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# Charge-remote fragmentation characteristics of monounsaturated fatty acids in resonance electron capture: differentiation between cis and trans isomers

Valery G. Voinov<sup>a,b</sup>, Magda Claeys<sup>a,\*</sup>

<sup>a</sup>University of Antwerp (UIA), Department of Pharmaceutical Sciences, Universiteitsplein 1, B-2610 Antwerp, Belgium

<sup>b</sup>Pacific Institute of Bioorganic Chemistry, Russian Academy of Sciences, 690022, Vladivostok, Russia

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## Abstract

In the present study we have examined cis and trans isomers of monounsaturated fatty acids (i.e. oleic, cis-vaccenic and erucic acids, and their trans isomers) using resonance electron capture (REC) at 7.2 eV. Differences between cis and trans isomers could be observed, which became more pronounced by lowering the electron energy within the limits of the resonance. The ion of diagnostic value does not belong to the regular charge-remote fragment ions corresponding to the combined loss of two hydrogen radicals and an alk(en)yl radical and is rationalized by a vinylic cleavage and loss of an alkyl radical directly from the excited molecular radical anion. In addition, collision-induced dissociation experiments have been performed on deprotonated cis- and trans-13-docosenoic acids generated by REC at 7.2 eV. These experiments demonstrated that the  $[M - H]^-$  ions were the precursors of all the regular charge-remote fragment ions observed in the REC spectra. The latter ions are rationalized by an initial loss of a hydrogen radical from different positions of the fatty acid chain yielding  $[M - H]^-$  ions (containing a radical site in the fatty acid chain) which fragment further by the combined loss of an alk(en)yl radical from the chain and a hydrogen radical from the carboxyl group. (Int J Mass Spectrom 205 (2001) 57–64) © 2001 Elsevier Science B.V.

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## 1. Introduction

Free fatty acids capture low-energy electrons in two energy regions, i.e. at 1.2 and 7.2 eV, giving rise to molecular radical anions, which are excited and unstable, and lose a hydrogen radical to form stable  $[M - H]^-$  ions [1–3]. In addition, resonance electron capture (REC) at 7.2 eV leads to charge-remote fragmentation (CRF) in the fatty acid chain, very

similar to what is observed for fatty acid carboxylate ions under high-energy collision activation conditions. Charge-remote fragmentation reactions were first described by Gross and co-workers (for a review, see [4]) for fatty acid carboxylate ions and functionalized alka(e)nes that contain a stable charge center and result in the formation of a homologous series of terminally unsaturated product ions corresponding to formal losses of elements of alka(e)nes. These reactions are analytically useful because they allow determination of the position of double bonds, hydroxy groups and branching in the aliphatic chain and are of

\* Corresponding author. E-mail: claeys@uia.ua.ac.be

Dedicated to Professor Aleksandar Stamatovic on the occasion of his 60th birthday.

fundamental interest because they represent a new type of gas phase ion reactions. Unlike most reactions which are initiated by either a radical or a charge site, charge-remote fragmentation reactions apparently do not involve a radical or charge site since they are noted in closed-shell precursor ions both positively and negatively charged. Previously reported results on the charge-remote fragmentation phenomenon have been interpreted in terms of a pericyclic process [5] or in terms of C–C bond cleavage, followed by  $\alpha$ -hydrogen abstraction [6]. Other work on alkali-cationized fatty acid esters has been interpreted in terms of C–H bond cleavage which is induced by a biradical from an electronically excited carbonyl group [7–9]. Support for the involvement of electronically excited states has come from work on dissociative resonance electron capture of fatty acids [1–3]. This work provides evidence for C–H bond cleavage at different positions along the alkyl chain as an initial step and is in agreement with a study demonstrating that the CRF process also leads to elimination of neutral alkene species [10]. Concerning the mechanism of CRF reactions observed for free saturated fatty acids in resonance electron capture at 7.2 eV, it has recently been proposed [3] that this technique leads to a doubly excited molecular anion species which is partially deactivated by loss of a hydrogen radical from different positions of the aliphatic chain. Subsequently, the  $[M - H]^-$  ions formed (containing a radical site in the fatty acid chain) can fragment further by loss of an alkyl radical from the chain and a hydrogen radical from the carboxyl group. Charge-remote fragmentation reactions can be considered as non-ergodic processes (or fragmentation directly from an excited state); if the resonance energy of 7.2 eV (or 161 kcal/mol) would be randomized over the whole fatty acid molecule the energy gained by individual C–C and C–H bonds becomes negligible.

In the present work, we have investigated cis and trans isomers of monounsaturated fatty acids using resonance electron capture at different electron energies within the limits of the resonance showing a maximum at 7.2 eV. REC spectra were recorded at different energies because previous experiments on saturated fatty acids demonstrated that the appearance

of the spectra is affected by the electron energy [3]. At the maximum of the resonance and higher energies (within the limits of the resonance) mainly ions corresponding to the combined loss of two hydrogen radicals and an alk(en)yl radical (formal combined loss of a hydrogen radical and elements of alk(e)anes) were detected, while at the lower energies of the resonance alkyl losses were also observed. By altering the electron energy within the limits of the 7.2 eV resonance differences have been found in the present study between cis and trans isomers of monounsaturated fatty acids, which became more pronounced at lower energy. To our knowledge, differences between cis and trans monounsaturated fatty acids cannot be detected using traditional ionization techniques such as electron or chemical ionization. Small differences, however, between cis and trans monounsaturated fatty acids have been reported by Jensen et al. [11] using fast atom bombardment ionization and high-energy collision-induced dissociation (CID).

The ion of diagnostic value for the differentiation of cis and trans monounsaturated fatty acids in REC can be rationalized by a distal vinylic cleavage in the alkenyl chain and loss of an alkyl radical directly from the molecular radical anion, and is more pronounced for trans than for cis isomers. Furthermore, it is demonstrated that the  $[M - H]^-$  ions (formed by loss of a hydrogen radical at different positions of the fatty acid chain) were the precursors of all the regular CRF ions corresponding to the combined loss of two hydrogen radicals and an alk(en)yl radical. These results support the hypothesis formulated earlier that C–H bond cleavage at different positions along the alkyl chain is a key step in the CRF phenomenon [7,8]. It has been proposed by Wysocki and Ross [6] for functionalized alka(e)nes under collision- and surface-induced dissociation conditions that charge-remote loss of alk(en)yl radicals is a lower energy process than the elimination of elements of alka(e)nes. The observations made in the present study on monounsaturated fatty acids in REC at 7.2 eV that losses of alkyl radicals occur at the lower limits of the resonance are in agreement with the latter study on the mechanism of charge-remote fragmentation reactions.

## 2. Experimental

### 2.1. Materials

Cis-9-octadecenoic (oleic, >99%), trans-9-octadecenoic (elaidic, >99%), cis-11-octadecenoic (cis-vaccenic, >97%), trans-11-octadecenoic (trans-vaccenic, >99%), cis-13-docosenoic (erucic, >99%) and trans-13-docosenoic (brassicidic, >98%) acids were purchased from Sigma Chemical Company (St. Louis, MO, USA). The isomeric purity of the fatty acids was checked after methylation with diazomethane by gas chromatography with flame ionization detection using a CP Sil 8CB capillary column (Chrompack, Middelburg, The Netherlands) which allowed for separation between cis and trans isomers.

### 2.2. Mass spectrometry

The resonance electron capture mass spectra were obtained on a VG70SEQ instrument (Micromass, Manchester, UK) modified for operation in the REC mode. To implement the REC mode on this instrument, some modifications were done as described in a previous study [3]. Briefly, the circuit of the filament power supply was changed; namely, the filament current was not set automatically in accordance with the electron trap (EI mode) or emission (CI mode) current but was adjusted manually using a potentiometer insulated from the high voltage. This configuration enabled us to operate at a total emission of up to 50  $\mu\text{A}$  without filament breakage. Electron energies (the energies of resonances) were set manually by a second additionally installed 10-turn potentiometer. The fatty acids were introduced through the direct inlet system. The instrumental conditions were as follows: accelerating voltage, 8 kV; emission current, 5  $\mu\text{A}$ ; analyzer pressure,  $(1\text{--}3)\times 10^{-7}$  Torr; ion chamber temperature, 180 °C.

CID spectra were obtained on an Autospec-oa-TOF instrument (Micromass, Manchester, UK) with an EBE-orthogonal acceleration-time-of-flight (TOF) configuration. For operating in the REC mode, instrumental conditions (software controlled parameters) for the CI mode instead of the EI mode were selected

but using an EI source instead of a CI source. This configuration enabled us to operate at a total emission current up to 250  $\mu\text{A}$  without filament breakage. The instrumental conditions were as follows: accelerating voltage, 8 kV; emission current, 50  $\mu\text{A}$ ; collision gas, methane; collision energy,  $E_{\text{lab}} = 800$  eV ( $E_{\text{com}} = 34$  eV); analyzer and TOF pressure,  $(3\text{--}4)\times 10^{-8}$  Torr; ion chamber temperature, 200 °C. The collision gas pressure was adjusted until the main beam intensity was reduced to 50% of its original value.

## 3. Results and discussion

Figs. 1 and 2 illustrate the REC spectra of erucic (cis) and brassidic (trans) acids at different electron energies between the limits of the 7.2 eV resonance (i.e. resonance showing a maximum at 7.2 eV). The top spectra (a) were obtained at an energy higher than 7.2 eV where a maximum was obtained for the  $[\text{M} - \text{H} - \text{H}_2]^-$  signal ( $m/z$  335), while the bottom spectra (b) were recorded at an energy lower than 7.2 eV where a maximum was obtained for the  $m/z$  225 signal. Comparison of the spectra reveals a clear difference for the abundance of the  $m/z$  225 ion which becomes more pronounced at lower energy.

This ion at  $m/z$  225, which is more abundant in the trans than in the cis isomer, can be rationalized by a vinylic cleavage and loss of a  $\text{C}_8\text{H}_{17}^\bullet$  radical from the molecular radical anion (Scheme 1). The negative molecular radical ion formed at 7.2 eV is believed to be in a doubly excited form (indicated by the two asterisks) and can be represented as a positive radical ion with two additional electrons in high-lying orbitals, where the first is the excited electron of an unshared pair of the carbonyl oxygen and the second is from the electron beam [1,2]. It is proposed that following the formation of a molecular radical anion an intramolecular interaction takes place between the double bond and the excited carboxyl group leading to the formation of a radical site at the C(13) position. Subsequently, a radical-induced loss of a  $\text{C}_8\text{H}_{17}^\bullet$  moiety can occur resulting in the formation of the ion at  $m/z$  225. In a similar manner a radical site can be generated at the C(14) position leading to the elimi-

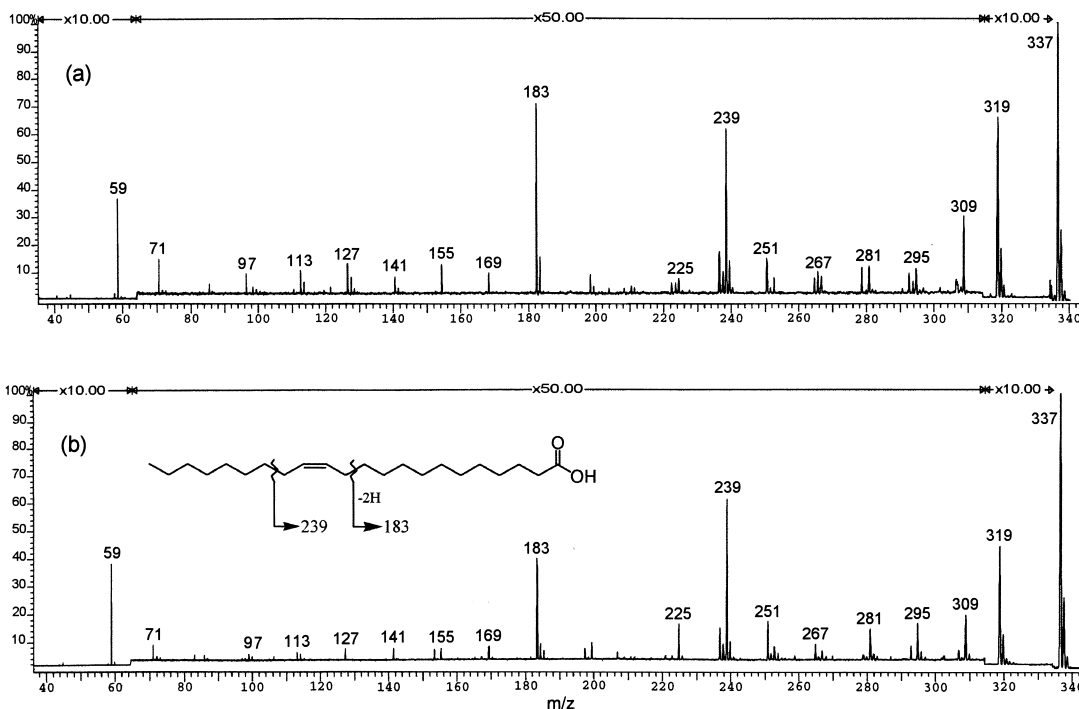


Fig. 1. REC spectra (7.2 eV resonance) obtained for erucic (cis-13-docosenoic) acid: (a) at an energy higher than 7.2 eV by optimising the  $[M - H - H_2]^-$  signal; and (b) at an energy lower than 7.2 eV by optimizing the ion at  $m/z$  225.

nation of a  $C_8H_{15}^\bullet$  radical and formation of an ion at  $m/z$  239. It is worth noting that this ion is also abundant in the spectra and is most abundant at the lower energy of the 7.2 eV resonance. Both the ions at  $m/z$  225 and 239 can be interpreted as formed directly from the excited molecular anion which is generated upon REC and thus do not belong to the regular CRF ions corresponding to the combined loss of two hydrogen radicals and an alk(en)yl radical. The latter are rationalized by an initial loss of a hydrogen radical from different positions of the fatty acid chain yielding  $[M - H]^-$  ions (containing a radical site in the fatty acid chain) which fragment further by the combined loss of an alk(en)yl radical from the chain and a hydrogen radical from the carboxyl group. Collision-induced dissociation (CID) experiments conducted at 7.2 eV on the  $[M - H]^-$  ions (formed by loss of a hydrogen radical at different positions of the fatty acid chain) indeed show that the  $m/z$  225 and 239 ions are absent from the  $[M - H]^-$  product ion

spectra (Fig. 3). In addition, it is worth noting that the ions at  $m/z$  281, 295, and 309 observed in the REC spectra (Figs. 1 and 2) are not detected in the  $[M - H]^-$  product ion spectra. The latter ions, therefore, can also be interpreted as formed directly from the molecular radical anion by loss of  $C_4H_9^\bullet$ ,  $C_3H_7^\bullet$ , and  $C_2H_5^\bullet$  radicals, respectively. Direct loss of alkyl radicals has been reported in a previous study [3] on the CRF behavior of saturated fatty acids in REC (7.2 eV resonance) and is in agreement with early work by Wysocki and Ross [6] on CRF characteristics of functionalized alkanes under collision- and surface-induced dissociation conditions.

The  $[M - H]^-$  product ion spectra of erucic and brassidic acids (Fig. 3) demonstrate that the  $[M - H]^-$  ions are the precursors of all the regular CRF ions observed in the REC spectra (Figs. 1 and 2) corresponding to the combined loss of two hydrogen radicals and an alk(en)yl radical. The formation of the ions at  $m/z$  183, 237, and 238, which define the gap

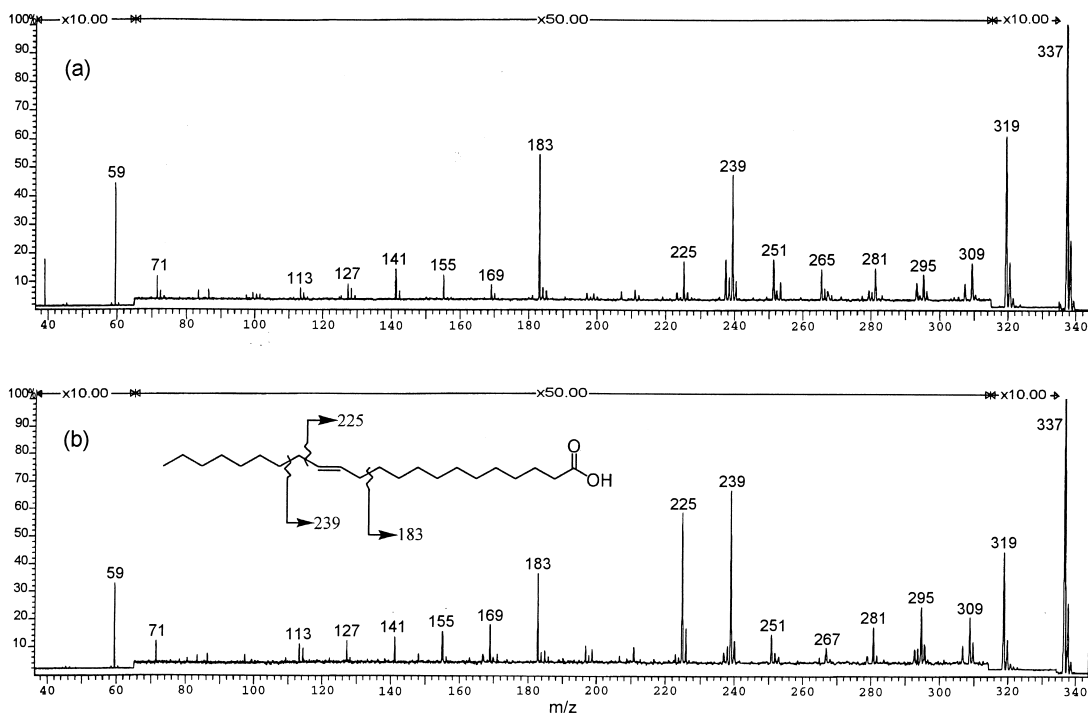
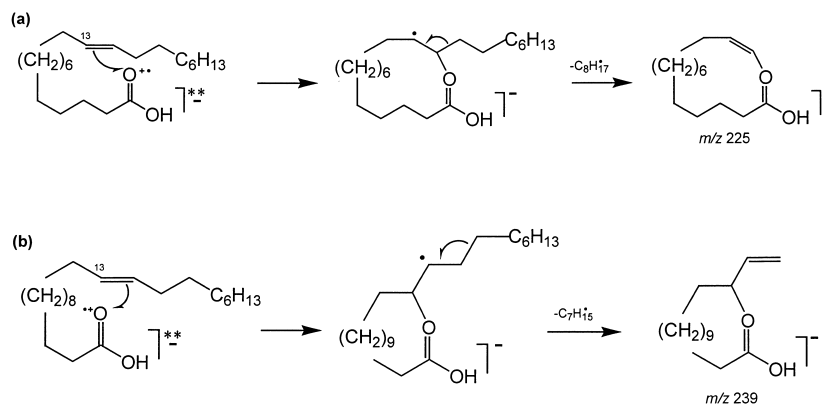


Fig. 2. REC spectra (7.2 eV resonance) obtained for brassidic (trans-13-docosenoic) acid: (a) at an energy higher than 7.2 eV by optimizing the  $[M - H - H_2]^-$  signal; and (b) at an energy lower than 7.2 eV by optimizing the ion at  $m/z$  225.

typical of the double bond at the C(13) position, is rationalized in Scheme 2. These ions and all the other CRF ions in the spectra ( $m/z$  113, 127, 141, 155, 169, 251, 265, 279, and 293) can be explained by a C–H bond cleavage at different positions along the alkenyl

chain which occurs during electron capture at 7.2 eV, followed by radical-induced processes.

In previous studies on alkali-cationized fatty acid esters [7,8] it has been proposed that C–H bond cleavage at different positions along the alkyl chain is



Scheme 1. Mechanistic proposal for the formation of the ions at  $m/z$  225 and 339 formed from cis- and trans-13-docosenoic acids in resonance electron capture (7.2 eV resonance).

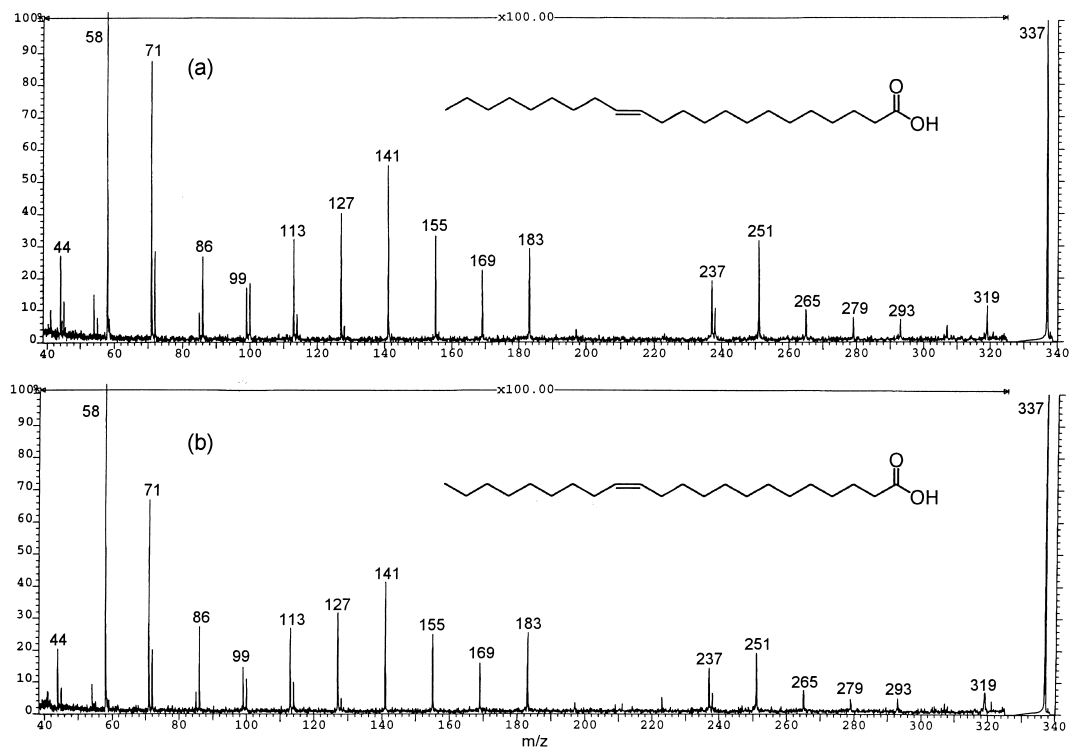
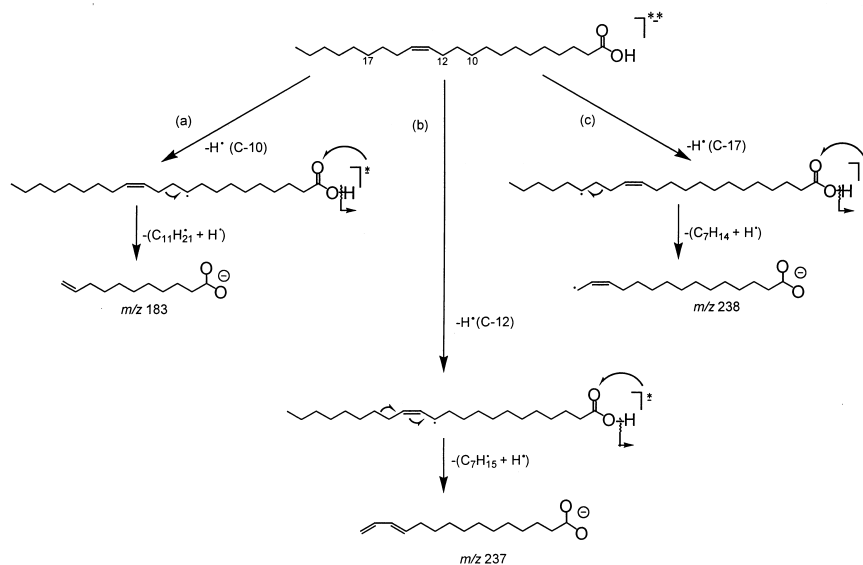


Fig. 3.  $[M - H]^-$  product ion spectra obtained for (a) erucic and (b) brassidic acids. For CID conditions, see Sec. 2.



Scheme 2. Mechanistic proposal for the formation of CRF ions ( $m/z$  183, 237, and 238) formed from 13-docosenoic acids in resonance electron capture (7.2 eV resonance).

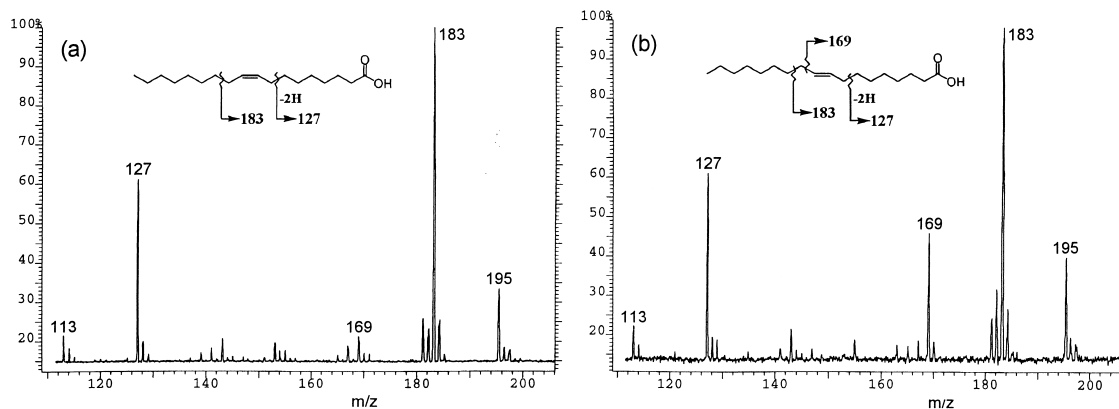


Fig. 4. REC spectra (mass region 130–230) obtained for (a) oleic (cis-9-octadecenoic) acid and (b) its trans isomer elaidic acid.

a key step in the CRF phenomenon. The observation made in the present study that the  $[M - H]^-$  ions formed by REC at the 7.2 eV resonance are the precursors of all the CRF ions involving combined loss of two hydrogen radicals and an alk(en)yl radical is in agreement with the latter hypothesis. Several other mechanisms have been advanced to rationalize the formation of CRF ions at high-energy CID of monounsaturated fatty acid ions, including 1,4-elimination of a vinylic hydrogen, a retro-ene reaction and a 1,4-conjugate elimination [12]. It is unlikely on the basis of results obtained in the present study that the latter reactions hold for CRF processes observed in REC (7.2 eV resonance). Furthermore, it is worth noting that the high-mass region of the  $[M - H]^-$  product ion spectra (Fig. 3) shows an ion corresponding to the loss of a molecule of water ( $m/z$  319) and that the low-mass region reveals odd-electron ions ( $m/z$  86, 100, 114, and 128). The latter ions formally correspond to the loss of elements of alkenes from the molecular radical anion, but, taking into account that they originate from the  $[M - H]^-$  precursors, can be interpreted by subsequent combined loss of a hydrogen radical and elements of alkadienes (by a radical-induced C–C cleavage in the direction of the charge). The differences in ion abundance ratios (i.e.  $[m/z$  113]/ $[m/z$  114] and  $[m/z$  127]/ $[m/z$  128]) observed between the first order REC spectra (Figs. 1 and 2) and between these spectra and the  $[M - H]^-$  product ion spectra (Fig. 3) likely result from differences in

the internal energy content of the fragmenting  $[M - H]^-$  ions.

In a next series of experiments, two pairs of cis/trans octadecenoic acids were examined in order to verify whether the observed differences between cis and trans 13-docosenoic acids also hold for other monounsaturated fatty acids. Figs. 4 and 5 illustrate the regions of interest of REC spectra obtained for cis and trans 9- and 11-octadecenoic acids, respectively. The spectra shown were obtained by optimizing the diagnostic peak (i.e. at  $m/z$  169 and 197, respectively) and demonstrate clear differences between cis and trans isomers.

#### 4. Conclusions

The results obtained in the present work demonstrate that monounsaturated fatty acids in resonance electron capture yield diagnostic charge-remote fragment ions which can be interpreted by loss of alkyl radicals directly from the excited molecular radical anion. The ion of diagnostic value for differentiation between cis and trans isomers involves a formal vinylic cleavage and is shown to be more abundant in trans than in cis isomers. No explanation has been given in the present study for this different behavior of cis and trans monounsaturated fatty acids in resonance electron capture. Molecular modeling studies



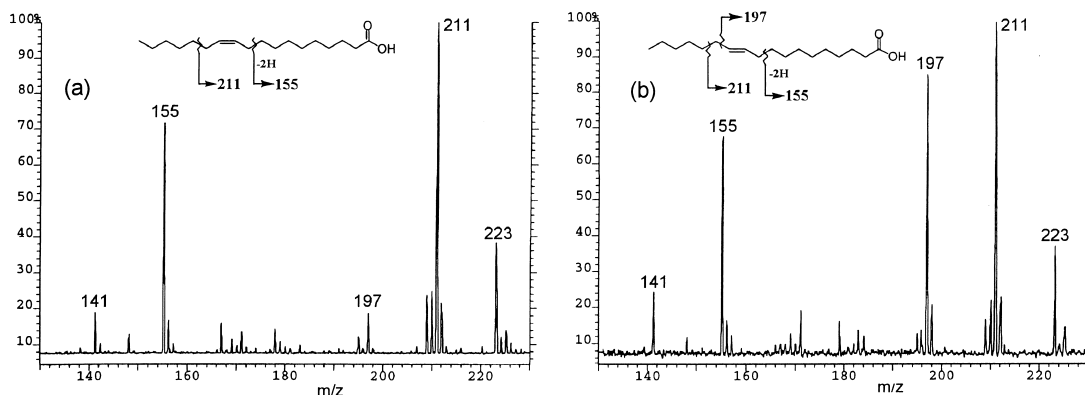


Fig. 5. REC spectra (mass region 130–230) obtained for (a) *cis*-vaccenic (11-octadecenoic) acid and (b) *trans*-vaccenic acid.

will be undertaken in future studies in order to cast more light on this isomer effect.

Furthermore, insight has been gained in the formation of regular charge-remote fragment ions observed in REC corresponding to the combined loss of two hydrogen radicals and an alk(en)yl radical. The  $[M - H]^-$  product ion spectra obtained for *cis*- and *trans*-13-docosenoic acids (Fig. 3) provide evidence that the  $[M - H]^-$  ions (formed by loss of a hydrogen radical at different positions in the fatty acid chain) are the precursors of all the regular CRF ions detected in REC. These results support a previously formulated hypothesis that C–H bond cleavage at different positions along the fatty acid chain is a key step in the CRF phenomenon [7,8].

In conclusion, the CRF process occurring in REC (and probably also in collision- and surface-induced dissociation) turns out to be more complex than originally thought and different types of processes have to be considered. Whereas some CRF ions can be interpreted in terms of C–H bond cleavage [7,8], followed by radical-induced reactions, others can be interpreted in terms of direct C–C bond cleavage [6] or indirect C–C bond cleavage caused by reaction with a double bond.

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