_{17ev}$ intensity ratios, namely 29/100, 8/100, 23/100, and 8/100 clearly indicated that components *1* and *3* were monoenes while components *2* and *4* were cyclopropanes.

Normal, Iso, and Anteiso Fatty Acid Esters

Ryhage and Stenhagen (5) have shown previously that in anteiso fatty acid methyl esters the P–29 ion is more intense than the P–31 ion. This is not so for either normal or iso esters. Because the considerably more intense P–43 ion is generated by elision of the α, β, and γ carbon atoms—i.e., by activity remote from the point of methyl branching in iso and anteiso esters—we determined whether the intensity ratio (P–29)/(P–31)/(P–43) is characteristic of the three series of compounds.

These ratios, measured at 17 ev (Table 3), can be seen to characterize members of the same isomeric series, and as before, ranges of expected values can be established. For methyl esters of fatty acids of carbon number 14–24 they are:

normal (16–28)/(32–60)/100;
iso (10–20)/(16–36)/100;
anteiso (32–58)/(16–38)/100.

The intensity ratios of fatty acid methyl esters with a methyl branch near the middle of the carbon chain resemble those of the iso series (Table 3). Mass spectrometric methods to distinguish between these two series of esters already exist (5).

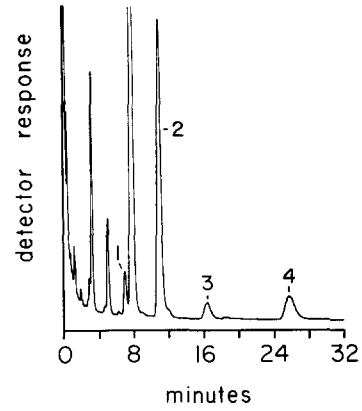

FIG. 1. GLC trace of fatty acid methyl esters from *Escherichia coli*. 3% OV-1 at 170°C. Compounds *1–4* all have retention times of esters of monoenoic acids (16:1–19:1), but *2* and *4* were shown by the intensity ratio method to be cyclopropanoid instead.

DISCUSSION

As a result of the work of Ryhage and Stenhagen (5, 6) and that described in this paper, it should now be possible to characterize unambiguously from a single injection into a GLC–MS unit the normal and monomethyl substituted fatty acid methyl esters, and to discriminate between monoenoic and cyclopropanoid fatty acid methyl esters. The value of this has already been demonstrated (2). The obvious extension of the work, namely the unambiguous determination by GLC–MS of position and stereochemistry in monoenoic and cyclopropanoid fatty acid esters without recourse to the formation of derivatives (7–9) is being further considered.

In this investigation doublet and triplet intensity ratios have been used to characterize specific compounds. This may prove a useful principle in GLC–MS

TABLE 3 $(P-29)/(P-31)/(P-43)_{17ev}$ INTENSITY RATIOS FOR METHYL ESTERS OF REPRESENTATIVE NORMAL, ISO, AND ANTEISO FATTY ACIDS

Carbon Number of Fatty Acid	Normal	Iso	Anteiso	Mid-Branched
14	19/47/100	11/19/100		
15	23/52/100		41/32/100	
16	25/53/100	19/28/100		
17	22/46/100		50/25/100	
18	20/46/100	15/19/100		22/29/100*
19	20/43/100		40/17/100	16/25/100†
20	21/43/100	17/22/100		
21			41/20/100	28/24/100‡
22	18/40/100	15/21/100		
23			32/20/100	15/21/100§
24	18/38/100	16/22/100		

Each value is the mean of at least six determinations.
* 9-Methylheptadecanoate.
† 10-Methyloctadecanoate.
‡ 12-Methyleicosanoate.
§ 14-Methyldocosanoate.

in which one is always faced with the problem that sample pressure—and consequently ion intensity—are continuously changing throughout the spectral run. It may be of more routine value to record and report the general fragmentation pattern of a molecule—e.g., that it loses units of mass 15, 18, 42, etc.—together with accurately measured intensity ratios of one or more groups of neighboring peaks, then to deal with bargraphs of the relative intensity of all the ions covered by the spectral scan. The former approach has the added advantage of being more readily incorporated into computerized data-processing systems.

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