JOURNAL OF MASS SPECTROMETRY, VOL. 32, 277–286 (1997)

Comparison of Low- and High-energy Collision-induced Dissociation Tandem Mass Spectrometry in the Analysis of Ricinoleic and Ricinelaidic Acid

Libérata Nizigiyimana, Hilde Van den Heuvel and Magda Claeys*
Department of Pharmaceutical Sciences, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Wilrijk-Antwerpen, Belgium

Ricinoleic acid and its *trans* isomer ricinelaidic acid, and their methyl esters, were analyzed by low- and high-energy collision-induced dissociation (CID) tandem mass spectrometry. It is shown that a stable charge centre is required to observe the rearrangement, which occurs in the β-hydroxyalkene part and results in the loss of heptaldehyde, and that this rearrangement corresponds to a low-energy CID reaction. Differences between the CID behaviour of ricinoleic acid and that of its *trans* isomer are related to the loss of water, which can also be regarded as a low-energy rearrangement reaction. Comparison of low- and high-energy CID spectra further revealed that high-energy CID gives rise to loss of a $H^{\cdot}$ radical and H_2 together with low-energy fragmentation. Examination of different molecule ions, including $[M-H]^-$, $[M+Li]^+$ and $[M-H+2Li]^+$ ions of free fatty acids and $[M+Li]^+$ ions of the methyl esters shows that charge-remote homolytic fragmentation is most pronounced for the $[M+Li]^+$ ions of the methyl esters. © **1997 by John Wiley & Sons, Ltd.**

J. Mass Spectrom. **32**, 277–286 (1997)
No. of Figures: 12 No. of Tables: 2 No. of Refs: 24

KEYWORDS: ricinoleic acid; fast atom bombardment; collision-induced dissociation; low-energy collision; high-energy collision

INTRODUCTION

In the framework of our studies on the structural characterization of fatty acid derivatives by fast atom bombardment (FAB), collision-induced dissociation (CID) and tandem mass spectrometry, we have compared the low- and high-energy CID behaviour of molecule ions of ricinoleic acid and its *trans* isomer ricinelaidic acid and their methyl esters. The main objectives of this study were to establish which of the fragmentations observed at high-energy CID correspond to low-energy reactions and, more specifically, to gain more insight into the structural requirements to observe the rearrangement which occurs in the *β*-hydroxyalkene part and results in the loss of heptaldehyde.

Gross and co-workers demonstrated that a stable charge centre is required to observe fragmentation remote from the charge for fatty acid molecule ions in high-energy CID (for reviews, see Refs 1 and 2). The occurrence of a rearrangement reaction, i.e. loss of heptaldehyde, in the *β*-hydroxyalkene part of the ricinoleic acid $[M-H+2Li]^+$ ion has been used by Adams and Gross[3] as an argument in favour of the 1,4-H_2 elimination mechanism which was proposed by Jensen *et al.*[4] to explain the formal loss of elements of alkanes in saturated fatty acid molecule ions containing a stable charge centre. However, other studies suggest that the occurrence of a rearrangement reaction in ricinoleic acid molecule ions may not be a valid argument to presume that a rearrangement involving 1,4-H_2 elimination operates in saturated fatty acid molecule ions in high-energy CID and favour homolytic mechanisms.[5–10] Wysocki and Ross[5] investigated the charge-remote fragmentation of functionalized alkanes and proposed a homolytic mechanism involving C—C cleavage based on their observation that FAB-generated precursor ions with a low internal energy lose radicals. Griffiths *et al.*[6] recently compared the charge-remote fragmentation of FAB- and electrospray (ES)-generated anions of sulphated and sulphonated lipids and also found an enhanced relative abundance of radical ions for the ES-generated anions which have a lower internal energy than the FAB-generated anions. An early account of the presence of radical ions formed by simple homolytic fragmentation is that of Bambagiotti-Alberti *et al.*,[7] who observed radical ions in the high-energy CID spectra of fatty acid carboxylate ions. Multi-step radical processes triggered homolytic C—O cleavages have also been considered by Domon *et al.*[8] to explain the charge-remote fragmentation of the $[M-H]^-$ ion of 12-acetoxystearic acid. A homolytic cleavage mechanism involving initial cleavage of C—H bonds all along the alkenyl chain was suggested by Claeys and Van den Heuvel[9] in order to rationalize the fragmentation observed in long-chain alkenyl salicylic acid $[M-H+2Li]^+$ ions. In a recent study, Claeys *et al.*[10] examined the charge-remote fragmentation of

* Correspondence to: M. Claeys.

CCC 1076–5174/97/030277–10 $17.50

Received 27 June 1996
Accepted 29 October 1996

$[M + Li]^+$ ions of deuterium-labelled *n*-butyl palmitate and oleate and presented evidence in support of the homolytic mechanism involving C—H cleavage as a rate-determining initial step.

In this paper, we show that the rearrangement which occurs in the β-hydroxyalkene part of ricinoleic acid and its *trans* isomer ricinelaidic acid, and their methyl esters, and which results in the loss of heptaldehyde, clearly corresponds to a low-energy CID reaction, and that a stable charge centre in the molecule ions is critical for observing this rearrangement. Low- and high-energy CID were also compared in order to examine whether (i) as found in previous studies,[10–13] loss of hydrogen occurs at high-energy CID together with a low-energy fragmentation reaction, and (ii) differences can be detected for the fragmentation of molecule ions of ricinoleic acid and its *trans* isomer and their methyl esters. Different types of molecule ions were examined in order to determine the structural requirements for observing charge-remote homolytic fragmentation.

EXPERIMENTAL

Mass spectrometry

Mass spectra were obtained on a VG70-SEQ hybrid mass spectrometer (Fisons, VGAnalytical) with an EBQQ configuration equipped with a caesium ion source. Cs^+ ions with an impact energy of $\sim$18 keV and a beam flux of 0.1 μA were used as the ionization beam. The accelerating voltage in the source was 8 kV. The samples were dissolved in dichloromethane (10 mg ml^{-1}) and 1 μl of the solution was mixed with 2 μl of the liquid matrix on the stainless-steel probe tip. As liquid matrices for liquid secondary ion mass spectrometry (LSIMS), *m*-nitrobenzyl alcohol (*m*-NBA) and *m*-nitrobenzyl alcohol saturated with LiI were used to produce $[M - H]^-/[M + H]^+$ and $[M + Li]^+/[M - H + 2Li]^+$ precursor ions, respectively. Low-energy CID was performed with argon in the quadrupole collision cell and scanning the quadrupole analyser. The gas pressure was 8×10^{-6} mbar (1 bar = 10^5 Pa) and the collision energy (laboratory frame) was 30 eV, conditions at which single collisions are expected to take place. High-energy CID spectra were acquired by linked scanning at constant B/E in the continuum mode of acquisition and accumulation of 15 scans. The helium pressure in the collision cell of the first field-free region was adjusted until the precursor selected ion beam was reduced to $\sim$50% of its original value. The ion abundances are based on three measurements and the associated error refers to three standard deviations. The most abundant product ion in the high-energy CID spectra was used for normalization. The multiplication factor was determined in a separate experiment for which the precursor ion signal was not saturated.

Ricinoleic and ricinelaidic acid were purchased from Sigma (St Louis, MO, USA); their methyl esters were prepared using diazomethane.

Nomenclature

The nomenclature for the fragmentation of fatty acid molecule ions in CID reactions introduced by Griffiths *et al.*[14] and modified by Claeys *et al.*[10] has been followed and has been extended in the present study for molecule ions containing the charge centre at the β-hydroxyalkene part. For clarity, the rules relevant for this study are briefly summarized below: (i) italic capital letters *C*, *A* and *H* are used to describe the nature of the bond broken with the charge retained on the carboxylate group and lower-case letters *c*, *a* and *h* are used for corresponding ions with the charge localized in the modified alkyl chain (*C* and *c* refer to a bond in a saturated part, *A* and *a* to an allylic bond and *H* and *h* to a homoallylic bond); (ii) a subscript to the right of the letter indicates the number of carbon atoms remaining in the fatty acyl part, e.g. C_n and c_n; and (iii) a prime to the left of the letter, e.g. $'A$, indicates that the product ion is deficient in one hydrogen compared with that product ion which would be formed by a homolytic fragmentation at the same point in the precursor molecule ion. It is pointed out that the last rule is a variation of that introduced by Griffiths et al.,[14] which was adapted by Claeys *et al.*[10] in order to make the nomenclature more general and also applicable to product ions formed from precursor molecule ions other than the $[M - H]^-$ ion, such as $[M + Li]^+$ and $[M - H + 2Li]^+$ ions.

RESULTS AND DISCUSSION

LSIMS of ricinoleic and ricinelaidic acid and their methyl esters

Figure 1 illustrates the molecular ion region of the LSI mass spectra obtained for ricinoleic acid and its *trans* isomer ricinelaidic acid using *m*-NBA as liquid matrix. Peaks are noted at *m/z* 337, 321, 299, 281 and 263, which correspond to $[M + K]^+$, $[M + Na]^+$, $[M + H]^+$, $[M + H - H_2O]^+$ and $[M + H - (2 \times H_2O)]^+$ ions, respectively. The ion abundance ratio $[M + H]^+/[M + H - H_2O]^+$ is different for the two isomers (13.2 $\pm$ 3.3% for the *cis* and 66.9 $\pm$ 2.5% for the *trans* isomer), indicating that the loss of water is favoured for the *cis* isomer. Loss of water can occur through several routes (Scheme 1); the present findings, however, may best be rationalized if route (b) is preferentially followed because loss of H_2O is more prevalent than loss of methanol for fragmentation of the $[M + H]^+$ ion of corresponding methyl esters (see below). The ion abundance ratio $[M + H - (2 \times H_2O)]^+/[M + H - H_2O]^+$ is also slightly different for the two isomers (17.8 $\pm$ 1.0% for the *cis* and 21.5 $\pm$ 1.2% for the *trans* isomer).

Similar results were obtained for the $[M + H]^+$ ion of the corresponding methyl esters (Fig. 2). The ion abundance ratios $[M + H]^+/[M + H - H_2O]^+$ and $[M + H - (H_2O + CH_3OH)]^+/[M + H - H_2O]^+$ are significantly different between the two isomers

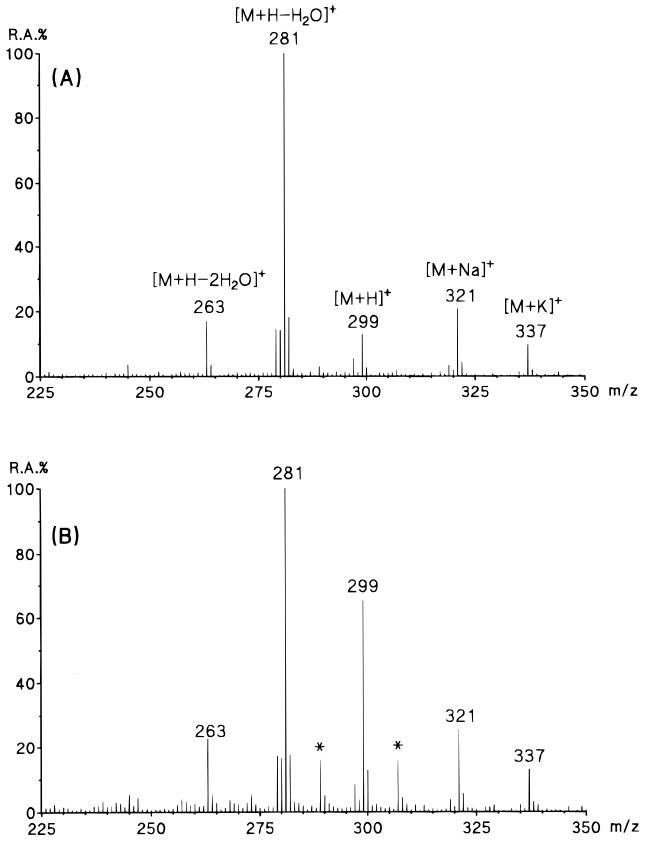


Figure 1. LSI mass spectra of (A) ricinoleic and (B) ricinelaidic acid obtained using *m*-NBA as matrix. Matrix peaks are indicated with asterisks.

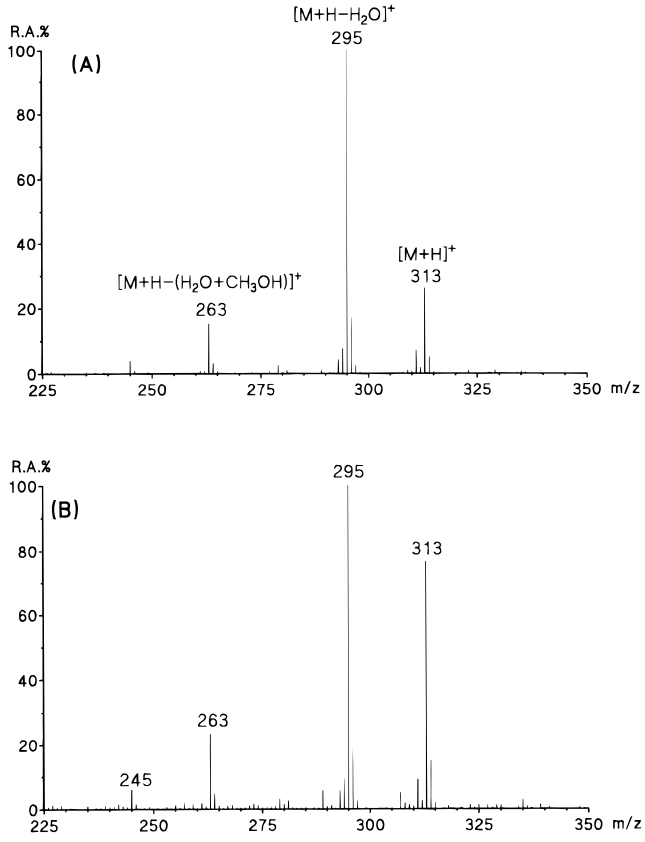


Figure 2. LSI mass spectra obtained for the methyl esters of (A) ricinoleic and (B) ricinelaidic acid using *m*-NBA as matrix.

(26.4 ± 0.3% for the *cis* and 70.7 ± 0.07% for the *trans* isomer and 15.2 ± 0.1% for the *cis* and 22.4 ± 1.0% for the *trans* isomer, respectively). Comparison of the LSI mass spectra obtained for the free fatty acids and their methyl esters indicates that the loss of water from the β-hydroxyalkene part is a prominent fragmentation route.

The LSI mass spectra obtained for ricinoleic acid and its *trans* isomer ricinelaidic acid using *m*-NBA saturated with LiI as the liquid matrix show $[M - H + 2Li]^+$ as well as $[M + Li]^+$ lithiated molecules (Fig. 3). The formation of an $[M + Li]^+$ molecule ion in the case of ricinoleic acid has briefly been mentioned by Adams and Gross.[3] In our opinion, the formation of the $[M + Li]^+$ ion is a typical feature of β-hydroxyalkene

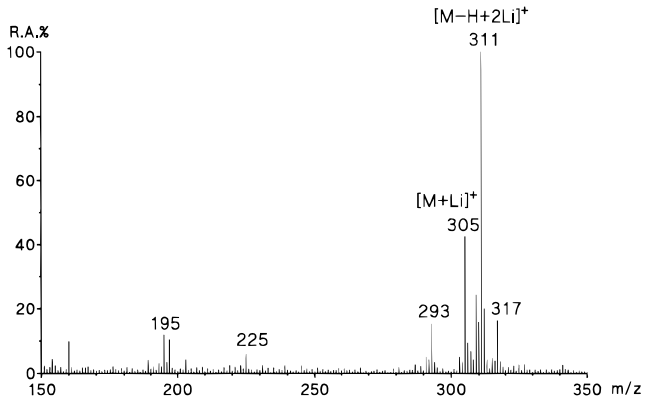
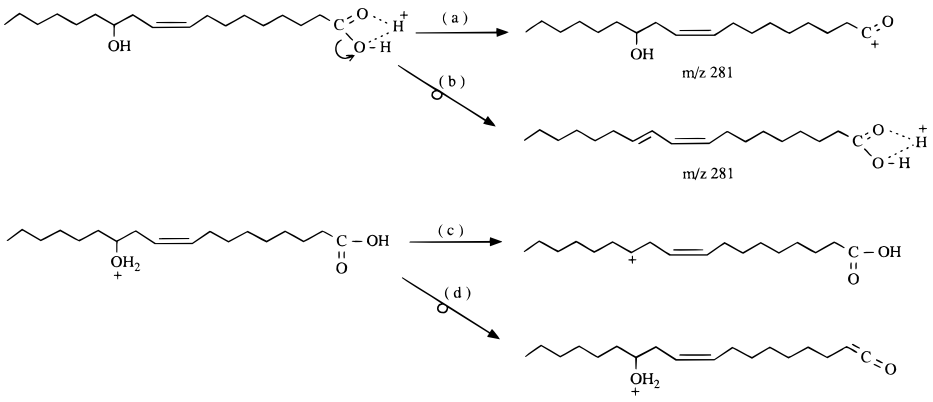


Figure 3. LSI mass spectrum of ricinoleic acid using *m*-NBA saturated with LiI as matrix.



Scheme 1

carboxylic acids and may be explained by Li^+ complex formation in the β-hydroxyalkene part; however, the $[M + Li]^+/[M\text{-}H + 2Li]^+$ ion abundance ratio was found not to be reproducible. The $[M + Li]^+$ ion is virtually not observed, for example, for oleic acid, which also contains a double bond at the C-9 position (not shown). As expected, the LSI mass spectra obtained for the corresponding methyl esters only show $[M + Li]^+$ ions (not shown).

Low-energy CID of $[M + H]^+$ ions

Table 1 lists the low-energy $[M + H]^+$ spectral data for ricinoleic acid and its *trans* isomer ricinelaidic acid. Product ions are noted at *m/z* 281 and 263, corresponding to the loss of one and two molecules of H_2O. The ion abundance ratios $[M + H - (2 \times H_2O)]^+/[M + H - H_2O]^+$ are found to be significantly different ($31.0 \pm 1.5\%$ for the *cis* and $48.6 \pm 1.0\%$ for the *trans* isomer). These results are in agreement with those obtained for the first-order LSI mass spectra, where a prevalent loss of one molecule of H_2O could also be observed for the *cis* isomer. The low-mass region shows ions at *m/z* 69, 83, 97, 111, 125 and 139, which are typical of fatty acyl chains.[15] The high-mass region shows a weak ion at *m/z* 245, which corresponds to the loss of three molecules of water; the loss of the third molecule of water, however, is difficult to explain but must occur in the carboxylic acid part.

With respect to the low-energy $[M + H]^+$ spectra of the methyl ester of ricinoleic acid and its *trans* isomer ricinelaidic acid (Table 1), product ions are observed at *m/z* 295 and 263, corresponding to the loss of H_2O and the combined loss of H_2O and CH_3OH, respectively. The ion abundance ratios $[M + H - (H_2O + CH_3OH)]^+/[M + H - H_2O]^+$ are found to be significantly different ($30.0 \pm 0.7\%$ for the *cis* and $43.2 \pm 2.7\%$ for the *trans* isomer). These results are consistent with those obtained for the free fatty acids, for which the loss of one molecule of H_2O appears to be favoured for the *cis* isomer. The results are also in agreement with those obtained from first-order LSI mass spectra (Fig. 2), which show similar although more pronounced differences for the $[M + H - (H_2O + CH_3OH)]^+/[M + H - H_2O]^+$ ion abundance ratios. As found for the free fatty acids, a series of carbocations is detected in the low mass range between *m/z* 69 and *m/z* 139. A small peak is also noted at *m/z* 245, which corresponds to the combined loss of CH_3OH and two molecules of H_2O.

It is pointed out that low-energy CID of $[M + H]^+$ protonated molecules only results in weak ions due to the loss of heptaldehyde ($C_7H_{14}O$) at *m/z* 185 and 199 for the free fatty acids and their methyl esters, respectively. This behaviour contrasts with that of $[M - H]^-$, $[M + Li]^+$ and $[M - H + 2Li]^+$ ions of the free fatty acids and $[M + Li]^+$ ions of the methyl esters (see below), where the loss of $C_7H_{14}O$ corresponds to a major fragmentation route. In the case of the methyl esters, the loss of water can be explained by a charge-remote rearrangement (Scheme 1, route b) or a charge-induced cleavage (route c), both of which can be regarded as low-energy processes.

High-energy CID of $[M + H]^+$ ions

The product ion spectra obtained for the $[M + H]^+$ protonated molecules of ricinoleic and ricinelaidic acid are summarized in Table 1. Major peaks observed in the high-mass range are at *m/z* 281, 279 and 263 and correspond to $[M + H - H_2O]^+$, $[M - H - H_2O]^+$ and $[M + H - (2 \times H_2O)]^+$ ions, respectively. The ion abundance ratios $[M + H - (2 \times H_2O)]^+/[M + H - H_2O]^+$ are different for the two isomers ($21.4 \pm 2.5\%$ for the *cis* and $33.7 \pm 2.7\%$ for the *trans* isomer). These results are in agreement with those obtained by first-order LSIMS and low-energy CID. The low-mass range contains a series of carbocations from *m/z* 55 to 167, where every peak is duplicated by another at 2 units lower. A peak is also found at *m/z* 183 which appears to be more intense for the *cis* isomer and corresponds to the combined loss of heptaldehyde and H_2. This phenomenon of H_2 loss is also evident for the carbocations in the low-mass range and the $[M + H - H_2O]^+$ ion (*m/z* 281).

The high-energy CID $[M + H]^+$ spectra obtained for the methyl esters of ricinoleic acid and its *trans* isomer (Table 1) show product ions in the high-mass range at *m/z* 295, 279, 263 and 245, corresponding to $[M + H - H_2O]^+$, $[M - H - CH_3OH]^+$, $[M + H - H_2O - CH_3OH]^+$ and $[M + H - 2H_2O\text{-}CH_3OH]^+$ ions, respectively. The ion abundance ratios $[M + H - (H_2O + CH_3OH)]^+/[M + H - H_2O]^+$ are significantly different between the two isomers ($59 \pm 2.7\%$ for the *cis* and $78.5 \pm 2.6\%$ for the *trans* isomer) and are in agreement with results obtained by first-order LSIMS and low-energy CID. The ion at *m/z* 279 corresponds to the combined loss of CH_3OH and H_2. Since this ion is not observed in the low-energy spectra (Table 1), these results indicate that the loss of H_2 corresponds to a high-energy CID reaction. Loss of H_2 appears to be very characteristic of high-energy CID and has been observed in cases where low-energy fragmentation also occurs, for example, for (i) *n*-alkenylsalicylic acid molecule ions ($[M - H]^-$ and $[M - H + 2Li]^+$), which show charge-proximate loss of CO_2,[11] (ii) protonated acylcarnitines and acylcarnitine esters, which show charge-induced elimination of $(CH_3)_3N$,[12] (iii) protonated monoacyl glycerides which show a charge-induced loss of H_2O and glycerol,[13] and (iv) lithiated *n*-butyl palmitate and oleate, which give rise to a charge-proximate rearrangement, i.e. loss of C_4H_8.[10]

A series of carbocations is observed, showing 14 unit intervals from *m/z* 55–223. Each peak is duplicated by another peak at 2 mass units lower. Two peaks not belonging to this series are noted at *m/z* 199 and 197, resulting from the loss of heptaldehyde and the combined loss of heptaldehyde and H_2, respectively. As observed for the free fatty acids (Table 1), the ion at *m/z* 197 is relatively more abundant for the *cis* isomer.

Low-energy CID of $[M - H + 2Li]^+$ and $[M + Li]^+$ ions

$[M - H + 2Li]^+$ precursor ions were examined because these species containing a stable charge centre have been shown to be suitable precursor ions in order

Table 1. Low- and high-energy CID spectral data obtained for protonated molecule species of ricinoleic acid (A) and its *trans* isomer ricinelaidic acid (B) and their corresponding methyl esters (Am and Bm)[a]

			Product ions				
CID regime	Compound	Precursor ion $[M+H]^+$ *m/z* (R.A., %)	$[M+H-H_2O]^+$ *m/z* (R.A., %) $[M-H-H_2O]^+$ *m/z* (R.A., %)	$[M+H-2H_2O]^+$ *m/z* (R.A., ‰)	$[M+H-H_2O-CH_3OH]^+$ *m/z* (R.A., ‰)	$[M+H-C_7H_{14}O]^+$ *m/z* (R.A., ‰) $[M-H-C_7H_{14}O]^+$ *m/z* (R.A., ‰)	Others *m/z* (λ R.A., ‰)
Low-energy	A	299 (251)	281 (100) 279 (—)	263 (31)		185 (10) 183 (4)	245 (6), 179 (6), 165 (7), 153 (5), 139 (8), 125 (14), 111 (16), 97 (20), 83 (16), 69 (5)
	B	299 (248)	281 (100) 279 (—)	263 (49)		185 (8) 183 (5)	245 (12), 179 (12), 165 (12), 153 (8), 139 (12), 125 (18), 111 (25), 97 (26), 83 (16), 69 (5)
	Am	313 (362)	295 (100) 293 (—)		263 (30)	199 (4) 197 (—)	245 (12), 179 (8), 165 (6), 139 (4), 125 (5), 111 (7), 97 (8), 83 (6)
	Bm	313 (262)	295 (100) 293 (—)		263 (45)	199 (8) 197 (4)	245 (14), 179 (11), 165 (8), 151 (6), 139 (6), 125 (8), 111 (10), 97 (10), 83 (7)
High-energy	A	299 (117)	281 (100) 279 (23)	263 (21)		185 (—) 183 (15)	165 (5), 139 (5), 125 (6), 123 (6), 111 (7), 109 (6), 97 (14), 95 (10), 83 (15), 81 (13), 69 (13), 69 (12), 67 (9), 55 (8)
	B	299 (206)	281 (100) 279 (19)	263 (34)		185 (—) 183 (—)	179 (8), 165 (7), 151 (7), 139 (7), 137 (8), 125 (8), 123 (10), 111 (13), 109 (10), 97 (24), 95 (21), 83 (25), 81 (28), 69 (21), 67 (15), 55 (13)
	Am	313 (197)	295 (100)		263 (62)	199 (10) 197 (19)	279 (74), 245 (18), 179 (13), 165 (16), 151 (11), 137 (12), 125 (10), 123 (16), 111 (7), 109 (18), 97 (33), 95 (38), 83 (32), 81 (38), 69 (26), 67 (21), 55 (20)
	Bm	313 (207)	295 (100)		263 (78)	199 (12) 197 (11)	279 (73), 245 (21), 179 (16), 165 (18), 151 (13), 137 (14), 125 (12), 123 (18), 111 (14), 109 (21), 97 (38), 95 (41), 83 (37), 81 (45), 69 (29), 67 (24), 55 (21)

[a] Ions with relative abundance (R.A.) ⩾ 4‰ have been listed. The $[M+H-H_2O]^+$ ion was used for normalization.

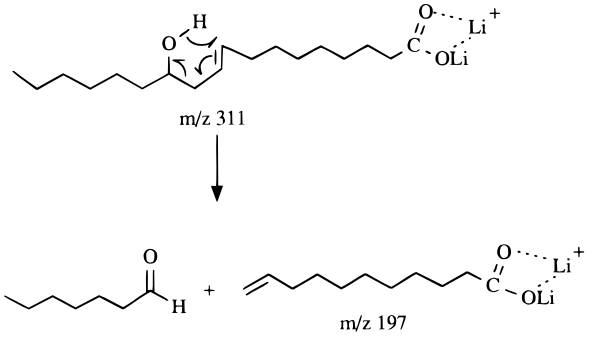
to observe charge-remote fragmentations.[11,16,17] Table 2 gives the low-energy $[M - H + 2Li]^+$ spectra of ricinoleic acid and its *trans* isomer ricinelaidic acid. Product ions are present at *m/z* 293 and 197, and are due to the loss of H_2O and heptaldehyde, respectively. The loss of $C_7H_{14}O$ corresponds to a rearrangement in the β-hydroxyalkene part (Scheme 2) and has been observed upon pyrolysis of β-hydroxyolefins.[18] The occurrence of this thermal rearrangement has also been well documented for high-energy CID by Adams and Gross.[3] It is pointed out that the present results indicate that low-energy CID is sufficient to observe this fragmentation. This behaviour is in contrast with the formal loss of elements of alkanes in long-chain fatty acid derivatives containing a stable charge centre, which is only noticed upon high-energy CID.[1,2,19–21] Comparison of the $[M - H + 2Li]^+$ spectra of the two isomers shows that the ion abundance ratio $[M - H + 2Li - H_2O]^+/[M - H + 2Li - C_7H_{14}O]^+$ is higher for the *cis* isomer (88.0 ± 1.6% for the *cis* and 48.4 ± 1.0% for the *trans* isomer). These results indicate that the loss of water, which corresponds to a charge-remote rearrangement, is again favoured for the *cis* isomer, and suggests that the loss of H_2O may be determined by the geometry of the double bond. Although it was not possible for $[M + H]^+$ ions of the free fatty acids and their methyl esters to determine whether the loss of H_2O occurs by a charge-proximate or a charge-remote reaction, these results indicate that charge-remote loss of H_2O is a major low-energy CID fragmentation pathway.

With regard to the low-energy $[M + Li]^+$ spectra of ricinoleic acid and its *trans* isomer ricinelaidic acid (Table 2), similar observations with regard to the ion abundance ratios $[M + Li - H_2O]^+/[M + Li - C_7H_{14}O]^+$ can be made (58.0 ± 0.7% for the *cis* and 34.2 ± 1.8% for the *trans* isomer). In addition to the ions observed at *m/z* 287 and 191, an ion is also noted at *m/z* 121, which can be explained by Li^+-adduct formation in the β-hydroxyalkene part followed by rearrangement (Scheme 3). The formation of the ion at *m/z* 121 indicates that Li^+-adduct formation may also occur in the β-hydroxyalkene part. On the basis of this observation, we believe it is reasonable to assume that the β-hydroxyalkene part may also be a possible protonation site. Comparison of the $[M + Li]^+$ spectra also shows that the formation of the *m/z* 121 ion does not appear to be affected by the geometry of the double bond.

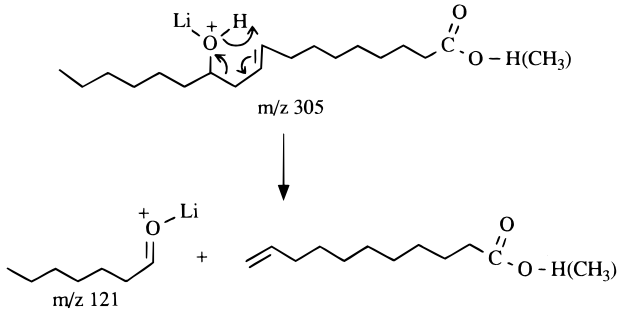
Similar results were obtained for the $[M + Li]^+$ ion spectra of the methyl esters of ricinoleic and ricinelaidic acid (Table 2). Product ions formed by loss of H_2O (*m/z* 301) and heptaldehyde (*m/z* 205) are observed and similar conclusions concerning the ion abundance ratios $[M + Li - H_2O]^+/[M + Li - C_7H_{14}O]^+$ can be made (17.5 ± 1.5% for the *cis* and 12.1 ± 2.0% for the *trans* isomer). The ion at *m/z* 121 is also noted; again, as found for the free fatty acids, its relative abundance does not appear to be dependent upon the geometry of the double bond.

High-energy CID of $[M - H + 2Li]^+$ and $[M + Li]^+$ ions

The high-energy CID $[M - H + 2Li]^+$ ion spectra obtained for ricinoleic acid and its *trans* isomer ricinel-



Scheme 2



Scheme 3

Table 2. Low-energy CID spectral data obtained for $[M - H + 2Li]^+$ and $[M + Li]^+$ precursor ions of ricinoleic acid (A) and its *trans* isomer ricinelaidic acid (B) and their corresponding methyl esters (Am and Bm)[a]

			Product ions		
Precursor ion	*m/z* (R.A., %)	Compound	$[P - H_2O]^+$ *m/z* (R.A., %)	$[P - C_7H_{14}O]^+$ *m/z* (R.A., %)	Other ion *m/z* (R.A., %)
$[M - H + 2Li]^+$	311 (602)	A	293 (88)	197 (100)	—
	311 (700)	B	293 (48)	197 (100)	—
$[M + Li]^+$	305 (1929)	A	287 (58)	191 (100)	121 (13)
	305 (1479)	B	287 (33)	191 (100)	121 (16)
$[M + Li]^+$	319 (243)	Am	301 (12)	205 (100)	121 (6)
	319 (248)	Bm	301 (7)	205 (100)	121 (6)

[a] P refers to the precursor ion. The $[P - C_7H_{14}O]^+$ ion was used for normalization. R.A. = relative abundance.

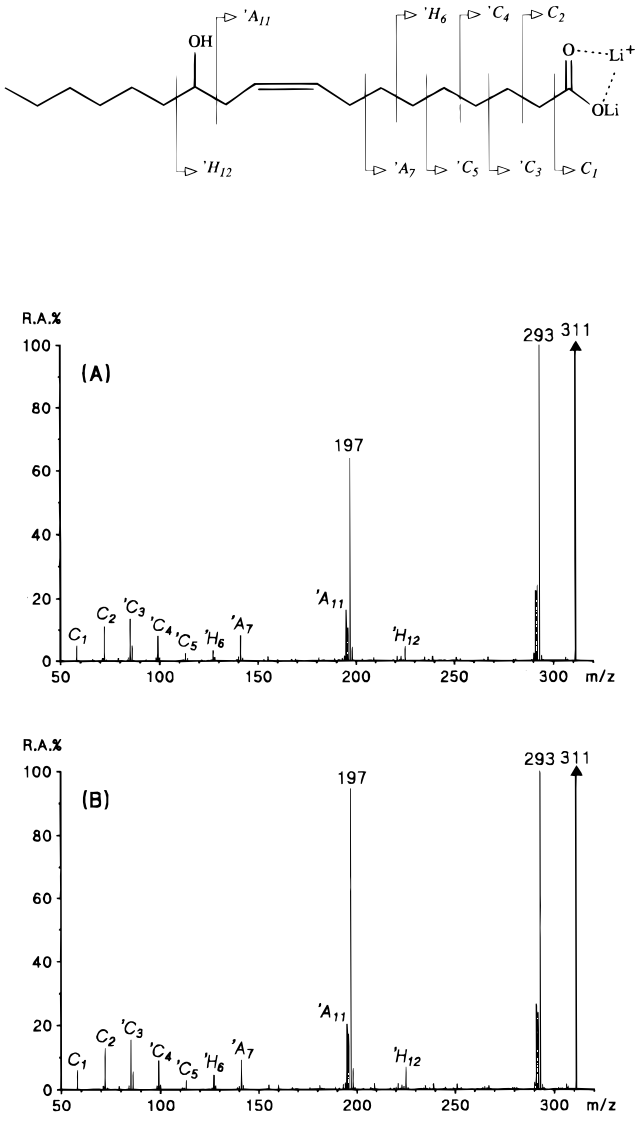


Figure 4. High-energy CID spectra obtained for $[M - H + 2Li]^+$ ions of (A) ricinoleic and (B) ricinelaidic acid at a beam attenuation of 50%. The spectra were normalized for the *m/z* 293 ion. The multiplication factor determined in a separate experiment was 19 and 16 for the *cis* and *trans* isomers, respectively.

aidic acid are illustrated in Fig. 4. As observed for low-energy CID, the ion abundance ratio $[M - H + 2Li - H_2O]^+/[M - H + 2Li - C_7H_{14}O]^+$ is significantly different for the two isomers ($64.3 \pm 1.7\%$ for the *cis* and $97.7 \pm 2.1\%$ for the *trans* isomer) and the loss of H_2O is more prevalent for the *cis* isomer. It is worth noting that both the ions at *m/z* 293 and 197 are accompanied by ions at one and two mass units lower, a feature which appears to be typical of high-energy CID. Product ions corresponding to fragmentation in the fatty acyl chain are noted in the low-mass range at *m/z* 141 ($'A_7$), 127 ($'H_6$), 113 ($'C_5$), 99 ($'C_4$), 85 ($'C_3$), 72 ($'C_2$) and 58 (C_1); these ions can best be rationalized by homolytic fragmentation, as discussed in detail in a previous study.[10] According to the latter proposal, the *C*-type ions are generated by H· radical abstraction at various positions along the alkyl chain followed by radical-induced C—C cleavage; in the case of the high-energy CID fragmentation of the *n*-butyl palmitate $[M + Li]^+$ ion, this mechanism could be supported by the finding that $[M + Li - H]^{+\cdot}$ ions serve as precursor ions for all the ions of the $'C$ series and that *n*-butyl [9,9-$^2H_2$]palmitate shows a decreased relative abundance for the $'C_{10}$ ion, consistent with C—H cleavage as a rate-determining initial step. The ion at *m/z* 225 ($'H_{12}$) belongs to the same ion series and can be readily explained by cleavage of the homoallylic C-12—C-13 bond following an initial cleavage of the allylic C-11—H bond.

Figure 5 shows the high-energy CID $[M + Li]^+$ spectra of ricinoleic acid and its *trans* isomer ricinelaidic acid. The fragmentation behaviour with respect to the loss of H_2O and heptaldehyde is essentially similar to that observed in the $[M - H + 2Li]^+$ spectra (Fig. 4). The ion abundance ratio $[M + Li - H_2O]^+/[M + Li$

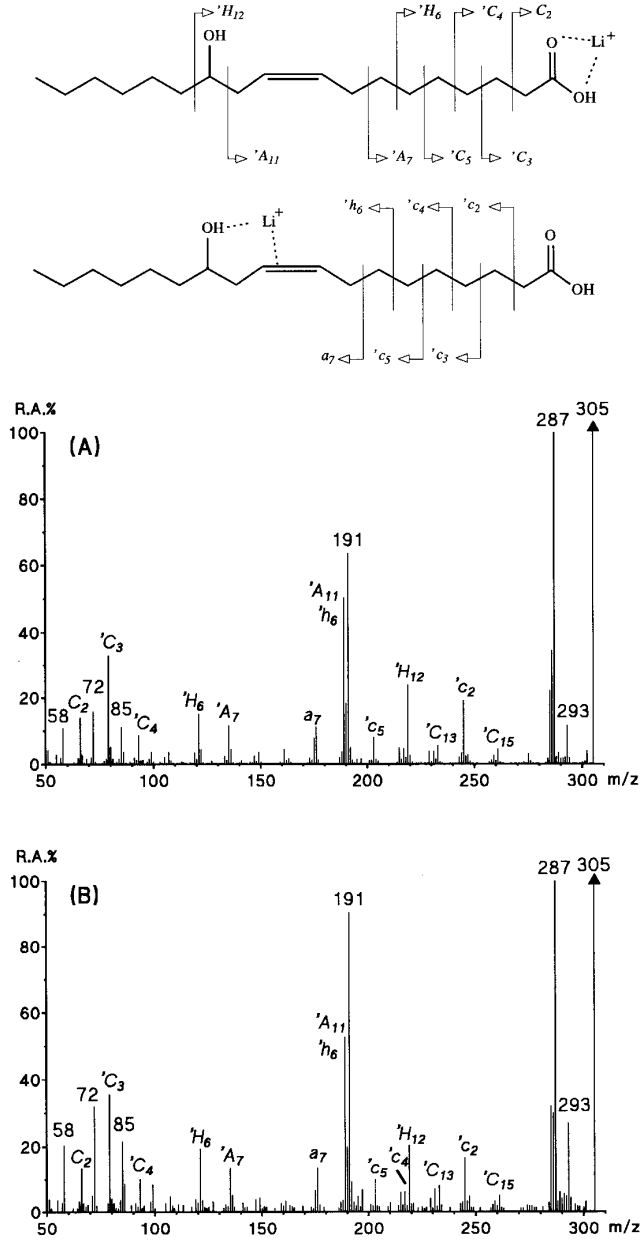


Figure 5. High-energy CID spectra obtained for $[M + Li]^+$ ions of (A) ricinoleic and (B) ricinelaidic acid at a beam attenuation of 50%. The spectra were normalized for the *m/z* 287 ion. The multiplication factor determined in a separate experiment was 49 and 68 for the *cis* and *trans* isomers, respectively.

$-C_7H_{14}O]^+$ is significantly different for the two isomers (65.9 ± 4.6% for the *cis* and 94.7 ± 4.6% for the *trans* isomer). The ions at *m/z* 191 and 287 again show satellite ions at one and two mass units lower. Ions corresponding to homolytic fragmentation in the fatty acyl chain are observed at *m/z* 66 (C_2), 79 ($'C_3$), 93 ($'C_4$), 121 ($'H_6$), 135 ($'A_7$), 189 ($'A_{11}$) and 219 ($'H_{12}$). The ion at *m/z* 121 can be attributed to $'H_6$ but most likely also originates from Li^+-adduct formation in the β-hydroxyalkene part and subsequent rearrangement (Scheme 3). The ion at *m/z* 189 corresponds to $'A_{11}$ but may also be rationalized by Li^+-adduct formation at the double bond and allylic cleavage of the C-8—H bond and radical-induced cleavage of the C-6—C-7 bond leading to $'h_6$ (Scheme 4). In a similar way, the ion at *m/z* 176 (a_7) is explained by Li^+-adduct formation in the β-hydroxyalkene part and allylic cleavage of the C-7—C-8 bond (Scheme 4). In addition, the ion at *m/z* 189 probably also has a contribution from the loss of heptaldehyde and associated loss of H_2.

The low mass region also reveals ions at *m/z* 58 ($[COOLi_2]^{+\bullet}$), *m/z* 72 ($[CH_2COOLi_2]^{+\bullet}$) and *m/z* 85 ($[CH_2{=}CHCOOLi_2]^+$); these ions are noted in the $[M - H + 2Li]^+$ spectra (Fig. 4) but are difficult to rationalize by fragmentation of $[M + Li]^+$ precursor ions. In addition, an ion is observed at *m/z* 293 which corresponds to loss of H_2O from the $[M - H + 2Li]^+$ ion. These results suggest that the $[M + Li]^+$ ion gives rise to interionic reactions under low-energy CID conditions to yield the $[M - H + 2Li]^+$ ion. The higher mass region also shows an ion at *m/z* 245 which may be attributed to $'c_2$; its enhanced relative abundance could be explained by the stability of the eliminated radical, which is stabilized by resonance.

The high-energy CID $[M + Li]^+$ spectra of the methyl esters of ricinoleic acid and its *trans* isomer ricinelaidic acid are illustrated in Fig. 6. Again, the fragmentation behaviour with regard to the loss of H_2O, heptaldehyde and associated loss of $H^\bullet$ and H_2 follows that observed in the $[M - H + 2Li]^+$ and $[M + Li]^+$ spectra of the free fatty acids (Figs 4 and 5). The ion abundance ratio $[M + Li - H_2O]^+/[M + Li - C_7H_{14}O]^+$ is significantly different for the two isomers (38.1 ± 0.5% for the *cis* and 28.1 ± 0.5% for the *trans* isomer).

Examination of the high-energy CID $[M + Li]^+$ spectra of the methyl esters is of interest because it enables one to obtain insight into the formation of ions

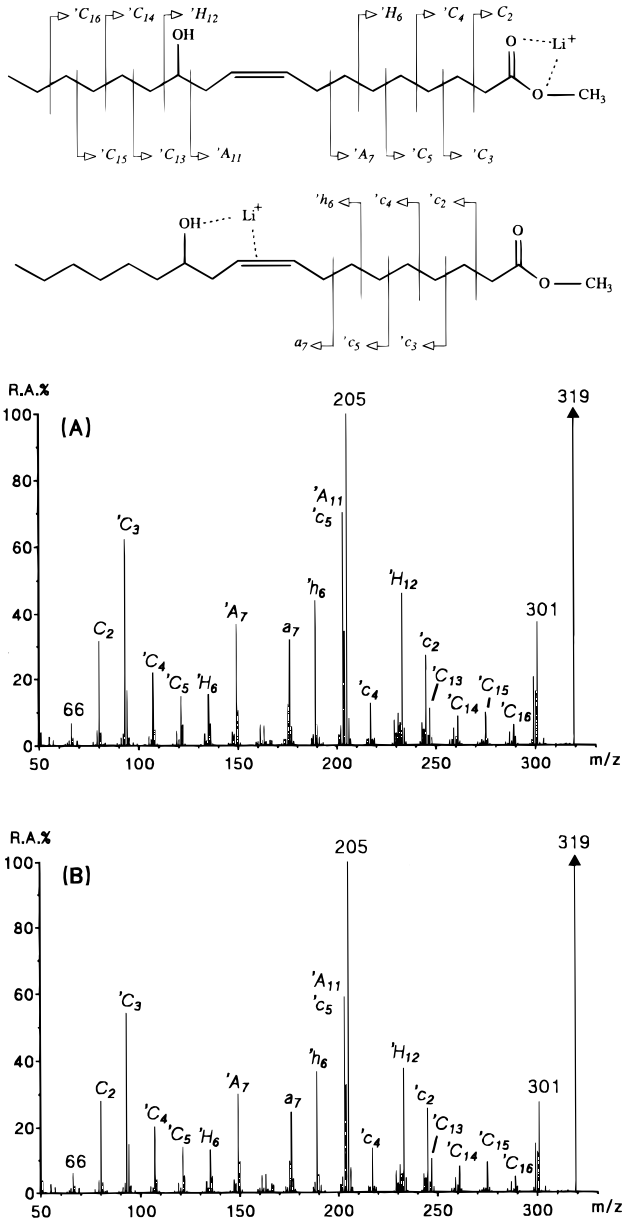
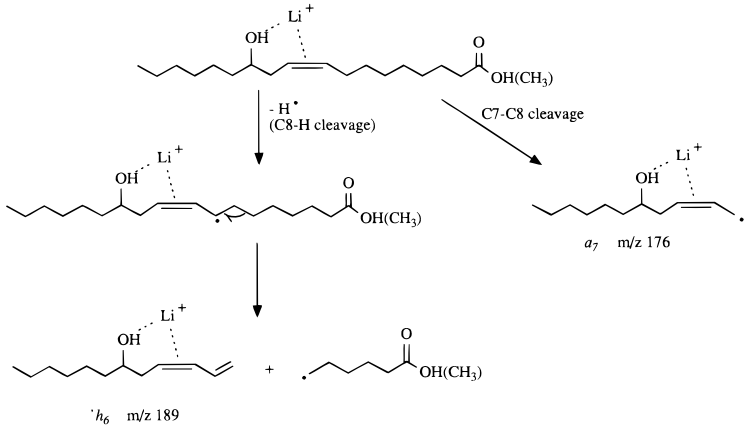


Figure 6. High-energy CID spectra obtained for $[M + Li]^+$ ions of the methyl esters of (A) ricinoleic and (B) ricinelaidic acid at a beam attenuation of 50%. The spectra were normalized for the *m/z* 205 ion. The multiplication factor determined in a separate experiment was 100 and 92 for the *cis* and *trans* isomers, respectively.



Scheme 4

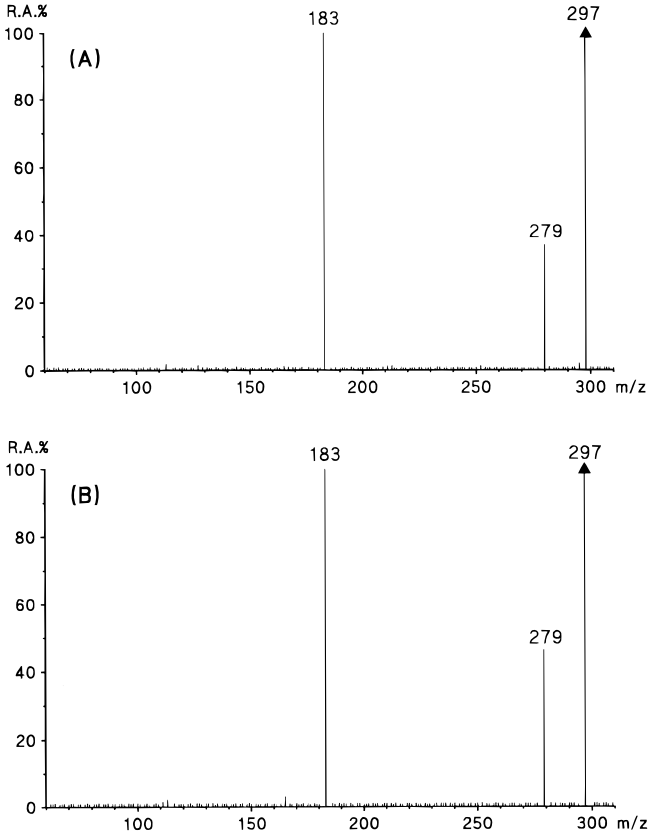


Figure 7. Low-energy CID spectra obtained for $[M-H]^-$ ions of (A) ricinoleic and (B) ricinelaidic acid at a gas pressure of 8×10^{-6} mbar and using a collision energy of 30 eV. The spectra were normalized for the *m/z* 183 ion. The multiplication factor determined in the same experiment was 60 and 52 for the *cis* and *trans* isomers, respectively.

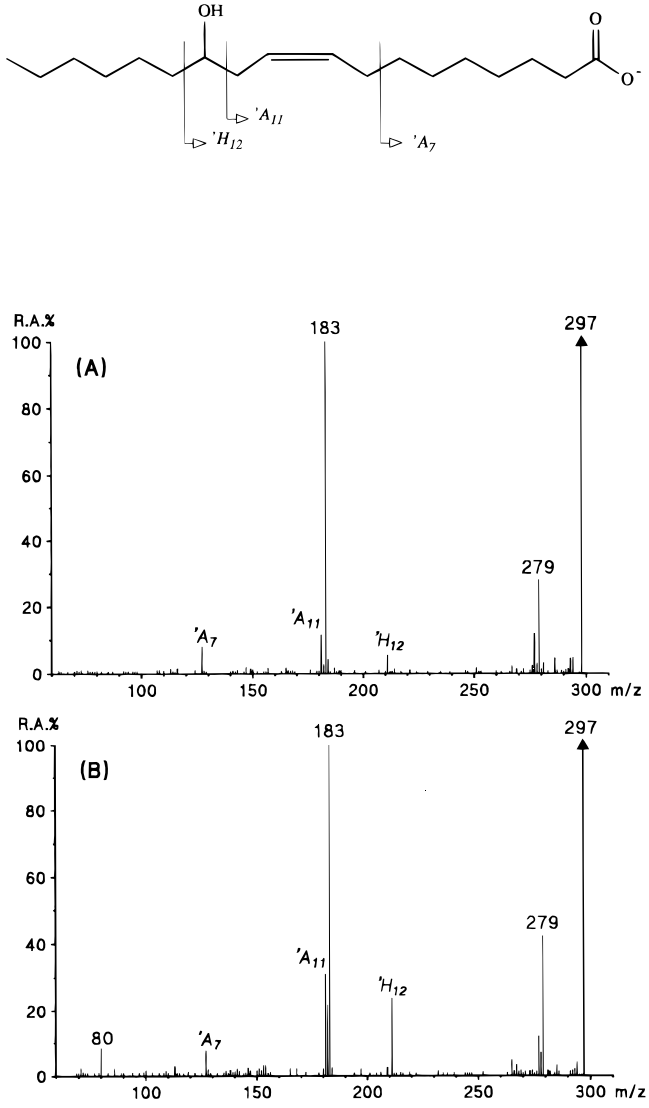


Figure 8. High-energy CID spectra obtained for $[M-H]^-$ ions of (A) ricinoleic and (B) ricinelaidic acid at a beam attenuation of 50%. The spectra were normalized for the *m/z* 183 ion. The multiplication factor determined in a separate experiment was 95 and 177 for the *cis* and *trans* isomers, respectively.

containing the *C*-terminus. Comparison with the $[M+Li]^+$ spectra of the free fatty acids show that the ions at *m/z* 176 (a_7) and 245 ($'c_2$) have not shifted, thus confirming that they contain the *C*-terminal part of the molecule. The high-energy CID $[M+Li]^+$ spectra of the methyl esters also clearly show two other *C*-terminal ions, more specifically $'h_6$ (*m/z* 189) and $'c_4$ (*m/z* 217); the $'c_5$ ion (*m/z* 203) however interferes with the $'A_{11}$ ion and the ion formed by combined loss of heptaldehyde and H_2.

Low-energy CID of $[M-H]^-$ ions

The negative-ion LSI mass spectra obtained for ricinoleic acid and its *trans* isomer ricinelaidic acid using *m*-NBA as the liquid matrix only showed the $[M-H]^-$ ion (spectra not shown). Figure 7 illustrates the low-energy CID spectra obtained for the $[M-H]^-$ ion of the two isomers. Two product ions are present at *m/z* 279 and 183, corresponding to the loss of water and heptaldehyde, respectively. Comparison of the low-energy CID spectra for the two isomers shows that the ion abundance ratio $[M-H-H_2O]^-/[M-H-C_7H_{14}O]^-$ is slightly different ($38.1 \pm 0.4\%$ for the *cis* and $45.2 \pm 0.9\%$ for the *trans* isomer). These results suggest that the loss of water occurs more readily for the *trans* isomer or, alternatively, that the competing *O*-hydro-*C*-allyl elimination is favoured in the *cis* isomer; this fragmentation behaviour is different from that observed for the $[M+H]^+$, $[M-H+2Li]^+$ and $[M+Li]^+$ ions of the free and methylated fatty acids, where the loss of H_2O is more prevalent for the cis isomer. The different behaviours of positively and negatively charged precursor ions with respect to the loss of H_2O (or alternatively, loss of heptaldehyde) in the *cis* and *trans* isomers suggest that the charge may have an effect on charge-remote rearrangement processes.

A similar low-energy CID spectrum of the ricinoleic $[M-H]^-$ ion has also recently been reported by Ponchaut *et al.*[22] Wheelan *et al.*[23] investigated the low-energy CID of the $[M-H]^-$ ion of ricinoleic acid and other monohydroxylated unsaturated fatty acids. In addition to loss of water, the combined loss of water and CO_2 was also observed by Wheelan *et al.*[23] for monohydroxylated unsaturated fatty acid carboxylate ions.

High-energy CID of $[M - H]^-$ ions

The high-energy CID spectra obtained for deprotonated molecules of ricinoleic acid and its *trans* isomer show peaks at *m/z* 279, 211, 183 and 127 (Fig. 8). The ion abundance ratio $[M - H - H_2O]^-/[M - H - C_7H_{14}O]^-$ is significantly different for the two isomers (24.8 ± 2.8% for the *cis* and 43.1 ± 0.4% for the *trans* isomer). As found for low-energy CID, the loss of water appears to be more favoured for the *trans* isomer. It is worth noting that the ions at *m/z* 183 and 279 are accompanied by ions at *m/z* values one and two units lower due to additional loss of $H^\cdot$ and H_2, respectively, a behaviour which appears to be characteristic of high-energy CID (see above). The ions at *m/z* 127 and 211 correspond to $'A_7$ and $'H_{12}$ ions, respectively, and may be rationalized by charge-remote homolytic cleavage in the fatty acyl chain. Whereas for the $[M - H]^-$ ions only $'A_7$ and $'H_{12}$ ions can be detected, $[M - H + 2Li]^+$ and $[M + Li]^+$ precursor ions are found to result in more extensive charge-remote homolytic fragmentation, e.g. fragmentation resulting in ions of the $'C$ and $'c$ type. These results indicate that charge-remote homolytic fragmentation in the fatty acyl chain is dependent upon the type of precursor ion subjected to high-energy CID and are in agreement with the results of Wysocki *et al.*,[24] who suggested that the internal energy required to record spectra which show remote-site fragmentation is compound dependent, i.e. strongly dependent upon the nature of the ionic group.

CONCLUSIONS

The results obtained in this study indicate that a stable charge centre in the carboxylic acid part is critical for observing the loss of heptaldehyde, which can be regarded as a low-energy CID charge-remote fragmentation reaction. Ricinoleic acid and its *trans* isomer ricinelaidic acid can be differentiated on the basis of the loss of H_2O, which is found to be prevalent in the *cis* isomer for positively charged precursor ions and can also best be rationalized by a charge-remote rearrangement. However, in some cases the ion abundance differences are subtle so that it would be essential to have both isomers as standards in order to determine the *cis/trans* geometry in an unkown sample unambiguously on the basis of the relative abundance of the ion formed by loss of water.

The fragmentation behaviour with respect to the loss of water and heptaldehyde is similar for high-and low-energy CID. Comparison of high-and low-energy CID spectra also reveals that associated loss of a hydrogen radical and hydrogen clearly corresponds to a high-energy CID fragmentation process. Examination of the high-energy CID spectra obtained for $[M + Li]^+$ and $[M - H + 2Li]^+$ precursor ions of free fatty acids and $[M + Li]^+$ ions of corresponding methyl esters reveals that homolytic fragmentation in the fatty acyl chain is most pronounced in the $[M + Li]^+$ spectra of the methyl esters of ricinoleic and ricinelaidic acid. Two series of charge-remote product ions are generated, more specifically, ions containing the stable charge (Li^+) at the carboxylic group or the β-hydroxyalkene part, which can both best be rationalized by charge-remote homolytic fragmentation. The high-energy CID fragmentation of $[M - H]^-$ ions is found to be limited to $H^\cdot$ and H_2 loss, which is associated with loss of water and heptaldehyde, and to charge-remote homolytic fragmentation at allylic and homoallylic positions.

Acknowledgements

L. Nizigiyimana is on leave from the University of Burundi and acknowledges the Belgian Ministry of Foreign Affairs (ABOS) for a doctoral research fellowship. M. Claeys is indebted to the Fund for Scientific Research (Flanders, Belgium) as a research director.

REFERENCES

1. J. Adams, *Mass Spectrom. Rev.* **9**, 141 (1990).
2. M. L. Gross, *Int. J. Mass Spectrom. Ion Processes* **118/119**, 137 (1992).
3. J. Adams and M. L. Gross, *J. Am. Chem. Soc.* **111**, 435 (1989).
4. N. J. Jensen, K. B. Tomer and M. L. Gross, *J. Am. Chem. Soc.* **107**, 1863 (1985).
5. V. H. Wysocki and M. M. Ross, Int. *J. Mass Spectrom. Ion Processes* **104**, 179 (1991).
6. W. J. Griffiths, A. Brown, R. Reimendal, Y. Yang, J. Zhang and J. Sjövall, *Rapid Commun. Mass Spectrom.* **10**, 1169 (1996).
7. M. Bambagiotti-Alberti, S. A. Coran, F. F. Vincieri and T. Petrucciani and P. Traldi, *Org. Mass Spectrom.* **21**, 485 (1986).
8. B. Domon, D. R. Müller and J. Richter, *Org. Mass Spectrom.* **27**, 1276 (1992).
9. M. Claeys and H. Van den Heuvel, *Biol. Mass Spectrom.* **23**, 20 (1994).
10. M. Claeys, L. Nizigiyimana and H. Van den Heuvel, *Rapid Commun. Mass Spectrom.* **10**, 770 (1996).
11. M. Claeys, H. Van den Heuvel, J. Claereboudt, J. Corthout, L. Pieters and A. J. Vlietinck, *Biol. Mass Spectrom.* **22**, 647 (1993).
12. J. F. Van Bocxlaer, M. Claeys, H. Van den Heuvel and A. P. De Leenheer, *J. Mass Spectrom.* **30**, 69 (1995).
13. L. Nizigiyimana, H. Van den Heuvel and M. Claeys, *J. Mass Spectrom. and Rapid Commun. Mass Spectrom.*, Special Volume, *Proceedings of the 3rd International Symposium on Applied Mass Spectrometry in the Health Sciences and the European Tandem Mass Spectrometry Conference*, Barcelona, 9–13 July, S19 (1995).
14. W. J. Griffiths, Y. Yang, J. Å. Lindgren and J. Sjövall, *Rapid Commun. Mass Spectrom.* **10**, 21 (1996).
15. K. L. Duffin, J. D. Henion and T. J. Shiek, Anal. Chem. **63**, 1781 (1991).
16. J. Adams and M. L. Gross, *Anal. Chem.* **59**, 1576 (1987).
17. M. J. Contado, J. Adams, N. J. Jensen and M. L. Gross, *J. Am. Soc. Mass Spectrom.* **2**, 180 (1991).
18. R. T. Arnold and G. Smolinsky, *J. Am. Chem. Soc.* **81**, 6443 (1959).
19. J. Adams and M. L. Gross, *J. Am. Chem. Soc.* **108**, 6915 (1986).
20. N. J. Jensen, F. W. Crow and M. L. Gross, *J. Am. Soc. Chem. Soc.* **105**, 5487 (1983).
21. N. J. Jensen, K. B. Tomer and M. L. Gross, *Anal. Chem.* **57**, 2018 (1985).
22. S. Ponchaut, K. Veitch, R. Libert, F. Van Hoof, L. Hue and E. de Hoffmann, *J. Am. Soc. Mass Spectrom.* **7**, 50 (1996).
23. P. Wheelan, J. A. Zirrolli and R. C. Murphy, *Biol. Mass Spectrom.* **22**, 465 (1993).
24. V. H. Wysocki, M. E. Bier, R. G. Cooks, *Org. Mass Spectrom.* **23**, 627 (1988).