Location of Double Bond Position in Unsaturated Fatty Acids by Negative Ion MS/MS

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Received March 25, 1983

A long-standing problem in the identification and structure proof of fatty acids and similar compounds has been the determination of the position of the double bond. The positive ion electron-ionization mass spectra of these compounds have been found to be uninformative due to extensive rearrangement and fragmentation.[1] On the other hand, negative ion mass spectra obtained in a CI mode also tend to be uninformative due to a lack of fragmentation. Thus, much of the work in this area has been directed toward derivatization of the double bond prior to analysis, e.g., by epoxidation,[2] or toward derivatization within the mass spectrometer source under CI conditions.[3] All of these methods suffer certain drawbacks, and current research continues to be focused on this problem.[4] Here we report a simple method that yields striking results for the location of double bonds in underivatized fatty acids. The method makes use of the collisional activated decomposition (CAD) spectra of the negative ions, $(M - H)^-$, of the fatty acids.

Mass spectra were obtained with a Kratos MS-50 triple analyzer tandem mass spectrometer, which has been recently described.[5] Briefly, the instrument consists of a high-resolution MS-I of Nier–Johnson geometry followed by an electrostatic analyzer used as MS-II. Fast atom bombardment (FAB)[6] was used to desorptively ionize the preformed conjugate bases from the basic matrix triethanolamine. CAD spectra were taken by activating the ions in the third field free region using helium gas (sufficient helium was added to suppress the ion beam by 50%) and scanning MS-II; 20–30 scans were signal averaged for each spectrum.

The mass spectra of the acids in the negative ion mode consist of $(M - H)^-$ ions with no apparent fragmentation (see Figure 1 for a typical spectrum). Upon collisional activation (see Figure 2 for a typical MS/MS spectrum), however, the $(M - H)^-$ ion gives rise to a rich diversity of fragment ions amounting to approximately 3% of the $(M - H)^-$ ion beam suppressed by collision. The observed peaks represent apparent successive losses of carbons initiated at the non-carboxy end of the molecule. It should be pointed out, however, that the mechanism is neither simply that

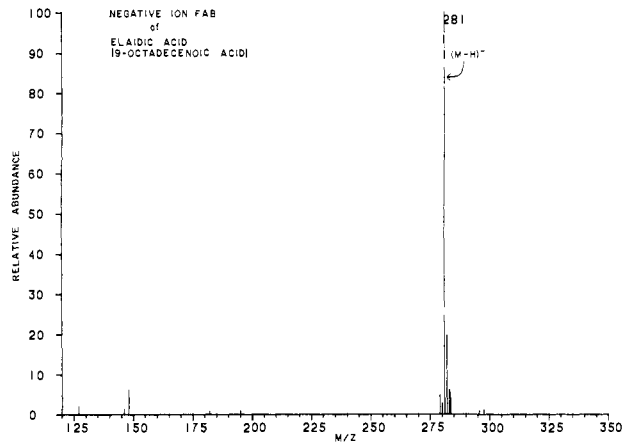

Figure 1. Negative ion mass spectrum of elaidic acid (9-octadecenoic acid).

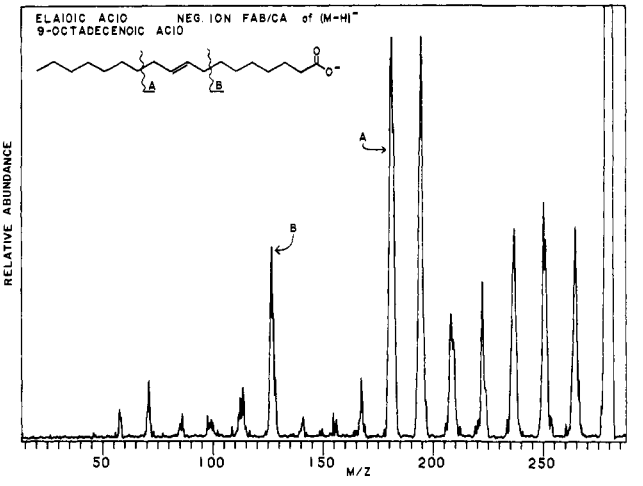

Figure 2. CAD spectrum of m/z 281 $[(M - H)^-]$ ion of elaidic acid.

of successive cleavages of CH_2 units nor competitive alkyl cleavages since the initial loss is of overlapping losses of 16, 17, and 18 amu with only a small contribution from loss of 15 amu, and the formal site of ionization is remote from the putative fragmentation. The large peak at m/z 182 is due to cleavage of the carbon–carbon bond allylic to the double bond on the CH_3 terminal side of the chain.[7] The next three lower mass peaks are dramatically less intense and are followed by a significantly greater intensity peak representing allylic cleavage on the carboxy side of the double bond.[8]

This pattern of two intense peaks corresponding to cleavage allylic to the double bond with three very minor intervening peaks appears to be a general characteristic of monounsaturated fatty acids and can be used to identify unequivocally the location of the double bond (see Figure 3 for the results in bar graph form for the 15 fatty acids we have studied). No differences were observed for cis and trans isomers.

When more than one double bond is present, the positions of the double bonds do not stand out as clearly as for monounsaturated acids. The same pattern, however, of preferential allylic cleavages is observed. This is illustrated by the bar graphs in Figure 4 for linoleic and linolenic acids. Also included in Figure 4 is the representation for ricinoleic acid, 12-hydroxyoleic acid. Here the allylic cleavage is greatly enhanced by the presence of

(1) See, e.g.: "Specialist Periodical Reports: Mass Spectrometry", Williams, D. H., Ed.; Chemical Society: London, 1971; Vol. 1, pp 144–146 and references therein.

(2) (a) Wolff, R. E.; Wolff, G.; McCloskey, J. A. *Tetrahedron* **1966**, *22*, 3093. (b) Eglinton, G.; Hunneman, D. H.; McCormick, A. *Org. Mass Spectrom.* **1968**, *1*, 593. (c) Niehaus, W. G., Jr.; Ryhage, R. *Anal. Chem.* **1968**, *40*, 1840. (d) Vocheron, M. J.; Michel, G.; Guilloy, R.; *Bull. Soc. Chim. Biol.* **1969**, *51*, 177. (e) Tumlinson, J. H.; Heath, R. R.; Doolittle, R. G. *Anal. Chem.* **1974**, *46*, 1309. (f) Reviewed by: Dommes, V.; Wirtz-Peltz, F.; Kunau, W.-H. *J. Chromatogr. Sci.* **1976**, *14*, 360. (g) Ariga, T.; Araki, E.; Murata, T. *Anal. Biochem.* **1977**, *83*, 474.

(3) (a) Hunt, D. F.; Crow, F. W.; Harvey, T. M. Abstracts of the 23rd Annual Conference on Mass Spectrometry and Allied Topics, May 1975, Houston, Texas, p 508. (b) Chai, R.; Harrison, A. G. *Anal. Chem.* **1981**, *53*, 34–37. (c) Ferrer-Correia, A. J.; Jennings, K. R.; Sensham, D. K. *Org. Mass. Spectrom.* **1976**, *11*, 807–872. (d) Ferrer-Correia, A. J.; Jennings, K. R.; Sensham, D. K. *Adv. Mass Spectrom.* **1978**, *7*, 287–294. (e) Budzikiewicz, H.; Busker, E. *Tetrahedron* **1980**, *36*, 255. (f) Ariga, T.; Araki, E.; Murata, T. *Chem. Phys. Lipids* **1977**, *19*, 14.

(4) (a) Kidwell, D. A.; Biemann, K. *Anal. Chem.* **1982**, *54*, 2462. (b) Greathead, R. J.; Jennings, K. R. *Org. Mass Spectrom.* **1980**, *15*, 431. (c) Bieri-Leonhardt, B. A.; Devilbiss, E. D.; Plimmer, J. R. *J. Chromatogr. Sci.* **1980**, *18*, 364.

(5) Gross, M. L.; Chess, E. K.; Lyon, P. A.; Crow, F. W.; Evans, S.; Tudge, H. *Int. J. Mass Spectrom. and Ion Phys.* **1982**, *42*, 243–254. MS-I is a standard Kratos MS-50 with an upper limit of mass resolving power of greater than 100 000. MS-II has an energy resolving power of 8000. The mass resolving power, however, is much less, ca. 100, due to unavoidable energy release in the collision process that serves to broaden the peaks.

(6) Barber, M.; Bordeli, R. S.; Sedgwick, R. D.; Tyler, A. M. *J. Chem. Soc., Chem. Commun.* **1981**, 325.

(7) The determination of the mechanism of the fragmentation must await further investigations involving, for example, isotopic labeling.

(8) Allylic cleavage has been observed in the field-ionization mass spectra of alkenes [Rang, S. A.; Müürisepp, A.-M. A.; Liitmaa, M. M.; Eisen, O. G. *Org. Mass Spectrom.* **1978**, *13*, 181] and in the electron-capture spectra of some C_7 and C_8 dienoic esters [Khvostenko, V. I.; Galkin, E. G.; Dzemiter, V. M.; Tolstikov, G. A.; Fal'ko, V. S.; *Izv. Akad. Nauk SSSR, Ser. Khim.* **1980**, *No. 7*, 1663–1665].

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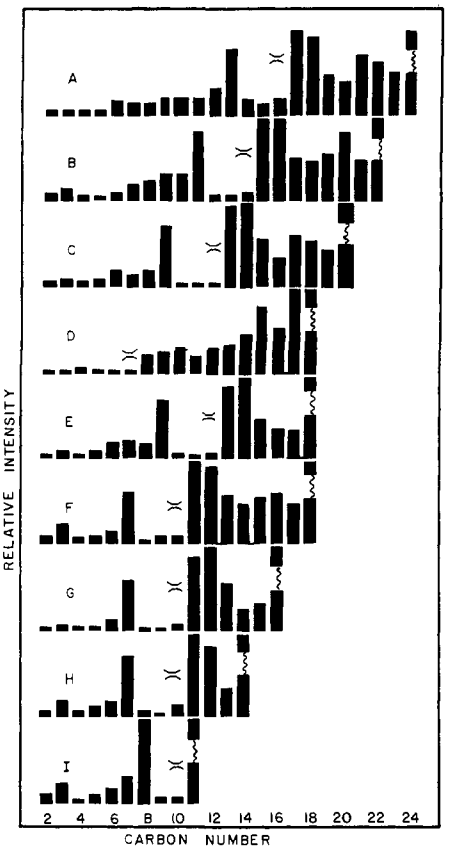

Figure 3. Bar graph representations of the CAD spectra of monounsaturated fatty acids (carbon one is the carboxylate terminus): (A) nervonic acid (*cis*-15-tetracosenoic acid); (B) erucic and brassidic acids (*cis*- and *trans*-13-docosenoic acids); (C) *cis*-11-eicosenoic acid; (D) petroselinic and petroselaidic acids (*cis*- and *trans*-6-octadecenoic acids); (E) *cis*- and *trans*-vaccenic acids (*cis*- and *trans*-11-octadecenoic acids); (F) oleic and elaidic acids (*cis*- and *trans*-9-octadecenoic acids); (G) palmitoleic and palmtolaidic acids (*cis*- and *trans*-9-hexadecenoic acids); (H) myristoleic acid (*cis*-9-tetradecenoic acid); (I) 10-undecenoic acid).

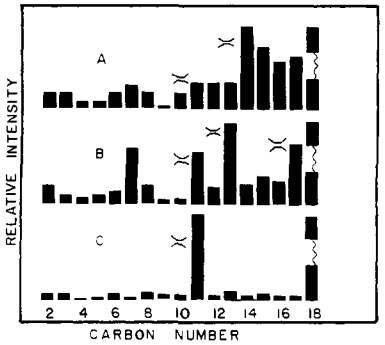

Figure 4. Bar graph representation of the CAD spectra of polyunsaturated and hydroxy fatty acids (carbon one is the carboxylate terminus): (A) linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid); (B) linolenic acid (*cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid); (C) ricinoleic acid (12-hydroxy-9-octadecenoic acid).

the alcohol functionality (α-cleavage).

The enhanced structural information obtained from dissociation reactions of negative ions compared to positive ions may be more general than for the fatty acids reported in this paper.[9] As a test, we are currently exploring the utility of this technique not only for more complex unsaturated acids but also for other unsaturated compounds. The mechanism of the CAD process is also under study.

(9) Specific fragmentation of fatty acid ester $(M - H)^-$ ions has also been recently reported: Bambagiotti, A. M.; Coran, S. A; Giannellini, V.; Vincieri, F. F.; Daolio, S.; Traldi, P. *Org. Mass Spectrom.* **1983**, *18*, 133-134.

Acknowledgment. This work was supported by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Instrumentation Facility (Grant No. CHE 82-11164).

Registry No. Elaidic acid, 112-79-8; nervonic acid, 506-37-6; erucic acid, 112-86-7; brassidic acid, 506-33-2; *cis*-11-eicosenoic acid, 5561-99-9; *cis*-6-octadecenoic acid, 593-39-5; *trans*-6-octadecenoic acid, 593-40-8; *cis*-11-octadecenoic acid, 506-17-2; *trans*-11-octadecenoic acid, 693-72-1; oleic acid, 112-80-1; palmitoleic acid, 373-49-9; palmtolaidic acid, 10030-73-6; myristoleic acid, 544-64-9; 10-undecenoic acid, 112-38-9; linoleic acid, 60-33-3; linolenic acid, 463-40-1; ricinoleic acid, 141-22-0.

Preparation and Structural Characterization of Acetylene(2,2′-dipyridylamine)copper(I) Tetrafluoroborate†

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Received March 21, 1983

Cuprous complexes with ethylene and acetylene have been of interest for many years. These complexes are, in general, unstable (to loss of C_2H_4 and C_2H_2) and only poorly characterized.[1-3] Our interest in this area arises from the proposed role of copper in the binding of the plant hormone ethylene to its receptor site.[4] Although the effects of ethylene on virtually every phase of plant development (germination, growth, flowering, fruit ripening, senescence, and abscission) are well established, the site of ethylene action remains unknown.[4,5] Binding and inhibition studies suggest that a copper ion may be involved.[4,5] We reported recently the synthesis and first structural characterization of stable Cu(I)–ethylene complexes, which established that the coordination chemistry of Cu(I)–monoolefin complexes is consistent with the

† Contribution No. 3211.

(1) (a) Jardine, F. H. *Adv. Inorg. Chem. Radiochem.* **1975**, *17*, 115-163. (b) Herberhold, M. "Metal π-Complexes"; Elsevier: New York, 1974; Vol. II, Part 2, pp 173-193. (c) Quinn, H. W.; Tsai, J. H. *Adv. Inorg. Chem. Radiochem.* **1969**, *12*, 217-373.

(2) (a) Tropsch, H.; Mattox, W. J. *J. Am. Chem. Soc.* **1935**, *57*, 1102-1103. (b) Gilliland, E. R.; Seebold, J. E.; Fitz-Hugh, J. R.; Morgan, P. S. *Ibid.* **1939**, *61*, 1960-1962. (c) Keefer, R. M.; Andrews, L. J.; Kepner, R. E. *Ibid.* **1949**, *71*, 2381-2383. (d) Haight, H. L.; Doyle, J. R.; Baenziger, N. C.; Richards, G. F. *Inorg. Chem* **1963**, *2*, 1301-1303. (e) Kawaguchi, S.; Ogura, T. *Ibid.* **1966**, *5*, 844-846. (f) Dines, M. B. *Ibid.* **1972**, *11*, 2949-2952. (g) Yanagihara, N.; Sampedro, J. A.; Casillas V., R.; Fernando, Q.; Ogura, T. *Inorg. Chem.* **1982**, *21*, 475-479. (h) Buxton, G. V.; Green, J. C.; Sellers, R. M. *J. Chem. Soc., Dalton Trans.* **1976**, 2160-2165. (i) Pasquali, M.; Floriani, C.; Gaetani-Manfredotti, A.; Chiesi-Villa, A. *Inorg. Chem.* **1979**, *18*, 3535-3542. (j) Pasquali, M.; Floriani, C.; Gaetani-Manfredotti, A.; Chiesi-Villa, A. *J. Am. Chem. Soc.* **1978**, *100*, 4918-4919. (k) Salomon, R. G.; Kochi, J. K. *J. Am. Chem. Soc.* **1973**, *95*, 1889-1897.

(3) (a) Camus, A.; Marsich, N.; Nardin, G.; Randaccio, L. *Inorg. Chim. Acta* **1977**, *23*, 131-144. (b) Gilliland, E. R.; Bliss, H. L.; Kip, C. E. *J. Am. Chem. Soc.* **1941**, *63*, 2088-2090. (c) Salah, O. M. A.; Bruce, M. I. *J. Chem. Soc., Chem. Commun.* **1972**, 858-859. (d) Salah, O. M. A.; Bruce, M. I. *J. Chem. Soc., Dalton Trans.* **1974**, 2302-2304. (e) Salah, O. M. A.; Bruce, M. I. *Ibid.* **1975**, 2311-2315. (f) Salah, O. M. A.; Bruce, M. I. *Aust. J. Chem.* **1976**, *29*, 531-541. (g) Pasquali, M.; Leoni, P.; Floriani, C.; Gaetani-Manfredotti, A. *Inorg. Chem.* **1982**, *21*, 4324-4328. (h) Pasquali, M.; Floriani, C.; Gaetani-Manfredotti, A.; Chiesi-Villa, A. *Inorg. Chem.* **1979**, *18*, 3535-3542.

(4) (a) Beyer, E. M.; Blomstrom, D. C. In "Plant Growth Substances 1979"; Skoog, F., Ed.; Springer-Verlag: New York, 1980; pp 208-218. (b) Beyer, E. M. *Plant Physiol.* **1976**, *58*, 268-271. (c) Burg, S. P.; Burg. E. A. *Ibid.* **1967**, *42*, 144-152. (d) Burg, S. P.; Burg, E. A. *Science (Washington, D.C.)* **1965**, *148*, 1190-1196.

(5) (a) Varner, J. E.; Ho, D. T.-H. In "Plant Biochemistry", 3rd ed.; Bonner, J., Varner, J. E., Eds.; Academic Press: New York, 1976; pp 713-770. (b) Abeles, F. B. *Annu. Rev. Plant Physiol.* **1972**, *23*, 259-292.

0002-7863/83/1505-5488$01.50/0