Identification of monoacyl- and monoalkylglycerols by gas–liquid chromatography–mass spectrometry using polar siloxane liquid phases

J. J. Myher, L. Marai, and A. Kuksis

Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, Toronto, Canada M5G 1L6

Abstract A comparative study was made of the spectra obtained after gas–liquid chromatography–mass spectrometry of the trimethylsilyl ethers of 1- and 2-monoacyl- and monoalkylglycerols. The glycerol derivatives were resolved on the basis of positional substitution and the degree of unsaturation on Silar-5CP (a cyanopropylphenylsiloxane) liquid phase, and the peaks were examined in a Varian MAT CH-5 single-focusing mass spectrometer. The 1-monoacyl- and 1-monoalkylglycerols possessed *m/e* M − 103 and *m/e* 205, respectively, as unique peaks, while the 2-monoacyl- and 2-monoalkylglycerols contained *m/e* 218 as the highly favored fragment. These differences were large and consistent enough to serve as a basis for identification and quantitation of the isomers in a mixture. The saturated and unsaturated monoacyl- and monoalkylglycerols differed markedly in the kind and intensity of the base peaks and other major fragments. In view of the effective gas–liquid chromatographic resolution of these compounds, the marked differences in the spectra of the trimethylsilyl ethers of the saturated and unsaturated species provided a distinct advantage for their identification by the gas–liquid chromatography–mass spectrometry system.

Supplementary key words 1-alk-1-enylglycerols · cyanopropylphenylsiloxane · quantitative analysis · positional isomers · saturated and unsaturated species · trimethylsilyl ethers

The GLC properties of monoacyl- (1, 2) and monoalkylglycerols (3) have been extensively studied, and improved conditions have recently been reported (4) for the separation of both positional isomers and saturated and unsaturated derivatives of short and long chain lengths. Likewise, the mass spectra of various monoacyl- (5) and monoalkylglycerols (6, 7) have been examined in detail, and characteristic differences between the positional isomers and among various homologs have been noted. Although several of the derivatives used for mass spectrometry are also suitable for GLC, no analyses using the combined technique have yet been reported for these glycerols.

The present work demonstrates that combined GLC–MS analysis of monoacyl- and monoalkylglycerols as the TMS ethers has major advantages over mass spectrometry with direct probe sampling. All the advantages are related to the improved homogeneity and structural purity of the sample made possible by the high resolving power of the polar siloxane liquid phase.

MATERIALS AND METHODS

Synthetic 1-, 2-, and 3-monoacyl-*sn*-glycerols of the common fatty acids were gifts from Dr. D. Buchnea or were purchased from Serdary Research Laboratories Inc., London, Canada, and were more than 95% single isomer. Mixtures of natural monoacylglycerols of better than 99% single isomer purity were prepared by Grignard degradation of corn, linseed, and cod liver oil triacylglycerols and TLC of the products on borate-treated silica gel, as previously described (4).

Chimyl, batyl, and selachyl alcohols, grade II, were obtained from Sigma Chemical Co., St. Louis, Mo. These materials were made up of the 1-monoalkylglycerols of saturated 16- and 18- and monounsaturated 18-carbon fatty chains along with smaller amounts of near homologs. Synthetic 2-monopalmitylglycerol and 2-monooleylglycerol were purchased from Serdary Research Laboratories and were more than 99% single isomer. Natural 1-alk-1-enyl glyceryl ethers were isolated from the total phosphatidylethanolamines of rabbit skeletal muscle by lithium aluminum hydride degradation and TLC (8). Details of the GLC properties of these materials have been described elsewhere (4).

For combined GLC–MS examination, the various monoacyl- and monoalkylglycerols were converted into the trimethylsilyl ethers as previously described (4). In order

Abbreviations: GLC, gas–liquid chromatography; GLC–MS, combined gas–liquid chromatography and mass spectrometry; TLC, thin-layer chromatography; TMS, trimethylsilyl.

TABLE 1. Equivalent chain lengths (ECL) of TMS ethers of monosubstituted glycerols[a]

Acid or Alcohol	Derivative of Glycerol			
	1- or 2-Alkyl	1-Alk-1-enyl[b]	2-Acyl	1-Acyl
16:0[c]	16.00	16.00	18.59	19.01
18:0	18.00	18.00	20.60	21.02
18:1	18.35	18.32	20.90	21.32
18:2			21.43	21.85
18:3			22.08	22.50

[a] 3% Silar-5CP at 200°C. All values are on a scale defined by the saturated alkyl homologs. 2 ECL units correspond to a retention ratio of 1.79.

[b] 1-Alk-1-enyl ethers are eluted ahead of alkyl ethers of corresponding carbon number when run as TMS ethers on SE-30 columns. The separation factor is 1.12 (4).

[c] Number of carbon atoms: number of double bonds.

to avoid isomerization, the monoacylglycerols were silylated with pyridine–hexamethyldisilazane–trimethylchlorosilane 12:5:2 (v/v/v).

The GLC–MS analyses were made with a Varian MAT CH-5 single-focusing mass spectrometer coupled to a Varian 2700 gas chromatograph and a Varian 620/i computer. The GLC was done with 180 cm × 2 mm ID stainless steel columns that contained about 30 cm of 3% OV-1 (a methyl siloxane) packing at the outlet end and 150 cm of a 3% Silar-5CP packing in the rest of the column. Other GLC columns of similar dimensions were packed with 3% Silar-5CP or 3% OV-1 alone. All the packings were from Applied Science Laboratories and contained Gas-Chrom Q (100–120 mesh) as the support. The carrier gas was helium at 10 ml/min. The GLC was done at 200°C isothermally, with the injector at 225°C and the transfer line at 250°C. The mass spectrometer was operated at an ionization voltage of 70 eV, an accelerating voltage of 3000 V, electron emission of 100 μA, and an ion source temperature of 270°C. The Watson-Biemann separator was kept at 270°C. Scanning was made at 4 sec/decade at a resolution of 800–1000. All spectra taken over the GLC peaks were corrected for total ion current variation. Also, spectra were taken of the column bleed and were subtracted from the spectra of the solutes by the computer, using Varian module SUB.

RESULTS AND DISCUSSION

Monoacylglycerols

Table 1 gives the GLC data on the TMS ethers of the monosubstituted glycerols. The equivalent chain length values indicate a complete resolution between any two solutes differing by more than 0.3 unit. Details of the GLC resolution of monoacyl- and monoalkylglycerols have been presented elsewhere (4).

TABLE 2. Mass spectra of TMS ethers of monoacylglycerols

Fragment Ion	Positional Isomer	Monoacylglycerols			
		16:0	18:0	18:1	18:2
		relative peak intensity			
M	1	0.8	1.3	14	12
	2		0.6	3	
M − 15	1	14	11	22	15
	2	11	12	5	
M − 90	1	2	4	47	32
	2	4	5	16	
M − (71 + 90)	1	2	5	7	4
	2	16	13	8	
M − 103	1	100	100	94	54
	2	2			
M − (103 + 90)	1			13	17
	2				
Acyl	1	20	22	24	12
	2	9	5	3	
Acyl − 1 (M − 236)	1			5	27
	2			2	
Acyl − 15	1				
	2				
Acid + 1	1	1			
	2	1			
Acid	1				
	2				
Acid − 1	1				2
	2			1	
218	1	2	4	3	6
	2	100	100	26	
205	1	14	14	19	12
	2	2	2	1	
203	1	12	23	32	18
	2	20	17	14	
201	1	2	5	28	2
	2	2	3	10	
191	1			2	3
	2	18	14	7	
147	1	29	23	58	50
	2	28	33	18	
129	1	12	26	100	100
	2	28	71	62	
103	1	12	14	48	39
	2	28	29	100	
73	1	34	38	99	51
	2	48	35	58	
69 (C_5H_9)	1	7	10	31	5
	2	5	5	12	
67 (C_5H_7)	1	4	5	23	45
	2	4	2	7	
57 (C_4H_9)	1	27	26	17	2
	2	25	22	5	
55 (C_4H_7)	1	14	15	51	14
	2	15	13	22	

Monoacylglycerols are identified by their component fatty acids; positional isomers refer to the 1(3)- and 2-positions in the glycerol molecule.

Table 2 summarizes the significant peaks in the GLC–MS spectra of the TMS ethers of 1- and 2-isomers of monopalmitoyl-, monostearoyl-, monooleoyl-, and monolinoleoylglycerols. The general nature of the spectra is very similar to that recorded by Johnson and Holman (5), who examined the TMS ethers of monoacylglycerols

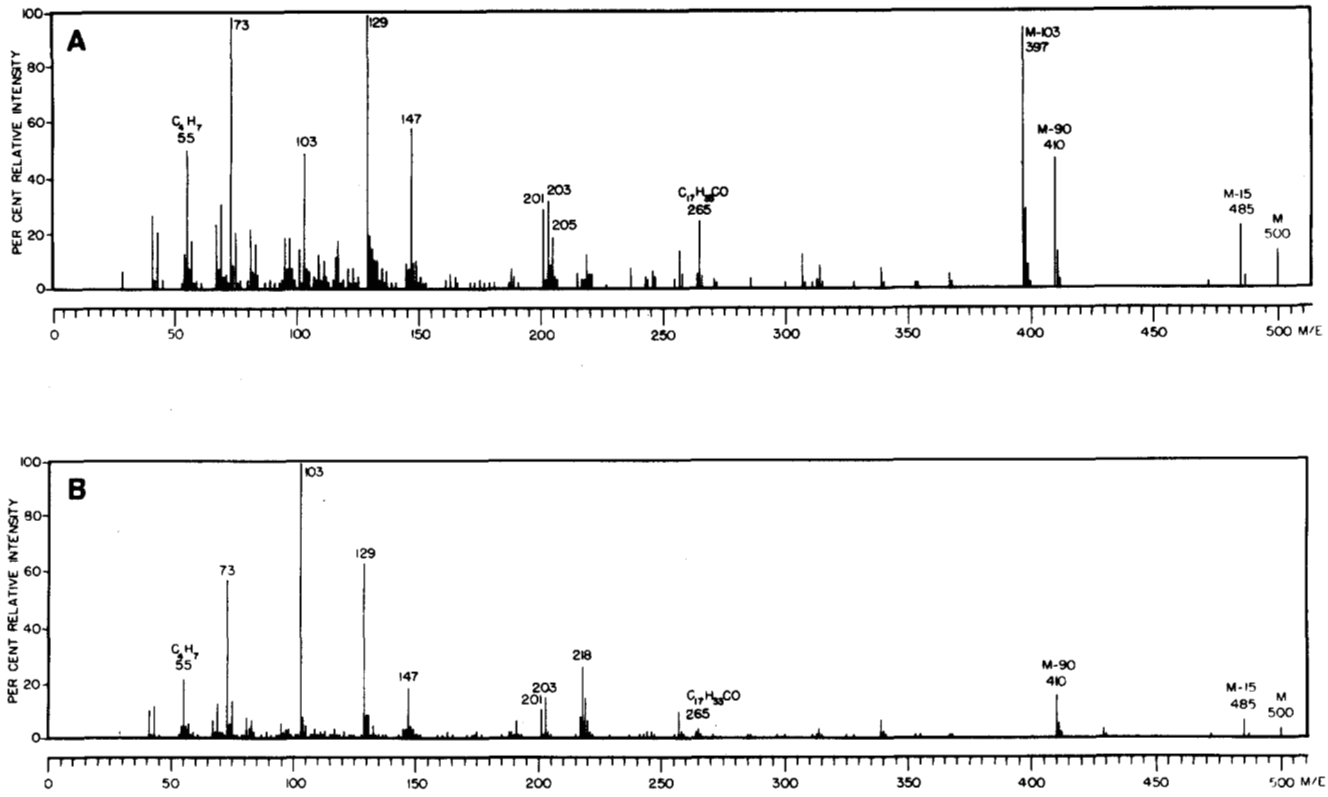

Fig. 1. GLC–MS spectra of TMS ethers of monooleoylglycerols as obtained with Silar-5CP. *A*, 1-monooleoylglycerol; *B*, 2-monooleoylglycerol. Operating conditions as given in text. Sample: 1 μl of a 1% solution of TMS ethers of mixed monooleoylglycerols in silylation mixture.

by direct probe mass spectrometry and concluded that this method can be used to distinguish isomers. The present study based on GLC purification and resolution of the isomers prior to mass spectrometry confirms the conclusions of Johnson and Holman (5) and demonstrates that the fragment M − 103 (*m/e* 371 for the TMS ether of 1-monopalmitoylglycerol) appears only in 1-monoacylglycerols. There was no unique fragment for the 2-monoacylglycerols, but *m/e* 218 was highly favored in all samples examined. The spectra of saturated 1-isomers had a 218 peak of less than 4% of the intensity of the 2-isomers. Other differences between the mass spectra of the 1- and 2-monoacylglycerols were seen in the proportions of the various other major intensity peaks. The 1-monoacylglycerols also gave a significantly higher proportion of the molecular ion. The peaks corresponding to acid and acid + 1 ions were very small or were absent from our runs, whereas Johnson and Holman (5) observed that some of these fragments contributed up to 18% of the total. **Fig. 1** compares the GLC–MS spectra of the TMS ethers of 1- and 2-monooleoylglycerols. The intensities of ions at *m/e* 73 and those corresponding to *m/e* 129 were about equal in all unsaturated 1-monoacylglycerols, whereas in the 2-monoacylglycerols only the ions *m/e* 73 and *m/e* 129 were of comparable intensity. The present study confirms the prediction of Johnson and Holman (5) that unsaturated monoacylglycerols would show the highest parent ion intensities. There were marked differences in the spectra of oleoyl- and linoleoylglycerols, but the contributions of the positional isomers to a mixture could still be calculated by reference to the characteristic intensity peaks. **Fig. 2** shows the reconstituted GLC elution pattern of the TMS ethers of 1-monooleoyl- and 2-monolinoleoylglycerols from a polar siloxane column on which these compounds overlap. The species can be resolved on the basis of molecular weight using the abundant M − 90, corresponding to M − $Si(CH_3)_3OH$ ions at *m/e* 410 and *m/e* 408, respectively. The contributions of each of the species to the total at each time of scanning was assessed by calibration of the appropriate intensities. The incompletely separated pair 1-monostearoylglycerol and 2-monooleoylglycerol could also be resolved by choosing characteristic peaks for each species.

It is possible that the less effective differentiation between the 1- and 2-monoacylglycerols in the direct probe studies was due to the presence of minor amounts of the 1-isomer in the 2-isomer and vice versa. Trimethylsilylation may bring about a partial isomerization of the monoacylglycerols, which require GLC for complete resolution (4). However, differences in the instrumentation and in the mode of entry could have also been responsible for the minor discrepancies.

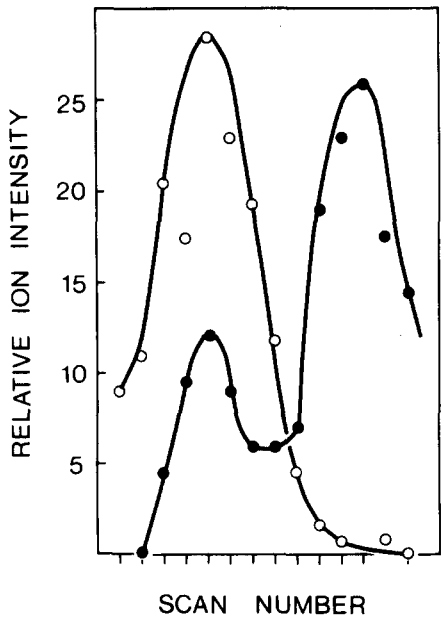

Fig. 2. Reconstituted GLC elution pattern of TMS ethers of 1-monooleoylglycerol (*open circles*) and 2-monolinoleoylglycerol (*closed circles*) from Silar-5CP. The contribution of each molecular species to the common GLC peak was determined by repeated scanning and measurement of the M − 90 fragment in the mass spectrometer. GLC–MS conditions as given in text. Sample: 1 μl of a 1% solution of TMS ethers of mixed monoacylglycerols in silylation mixture.

Monoalkylglycerols

Complete GLC–MS spectra were also recorded for the TMS ethers of the monoalkylglycerols, and **Table 3** provides a summary of the significant peaks in the 1- and 2-isomers of monopalmityl-, monostearyl-, and monooleylglycerols. The general nature of the spectra of the 1-monoalkylglycerols when run as the TMS ethers is similar to that recorded by Hallgren and Larsson (6), who examined the dimethoxyderivatives of various 1-monoalkylglycerols by direct probe mass spectrometry. There have been no previous studies of the mass spectrometric behavior of the 2-monoalkylglycerols. Like their monoacyl counterparts, the 1- and 2-isomers of the monoglyceryl ethers exhibit distinct differences in their fragmentation patterns. The major ones are the location of the base peak at *m/e* 218 in the 2-isomer and at *m/e* 205 in the 1-isomer. Furthermore, the *m/e* 218 peak does not appear in the 1-isomer at all, while that of *m/e* 205 appears in the 2-isomer to the extent of only 10–15%. Another peak appearing exclusively in the 2-isomer is the *m/e* 191 ion. There are other lesser differences in the proportion of the peak intensities between the 1- and 2-monoalkylglycerols. Thus, the saturated 2-isomer possessed distinctly less of the M − 15 and M − 90 peaks and more of the M − 104 peaks. Like the monoacylglycerols, the TMS ethers of the saturated monoalkylglycerols gave no or very little molecular ion. In contrast to the saturated species, the monounsaturated monoalkylglycerols exhibited a prominent molecular ion, which was especially intense in the 2-isomers. A much higher proportion of molecular ion in the unsaturated than in the saturated monoalkylglycerols has been previously reported by Hallgren and Larsson (6); this again is consistent with the behavior of the monoacylglycerols. In addition to the characteristic differences in the location of the base peaks in the unsaturated 1- and 2-monoalkylglycerols, there were also differences in the distribution and intensity of the *m/e* 191 and M − (103 + 90) fragments. The saturated and unsaturated monoalkylglycerols also differed in the fragmentation patterns of the hydrocarbon chains, as already noted for the TMS ethers of the

TABLE 3. Mass spectra of the TMS ethers of monoalkylglycerols

Fragment Ion	Positional Isomer	Monoalkylglycerols 16:0	18:0	18:1
		relative peak intensity		
M	1			13
	2			30
M − 15	1	7	2	4
	2	2		3
M − 90	1	8	2	2
	2	1		1
M − 104	1	3	1	2
	2	10		11
M − (73 + 74)	1	13	4	1
	2	2		1
M − (90 + 90)	1	5	1	2
	2			
M − (103 + 90)	1			7
	2			25
M − 235	1	2		
	2	2		3
218	1			
	2	100		100
205	1	100	100	100
	2	11		15
191	1			
	2	15		19
147	1	34	25	47
	2	15		28
133	1	39	27	14
	2	83		39
131	1	20	7	21
	2	12		21
130	1	37	18	12
	2	33		47
129	1	12	4	19
	2	23		48
117	1	31	22	33
	2	52		44
103	1	15	5	25
	2	28		87
73	1	34	22	59
	2	38		73
57 (C_4H_9)	1	26	18	22
	2	39		40
55 (C_4H_7)	1	12	10	39
	2	16		53

Monoalkylglycerols are identified by their component fatty chains; positional isomers refer to the 1- and 2-positions in the glycerol molecule; M − 235 ion corresponds to the alkyl hydrocarbon radical.

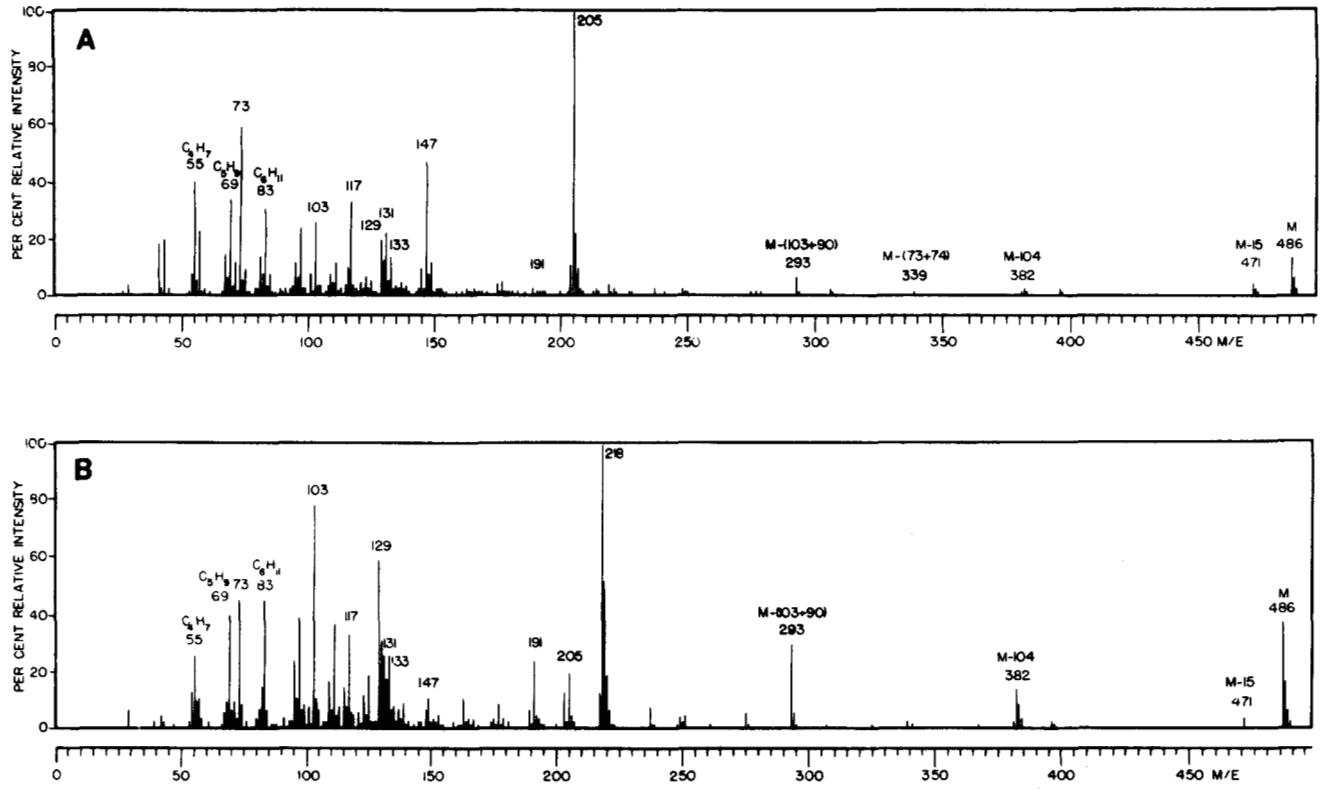

Fig. 3. GLC–MS spectra of TMS ethers of monooleylglycerols as obtained with Silar-5CP. *A*, 1-monooleylglycerol; *B*, 2-monooleylglycerol. Operating conditions as given in text. Sample: 1 μl of a 1% solution of TMS ethers of mixed monooleylglycerols in silylation mixture.

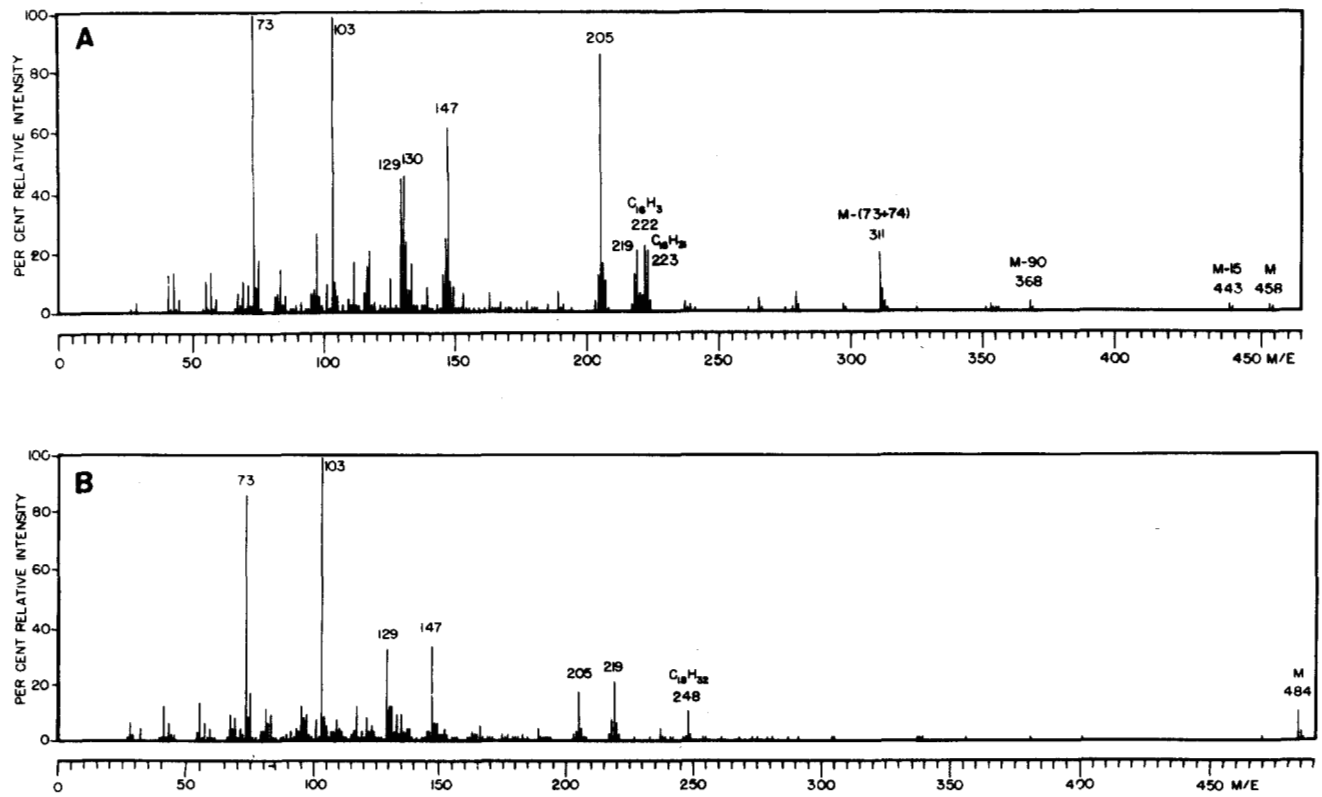

Fig. 4. GLC–MS spectra of TMS ethers of alkenylglycerols as obtained with Silar-5CP. *A*, 1-hexadec-1-enylglycerol; *B*, 1-octadecadi-1,9-enylglycerol. Operating conditions as given in text. Sample: 1 μl of a 1% solution of mixed 1-alk-1-enylglycerols in silylation mixture.

TABLE 4. Mass spectra of TMS ethers of 1-monoalk-1-enylglycerols

Fragment Ion	Monoalkenylglycerols[a] 16:0A[b]	18:0A[b]	18:1A[b]
	relative peak intensity		
M	2	0.8	10
M − 15	2	1	1
M − 90	3	2	
M − (73 + 74)	7	3	2
M − (89 + 90)	6	2	1
M − 236	22	10	10
219	5	2	2
205	85	45	18
147	62	56	33
130	46	35	12
129	44	39	33
103	98	94	100
73	100	100	85

[a] The 1-monoalk-1-enylglycerols are identified by the number of carbons and double bonds in their fatty chains. M − 236 ion represents the [alkenyl hydrocarbon − 1] fragment.

[b] "A" denotes the alkenyl function of the different monoalkenylglycerols.

saturated and unsaturated monoacylglycerols. The most abundant hydrocarbon fragment in the saturated species was the saturated C_4H_9 ion at *m/e* 57, while in the monounsaturated alkylglycerols the C_4 to C_7 monounsaturated ions at *m/e* 55, 69, 83, and 97 were the most abundant. **Fig. 3** compares the GLC–MS spectra of the monooleylglycerols.

Monoalkenylglycerols

Fig. 4 compares the GLC–MS spectra of the TMS ethers of 1-hexadec-1-enylglycerol (16:0A) and 1-octadecadi-1,9-enylglycerol (18:1A), and **Table 4** gives a summary of the major fragments of the saturated and unsaturated monoalkenylglycerols examined in the present study. These molecules undergo a much more extensive disintegration under the impact of electron bombardment. The two compounds have relative intensities of 80–100 for both the *m/e* 73 and *m/e* 103 fragments, which represent the $Si(CH_3)_3$ and the $CH_2Si(CH_3)_3$ radicals. Nevertheless, the alkenyl ethers containing the second double bond in the chain exhibit a more pronounced molecular ion and reduced *m/e* 205 ion. Some of the differences in the spectra can be rationalized on the basis of previous experience with the interpretation of the mass spectra of saturated and unsaturated monoacyl- and monoalkylglycerols. There have been no previous reports on the fragmentation patterns of the alkenylglycerols, although cyclic acetals of the plasmalogen type have been examined (9). It is obvious that the mass spectra recorded for the TMS ethers of the alkenylglycerols differ markedly from those of the cyclic acetals and can be used to distinguish between them and fatty acid methyl esters and dimethylacetals as well as monoacyl- and monoalkylglycerols. The 1-alk-1-enylglycerols possess a characteristic but small fragment at *m/e* 219 that is totally absent in 1-alkylglycerols although present in 2-alkylglycerols due to an isotope contribution from the 218 peak. The abundant $CH=CH(CH_2)_nCH_3$ ion at M − 236 from all alkenylglycerols may also be distinguished from the similar trace ion at M − 235 from alkylglycerols. On the other hand, the ion at *m/e* 117 is abundant only in alkylglycerols. These fragments may serve as a means of quantitative estimation of both alkenyl- and alkylglycerol components in a glycerol ether peak in which they may occur together. Monounsaturated alkyl ethers are separated on a Silar-5CP column from species having only the alkenyl double bond. However, the *m/e* M − 236 alkenylglycerol ion could still serve to identify any alkenyl component. Both alkyl- and alkenylglycerols are well resolved from acylglycerols by GLC on Silar-5CP. The differences between the TMS ethers of the alkenyl acetals and cyclic acetals produced by acid catalysis are best seen from the molecular ion of these molecules and the fragments indicative of the two TMS ether groups in the alkenyl ether molecules. The identity of the alkenyl ethers in this study was substantiated by an essentially quantitative conversion of these compounds into the dimethylacetals, which could be effectively identified by GLC–MS as described elsewhere (10). In the absence of appropriate 2-isomers of the alkenyl ethers, it is not possible to report whether or not mass spectrometry can distinguish between the 1- and 2-isomers of the alkenylglycerols, but on the basis of the present work one would expect a very large *m/e* 218 or *m/e* 219 and a much reduced *m/e* 205 in the 2-isomer.

The present study demonstrates that a combined GLC–MS analysis has distinct advantages over mass spectrometry with direct probe sampling for the identification of monoacylglycerols. It provides pure derivatives of single positional isomers, which cannot be achieved by direct probe sampling without extensive prior purification of the derivatives. The TMS ethers that are best suited for mass spectrometry of the monoacylglycerols cannot be effectively purified except by GLC. As a result of the systematic and essentially complete resolution of the various positional isomers and unsaturation homologs of monoacylglycerols on the Silar-5CP columns, it was possible to recognize clearly the fragments that were characteristic of each positional isomer. The results obtained with the 1- and 2-monoacylglycerols were fully supported by the data derived from analyses of the corresponding alkylglycerols. The *m/e* M − 103 fragment appeared only in the 1-monoacylglycerols, and the *m/e* 205 only in 1-monoalkylglycerols, while the fragment *m/e* 218 was greatly favored in the 2-monoacyl and 2-monoalkyl isomers. In all instances the GLC–MS analysis provides the additional

possibility of clearly differentiating between saturated and unsaturated species. The system is suitable for the determination of monoacyl- and monoalkylglycerols in natural mixtures.

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