Pyrrolidides for Mass Spectrometric Determination of the Position of the Double Bond in Monounsaturated Fatty Acids

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ABSTRACT

Mass spectra of pyrrolidides of monounsaturated straight chain fatty acids are presented and discussed. The spectra of pyrrolidides contain mainly ions from the polar part of the molecule. This gives simple spectra from which double bond positions can be deduced directly. If an interval of 12 atomic mass units is observed between the most intense peaks of clusters of fragments containing n and n-1 carbon atoms of the acid moiety, the double bond occurs between carbons n and n+1 in the molecule This rule is valid for double bonds occurring at positions Δ^5-Δ^{15} in an 18-carbon chain and has been applied to acids having 10-24 carbon atoms.

INTRODUCTION

The location of double bonds in fatty acids by mass spectrometry has been approached in many ways which have been summarized in reviews (1, 2). Under electron impact, double bonds have a tendency to migrate (3), so it has not been possible previously to locate the unsaturation directly without chemical modification at the double bond. Vetter, et al., (4) suggested a derivatization of fatty acids with pyrrolidine as a possible solution, because the amide group has a charge stabilization effect upon the fatty acid moiety. Recently Bohlmann and Zdero (5) studied the mass spectra of piperidides to deduce the structure of naturally

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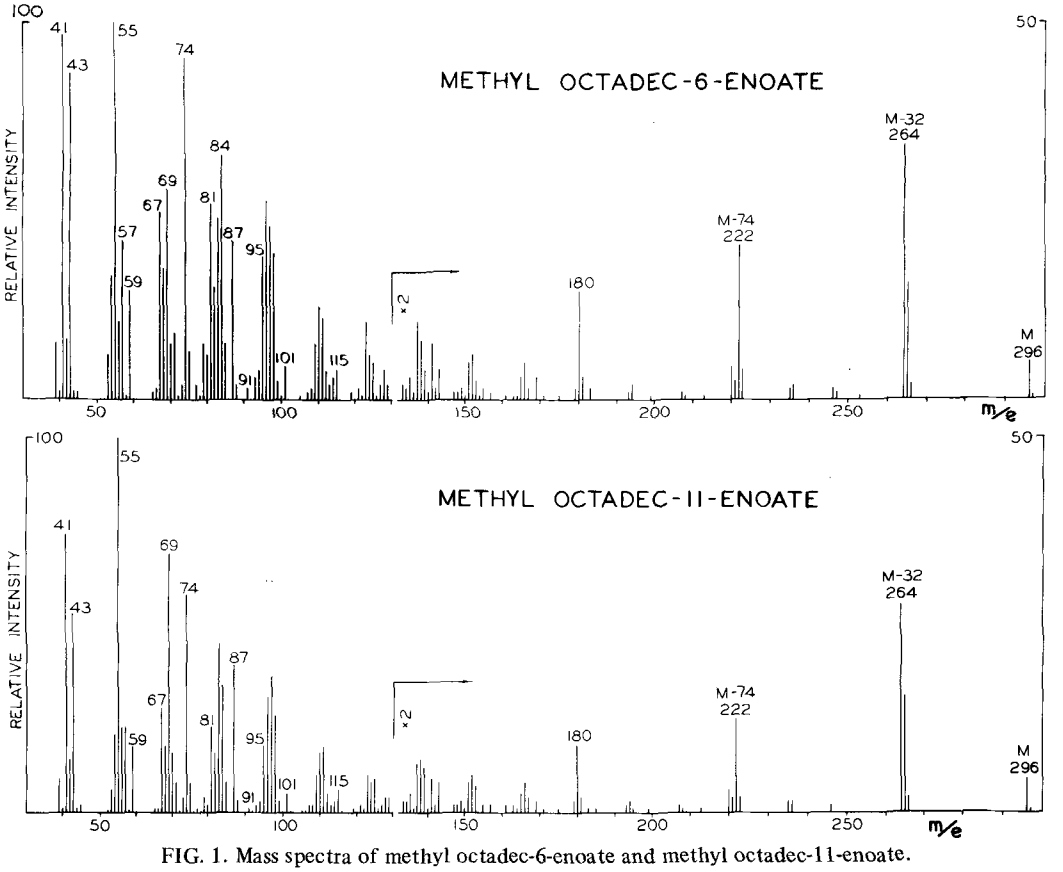


FIG. 1. Mass spectra of methyl octadec-6-enoate and methyl octadec-11-enoate.

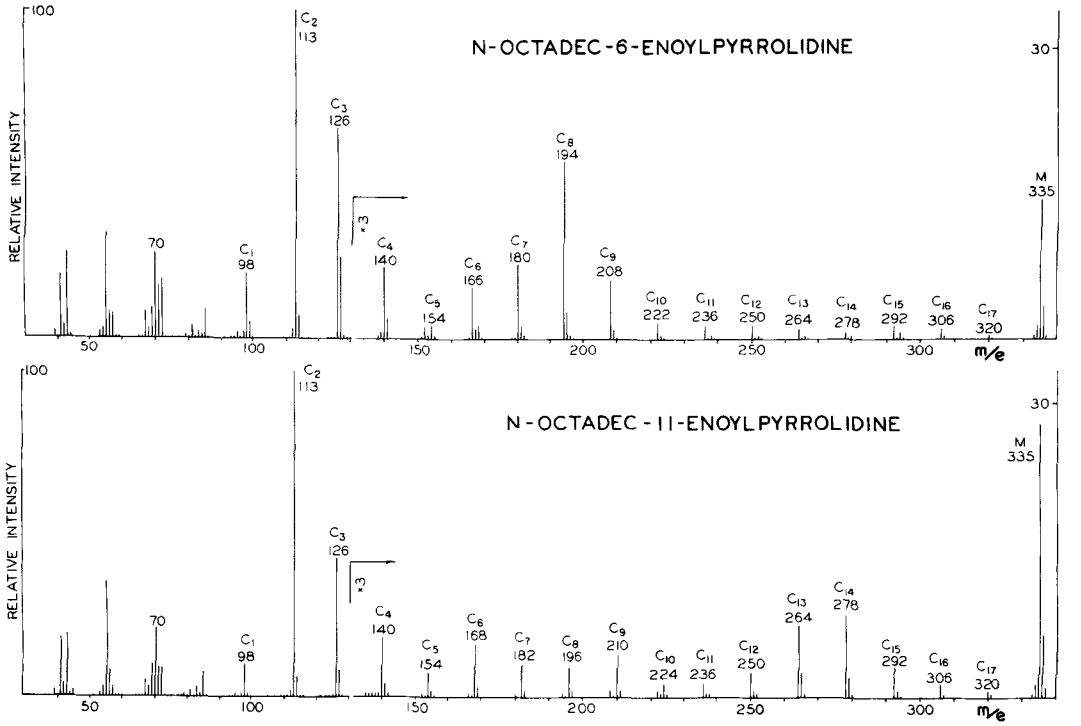


FIG. 2. Mass spectra of N-octadec-6-enoylpyrrolidine and N-octadec-11-enoylpyrrolidine.

occurring amides of conjugated unsaturated compounds. We report here a study of the low resolution mass spectra of pyrrolidine derivatives of a series of isomeric straight chain unsaturated fatty acids which confirms and implements the prediction of Vetter (4).

EXPERIMENTAL METHODS

Most of the methyl esters of the unsaturated fatty acids were supplied by the Lipids Preparation Laboratory, The Hormel Institute, Austin, Minn., although a few of the octadecenoic acid isomers were obtained from the preparations of Gunstone and Ismail (6).

The pyrrolidides were prepared in a quantitative yield on a microscale in the following way: 10 μl fatty acid methyl ester was dissolved in 1 ml freshly distilled pyrrolidine (Aldrich Chemical Co., Milwaukee, Wisc.) and 0.1 ml acetic acid. The mixture was heated to 100 C in a sealed tube for half an hr and cooled to room temperature. The conversion from methyl ester to amide is followed conveniently by gas liquid chromatography (GLC). The amide so formed is taken up in methylene chloride and washed with dilute hydrochloric acid and with water. After drying with magnesium sulfate, evaporation, and purity check by thin layer chromatography (TLC), the amide was ready for mass spectrometry (MS).

The mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6D single focusing instrument operating at an ionization potential of 70 eV. The samples were introduced through an all glass heated inlet system at 175 C. The gas chromatograph was a Barber Coleman 5000 instrument equipped with an all glass 1.80 m x 2 mm column containing 3% OV-1 on Chromosorb W (HP), 80-100 mesh. Column temperature was 230 C and the flow 30 ml argon/min. The GLC-MS combination used GLC conditions the same as mentioned above. The pyrrolidine reaction mixture was injected directly onto the column and unreacted methyl ester separated readily from the pyrrolidine derivative (4).

RESULTS AND DISCUSSION

The mass spectra of the methyl ester and of the pyrrolidide of the same saturated fatty acid show similar cleavage patterns in the high mass region with peaks 14 atomic mass units apart derived from fragmentations at each bond (4). If the fatty acid is unsaturated, the spectrum of the methyl ester becomes more complicated, whereas the spectrum of the pyrrolidide remains simple.

Mass spectra of the methyl esters of petro-

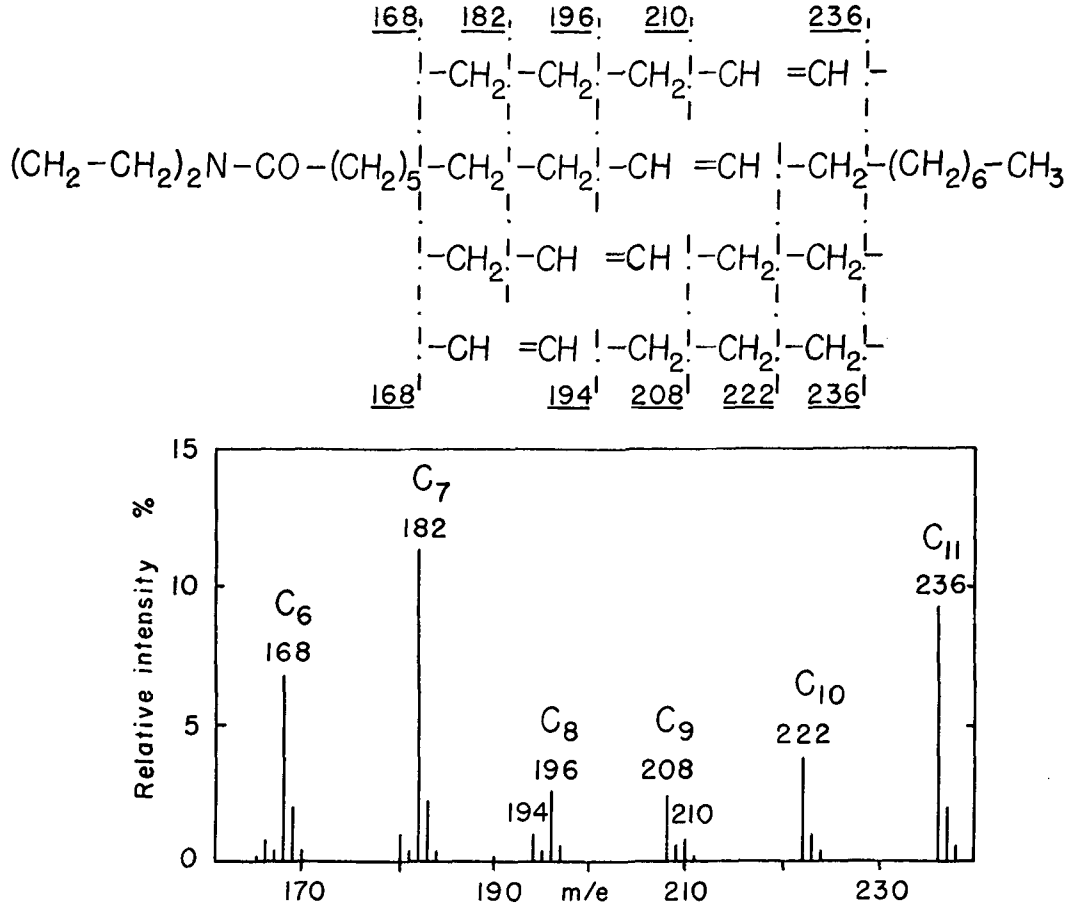


FIG. 3. Postulated fragmentations in N-octadec-9-enoylpyrrolidine and in its isomers in which the double bond has migrated after electron impact, shown above the partial spectrum obtained from N-octadec-9-enoylpyrrolidine.

selinic acid (6-18:1) and vaccenic acid (11-18:1) (7) are very much the same (Fig. 1) and very similar to the spectra of other isomeric monounsaturated fatty acid methyl esters (8-11 and B.Å. Andersson and R.T. Holman unpublished results).

If one compares the spectra of the pyrrolidide derivatives of the two acids (Fig. 2), there are clear differences. The spectra of both isomers show very pronounced fragments containing the polar part of the molecule (12, 13). No other main fragmentations disturb this pattern. In the case of the 6-18:1 isomer, the molecular ion *m/e* 335 yields a series of ions *m/e* 320, 306, 292, 278, 264, 250, 236, 222, 208, 194, 180, 166, 154, 140, 126, 113, and 98. In the case of the 11-18:1 isomer, the series is *m/e* 320, 306, 292, 278, 264, 250, 236, 224, 210, 196, 182, 168, 154, 140, 126, 113, and 98. Considering 6-18:1 and only the most prominent ion of each cluster, the interval between the fragments containing 5- and 6-carbon atoms from the fatty acid moiety (*m/e* 154 and 166) is 12 atomic mass units. For the 11-18:1 isomer, the same interval of 12 atomic mass units occurs between fragments which include carbons 10 and 11 of the fatty acid (*m/e* 224 and 236). From the mass spectra of all isomers of 18:1 from Δ^5-Δ^{15}, the following rule has been formulated: *If an interval of 12 atomic mass units, instead of the regular 14, is observed between the most intense peaks of clusters of fragments containing n and n-1 carbon atoms of the acid moiety, a double bond occurs between carbon n and n+1 in the molecule.* The 4-, 16- and 17-18:1 isomers have unique fragments that identify them. The characteristic ions are listed for each isomer in Table I. The mass spectra do not distinguish *cis*- from *trans*-isomers, exemplified in the case of the 9-18:1 isomers.

The simple cleavage pattern for the pyrro-

TABLE I

Key Fragments in the Spectra of Pyrrolidides of Monounsaturated Fatty Acids

Pyrrolidide	*m/e*	Relative intensity	*m/e*	Relative intensity	*m/e*	Relative intensity	*m/e*	Relative intensity	*m/e*	Relative intensity	*m/e*	Relative intensity	Molecular peak *m/e*	Molecular peak Relative intensity
cis-4-18:1	124	1.8	126	10.8	138	2.9	139	4.2	152	13.9	166	57.0	335	17.2
cis-5-18:1	126	6.3	138	.3	140	.6	152	.5	166	1.6	180	2.5	335	5.3
cis-6-18:1	140	7.7	152	1.5	154	1.7	166	5.6	168	1.5	180	8.1	335	14.3
cis-7-18:1	154	9.8	166	2.5	168	4.4	180	6.5	182	2.5	194	7.2	335	16.6
cis-8-18:1	168	13.2	180	2.0	182	4.5	194	2.4	196	.9	208	4.2	335	18.1
cis-9-18:1	182	11.4	194	1.0	196	2.5	208	2.3	210	.7	222	3.6	335	24.8
trans-9-18:1	182	11.0	194	1.2	196	2.4	208	2.2	210	.8	222	3.6	335	24.0
cis-10-18:1	196	7.4	208	1.0	210	2.0	222	1.8	224	.7	236	3.3	335	29.8
cis-11-18:1	210	4.9	222	1.1	224	1.6	236	1.5	238	.7	250	2.7	335	29.0
cis-12-18:1	224	4.8	236	.7	238	1.5	250	1.2	252	.8	264	3.0	335	33.8
cis-13-18:1	238	4.5	250	.6	252	1.3	264	1.1	266	.5	278	2.9	335	33.0
cis-14-18:1	252	3.9	264	.4	266	.9	278	1.2	280	.5	292	2.6	335	28.8
cis-15-18:1	266	3.1	278	.5	280	1.0	292	1.3	306	2.4	320	2.9	335	28.4
cis-16-18:1	266	2.3	278	.6	280	3.3	292	1.1	294	.7	306	1.2	335	26.0
cis-17-18:1	280	.9	292	.6	294	1.6	306	.4	308	.2	320	.4	335	8.1
cis-4-10:1	124	3.5	126	6.9	138	6.0	139	6.1	152	26.0	166	99.0	223	33.5
cis-9-14:1	182	12.0	194	1.4	196	2.7	208	2.6	210	1.0	222	4.0	279	28.0
cis-9-16:1	182	11.0	194	1.4	196	2.2	208	3.3	210	.8	222	3.8	307	26.0
cis-11-20:1	210	4.4	222	.9	224	1.8	236	1.4	238	.9	250	2.7	363	28.0
cis-13-22:1	238	4.4	250	2.1	252	2.8	264	2.0	266	1.2	278	4.2	391	37.0
cis-15-24:1	266	2.3	278	.6	280	1.1	292	1.0	294	.6	306	2.9	419	34.3

TABLE II

Metastable Peaks and Proposed Fragmentation Pathways of Pyrrolidides of 6-18:1 and 11-18:1

Petroselinic pyrrolidide		Vaccenic pyrrolidide		Fragmentation pathway
Calculated	Found	Calculated	Found	
305.7	305.8	305.7	305.8	335 → 320
279.5	279.6	279.5	279.7	335 → 306
254.5	254.6	254.5	254.6	335 → 292
230.7	230.9	230.7	230.9	335 → 278
208.1	208.2	208.1	208.3	335 → 264
186.6	186.8	186.6	186.8	335 → 250
166.3	166.5	166.3	166.4	335 → 236
		149.8	150.0	335 → 224
147.1	147.3	147.1	147.2	335 → 222
		131.6	131.8	335 → 210
129.2	129.4			335 → 208
		114.7	114.7	335 → 196
112.3	112.3			335 → 194
		98.9	99.0	335 → 182
96.7	96.9			335 → 180
		84.3	84.5	335 → 168
82.3	82.4			335 → 166
47.4	47.5	47.4	47.6	335 → 126
38.1	38.2	38.1	38.2	335 → 113

lidides can be explained in the following way: Metastable peaks (Table II) strongly indicate a direct cleavage from the molecular ion to each principal fragment in a cluster, all including the pyrrolidide group. However, no metastables were detectable for stepwise degradations. The double bond seems to move before the fragmentation occurs, preferentially towards the polar part of the molecule by one or more steps, as is shown in Figure 3 for oleoylpyrrolidide. However, a competitive fragmentation can occur if the amine group is removed (*m/e* 265) and ions of type $R\text{-}C{\equiv}O^+$ are formed. This fragmentation should be of minor influence (12) but must be investigated by high resolution MS.

If positional isomers were present in the pyrrolidide, their fragmentation patterns would contribute to these peaks in the spectrum which we interpret to be caused by isomerization under electron impact. Therefore, methyl octadec-6-enoate and the pyrrolidide derived from it were both ozonized and reduced to aldehydes (14). GLC of the products revealed dodecanal to be the only significant aldehyde product arising from the hydrocarbon end of the molecule. The two preparations had the same proportions of minor products occurring in the vicinity of possible homologous aldehydes, indicating that formation of the pyrrolidide had not measurably isomerized the acid moiety. This conclusion was confirmed by the observations that pyrrolidides synthesized via the acid chloride or via carbodiimide coupling had the same mass spectra as pyrrolidide synthesized as described above. Moreover, other tertiary amides of the same unsaturated acid have the same fragmentation pattern as shown here for pyrrolidides (B.Å. Andersson, W.H. Heimermann, and R.T. Holman, unpublished data). Thus, the conditions during the formation of the pyrrolidide do not appear to shift the double bond nor explain the ions which we believe to arise from isomerization under electron impact.

The 4-18:1 isomer has its characteristic series of fragments *m/e* 126, 139, 152. The 15- and 16-18:1 isomers have almost identical fragmentation patterns, the only difference being that in the case of the 15-18:1 isomer, the fragment, including the carbon atom in position 14 (*m/e* 280), is smaller than the fragment with 1 carbon less (*m/e* 266); and, for the 16-18:1 isomer, the intensities of the mentioned fragments are in reverse order. The 17-18:1 isomer has its series of fragments *m/e* 294, 306, and 320 which distinguishes it from the other isomers.

The rules developed on the isomers of 18:1 are true for other unsaturated acids. The spectra of the pyrrolidides of 4-10:1, 9-14:1, 9-16:1, 11-20:1, 13-22:1, and 15-24:1 were all interpretable by the same rules (Table I). The base peak in all spectra of the pyrrolidides is *m/e* 113. It is formed by a McLafferty rearrangement (Fig. 4) which was proven by Duffield and Djerassi (12) who used deuterium labeling of very short chain fatty acids. We have

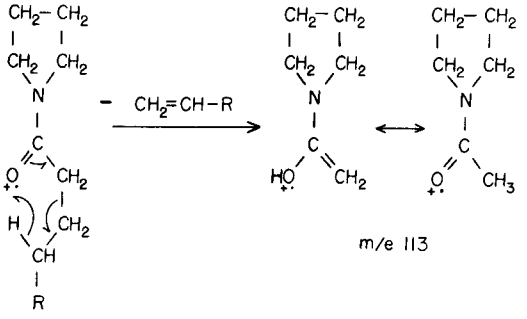
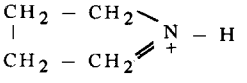
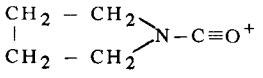


FIG. 4. McLafferty rearrangement in a fatty acid pyrrolidide.

confirmed this rearrangement by study of the spectrum of the pyrrolidide of 4,4-dideuterio octadecanoic acid. A metastable peak at *m/e* 38.1 indicates also the pathway $335^+ \rightarrow 113^+ + 222$. This peak moves to *m/e* 38.3 for 9,12-18:2, indicating the rearrangement $333^+ \rightarrow 113^+ + 220$. The fragment *m/e* 70 has been shown to have the structure (12):



and the *m/e* 98 fragment to be:



Other major peaks in the low mass region derived mainly from cleavage of the pyrrolidide ring have been discussed by other authors (12, 13).

Pyrrolidides offer several advantages for the structural analysis of fatty acids. The derivative is prepared easily quantitatively in a one step reaction on less than mg quantities. By derivatizing the carboxyl group, the reaction is equally quantitative regardless of the number of double bonds or other groups in the molecule. Solubility problems set no limits on the extent of the reaction as is the case with oxidative derivatization of polyunsaturated acids. In our hand, preparation of pyrrolidides of polyunsaturated acids having up to four double bonds offered no difficulties. The rules for interpreting mass spectra of pyrrolidides apply to a wide range of isomers and homologs of monoenoic acids. In unpublished work from this laboratory, these rules have been applicable to dienoic and trienoic acids. Mass spectra of pyrrolidides of acetylenic, cyclopropane-, branched, deuterated, and other fatty acids are more easily interpreted than are spectra of corresponding methyl esters, because only one fragmentation pattern occurs. Thus, pyrrolidides may become a general analytical tool, permitting GLC-MS structural analysis of a wide range of structures occurring in a single sample, minimizing purification steps and derivatization procedures.

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