Tetrahedron Letters No.49, pp. 5021-5026, 1967. Pergamon Press Ltd. Printed in Great Britain.

DETERMINATION OF DOUBLE BOND POSITIONS IN POLYUNSATURATED FATTY ACIDS USING COMBINATION GAS CHROMATOGRAPHY - MASS SPECTROMETRY

Walter G. Niehaus, Jr.[1] and Ragnar Ryhage
Department of Chemistry and Laboratory for Mass Spectrometry
Karolinska Institute, Stockholm, Sweden

(Received in UK 31 August 1967)

The determination of the positions of ethylenic bonds in multiply unsaturated acyclic compounds is a formidable problem. Classical approaches have involved oxidative cleavage of the double bonds, followed by characterization of the fragments produced, usually by gas chromatography.

The use of mass spectrometry for determination of double bond position has not thus far yielded much useful information. Compounds isomeric with respect to double bond positions give very similar or identical spectra (1). A number of derivatives of unsaturated fatty acids have been prepared and studied by mass spectrometry. Catalytic deuteration of the double bonds is not satisfactory due to extensive incorporation and exchange of deuterium (2). Reduction with deuterio - hydrazine gives somewhat more satisfactory results (3), but is complicated by hydrogen-deuterium exchange reactions (4), and by rearrangements during the electronic bombardment (5). Epoxidation of a monoenoic acid followed by treatment with sodium iodide yields a mixture of two isomeric keto-fatty acids. α and β cleavage of the ketones gives rise to fragments which allow the determination of the double bond position (6). Dihydroxy fatty acids, produced by permanganate oxidation of monounsaturated fatty acids, undergo a characteristic cleavage between the carbon atoms bearing the hydroxyl groups (7).

Recently, combined gas chromatography - mass spectrometry has been employed using isopropylidene derivatives of the dihydroxy acids formed on oxidation of the double bond. The mass spectra contain peaks corresponding to cleavages of bonds α to the 1, 3-dioxolane ring (8). A similar study has been performed with hydrocarbons (9).

Although several of these methods give satisfactory results with monounsaturated fatty acids, the localization of double bond positions in more highly unsaturated compounds has not been reported.

We have developed a method for localization of double bond positions in fatty acids containing 1 to 4 double bonds, and present here the results obtained with the isomeric octadecatrienoic acids, α -linolenic and γ -linolenic acids.

---

[1] Recipient of a Postdoctoral Research Fellowship from the National Heart Institute, USPHS.

EXPERIMENTAL

The unsaturated fatty acids were oxidized to the corresponding polyhydroxy compounds essentially as described by McCloskey and McClelland (8). The polyhydroxy fatty acids were methylated using dimethylsulfinyl carbanion and methyl iodide, as described by Hakomori (10). The resulting polymethoxy fatty acid methyl esters were analyzed directly, without prior purification, using the LKB 9000 Gas Chromatograph - Mass Spectrometer. A column of 1% SE-30 on Gas-Chrom P was operated at a temperature between 200 and 220°. The energy of the bombardment electrons was kept at 70 eV. The ion outdrawings potential was increased from a normal value of 4 volts to about 8 volts which gave a higher intensity to the diffuse peaks due to metastable ions. Magnetic scanning was used and the mass spectra were obtained in about 5 seconds.

RESULTS

The mass spectrum of 9, 10, 12, 13, 15, 16-hexamethoxy - methyl octadecanoate, derived from α -linolenic acid, is shown in Fig. 1. The parent molecular ion of 478 is absent. Small peaks are present at m/e 414 and 415, corresponding to M-2x32 and M-(31+32). The next highest mass observed is 405, which corresponds to a simple cleavage of the bond between C-15 and C-16, and the loss of 73 mass units. The fragment m/e 405 loses several methoxy groups as methanol ($-OCH_3$ + H), as is also shown in Table 1. This fragmentation is confirmed by the diffuse peaks due to metastable transitions. Similar fragmentation occurs between the other methoxy substituted carbon atoms. Both fragments produced from each cleavage give rise to major peaks in the spectrum (Fig. 1 and Table 1).

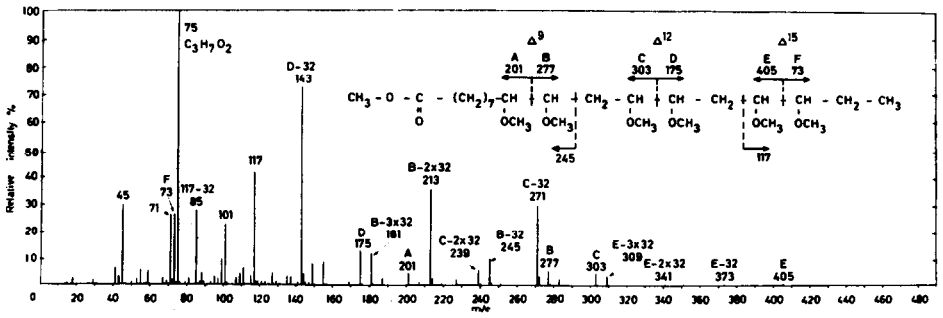

FIG. 1
Derivative of α -linolenic acid

The mass spectrum of 6, 7, 9, 10, 12, 13 - hexamethoxy - methyl octadecanoate, derived from γ -linolenic acid, also shows characteristic fragmentation between the methoxy substituted carbon atoms (Fig. 2 and Table 1).

TABLE 1

Principal Ion Fragments

α - Linolenic Acid Derivative				γ - Linolenic Acid Derivative			
m/e		Per cent of base peak	Double bond position	m/e		Per cent of base peak	Double bond position
201	A[1]	5.7	Δ^9	159	A[2,3]	53.9	Δ^6
169	A-32	2.5		127	A-32	31.7	
137	A-2x32	4.1		95	A-2x32	15.0	
277	B[3]	6.1	Δ^9	319	B	0.9	Δ^6
245	B-32[3]	10.4		287	B-32	5.4	
213	B-2x32	35.7		255	B-2x32	6.7	
181	B-3x32	12.9		223	B-3x32	15.6	
149	B-4x32	8.9		191	B-4x32	3.6	
303	C	5.4	Δ^{12}	261	C	13.8	Δ^9
271	C-32	30.4		229	C-32	38.9	
239	C-2x32	6.4		197	C-2x32	29.9	
207	C-3x32	2.1		165	C-3x32	5.1	
175	D	13.8	Δ^{12}	217	D	16.8	Δ^9
143	D-32	73.2		185	D-32	95.8	
111	D-2x32	7.3		153	D-2x32	4.7	
405	E	0.7	Δ^{15}	363	E	2.4	Δ^{12}
373	E-32	1.2		331	E-32	3.8	
341	E-2x32	1.7		299	E-2x32	4.0	
309	E-3x32	4.3		267	E-3x32	13.8	
277	E-4x32[3]	6.1		235	E-4x32	7.1	
73	F	26.8	Δ^{15}	115	F	21.6	Δ^{12}
				83	F-32	8.9	

1. See Fig. 1
2. See Fig. 2
3. See discussion

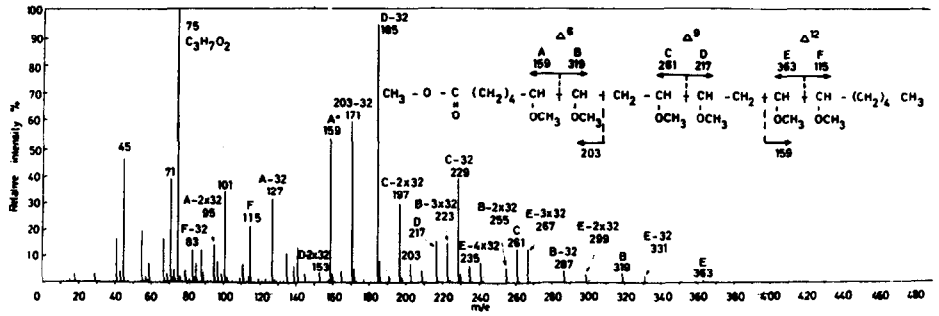

FIG. 2
Derivative of γ -linolenic acid

DISCUSSION

As is shown in the figures and table, nearly all of the major fragments arise from cleavage of the bond between the carbon atoms bearing the methoxyl groups, and each forms a series resulting from subsequent losses of methanol from the parent fragment.

One parent fragment arising from cleavage of the bond between C-9 and C-10 (A) has the general formula $\left[CH_3\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}(CH_2)_a\text{-}\underset{\underset{OCH_3}{|}}{CH}\text{-}\right]^+$ for which m/e = (103 + 14a). Similar parent fragments (C, E) arising from cleavage of bonds between the other methoxyl-bearing carbon atoms will have m/e = (103 + 14a) + 102x, where x refers to the number of additional $\left[\text{-}\underset{\underset{OCH_3}{|}}{CH}\text{-}CH_2\text{-}\underset{\underset{OCH_3}{|}}{CH}\text{-}\right]$ groups in the fragment. The other parent fragment arising from cleavage of the bond between C-15 and C-16 (F) has the general formula $\left[\text{-}\underset{\underset{OCH_3}{|}}{CH}\text{-}(CH_2)_b\text{-}CH_3\right]^+$ for which m/e = 59 + 14b. Similar parent fragments (B, D) arising from cleavage of bonds between the other methoxyl-bearing carbon atoms will have m/e = (59 + 14b) + 102x.

The only other important fragments arise from cleavage α to the first and last pair of methoxyl-bearing carbon atoms (Figs 1 and 2) and have the following general structures: $\left[CH_3\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}(CH_2)_a\text{-}\underset{\underset{OCH_3}{|}}{CH}\text{-}\underset{\underset{OCH_3}{|}}{CH}\right]^+$ m/e (351 + 14a), $\left[\text{-}\underset{\underset{OCH_3}{|}}{CH}\text{-}\underset{\underset{OCH_3}{|}}{CH}\text{-}(CH_2)_b\text{-}CH_3\right]^+$ m/e (307 + 14b).

Therefore, after grouping the major fragments into their respective series, consisting of the parent fragment plus secondary fragments resulting from successive losses of methanol (Table 1), it is relatively easy to determine the values of a and b, and thus to determine the positions of the double bonds in the original compound.

Fatty acids with double bonds interrupted by more than one methylene group would be expected to undergo a similar fragmentation, and the positions of the original double bonds could be determined in an analogous manner.

Several peaks in the spectrum of the γ-linolenic acid derivative could arise from cleavage of either of two bonds, producing different fragments with the same mass number. For example, the peak at m/e 159 could arise from splitting of the bond between the first pair of methoxyl-bearing carbon atoms (A), or from splitting α to the last pair of methoxyl-bearing carbon atoms (Fig. 2). Fragment A has the emperical formula ($C_8H_{15}O_3$) for which m/e = 159.1021. The other fragment has the emperical formula ($C_9H_{19}O_2$) for which m/e = 159.1385.

To distinguish between these two possible fragments, which comprise an ($O-CH_4$) doublet, a peak matching accessory of the LKB 9000 single focusing instrument was employed (11). The sample was introduced via the direct probe. The experimentally determined value for this peak was m/e = 159.1325.

Using the peak matching device with a resolution $\frac{M}{\Delta M}$ of 1500, the value of m/e can be determined with an accuracy of 10 parts per million, This corresponds to a value of 159.1325 $\pm$ 0.0016. However, the observed value is 0.006 less than the actual value for $C_9H_{19}O_2$, and 0.03 greater than the actual value for $C_8H_{15}O_3$. It is therefore apparent that the peak seen at m/e 159 must consist of a mixture of the two fragments comprising the ($O-CH_4$) doublet.

The major contribution to the peak must be from the fragment $C_9H_{19}O_2$, resulting from cleavage α to the final pair of methoxyl-bearing carbon atoms, and only a minor contribution is from $C_8H_{15}O_3$ (fragment A). This finding resolves the apparent difference in fragmentation between the two derivatives, i.e. the greater relative intensity of fragment A from the γ-linolenic acid derivative. Both spectra are now seen to contain a large peak (greater than 40% of the base peak) corresponding to cleavage α to the final pair of methoxyl bearing carbon atoms (m/e 117 and 159, respectively) and a much less intense peak corresponding to fragment A.

Using the same method, the ($O-CH_4$) doublets at m/e 319, 261, 217 and 363 were shown to arise primarily from cleavage between the corresponding pairs of methoxyl-bearing carbon atoms, i.e. fragments B, C, D and E. These data will be published in detail in a later communication.

We assume that in the spectrum of the α-linolenic acid derivative, the peak at m/e 245 is also a mixture of (B-32) and the fragment arising from cleavage α to the first pair of methoxyl-bearing carbon atoms (Fig. 1). Peak matching will also be performed on this derivative to resolve this question.

Diffuse peaks due to metastable ions are present for all the transitions shown in Table 1, and help in the interpretation of the fragmentation. For example, in the spectrum of the α-linolenic acid derivative, the peak at m/e 277 corresponds to fragment B; but also to

the fragment (E - 4x32), since a metastable peak is present at m/e 248.5, corresponding to the transition $309^{+} \rightarrow 277^{+} + 32$. These findings resolve the apparent differences in relative intensity of the peaks corresponding to B, B - 32, and B - 2x32 in the two spectra, and will be discussed in detail in a later communication.

REFERENCES

1. B. Hallgren, R. Ryhage, and E. Stenhagen, Acta Chem. Scand., 13, 845 (1959).
2. N. Dinh-Nguyen, and R. Ryhage, Journal of Res. Inst. for Catalysis, Hokkaido Univ. 8, 73 (1960).
3. N. Dinh-Nguyen, R. Ryhage and S. Ställberg-Stenhagen, Arkiv för Kemi, 15, 433 (1960).
4. N. Dinh-Nguyen, R. Ryhage and S. Ställberg-Stenhagen, Arkiv för Kemi, 18, 393 (1961).
5. R. Ryhage and E. Stenhagen, Mass Spectrometry of Organic Ions, Academic Press, New York, 399 (1963).
6. G. Kenner, and E. Stenhagen, Acta Chem. Scand., 18, 1551 (1964).
7. R. Ryhage and E. Stenhagen, Arkiv för Kemi, 15, 545 (1960).
8. J. McCloskey and M. McClelland, J. Am. Chem. Soc., 87, 5090 (1965).
9. R. Wolff, G. Wolff and J. McCloskey, Tetrahedron, 22, 3093 (1966).
10. S. Hakomori, J. Biochem, 55, 205 (1964).
11. R. Ryhage, 15th Annual Conference on Mass Spectrometry and Allied Topics. ASTM Committees E-14, Denver, (1967).