

Charge-remote and Charge-proximate Fragmentation Processes in Alkali-cationized Fatty Acid Esters upon High-energy Collisional Activation. A New Mechanistic Proposal

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The effect of the metal ion on the high-energy collision-induced dissociation (CID) of alkali metal-cationized *n*-butyl and methyl ester derivatives of palmitic and oleic acid was examined. The results show that the alkali metal ion has a pronounced effect and does not act as a mere 'spectator' ion with respect to the fragmentation process. While C–H cleavage is a dominant process for $[M + Li]^+$ as well as $[M + Na]^+$ precursor ions, C–C cleavage is also significant for the $[M + Na]^+$ ions. Homolytic mechanisms involving the formation of a transient biradical cation are proposed which enable us to rationalize in a straightforward manner all product ions formed by both charge-remote and charge-proximate fragmentations. The mechanistic proposal is discussed in view of available knowledge on electron impact, CID and related processes. In order to predict how the alkali metal ion could affect the reactivity of the postulated biradical state formed following electronic excitation of the alkali metal-cationized molecules, quantum chemical calculations were performed on methyl and *n*-butyl acetate as model substances. The decreased spin density at the carbonyl oxygen atom in the biradical state may provide an explanation for the greater tendency towards C–C cleavage reactions of the sodium-cationized fatty acid esters relative to the corresponding lithium complexes. © 1988 John Wiley & Sons, Ltd.

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INTRODUCTION

Since the development of soft ionization techniques capable of producing protonated, deprotonated or cationized molecules, many studies have been devoted to collision-induced dissociation (CID) processes of even-electron molecular ions. Early studies by Röllgen and co-workers^{1–3} dealing with high-energy CID reactions of small organic molecules and monosaccharides, which were cationized with an alkali metal ion (Li^+ , Na^+ or K^+) under field desorption or surface ionization conditions, demonstrated that the alkali metal ion acts predominantly as a charge carrier in the qualification of a 'spectator' ion with respect to the fragmentation process. It was also noted that molecules cationized with lithium behave to some extent as odd-electron molecular ions which are formed in electron ionization and as excited neutral molecules.¹ Investigation of the CID of even-electron molecular ions enjoyed a revival when the potential of CID in combination with tandem

mass spectrometric techniques to determine structural features in biomolecules such as fatty acids was recognized by Gross and co-workers (for reviews, see Refs 4 and 5). They reported that, in closed-shell ions containing a stable charge center, structurally informative fragmentation, termed charge-remote fragmentation (CRF), occurs at sites remote from the charge. Because similar fragmentation was observed for both positively and negatively charged closed-shell precursor ions, the logical assumption was made that the charge is not involved in CRF reactions. In the present study, attention was focused on some new insights into the mechanism of CRF in the hope that further progress can be made towards a physico-chemical understanding of this process and, more generally, of high-energy CID processes of even-electron ions.

A great number of studies have dealt with fundamental aspects of charge-remote fragmentation and its analytical application to biomolecules. The mechanism of CRF has been investigated for molecular ions of saturated fatty acids ($[M - H]^-$ and $[M - H + 2Li]^+$) and functionalized alkanes, but has remained a subject of controversy.⁵ A concerted 1,4- H_2 -elimination mechanism resulting in terminally unsaturated product ions has been formulated by Jensen *et al.*,⁶ while a homolytic C–C cleavage mechanism has been proposed by Wysocki and Ross.⁷ In recent studies, evidence for an alternative homolytic mechanism involving an initial C–H cleavage as a product-determining reaction has

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been obtained for fatty acid esters cationized with lithium.^{8–10} It was demonstrated that this mechanism not only applies to carbon positions remote from the charge but also holds equally well for proximate positions.⁹ The latter mechanism involving C–H cleavage was originally proposed in a study by Claeys and Van den Heuvel¹¹ of the CRF behavior of long-chain alkenylsalicylic acids. Whereas for saturated fatty acid esters C–H cleavage appears to be a major pathway, allylic C–H and C–C cleavages are found to be predominant pathways for monounsaturated and 1,4-diunsaturated fatty acid derivatives.^{8,10} It is worth mentioning that C–H cleavage has also been reported to be important in the CID of Li⁺-cationized tripeptides by Leary *et al.*,¹² more specifically, in the formation of $[A_n + Li - H]^+$ -type ions.

In the present study we compared the fragmentation of Li⁺ and Na⁺ cationized methyl and *n*-butyl palmitate and oleate with the aim of examining the effect of the alkali metal ion on CID processes. The results show that the alkali metal ion has an effect and does not act as a mere 'spectator' ion: while C–H cleavage is a dominant process for $[M + Li]^+$ and for $[M + Na]^+$ ions, C–C cleavage is also significant for the $[M + Na]^+$ ions. Mechanisms involving a transient biradical cation are proposed which enable us to rationalize in a straightforward manner all product ions formed. They account for the differences observed between the fragmentation of $[M + Li]^+$ and $[M + Na]^+$ ions of saturated fatty acid esters and also provide an explanation for the peculiar CID behavior of cationized mono-unsaturated fatty acid derivatives. In order to predict how the alkali metal ion could affect the reactivity of the postulated excited transition state, quantum chemical calculations were performed on methyl and *n*-butyl acetate as model substances. Some of the results obtained in this study were presented at the 14th International Mass Spectrometry Conference (25–29 August 1997, Tampere, Finland) and a brief preliminary account will be published in the proceedings of that conference.¹³

EXPERIMENTAL

Mass spectrometry

High-energy CID spectra were obtained on a VG70SEQ hybrid mass spectrometer of EBqQ design equipped with a cesium ion source (Micromass, Manchester, UK). Cesium ions with an impact energy of ~18 keV and a beam flux of 0.3 μ A were used as the ionization beam. The accelerating potential in the source was 8 kV. The helium pressure in the collision cell of the first field-free region was adjusted until the mass-selected ion beam was reduced to ~50% of its original value. The product ion spectra were acquired by linked scanning at constant *B/E* at a scan rate of 8 s per decade and were obtained in triplicate. The samples were dissolved in dichloromethane (2.5 mg ml⁻¹) and 1 μ l of the solution was mixed with 2 μ l of the liquid matrix on the stainless-steel probe tip. To produce $[M + Li]^+$ or $[M + Na]^+$ precursor ions, the liquid

matrix, *m*-nitrobenzyl alcohol, was saturated with LiI or NaI, respectively.

The fragmentation yield was determined by expressing the sum of the abundances of the C_2 , C_x and C'_x ($3 \leq x \leq n - 1$, where *n* is the number of carbon atoms in the fatty acyl chain) ions as a percentage of the sum of the abundances of these ions and the $[M + Li]^+$ or $[M + Na]^+$ precursor ion, under experimental conditions such that the precursor ion signal did not become saturated.

Ion nomenclature

The nomenclature for the fragmentation of fatty acid molecular ions in CID reactions introduced by Griffiths *et al.*¹⁴ and modified by Claeys *et al.*⁸ has been followed. For clarity, the rules relevant to this study are briefly summarized as follows: (i) the italicized capital letters *C*, *A* and *H* are used to describe the nature of the bond broken with the charge retained on the carboxylate group; *C* refers to a bond in a saturated part whereas *A* and *H* refer to an allylic and a homoallylic bond respectively; (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 (iii) a prime to the left of the letter indicates that the product ion is deficient in one hydrogen compared with that product ion which would be formed by homolytic fragmentation at the same point in the precursor molecular ion.

It is pointed out that the last rule is a variation of that introduced by Griffiths *et al.*,¹⁴ which was adapted in order to make the nomenclature more general and also applicable to product ions formed from precursor molecular ions other than $[M - H]^-$ ions such as $[M + Li]^+$ and $[M - H + 2Li]^+$ ions.⁸ The *C* ions are formed by cleavage of a C–C bond and can be regarded as odd-electron (OE) ions. The terminally unsaturated ions C'_n are generated by C–H cleavage at various positions in the alkyl chain and subsequent radical-induced C–C cleavage and correspond to even-electron (EE) ions.

Materials

Palmitic acid and oleic acid were purchased from Sigma (St Louis, MO, USA). [9,9-²H₂]Palmitic acid was obtained from Merck, Sharp and Dohme (Montreal, Canada).

Derivatization

Methyl esters were prepared using diazomethane and *n*-butyl esters were obtained following the method reported by Greeley.¹⁵ Briefly, *n*-butyl esters were prepared by dissolving 2.5 mg of fatty acid in 95 μ l of methanol and adding 2 mg of tetramethylammonium hydroxide in 5 μ l of methanol and 10 μ l of 1-iodobutane. The mixture was allowed to react at room temperature for 15 min and then filtered in order to remove tetramethylammonium iodide. The filtrate was evaporated under nitrogen and the residue was dissolved in 1 ml of dichloromethane.

Theoretical calculations

All geometries were optimized with *ab initio* calculation using the STO-3G and 6-31G* basis sets (GAMESS-UK molecular orbital package). The calculations were performed as follows: in a first step, the geometry of the uncomplexed ester (R) was optimized; in a second step, the geometry optimization was done for the alkali metal ion complex (RMe^+); and subsequently, quantum chemical properties of the RMe^+ complex biradical species were calculated at the unrestricted and restricted Hartree-Fock (HF) SCF level. The calculations at the restricted HF level were performed following those at the unrestricted HF level to determine the final transition-state energy. The convergence criterion was that the norm of the gradient is less than 0.000 75 and of the average gradient is less than 0.0005.

Two specific sites of Me^+ attachment to methyl and *n*-butyl acetate were found by total optimization using the STO-3G basis set. The lowest total energies of different conformers formed by Li^+/Na^+ attachment were determined. In the case of Li^+ methyl acetate complexes, the structures of the complexes were also fully optimized at the HF/6-31G* level and the geometries were refined at the MP2/6-31G* level. In addition, the zero-point vibrational energy (ZPVE) correction was added for the lowest vibrational level of a ground electronic state. Calculation at the MP2/6-31G* level also allowed the definition of two stable conformers for the Li^+ methyl acetate complexes.

RESULTS AND DISCUSSION

CID of *n*-butyl and methyl palmitate $[\text{M} + \text{Li}]^+$ and $[\text{M} + \text{Na}]^+$ ions

The CID spectra of the $[\text{M} + \text{Li}]^+$ and $[\text{M} + \text{Na}]^+$ ions of *n*-butyl and methyl palmitate are illustrated in Figs 1 and 2. The fragmentation of the *n*-butyl palmitate $[\text{M} + \text{Li}]^+$ ion has been discussed in detail in previous studies^{8,9} but is briefly summarized here in order to facilitate the discussion of the results obtained in the present study for the *n*-butyl palmitate $[\text{M} + \text{Na}]^+$ ion and methyl palmitate $[\text{M} + \text{Li}]^+$ and $[\text{M} + \text{Na}]^+$ ions. Product ions formed by high-energy homolytic cleavage reactions and also a low-energy rearrangement cleavage are present in the high-energy CID product ion spectrum of the *n*-butyl palmitate $[\text{M} + \text{Li}]^+$ ion [Fig. 1(A)]. The ion at m/z 263 is formed by a low-energy rearrangement reaction, more specifically, a McLafferty-type rearrangement in the *n*-butyl ester part. The C_2 ion and the ions of the 'C series correspond to ions characteristic of high-energy CID, which can all be rationalized by initial C-H cleavage reactions. The fragmentation of the $[\text{M} + \text{Li}]^+$ ion of methyl palmitate [Fig. 2(A)] is similar to that of *n*-butyl palmitate, except that the ion due to the loss of 1-butene corresponding to a McLafferty-type rearrangement in the ester part is absent from the spectrum. Compared with the $[\text{M} + \text{Li}]^+$ spectra, the $[\text{M} + \text{Na}]^+$ spectra [Figs 1(B) and 2(B)] clearly reveal a different product ion

profile: in addition to the ions of the 'C series observed for the fragmentation of the *n*-butyl and methyl $[\text{M} + \text{Li}]^+$ ions, the enhanced relative abundance of C-type ions, corresponding to C-C cleavage in the acyl chain, is a striking feature of the $[\text{M} + \text{Na}]^+$ spectra. It is pointed out that the ion due to the loss of 1-butene is absent from the *n*-butyl palmitate $[\text{M} + \text{Na}]^+$ spectrum, suggesting that the alkali metal ion also has an effect on the latter fragmentation reaction.

With respect to the formation of C-type ions, it is worth mentioning that this fragmentation behavior has also been reported for fatty acid carboxylate ions^{14,16} and for triacylglycerols cationized with NH_4^+ .¹⁷ Bambagiotti-Alberti *et al.*¹⁶ observed C-type ions upon high-energy CID of fatty acid carboxylate ions formed by dissociative electron capture of fatty acid methyl esters, while Griffiths *et al.*¹⁴ noted C-type ions employing an intermediate collision energy (400 eV, laboratory frame) for electrospray-generated fatty acid carboxylate ions. Pittenauer *et al.*¹⁷ reported similar homolytic fragmentation behavior for electrospray-generated ammoniated triacylglycerols which were collisionally activated on high-energy CID. Furthermore, C-type ions formed by distal cleavages in monounsaturated and 1,4-diunsaturated chains have recently also been shown to be of high diagnostic value in the structure characterization of isomeric 5-alk(adi)enylresorcinols by Suzuki *et al.*¹⁸ for determining the locations of double bonds in positional isomers present in a mixture.

It has been demonstrated in previous studies that C-H cleavage in the acyl chain at both charge-remote and charge-proximate positions is a decisive product-determining cleavage when the $[\text{M} + \text{Li}]^+$ ion of *n*-butyl palmitate is collisionally activated.^{8,9} These former results together with observations on Li^+ - and Na^+ -cationized fatty acid *n*-butyl and methyl esters made in the present study, which indicate that the alkali metal ion influences the fragmentation, prompted us to consider mechanisms involving a biradical cation formed by excitation of the alkali-cationized molecule. Pathways for the high-energy CID fragmentation of *n*-butyl palmitate $[\text{M} + \text{Li}]^+$ and $[\text{M} + \text{Na}]^+$ ions are given in Schemes 1 and 2. The proposed pathways not only account for the formation of 'C-type ions (pathway a/b/c), but also enable us to formulate the C_2 ion and the other ions of the C series in a logical manner (pathways d and e/f). The proposed pathway (a/b/c) for the formation of 'C-type ions takes into account the finding by Cordero and Wesdemiotis¹⁹ that alkenes are eliminated during high-energy CID of lithium-cationized fatty acids. However, with regard to sodium-cationized fatty acid derivatives, the latter process needs to be confirmed. Previous deuterium labeling results obtained for lithium-cationized *n*-butyl [9,9-²H₂]palmitate indicate that step a is product-determining.⁸ Formation of the 'C₅ ion may compete with that of the C_2 ion, providing a reasonable explanation for the decreased relative abundance of the 'C₅ ion compared with other ions of the 'C series, which is a typical feature of the high-energy CID product ion spectra. The enhanced relative abundance of the C_2 ion and ions of the C-type in the $[\text{M} + \text{Na}]^+$ spectra may be explained by a decreased spin density at the carbonyl O atom of the biradical cation (see below) formed upon

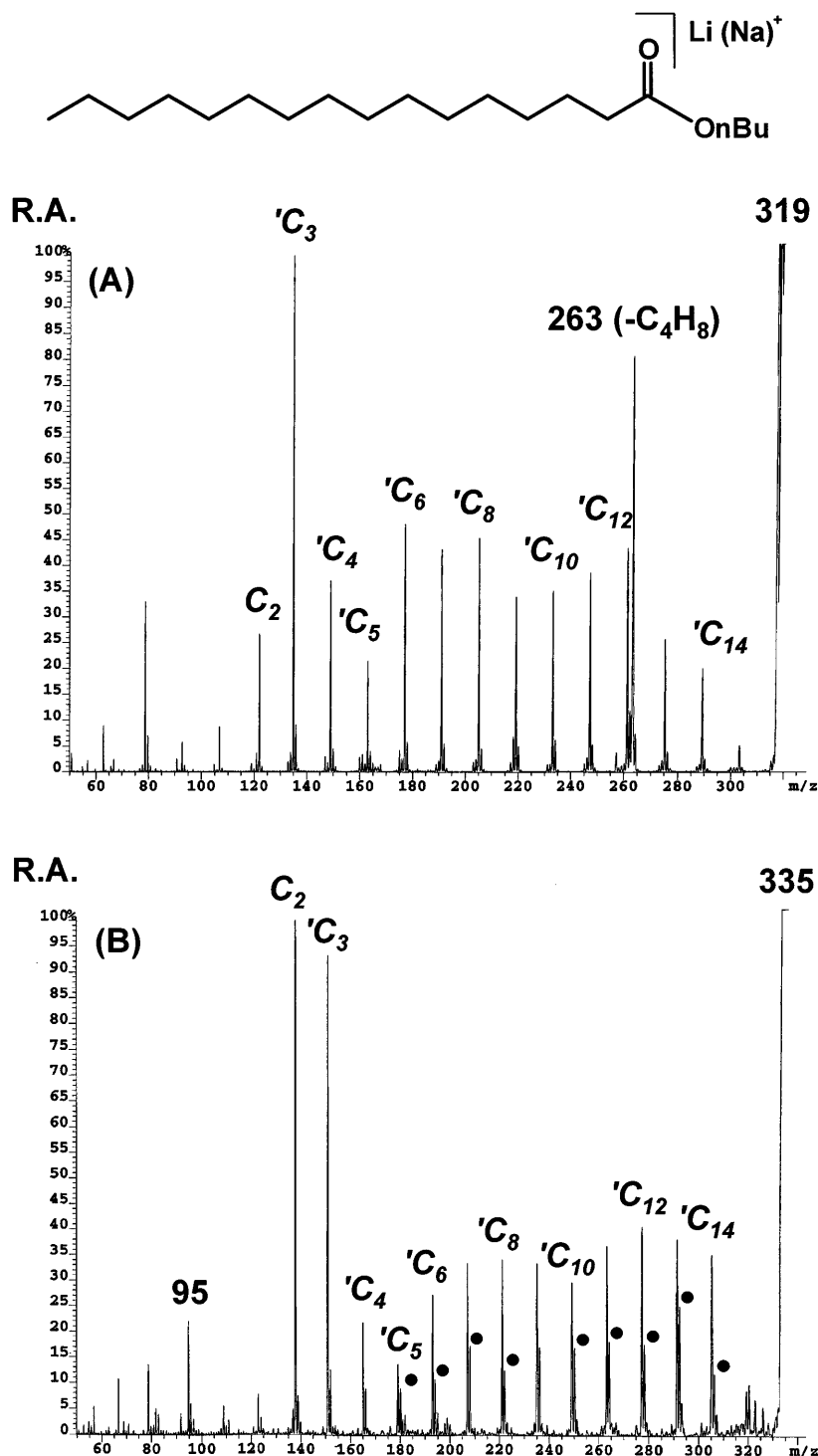


Figure 1. High-energy CID spectra of *n*-butyl palmitate (A) $[\text{M} + \text{Li}]^+$ and (B) $[\text{M} + \text{Na}]^+$ ions. The peaks marked with ● refer to C-type ions.

collisional activation as a first excited transition state, favoring C–C cleavage at positions all along the acyl chain (pathways d and e/f), which can be regarded as competitive pathways. Deuterium labeling results obtained for the *n*-butyl and methyl $[9,9\text{-}^2\text{H}_2]$ palmitate $[\text{M} + \text{Na}]^+$ ions (not shown) are consistent with alkyl migration because a kinetic isotope effect could only be detected for the C_{10} ion. It can also be noted that the fragmentation efficiency is higher for the $[\text{M} + \text{Li}]^+$ ions than the $[\text{M} + \text{Na}]^+$ ions. For example, in the case

of methyl palmitate, the fragmentation efficiency is 6.4% for the $[\text{M} + \text{Li}]^+$ ion *vs.* 4.3% for the $[\text{M} + \text{Na}]^+$ ion. This result can also be rationalized by the lower reactivity of the postulated biradical species in the case of Na^+ cationization. As alternative mechanisms to those depicted in Schemes 1 and 2, direct hydrogen and alkyl radical release, which is expected to be energetically more demanding, could also be considered.

Furthermore, comparison of the results obtained for the *n*-butyl and methyl palmitate $[\text{M} + \text{Li}]^+$ and

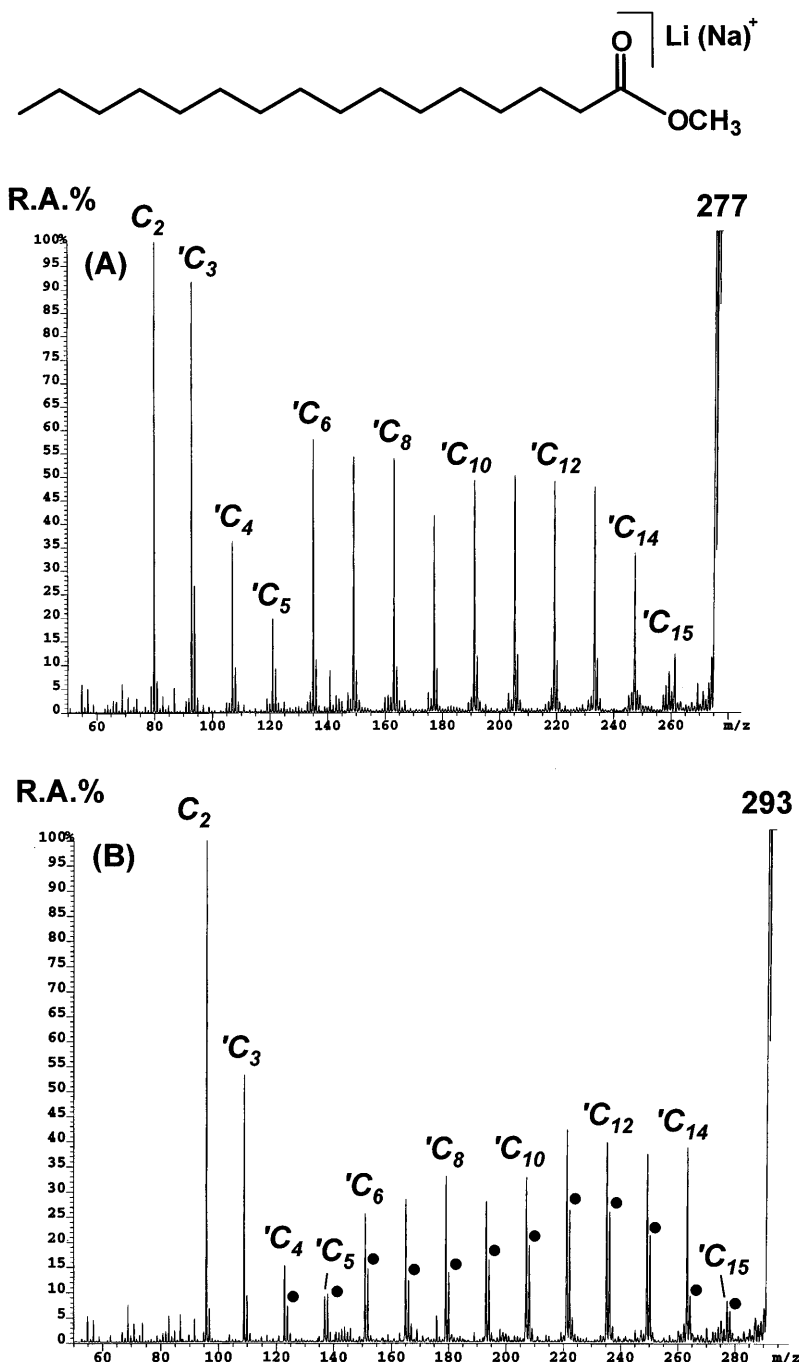
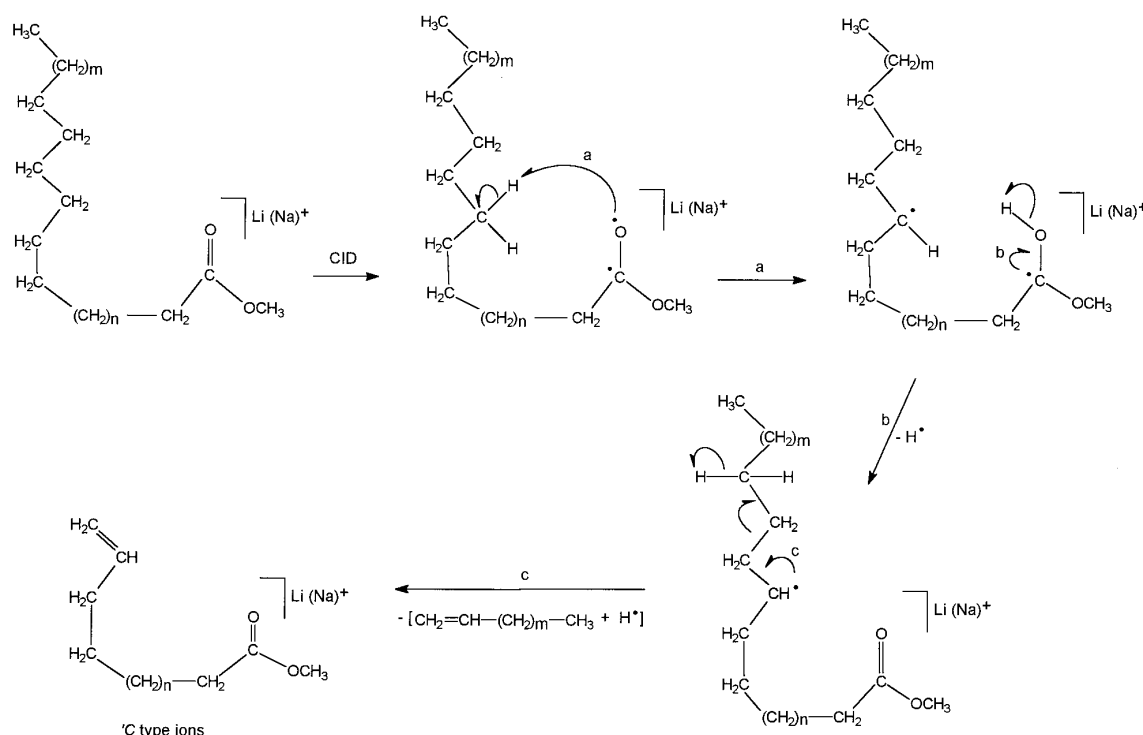


Figure 2. High-energy CID spectra of methyl palmitate (A) $[M + Li]^+$ and (B) $[M + Na]^+$ ions. The peaks marked with ● refer to C-type ions.

$[M + Na]^+$ ions indicates that the ester alkyl group also has an effect on high-energy CID processes. The C_2 ion and the other ions of the C series have an increased relative abundance in Li^+ cationized methyl palmitate compared with Li^+ -cationized *n*-butyl palmitate. With respect to the behavior of Na^+ -cationized methyl and *n*-butyl palmitate, the effect of the ester alkyl group can only be noted for the C_2 ion. These results suggest that the ester alkyl group may in addition to the alkali metal ion also influence the reactivity of the postulated excited transition state (see below).

As mentioned already, formation of C-type ions has been demonstrated for electrospray-generated fatty acid carboxylate ions.¹⁴ Whereas for fast atom bombard-

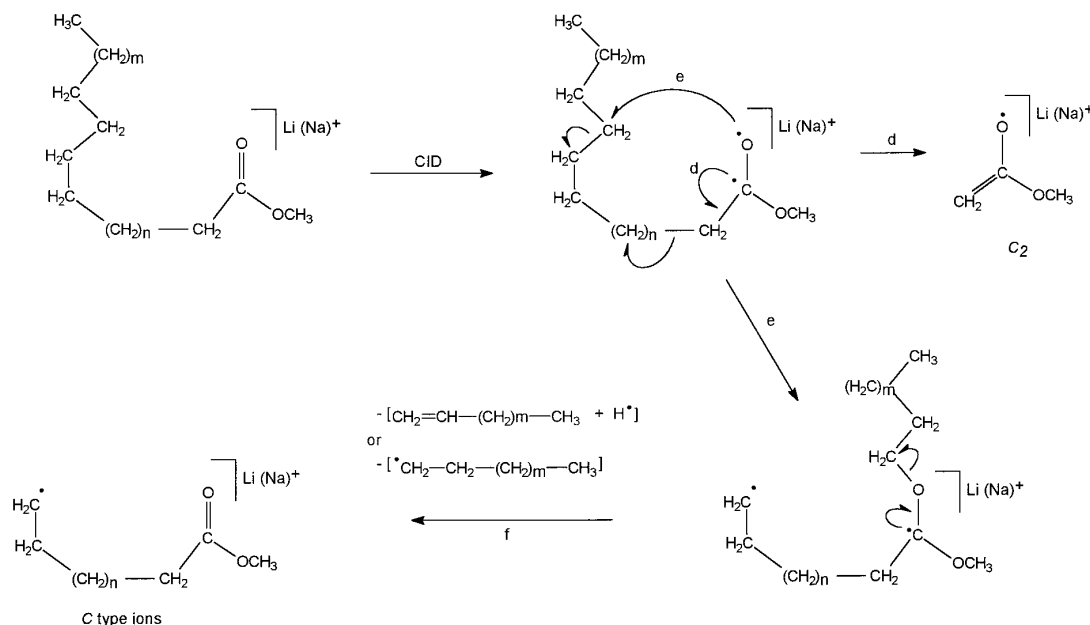
ment (FAB)-generated fatty acid carboxylate ions mainly $'C$ -type ions are formed, employing an intermediate collision energy (400 eV, laboratory frame) electrospray-generated ions also result in a regular series of C-type ions, suggesting that the internal energy of the precursor ions has an effect on CID fragmentation processes.²⁰ The formation of a homologous series of odd-electron ions, which can be compared with C-type ions, was discussed in detail by Wysocki and Ross⁷ for protonated 4-pentadecylpyridine and trimethyloctadecylammonium ions which were collisionally activated by low-energy surface-induced dissociation. While FAB-generated protonated 4-pentadecylpyridine mainly gave rise to terminally



Scheme 1. Mechanism proposed to rationalize the 'C type ions formed at high-energy CID of methyl palmitate cationized with Li^+ or Na^+ . C-H cleavage (route a) occurs at positions all along the acyl chain.

unsaturated product ions, the isobutane chemical ionization-generated precursor ions yielded an additional series of odd-electron ions at masses 1 unit higher. Similar findings were reported for trimethyloctadecylammonium ions, which were found to result in a homologous series of odd-electron ions when they were produced in a high-pressure FAB source instead of in a regular FAB source. With respect to possible transitions in the carbonyl group, both $n \rightarrow \pi$ and $\pi \rightarrow \pi$ transitions may be considered. The n, π excited state, which requires a lower activation energy than the π, π state, is probably responsible for the loss of 1-butene in the ester part of lithium-cationized *n*-butyl palmitate,

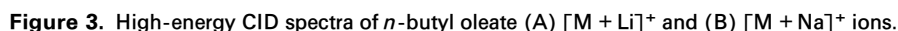
which corresponds to a McLafferty-type rearrangement and is observed at a low collision energy (50 eV, laboratory frame).⁸ The latter fragmentation reaction may be compared with the Norrish type 2 photochemical reaction of ketones, which is known to involve an n, π excited state capable of abstracting a hydrogen atom from the γ -position.²¹ Concerning the mechanism of the McLafferty-type rearrangement in even-electron ions, it is relevant to mention here that Budzikiewicz and Bold²² have proposed the formation of an excited species, more specifically, a transient biradical, for the McLafferty-type fragmentation of methyleneiminium ions. Their proposal for the latter rearrangement,



Scheme 2. Mechanism proposed to rationalize the C type ions at high-energy CID of methyl palmitate cationized with Li^+ or Na^+ involving C-C cleavage. It is pointed out that pathway e/f is mainly followed in the case of Na^+ cationization,

in a previous study,⁸ but is briefly summarized here in order to facilitate the discussion of the results obtained in the present study for the *n*-butyl oleate $[M + Na]^+$ ion and methyl oleate $[M + Li]^+$ and $[M + Na]^+$ ions. Comparison of the $[M + Li]^+$ spectra with that of the $[M - H + 2Li]^+$ molecular ion of oleic acid, reported by Adams and Gross,²⁴ shows the same homologous ion series in each case. The gap of 54 units between the ions at m/z 191 (A_7) and 245 (A_{11}), which is typical of an unsaturation at the C(9) position, can be clearly discerned. The A_{11} ion is accompanied by a significant A_{11} ion (m/z 246) of the OE type, formed by a distal

The CID spectra of the $[M + Li]^+$ and $[M + Na]^+$ ion of *n*-butyl and methyl oleate are illustrated in Figs 3 and 4. The fragmentation of the *n*-butyl oleate $[M + Li]^+$ ion [Fig. 3(A)] has been discussed in detail



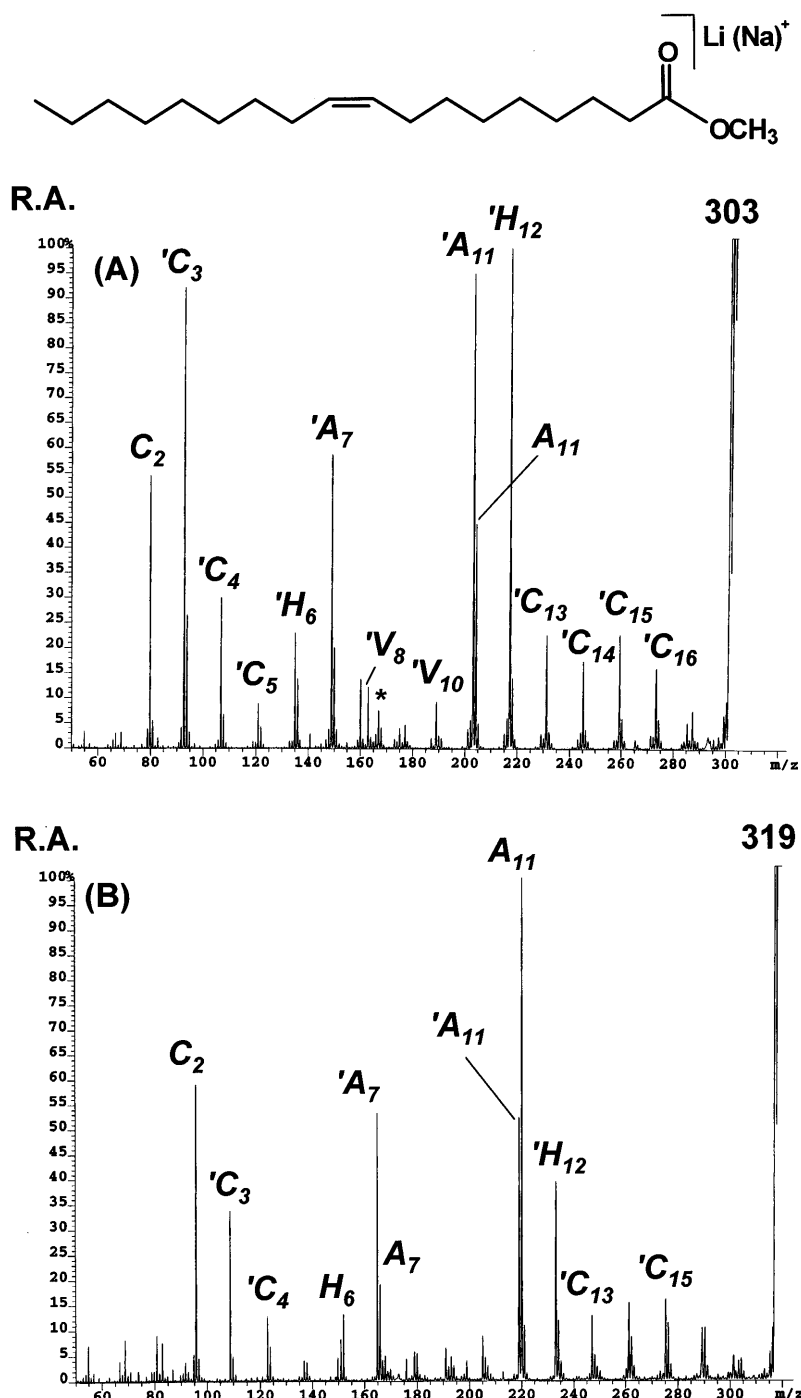


Figure 4. High-energy CID spectra of methyl oleate (A) $[M + Li]^+$ and (B) $[M + Na]^+$ ions.

allylic cleavage. The $'H_{12}$ ion (m/z 259) has an abundance comparable to those of the $'A_7$ and $'A_{11}$ ions, a feature which is also typical of an unsaturation at the C(9) position. The n -butyl oleate $[M + Li]^+$ spectrum shows the characteristics of a n -butyl ester derivative, which include the loss of C_4H_8 (m/z 289), the combined loss of C_4H_8 and H_2 (m/z 287) and the formation of the ion at m/z 79. In addition, an ion is present at m/z 160, which can be explained by Li^+ adduct formation at the double bond followed by homolytic C(7)–C(8) cleavage and can be regarded as diagnostic of a C(9) double bond. The fragmentation of the $[M + Li]^+$ ion of methyl oleate [Fig. 4(A)] is similar to that of n -butyl

oleate, except that the ion due to the loss of 1-butene corresponding to a McLafferty-type rearrangement in the ester part is absent from the spectrum.

As found for the $[M + Na]^+$ spectra of n -butyl and methyl palmitate [Figs 1(B) and 2(B)], the $[M + Na]^+$ spectra of n -butyl and methyl oleate [Figs 3(B) and 4(B)] show a different product ion profile: in addition to the ions of the $'C$, $'A$ and $'H$ series observed for the fragmentation of the n -butyl and methyl oleate $[M + Li]^+$ ions, the enhanced relative abundance of some C -, A - and H -type ions, corresponding to C–C cleavage in the acyl chain, is a striking feature of the $[M + Na]^+$ spectra. In addition, as noted for the frag-

mentation of the methyl palmitate $[M + Na]^+$ ion, the ion due to the loss of 1-butene is absent from the methyl oleate $[M + Na]^+$ spectrum.

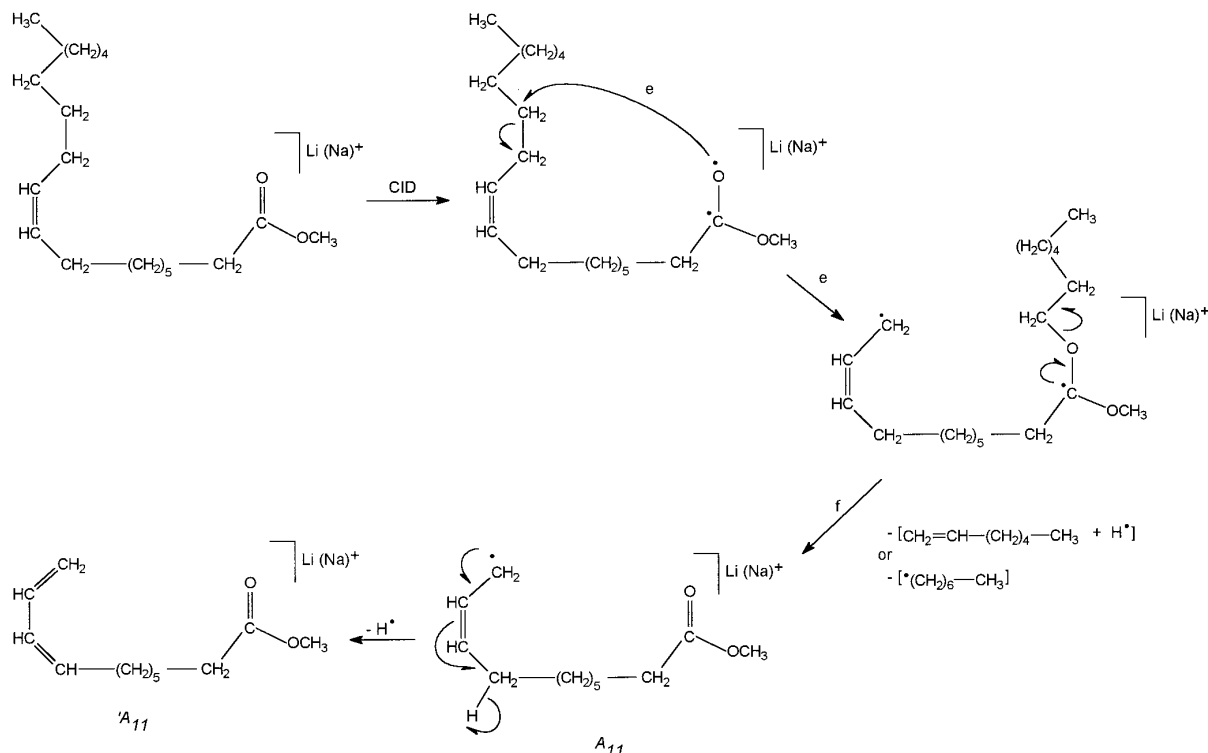
It has been demonstrated by Antoine and Adams²⁵ that the alkali metal ion has a pronounced effect on the relative abundance of *A*-type ions corresponding to a distal allylic cleavage for monounsaturated fatty acids cationized with Li^+ , Na^+ and K^+ . In agreement with the latter study, we also noted that the relative abundance of the A_{11} ion is higher in the spectra of the *n*-butyl and methyl oleate $[M + Na]^+$ ion than in those obtained for the $[M + Li]^+$ ion (the $[A_{11}]/[M]^+$ ratios are 2.6 and 1.9 in the $[M + Li]^+$ spectra of *n*-butyl and methyl oleate, respectively *vs.* 0.6 and 0.6 in the corresponding $[M + Na]^+$ spectra). A mechanism involving direct C(11)–C(12) cleavage (Scheme 3) enables us to formulate the $'A_{11}$ and A_{11} ions; again, the decreased spin density at the carbonyl O atom of the excited transition state formed upon CID (see below) allows us to rationalize the increased relative abundance of the A_{11} ion in the $[M + Na]^+$ spectra.

Theoretical calculations

Theoretical calculations were performed on Li^+ - and Na^+ -cationized methyl and *n*-butyl acetate with the aim of evaluating whether predicted electronic properties such as the spin density could be correlated with experimental data. In the case of Li^+ - and Na^+ -cationized fatty acid esters, the metal ion behaves as an electron acceptor for the π -bonded ester group. The complexes are formed between electron-enriched metal atoms and electron-poor atoms (O, C) and should therefore be regarded as 'inverse' alkali metal-bonded complexes.

The minimum basis set STO-3G was used to compare the geometric and electronic properties of Li^+ and Na^+ complexes because of the size of the complexes. The calculation of shielding tensors of ^{13}C and ^{17}O in Li^+/Na^+ complexes using STO-3G gave values which are closer to experimental values than those obtained with a higher level 6-31G** basis set.²⁶ It was also found that computation employing STO-3G afforded adequate geometric information in the case of large Li clusters and allowed to predict basic trends in their properties.²⁷ The optimized geometric values calculated for Li^+/Na^+ -cationized methyl and *n*-butyl acetate are given in Table 1. Two specific sites of Me^+ attachment to methyl and *n*-butyl acetate were found by total optimization using the STO-3G basis set. Calculation at the MP2/6-31G* level also allowed us to define two stable conformers for the Li^+ methyl acetate complexes. The main differences for the Li^+ and Na^+ complexes (Table 1) relate to the $Me^+ - O^5 = C^3$ region. More specifically, the $Me^+ - O^5$ bond is higher in the Na^+ complex, owing to the small metal chelation effect, compared with the Li^+ complex [for example, in the case of methyl acetate, the bond lengths are 2.161 Å in the Na^+ complex (3a) *vs.* 1.579 Å in the Li^+ complex (2a)], where it can be considered covalent-like.

The calculated electronic properties of the Li^+/Na^+ complexes of methyl and *n*-butyl acetate are summarized in Table 2. Based on the calculated total energy using a higher basis set (6-31G* with ZPVE correction), it could be confirmed that the a form of the Li^+ complex, with the lithium–oxygen bond colinear with the ester carbonyl bond, is more stable than the b form, which is of the bidentate type. The linear a form of the methyl acetate– Li^+ complex, for example, is favored over the bidentate b form by about 1.3 kcal mol⁻¹. The mode of bonding in Li^+ complexes has



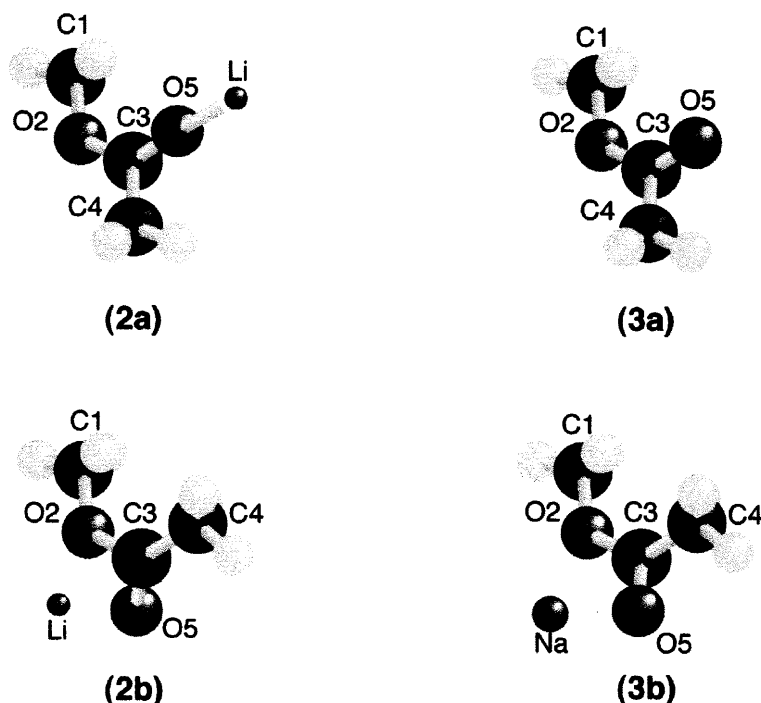
Scheme 3. Mechanism proposed to rationalize the A_{11} and $'A_{11}$ ions at high-energy CID of methyl oleate cationized with Li^+ or Na^+ involving C–C cleavage.

Table 1. *Ab initio* STO-3G optimized geometrical values (see Fig. 5 for definition of bonds (in Å) and angles in degrees))

No.	Compound	C(1)—O(2)	O(2)—C(3)	C(3)—O(5)	O(5)—Me ⁺	Parameters		Me ⁺ —O(5)—O(3)	O(5)—C(3)—O(2)	O(2)—Me ⁺
						C(3)—C(4)				
1	C ₃ H ₆ O ₂	1.437	1.395	1.215	—	1.538		—	123.1	—
2a	C ₃ H ₆ O ₂ Li ⁺	1.451	1.334	1.258	1.579	1.536		174.9	122.5	3.766
2b	C ₃ H ₆ O ₂ Li ⁺	1.447	1.397	1.232	1.830	1.532		91.6	110.2	1.838
2c	C ₆ H ₁₂ O ₂ Li ⁺	1.458	1.320	1.574	1.574	1.536		175.9	122.6	3.754
3a	C ₃ H ₆ O ₂ Na ⁺	1.429	1.295	1.213	2.161	1.499		163.1	122.9	4.339
3b	C ₃ H ₆ O ₂ Na ⁺	1.432	1.301	1.213	2.132	1.505		145.9	118.1	3.590
3c	C ₆ H ₁₂ O ₂ Na ⁺	1.450	1.356	1.235	1.982	1.539		141.1	122.6	4.243

been evaluated for sulfones and sulfonates by Gal *et al.*²⁸ It is interesting to note that for these compounds, two stable conformers were predicted by theoretical calculations, a linear adduct with the lithium–oxygen bond colinear with the sulfoxide bond and a bidentate form.

The nature of the bonding in the Li⁺/Na⁺ complexes may be evaluated using a Mulliken population analysis. Table 2 shows that there is a very small charge transfer in the Li⁺ complex; for example, in the case of the methyl acetate–Li⁺ complex, the Li⁺ ion retains 0.64

**Figure 5.** *Ab initio* STO-3G optimized conformers of Li⁺ and Na⁺ methyl acetate complexes.**Table 2.** Calculated spin densities and Mulliken charges for Me⁺-cationized methyl and *n*-butyl acetate (values discussed in the text are in bold)

No.	Compound	C(1)	O(2)	Spin density		O(5)	Mulliken charge on the metal atom
				C(3)	C(4)		
2a	C ₃ H ₆ O ₂ Li ⁺						(0.644)
	C ₃ H ₆ O ₂ Li ⁺	0.0023	0.0002	0.0013	0.0008	0.0529	
2b	C ₃ H ₆ O ₂ Li ⁺						(1.427)
	C ₃ H ₆ O ₂ Li ⁺	0.0000	0.0707	0.0031	0.0018	0.0153	
4a	C ₆ H ₁₂ O ₂ Li ⁺						(0.579)
	C ₆ H ₁₂ O ₂ Li ⁺	0.0023	0.0041	0.0035	0.0084	0.0659	
3a	C ₃ H ₆ O ₂ Na ⁺						(2.077)
	C ₃ H ₆ O ₂ Na ⁺	0.0001	0.0047	0.0000	0.0122	0.0159	
3b	C ₃ H ₆ O ₂ Na ⁺						(2.917)
	C ₃ H ₆ O ₂ Na ⁺	0.0016	0.0306	0.0055	0.0084	0.0296	
5a	C ₆ H ₁₂ O ₂ Na ⁺						(0.869)
	C ₆ H ₁₂ O ₂ Na ⁺	0.0001	0.0047	0.0000	0.0126	0.0179	

electronic units of its positive charge in complex **2a** and 1.43 units in complex **2b**. The latter result indicates that bonding in complex **2b** is mostly due to electrostatic interaction and that the major contribution is made by ion–dipole interactions. The calculated Mulliken populations for the Na^+ complexes reveal higher values for the two conformers, due to a stronger electrostatic interaction associated with the charge on Na.

The reactive sites for radical attack may be indicated by the spin density. The reactive nature of the excited transition state of the lithiated cation could be due to the nature of the particular radical localization (Table 2). It is worth noting that there is a significant difference in the spin density on the carbonyl O atom for the a conformers of the Li^+ and Na^+ complexes of methyl acetate (0.0529 *vs.* 0.0159). Furthermore, comparison of the values for the a conformers of the Li^+ complex of methyl and *n*-butyl acetate reveals a higher spin density on the carbonyl O atom in the case of *n*-butyl acetate (0.0529 *vs.* 0.0659). This result is in agreement with the finding that the nature of the ester alkyl group also has an effect on CID processes and follows the experimental trend that *n*-butyl esters show more pronounced C–H cleavage reactions relative to methyl esters. Hence it appears that the high tendency of Li^+ -cationized fatty acid esters to undergo C–H cleavage following high-energy collisional activation could be correlated with a high radical localization on the carbonyl O atom in the excited transition state.

How can the new mechanistic proposal be reconciled with available knowledge on electron impact, CID and related processes?

The proposed mechanism for charge-remote fragmentation in lithium-cationized fatty acid esters involving C–H cleavage corresponds to formal 1,4-eliminations of H_2 and can thus be reconciled with the simple mechanism proposed earlier by Gross and co-workers.⁶ It is also worth noting that the high-energy CID behavior of lithium-cationized methyl and *n*-butyl palmitate and oleate is very comparable to the EI behavior of the picolinyl derivative of palmitic and oleic acid reported by Harvey.^{29,30} The mechanism of the EI fragmentation of the picolinyl derivatives of fatty acids relates to a pyridinium radical cation-induced abstraction of a hydrogen radical at positions all along the alkyl or alkenyl chain and is similar to those proposed in the present study for high-energy CID, in which radical attack at various positions along the chain is triggered by the excited transition state of the ester carbonyl group. Furthermore, it is worth mentioning that EI of fatty acid methyl esters gives rise to a homologous series of carbomethoxy ions which were extensively investigated by Dinh-Nguyen³¹ using deuterium labeling and could be partly rationalized by initial hydrogen transfer to the carbonyl group followed by a radical-induced C–C cleavage in the acyl chain. This carbomethoxy ion series was also noted by Zirrolli and Murphy³² on low-energy CID of EI-generated molecular ions of fatty acid methyl esters and was shown to be of high diagnostic value for the structural determination of branched-chain fatty acids. With respect to the pro-

posed mechanism for charge-remote fragmentation in sodium-cationized fatty acid esters involving alkyl migration to the cationized ester group, similarity exists with EI fragmentation mechanisms of fatty acid methyl esters, as, for example, with that proposed by Budzikiewicz *et al.*³³ to rationalize the $[\text{M} - 43]^+$ ion involving a complex scission of an internal methylene part with a hydrogen transfer and rearrangement of the carbon skeleton.

As regards participation of the charge in high-energy CID reactions, it should be mentioned that Tuinman and co-workers^{34,35} observed a peculiar CID fragmentation of alkyltrimethylammonium ions with a perdeuterated alkyl chain. They described mixed-site fragmentation reactions, in which the fixed charge site on the nitrogen atom interacts with backbone σ -bonds, as competitive reactions with CRF, resulting in a second series of product ions accompanying the predicted CRF product ions, but at masses 3 units higher. A similar high-energy CID behavior of alkyltrimethylammonium ions with deuterated methyl groups was also reported by Bambagiotti-Alberti *et al.*³⁶ Mixed-site fragmentation reactions were recently investigated in more detail by Whalen *et al.*,³⁷ who considered the involvement of a distonic diradical cation to explain the CID fragmentation of alkyltrimethylammonium ions with a perdeuterated alkyl chain. The latter distonic biradical cation may be formed following an excitation, resulting in a transient excited species with the active site located at an N–C bond, which can induce cleavage of backbone C–C bonds. The regular series of CRF product ions in alkyltrimethylammonium ions (loss of $\text{C}_n\text{H}_{2n} + \text{H}_2$) can be rationalized from the same excited transition state, which can trigger C–H cleavage at carbon positions all along the alkyl chain.

With respect to the energetics, it has been demonstrated by Wysocki *et al.*³⁸ that CRF reactions are already occurring at low excitation energies in the eV range under surface-induced dissociation conditions. An extreme case was reported by Cody,³⁹ who examined 4-pentadecylpyridine and observed CRF reactions using a Fourier transform ion cyclotron resonance spectrometer and collision energies as low as 2.5 eV. In contrast, a large activation energy (>10 eV) was needed to observe CRF for the stearate anion and octadecylammonium ion under surface-induced dissociation conditions.³⁸ The energy required to generate an excited transition state will be dependent on the nature of the functional group participating in CRF and may either concern an $n \rightarrow \pi$, $\pi \rightarrow \pi$ or $\sigma \rightarrow \sigma$ transition. The proposed mechanism based on the formation of a first excited transition state can, therefore, also be reconciled with experimental findings by Wysocki *et al.*³⁸ that the internal energy required to record spectra which show CRF is compound dependent. Other observations relevant to the energetics of CRF processes are those made by Voinov *et al.*,⁴⁰ who demonstrated that saturated fatty acids form $[\text{M} - \text{H}]^-$ ions by electron capture in two energy regions, 1.2 and 7 eV, and that those formed by electron capture at 7 eV exhibit CRF behavior. In contrast, the $[\text{M} - \text{H}]^-$ ions formed by electron capture at 1.2 eV found to correspond to fatty acid carboxylate anions revealed a different fragmentation behavior (i.e. loss of H_2 and H_2O , and formation of an

ion at m/z 59). Structure investigation of the $[M - H]^-$ ion formed by electron capture at 7 eV employing deuterium-labeled isotopomers suggests an electronically excited state of the molecule which has retained the hydrogen atoms from the hydroxyl group and the C(2) position.⁴¹ The energetics of charge-remote fragmentation were recently studied in carbocyanine dyes using collision- and surface-induced dissociation mass spectrometry by Melnyk *et al.*⁴² This study clearly showed differences in CRF dissociation processes with varying internal energy deposition.

The mechanism of collisional energy transfer to 8 keV macromolecular ions has been addressed by Uggerud and Derrick,⁴³ who questioned whether electronic excitation is an important mechanism and proposed a simple model for impulsive energy transfer between a single atom in a macromolecular ion and a gas atom. The experimental results obtained in the present study for alkali-cationized fatty acid esters, however, favour electronic excitation resulting in the formation of a transient biradical located in the ester carbonyl group.

CONCLUSIONS

Recent results obtained for the high-energy CID fragmentation of long-chain fatty acid methyl and *n*-butyl esters cationized with Li^+ and Na^+ as well as other

considerations have led to the proposal of new homolytic mechanisms corresponding to formal 1,4-eliminations of H_2 and to eliminations of alkyl radicals.

The proposed mechanisms involve the formation of a biradical cation as a first excited transition state with a biradical site located in the ester carbonyl group, which interacts with σ -bonds of the fatty acyl chain at both remote and proximate carbon positions. The results obtained in the present study for charge-remote and charge-proximate fragmentation in alkali-cationized fatty acid esters are consistent with participation of the alkali-cationized ester group in the fragmentation process and point to electronic excitation resulting in a transient biradical species during high-energy collisional activation of these compounds. As a consequence, an odd-electron ion behavior holds for the high-energy CID fragmentation of their even-electron molecular ions.

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REFERENCES

1. F. W. Röllgen, F. Borchers, U. Giessmann and K. Levsen, *Org. Mass Spectrom.* **12**, 541 (1977).
2. F. W. Röllgen, U. Giessmann, F. Borchers and K. Levsen, *Org. Mass Spectrom.* **13**, 459 (1978).
3. F. W. Röllgen, U. Giessmann and K. Levsen, *Adv. Mass Spectrom.* **8A**, 997 (1980).
4. J. Adams, *Mass Spectrom. Rev.* **9**, 141 (1990).
5. M. L. Gross, *Int. J. Mass Spectrom. Ion Processes* **118/119**, 137 (1992).
6. N. J. Jensen, K. B. Tomer and M. L. Gross, *J. Am. Chem. Soc.* **107**, 1863 (1985).
7. V. H. Wysocki and M. M. Ross, *Int. J. Mass Spectrom. Ion Processes* **104**, 179 (1991).
8. M. Claeys, L. Nizigiyimana, H. Van den Heuvel and P. J. Derrick, *Rapid Commun. Mass Spectrom.* **10**, 770 (1996).
9. L. Nizigiyimana, P. K. Rajan, A. Haemers, M. Claeys and P. J. Derrick, *Rapid Commun. Mass Spectrom.* **11**, 1808 (1997).
10. L. Nizigiyimana, H. Van den Heuvel and M. Claeys, *Bull. Soc. Chim. Belg.* **106**, 439, 1997.
11. M. Claeys and H. Van den Heuvel, *Biol. Mass Spectrom.* **23**, 20 (1994).
12. J. A. Leary, Z. Zhou, S. A. Ogden and T. D. Williams, *J. Am. Soc. Mass Spectrom.* **1**, 473 (1990).
13. M. Claeys, L. Nizigiyimana and H. Van den Heuvel, in *Proceedings of the 14th International Mass Spectrometry Conference*, Tampere, Finland, 25–29 August 1997, in press.
14. W. J. Griffiths, Y. Yang, J. Å. Lindgren and J. Sjövall, *Rapid Commun. Mass Spectrom.* **10**, 21 (1996).
15. R. H. Greeley, *J. Chromatogr.* **88**, 229 (1974).
16. M. Bambagiotti-Alberti, S. A. Coran, F. F. Vincieri, T. Petrucciani and P. Traldi, *Org. Mass Spectrom.* **21**, 485 (1986).
17. E. Pittenauer, T. Aichinger, K. de Hueber and J. Bailer, in *Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics*, Portland, OR, 12–16 May 1996, p. 928.
18. Y. Suzuki, Y. Esumi, M. Uramoto, Y. Kono and A. Sakurai, *Biosci. Biotech. Biochem.* **61**, 480 (1997).
19. M. M. Cordero and C. Wesdemiotis, *Anal. Chem.* **66**, 861 (1994).
20. W. J. Griffiths, A. Brown, R. Reimendal, Y. Yang, J. Zhang and J. Sjövall, *Rapid Commun. Mass Spectrom.* **10**, 1169 (1996).
21. J. D. Coyle, *Introduction to Organic Photochemistry*, pp.106–140. Wiley, Chichester (1986).
22. H. Budzikiewicz and P. Bold, *Org. Mass Spectrom.* **26**, 709 (1991).
23. H. J. Veith and J. H. Gross, *Org. Mass Spectrom.* **26**, 1097 (1991).
24. J. Adams and M. L. Gross, *Anal. Chem.* **59**, 1576 (1987).
25. M. Antoine and J. Adams, *J. Am. Soc. Mass Spectrom.* **3**, 776 (1992).
26. R. Nizmal and S. Subramanian, *Chem. Phys. Letters* **166**, 326 (1990).
27. A. K. Ray, *J. Phys. B* **19**, 1253 (1986).
28. J.-F. Gal, I. Koppel, R. Kurg and P.-C. Maria, *Int. J. Quantum Chem.* **59**, 409 (1996).
29. D. J. Harvey, *Biomed. Mass Spectrom.* **11**, 340 (1984).
30. D. J. Harvey, *Biomed. Mass Spectrom.* **9**, 33 (1982).
31. N. Dinh-Nguyen, *Ark. Kemi* **28**, 289 (1968).
32. J. A. Zirrolli and R. C. Murphy, *J. Am. Soc. Mass Spectrom.* **4**, 223 (1993).
33. H. Budzikiewicz, C. Djerassi and D. H. Williams, *Mass Spectrometry of Organic Compounds*, pp. 3–17. Holden Day, San Francisco (1967).
34. A. A. Tuinman and K. D. Cook, in *Proceedings of the 38th ASMS Conference on Mass Spectrometry and Allied Topics*, Tucson, AZ, 1990, p. 938.
35. A. A. Tuinman, K. D. Cook and L. J. Magid, *J. Am. Soc. Mass Spectrom.* **1**, 85 (1989).
36. M. Bambagiotti-Alberti, S. A. Coran, F. Benvenuti, P. Lonostro, S. Catinella, D. Favretto and P. Traldi, *J. Mass Spectrom.* **30**, 1742 (1995).
37. K. Whalen, J. S. Grossert, J. M. Curtis and R. K. Boyd, *Int. J. Mass Spectrom. Ion Processes* **160**, 223 (1997).

38. V. H. Wysocki, M. E. Bier and R. G. Cooks, *Org. Mass Spectrom.* **23**, 627 (1988).
39. R. B. Cody, *Rapid Commun. Mass Spectrom.* **2**, 260 (1988).
40. V. G. Voinov, V. M. Boguslavskiy and Y. N. Elkin, *Org. Mass Spectrom.* **29**, 641 (1994).
41. V. G. Voinov, N. E. Petrachenko, V. E. Dorofeyev, H. Nakamatsu and T. Mukoyama, *Int. J. Mass Spectrom. Ion. Processes* **137**, 31 (1994).
42. M. C. Melnyk, R. E. Carlson, K. L. Busch, K. L. Schey and M. G. Bartlett, *J. Mass Spectrom.* **33**, 75 (1998).
43. E. Uggerud and P. J. Derrick, *J. Phys. Chem.* **95**, 1430 (1991).