Mass Spectrometric Determination of Positions of Double Bonds in Polyunsaturated Fatty Acid Pyrrolidides

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ABSTRACT

Low resolution mass spectra of pyrrolidides of isomeric octadecadienoic acids and other polyunsaturated straight chain fatty acids are presented and discussed. The spectra of the pyrrolidides contain mainly ions from the polar part of the molecule and give spectra that are specific for each isomer. The interpretation follows, in most cases, the rule developed for monounsaturated fatty acid pyrrolidides.

INTRODUCTION

The location of double bonds in fatty acids by mass spectrometry has been approached in many ways which have been reviewed by Zeman, et al., (1,2). Pyrrolidides recently were suggested as suitable derivatives for mass spectrometry of unsaturated fatty acids (3), and the mass spectra of a series of pyrrolidides of monoenoic fatty acids recently was investigated (4). Later work showed that several tertiary amides (5,6) give easily interpretable mass spectra from which the double bond position could be deduced easily by the rule developed for pyrrolidides of monoenoic fatty acids (4). Of the amides studied, pyrrolidides were most suitable (6) for further investigation of fatty acids. This paper discusses the mass spectra of pyrrolidides of some polyenoic fatty acids with special reference to the isomeric series of methylene interrupted octadecadienoic fatty acids.

EXPERIMENTAL PROCEDURES

Methyl esters of methylene interrupted 18:2 fatty acids were obtained from the preparations of Christie and Holman (7); the *cis,cis*-9,15-18:2 and *trans,trans*-9,11-18:2 were submitted by H.J. Dutton; and the remaining polyenoic fatty acids were prepared by the Lipids Preparation Laboratory, The Hormel Institute, Austin, Minn. The pyrrolidides were prepared in quantitative yield on a microscale by a procedure described previously (4). Mass spectra were obtained on Hitachi Perkin-Elmer RMU-6D and LKB 9000 single focusing instruments operating at an ionization potential of 70 eV.

The samples were introduced through an all glass heated inlet system at 170-190 C (RMU-6D) or via a gas liquid chromatography-mass spectrometry (GC-MS) combination (LKB 9000) at 240 C. The GLC was performed on a F & M model 810 instrument furnished with an 8 ft x 1/8 in. aluminum column packed with 10% Silar 10C on 100/120 Gas Chrom Q (Applied Science Laboratories, State College, Pa.). Column temperatures were kept isothermal between 230 and 250 C, and the flow rate was 15 ml argon/min. The GLC conditions on the LKB 9000 instrument were: a 6 ft x 1/8 in. glass column filled with 3% OV-1 on Chromosorb W (HP) 80/100 (Applied Science Laboratories) at 230 C and helium as carrier gas.

RESULTS AND DISCUSSION

All mass spectra showed simple cleavage patterns, as was the case with monoenoic acids (3,4), with the base peak *m/e* 113 obtained through a McLafferty rearrangement (3). Each fragment in the high mass region was derived through a direct cleavage from the molecular ion which was indicated by mestastable ions (4). The molecular ions varied in intensity from 7% (5,8-14:2) to 47% (9,12-18:2) of the base peak. When the 18:1 series was compared with the methylene interrupted 18:2 series, it was obvious that the patterns of intensities of the molecular ions were similar and were governed by the positions of the double bonds. Thus, the lowest intensities for the molecular ions of the two series occurred for 5-18:1 (5.3%) and 5,8-18:2 (8.8%). In the 18:2 series, each isomer gave a fragmentation pattern which distinguished it from other isomers. Key fragments for all compounds investigated are given in Table I. In Figure 1, the spectra of *cis,cis*-6,9-18:2 and *cis,cis*-9,12-18:2 are shown. Here, the double bonds were indicated by the intervals of 12 atomic mass units (amu) within the regular series of 14 amu intervals according to the rule, which was formulated in a previous paper (4), for pyrrolidides of monoenoic fatty acids: *"If an interval of 12 atomic mass units, instead of*

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TABLE I

Key Fragments in Spectra of Pyrrolidides of Polyunsaturated Fatty Acids

Pyrrolidide	m/e	Rel. int.[a]	m/e	Rel. int.	m/e	Rel. int.	m/e	Rel. int.	m/e	Rel. int.	m/e	Rel. int.	m/e	Rel. int.	m/e	Rel. int.	m/e	Rel. int.	Molecular peak m/e	Molecular peak Rel. int.
cis,cis-4,7-18:2	124	1.3	126	3.3	138	1.3	139	2.3	152	11.0	153	7.8	166	4.7	178	2.4	192	3.5	333	16.0
cis,cis-5,8-18:2	140	1.1	152	.7	154	.6	166	2.9	168	1.1	178	.6	180	1.2	192	.5	206	.8	333	8.8
cis,cis-6,9-18:2	154	3.8	166	3.9	168	3.2	178	1.0	180	10.0	192	1.0	194	6.3	206	2.4	220	3.8	333	27.0
cis,cis-7,10-18:2	168	4.5	180	4.7	182	3.3	192	1.0	194	13.0	206	1.4	208	6.5	220	2.7	234	4.5	333	28.0
cis,cis-8,11-18:2	182	5.4	194	2.9	196	2.3	206	1.6	208	15.0	220	2.4	222	7.4	234	3.2	248	3.1	333	43.0
cis,cis-9,12-18:2	196	3.4	208	2.8	210	1.9	220	1.9	222	10.0	234	2.7	236	6.8	248	2.4	262	3.1	333	47.0
cis,cis-10,13-18:2	210	2.4	222	1.9	224	1.6	234	1.9	236	7.8	248	1.5	250	6.8	262	1.7	276	2.3	333	40.0
cis,cis-11,14-18:2	224	2.4	236	1.6	238	1.8	248	1.2	250	6.0	262	1.4	264	5.8	276	1.9	290	2.7	333	41.0
cis,cis-12,15-18:2	238	2.4	250	1.4	252	1.5	262	1.2	264	5.9	265	2.7	290	2.2	291	.7	304	3.0	333	45.0
cis,cis-13,16-18:2	238	5.2	250	1.0	252	1.9	262	.9	264	1.5	265	.6	290	3.5	291	1.0	304	1.8	333	43.0
cis,cis-14,17-18:2	252	5.4	260	.8	262	.8	264	.8	276	1.0	278	1.3	290	1.3	292	4.4	304	1.6	333	8.4
cis,cis-5,8-14:2	140	1.2	152	.9	154	.8	166	2.2	168	1.0	178	.5	180	1.1	192	.5	206	.8	277	7.1
cis,cis-7,10-16:2	168	4.9	180	4.0	182	2.7	192	.8	194	12.0	206	1.0	208	5.2	220	2.4	234	3.0	305	16.0
cis,cis-9,15-18:2	196	2.6	208	2.2	210	1.8	222	3.4	264	7.7	276	1.0	278	1.5	290	1.8	304	2.5	333	19.0
cis,cis,cis-6,9,12-18:3	154	3.9	166	3.4	180	6.5	194	8.7	206	2.5	220	8.8	234	3.1	246	3.8	260	3.4	331	23.0
cis,cis,cis-9,12,15-18:3	196	3.3	208	2.6	222	5.3	236	5.1	248	2.7	262	7.7	276	2.9	288	3.5	302	3.1	331	40.4
cis,cis,cis-8,11,14-20:3	182	4.5	194	3.2	208	9.2	222	6.3	234	3.2	248	8.6	262	3.5	274	4.1	288	3.2	359	38.0
trans,trans-8,10-18:2	180	1.9	182	2.1	192	.5	194	1.5	196	1.0	206	1.1	208	1.3	210	.7	220	2.1	333	23.0
trans,trans-9,11-18:2	194	.7	196	1.1	206	.6	208	1.0	210	.5	220	.8	222	.7	224	.6	234	1.4	333	10.0
trans,trans-10,12-18:2	208	2.0	210	3.1	220	1.8	222	2.1	224	2.2	234	2.4	236	2.1	238	1.6	248	2.1	333	23.0
cis,cis,cis,cis-5,8,11,14-20:4	140	1.7	152	.9	154	1.0	166	2.8	168	1.6	180	3.4	192	.6	194	.7	206	2.4		
	208	1.6	218	.8	220	1.5	232	2.1	246	2.4	258	.5	260	.7	272	1.0	286	.9	357	6.5

[a]Rel. int. = relative intensity.

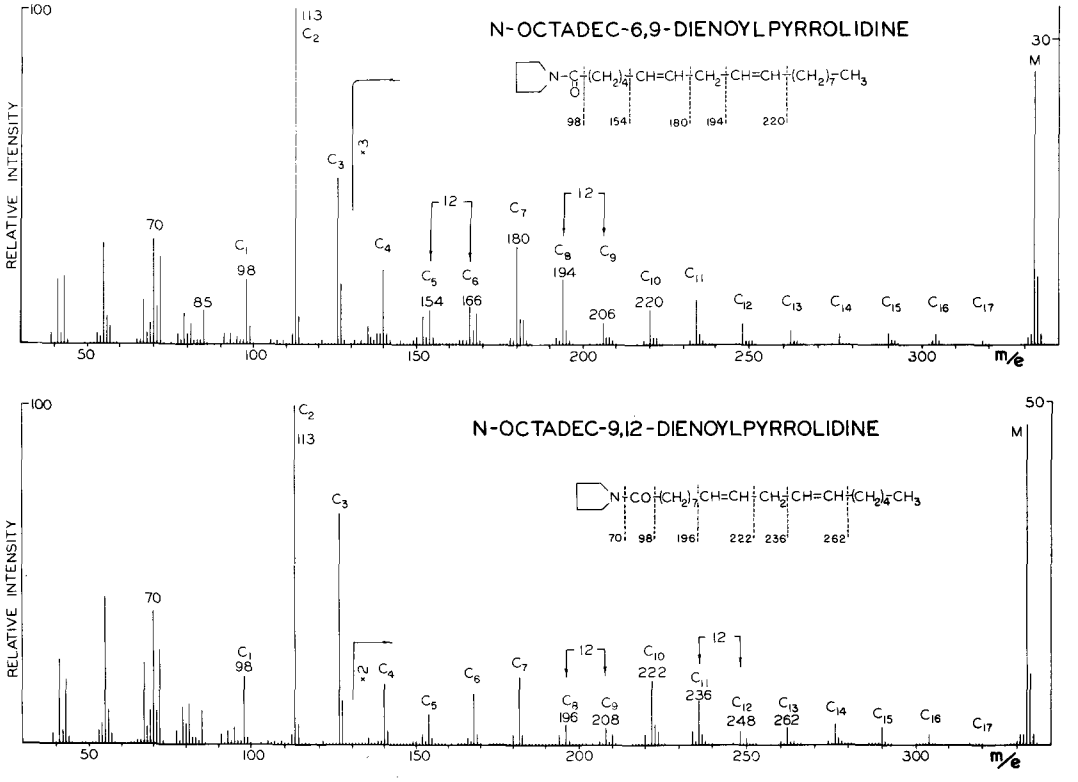

FIG. 1. Mass spectra of N-octadec-6,9-dienoylpyrrolidine and N-octadec-9,12-dienoylpyrrolidine.

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." This rule could be applied to most of the compounds listed in Table I, the exceptions being the dienoic pyrrolidides which have a double bond in positions 4,15,16 or 17 in a methylene interrupted system.

The spectrum of the 4,7-18:2 isomer had characteristic peaks at *m/e* 124 and *m/e* 126 together with *m/e* 139 and *m/e* 152 indicating the double bond in position 4. This was likewise true for the pyrrolidides of 4-18:1 and 4-10:1 (4). The double bond in position 7 was shown by the interval of 12 amu between carbons 6 and 7 (*m/e* 166 and *m/e* 178) according to the rule. The 12,15-18:2, 13,16-18:2 and 14,17-18:2 isomers had spectra which distinguished them from each other. The 12,15-18:2 isomer followed the rule, but the key fragments, representing 12 carbons of the chain, *m/e* 250 and *m/e* 252, were of almost the same intensity. Other details that distinguished the two isomers from each other were: the cluster of peaks that contained 13 carbons of the fatty acid chain of 12,15-18:2 was stronger than the corresponding peaks, e.g. *m/e* 265, for the 13,16-18:2 isomer. In the spectrum of the 13,16-18:2 isomer, the cluster of peaks that contained 15 carbons of the chain, e.g. *m/e* 291, was stronger than the corresponding cluster for the 12,15-18:2 isomer. Finally, for the spectrum of the 14,17-18:2 isomer, the interval of 12 amu, typical for the 12,15-18:2 and 13,16-18:2 isomers, was moved one carbon closer to the methyl end, e.g. *m/e* 278 and *m/e* 290 to *m/e* 292 and *m/e* 304.

The spectra of lower homologues of methylene interrupted dienoic acids represented by 5,8-14:2 and 7,10-16:2 followed the rule completely as can be seen in Table I. Also, a fatty acid with the two double bonds separated by four methylene groups, 9,15-18:2, gave a mass spectrum (Fig. 2) from which the double bonds could be located via the rule.

The mass spectra of pyrrolidides of three trienoic acids, 6,9,12-18:3, 9,12,15-18:3, and 8,11,14-20:3, were also interpretable according to the rule developed for monoenoic acids. This is shown for the 9,12,15-18:3 isomer in Figure 2. However, some of the key fragments, e.g. *m/e* 248 and *m/e* 250, in the spectrum of 6,9,12-18:3 were of intensities almost equal to those of the spectrum of the 12,15-18:2 isomer.

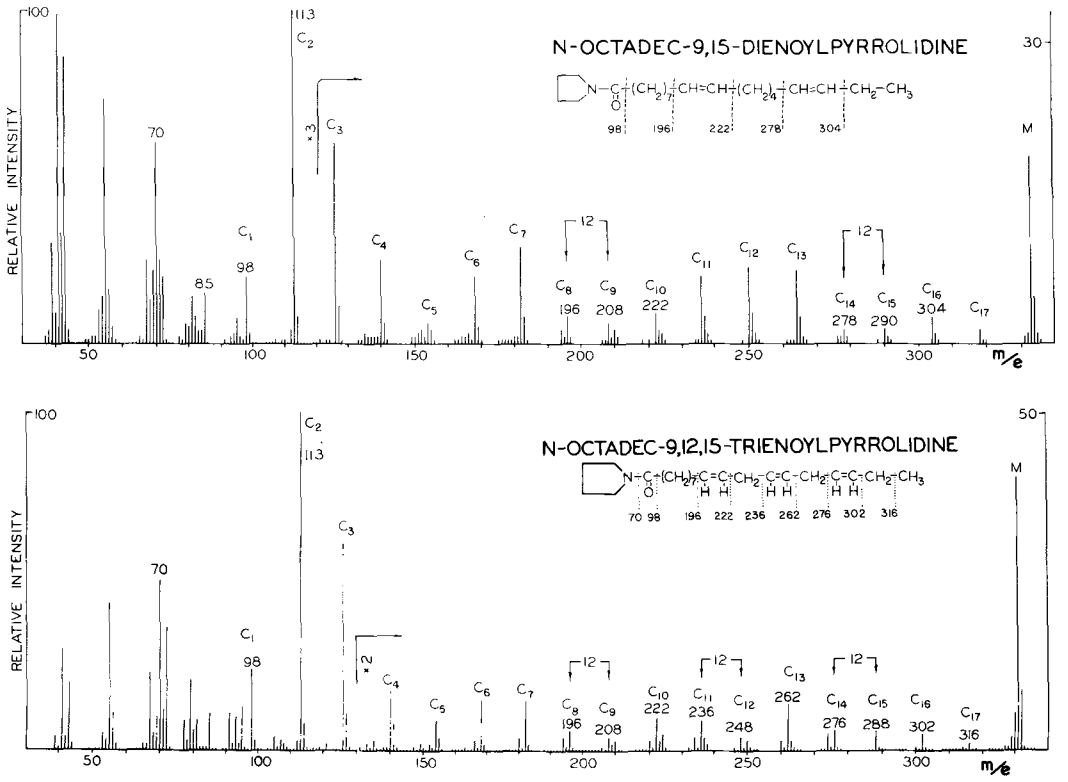

FIG. 2. Mass spectra of N-octadec-9,15-dienoylpyrrolidine and N-octadec-9,12,15-trienoylpyrrolidine.

A tetraenoic fatty acid, 5,8,11,14-20:4, also was investigated, and its pyrrolidide derivative gave a mass spectrum which could be interpreted partially according to the rule. In this case, key diagnostic fragments, e.g. *m/e* 152 vs *m/e* 154 and *m/e* 192 vs *m/e* 194, were of almost equal intensities.

In the spectra of pyrrolidides which have methylene interrupted double bonds in the middle of the chain, the peak cluster lying between the intervals of 12 amu was observed to be more intense than its surrounding peaks. For example, *m/e* 180 in the spectrum of the pyrrolidide of 6,9-18:2; *m/e* 222 in the spectrum of the pyrrolidide of 9,12-18:2; and *m/e* 222 and 262 in the spectrum of the pyrrolidide of 9,12,15-18:3 were high peaks in the general profile of the mass spectrum range involving the double bonds. This phenomenon facilitates the location of methylene interrupted polyunsaturation when it is difficult to distinguish whether intervals are 12 or 14 amu. In the mass spectrum of the pyrrolidides of a polyunsaturated fatty acid, *the presence of a peak relatively more intense than the peak clusters which flank it and which are involved in probable intervals of 12 amu indicates the presence of a methylene interrupted system. If the prominent peak contains m carbons of the fatty acid residue, the methylene carbon in the molecule was at position m + 1.*

Three fatty acids with conjugated unsaturation in the middle of the chain, i.e. 8,10-18:2, 9,11-18:2, and 10,12-18:2, also were investigated. In Table I, the key fragments in their spectra are listed. In each case, the double bond closer to the carbonyl was indicated by the interval of 12 amu according to the rule, but the remote double bond was, however, not in agreement with the rule. The conjugated system was indicated by another interval of 12 amu immediately following the first interval of 12 amu.

To test the use of pyrrolidides in the interpretation of unknown fatty acids with GLC and MS, corn oil triglycerides were investigated in a previous paper (6), and the double bonds were located without difficulties by using the rule developed for the monoenoic fatty acids. An 18 carbon fatty acid with one actylenic bond and three double bonds also was investigated (8), and, by applying our rule, the double bonds were found at positions 9,12 and 15, which later was confirmed by other analytical

methods. Interpretation of the mass spectrum of a triglyceride of this fatty acid revealed the same double bond positions when the same logic as used with pyrrolidides was applied. Recently, the mass spectrum of the pyrrolidide of an unknown polyunsaturated acid was studied by Joseph (9). Interpretation of the spectrum suggested the structure to be 3,6,9,12,15-18:5, and this structure was confirmed by other analytical methods.

This work shows that pyrrolidides offer advantages for the mass spectrometric analysis of polyenoic fatty acids. The derivatives are easy to prepare, and no chemical modification of the unsaturation in the fatty acid chain is necessary, as is the case with most of the other methods developed for this purpose (1,2). Although spectra of all the polyunsaturated fatty acid pyrrolidides are not interpretable according to the rule developed for the monoenoic acids, it is noteworthy that all spectra differ from each other. With enough reference compounds, there should be no problem in identifying each positional isomer by mass spectrometry.

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