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Distinguishing between Cis/Trans Isomers of Monounsaturated Fatty Acids by FAB MS

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Fast-atom bombardment (FAB) mass spectrometry in the negative ion mode can be used to unambiguously distinguish between cis and trans isomers of monounsaturated fatty acids by the relative signal strengths of an intense pair of ion signals. Under normal FAB ionization/desorption conditions, the deprotonated molecules (i.e., [M − H]$^-$) of six fatty acids underwent charge remote fragmentation. A characteristic fragmentation pattern of two intense peak clusters of peaks with three weak intervening clusters of peaks are used in each case to identify the position of the double bond. The possibility of resonance electron capture occurring during the FAB process is discussed.

Owing to their biological importance, the identification and structural determination of unsaturated fatty acids have attracted attention for decades. Early mass spectrometric efforts using electron impact or chemical ionization, either in the positive or negative ion mode, failed to provide useful structural information. Both of these ionization techniques required derivatization of the fatty acids prior to analysis; thus, the structural information obtained from these studies was indirect.

Tomer et al. were the first to apply negative fast-atom bombardment (FAB) MS/MS to the structural determination of unsaturated fatty acids.[1] In this seminal study, they demonstrated that structural information could be obtained from the intensity distribution of a series of product ions induced by charge remote fragmentation (CRF) of the deprotonated molecule [M − H]$^-$ (for reviews, see refs 2 and 3). Specifically, the position of a double bond in an unsaturated fatty acid is indicated by a characteristic pattern of two intense peaks, corresponding to cleavage of allylic bonds, and three very much lower intensity peaks in the mass spectrum.[1]

In spite of being able to locate the position of double bonds in unsaturated fatty acids, use of MS to distinguish cis and trans isomers of these compounds has remained challenging and thus little used. To date, relatively little effort has been directed toward improving the utility of the method. Jensen et al.,[4] using FAB ionization/desorption coupled in conjunction with high-energy collision-induced dissociation (CID) on a four-sector mass spectrometer, found that cis and trans isomers could be distinguished by analyzing the relative intensities of two adjacent peaks resulting from cleavage of the allylic bond on the side distal to the double bond. Unfortunately, due most likely to the four-sector's relatively rapid obsolescence, work on distinguishing cis/trans geometric isomers was not pursued further, and its principle finding was largely forgotten. In a more recent study on unsaturated fatty acids using resonance electron capture (REC) at 7.2-eV electron energy, Voinov and Claeys[5] reported an ion peak that is formed by a radical-induced fragmentation that shows different abundances for cis and trans isomers. In principle, this diagnostic ion peak could be used as an indicator of unsaturated fatty acid geometric isomerism; however, the requirement for controlling the electron energy seems thus far to have hindered general application of this method.

CRF of fatty acid carboxylate anions on a commercial double sector (E/B) mass spectrometer operated under normal FAB conditions has recently been observed.[6] In the present study, it is shown how this phenomenon can be exploited to produce spectra under normal FAB conditions that can be used for locating the double bond's position and for differentiating between geometric isomers of long-chain monounsaturated fatty acids.

EXPERIMENTAL SECTION

The fatty acids, *cis*-9-octadecenoic acid (oleic acid, >99%), *trans*-9-octadecenoic acid (elaidic acid, >99%), *cis*-11-octadecenoic acid (*cis*-vaccenic acid, ≥97%), *trans*-11-octadecenoic acid (trans-vaccenic acid, >99%), *cis*-13-docosenoic acid (erucic acid, ≥99%), *trans*-13-docosenoic acid (brassidic acid, >98%), and triethanolamine (≥99%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and used without further purification.

The FAB spectra were obtained with a JEOL MS Route JMS 600H mass spectrometer. Triethanolamine was used as matrix. A small amount of fatty acid sample was mixed with matrix on the stainless steel tip of the FAB probe before being introduced into the ionization chamber. Xe atoms were used as primary particles; their energy was 4 keV, and their flux equivalent was 5 mA. The secondary ions were accelerated to 3 keV. The temperature of the ionization chamber was kept below 50 °C. The magnetic sector

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was scanned at 5 s/decade, and each spectrum comprised 5−10 scans averaged together. Matrix background was subtracted before assessing the relative intensities of peaks for diagnostic purposes.

RESULTS AND DISCUSSION

Structural analysis of unsaturated fatty acids by mass spectrometry was first attempted on a three-sector instrument using CID.[1] The resolving power (~100−200) of this instrument was insufficient to distinguish the product ion peaks within the cluster that located the position of the double bond. A doublet peak that made it possible to distinguish the geometrical isomers of unsaturated fatty acids was later observed on a four-sector mass spectrometer,[4] but this form of instrument disappeared quickly when more practical configurations for MS/MS analyses became available in the early 1990s, and the potentially useful analytical approach for cis/trans isomerism was neglected and soon forgotten.

In a previous study, CRF of fatty acids was observed under regular negative FAB operating conditions.[6] This discovery makes it possible in principle to conduct structural analysis of long-chain compounds with good sensitivity and resolution on a commercial, double-focusing, E/B-sector instrument. The resolving power of the instrument used in the present study was set at 700, which was more than sufficient to distinguish clusters of peak in the mass spectra of the monounsaturated fatty acids (M_r < 500) while maintaining high sensitivity. Fragment ion peaks were readily apparent in the mass spectra even without averaging (spectra not shown).

The dominant $[M - H]^-$ signal in each of the negative ion FAB mass spectra of the three cis isomers of the monounsaturated fatty acids (Figure 1) is accompanied by a series of CRF peaks. The yield of fragment ions is 1−2.5% of the corresponding $[M - H]^-$ species, depending on the compound. Within each series of fragments, a characteristic pattern, comprising two intense clusters of peaks separated by three weak intervening ones, is evident. No noticeable differences in this general pattern were observed between the cis and trans isomers (latter spectra not shown). This observation agrees well with that of Tomer et al.,[1] who determined that the more massive of the two intense clusters of peaks is due to cleavage of the allylic carbon−carbon bond on the CH_3 terminal side of the chain whereas the less massive of the two clusters results from allylic cleavage on the carboxyl side of the double bond. Thus, this characteristic pattern enabled the positions of the double bonds to be readily located in all three unsaturated fatty acids analyzed. Specifically, the CRF peaks with *m/z* 127 and 181 could be used to locate the double bond position at C_9 in oleic (Figure 1a) and elaidic acids. Similarly, the peaks with *m/z* 155 and 209 for *cis*- (Figure 1b) and *trans*-vaccenic acid and with *m/z* 181 and 237 for erucic (Figure 1c) and brassidic acids indicated a double bond at C_{11} and C_{13}, respectively.

Examination of the more massive of the two intense peak clusters in the mass spectral pattern described in the preceding paragraph reveals an interesting series of three peaks (Figure 2) that was first observed by Jensen et al.[4] in their studies with a four-sector instrument. According to our latest study,[6] the smaller *m/z* peak in the series is due to a diradical ion (odd *m/z*), which can be attributed to a H loss followed by a loss of a C_nH_{2n+1} species. Jensen et al. proposed that the middle *m/z*-peak of the

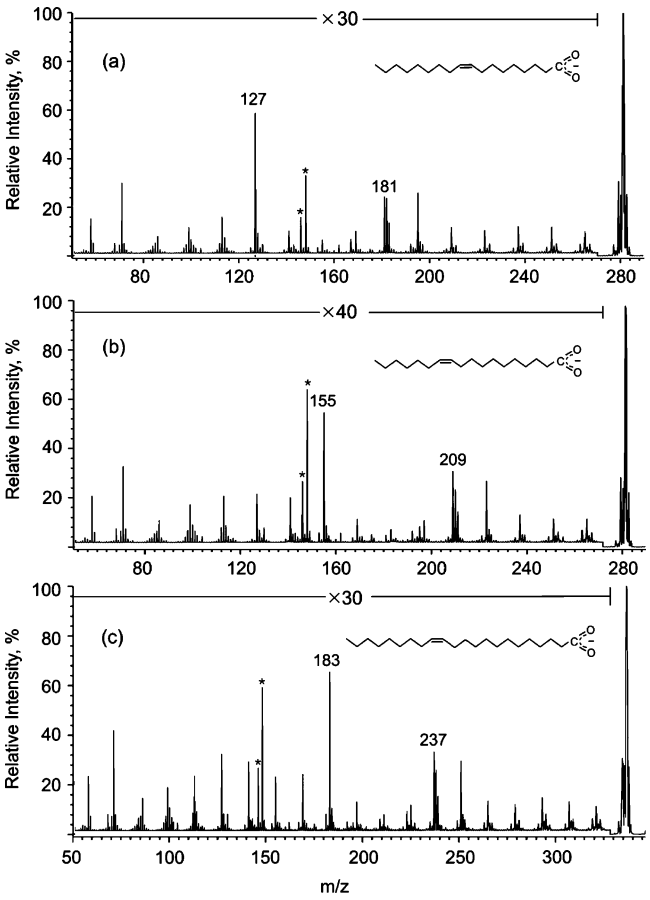

Figure 1. Negative ion FAB spectra of (a) oleic (*cis*-9-octadecenoic) acid, (b) *cis*-vaccenic (*cis*-11-octadecenoic) acid, and (c) erucic (*cis*-13-docosenoic) acid. Matrix peaks are marked with asterisks.

doublet is due to the odd-electron radical ion (even *m/z*), which may be formed by an alkyl cleavage leading to the loss of a C_nH_{2n+1} species.[4] Of particular interest here is the fact that the relative intensity of the two peaks in the doublet differs systematically for the cis and trans isomers. Specifically, the mass peak with lower *m/z* in the series is more intense than the mass peak with the higher *m/z* for all of the cis isomers (Figure 2a, c, e). By contrast, the mass peak with lower *m/z* in the doublet is weaker than the mass peak at the higher *m/z* value for all trans isomers (Figure 2b, d, f). This mass spectral pattern is reproducible; thus, it should be possible to use it to distinguish the cis and trans isomers of monounsaturated fatty acids.

A third relatively intense peak appears on the high mass side of the cis/trans series in each spectrum (Figures 2). This peak is intriguing because it was barely observable in Jensen et al.'s study, in which CID was used to fragment the $[M - H]^-$ precursor. One possible explanation for formation of the third ion is that it results from a molecular radical anion through REC during FAB. It is known that neutrals compose a major part of the sputtered material under FAB conditions[7,8] whereas ions compose only a fraction ($\sim 10^{-6}-10^{-1}$) of the total ejecta. In the present study, the existence of free electrons in the ion source was confirmed

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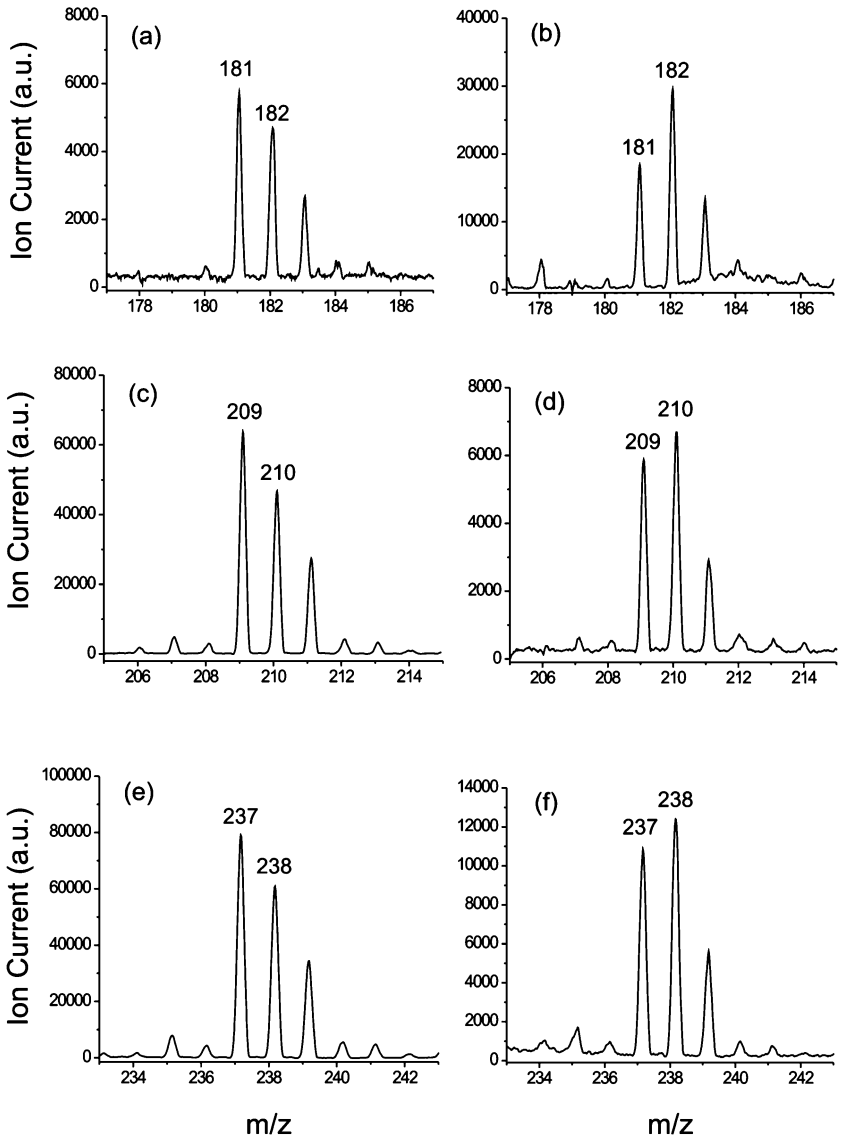

Figure 2. Expanded negative ion FAB spectra showing the more massive of the two intense peak clusters associated with the double bond of (a) oleic (*cis*-9-octadecenoic) acid, (b) elaidic (*trans*-9-octadecenoic) acid, (c) *cis*-vaccenic (*cis*-11-octadecenoic) acid, (d) *trans*-vaccenic (*trans*-11-octadecenoic) acid, (e) erucic (*cis*-13-docosenoic) acid, and (f) brassidic (*trans*-13-docosenoic) acid.

by loading the sample probe with matrix only, introducing chloroform through the standard sample inlet, and monitoring Cl^- ion in the negative ion FAB mode. The appearance of Cl^- in this experiment indicated that a fraction of the electrons released by FAB of the matrix matched the resonance capture energy of the chloroform that leads to the formation of Cl^-. Evidence for electron capture by oligonucleotides in the negative ion FAB mode was reported by Laramée et al.,[9] and more recently, REC by unsaturated fatty acids was observed by Voinov and Claeys.[5] At an electron energy of 7.2 eV, these investigators recorded the REC mass spectra of three pairs of monounsaturated fatty acid isomers and observed ions with m/z 183 for oleic acid and elaidic acid, m/z 211 for *cis*- and *trans*-vaccenic acid, and m/z 239 for erucic acid and brassidic acid that were much less abundant in the regular CID spectra of $[M - H]^-$. It was proposed that, rather than being caused by regular CRF, these ions were formed directly by an alkyl radical loss from excited molecular anions produced by REC. Since both of these ions are present in the corresponding FAB spectra recorded in the present study (Figure 2) and the availability of low-energy electrons for REC is established by the presence of Cl^- when chloroform was introduced into the ion source, it seems likely that the ion signal on the high m/z side of the cis/trans series is due to a REC product ion. Since the resonant electron energy cannot be measured in this instance, however, this argument remains speculative and further study is needed to elucidate the mechanism by which these ions are formed.

In summary, three pairs of cis/trans fatty acid isomers, under regular negative ion FAB ionization/desorption conditions, showed that the $[M - H]^-$ precursors of these compounds underwent charge remote fragmentation to produce a pattern in their mass spectra composed of two intense clusters of peak with three intervening weak ones. As previously demonstrated,[1] this characteristic pattern enabled the double bond position in these compounds to be unambiguously located. The cis and trans

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isomers of the fatty acids could be readily distinguished by observing the relative intensities of two characteristic peaks in their mass spectra. These two peaks were reported in a study by Jensen et al.,[4] in which the double bond in all four of the monounsaturated fatty acids analyzed was located at C_9. In the presented study, the trials were extended to compounds that had double bonds at carbons 9, 11, and 13. The mass spectral differences between the cis and trans isomers remained the same regardless of the position of the double bond, strongly suggesting that negative ion FAB mass spectra can be used to unambiguously identify the cis/trans isomerism of monounsaturated fatty acids.

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