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Utilization of charge and mass labeling for the structural identification of heterocyclic quaternary salts by mass spectrometry

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Dedicated to Professor Y. Hoppilliard.

Abstract

Heterocyclic compounds of general formula $C^{++}2Br^{-}$ were studied by tandem mass spectrometry. Fragmentations taking place from the $[C–H]^{+}$ and $[CBr]^{+}$ ions allowed identification of their structures. The two fundamental data of mass spectrometry, mass and charge, have been employed for characterization purposes. (Int J Mass Spectrom 219 (2002) 391–401) © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Mass spectrometry; Heterocyclic chemistry

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1. Introduction

When used as an analytical tool, one important information that a mass spectrum provides is the value of the molecular weight. However, this is not the sole data available to chemists: structural knowledge can be gained from the study of fragment ions. Soft ionization techniques allow the characterization of polar or labile compounds. Generally, fragment ions are scarcely produced under such ionization conditions. So, fragmentations are induced by collision activated dissociation (CAD) through MS/MS experiments. Coupling liquid chromatography to mass spectrometry (LC/MS) enlarges the application domain to the mixture analyses. Ideally, a LC/MS/MS procedure should provide three pieces of information: (i) purity of the compound is determined from the LC/UV chromatogram; (ii) molecular weight is deduced from the mass spectrum; (iii) structural knowledge is gathered from the CAD spectrum of the selected parent ion, usually but not necessarily the protonated molecular ion $[MH]^{+}$.

Azolium derivatives are used as herbicides [1] and we have described the synthesis and physico-chemical properties of 2-pyrazolinium salts related to dibenzoquat DF [2]. We have already described the fast atom bombardment (FAB) mass spectrometry of azolium

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salts [3–5] and more recently the subject has been taken up by Schäfer and Budzikiewicz [6]. These publications deal exclusively with the study of ions related to the pre-existing cation. In particular, the reactivity due to the matrix used in FAB experiments was explored; reduction processes corresponding to the fixation of a hydride [4] or an electron [5] were established.

The aim of the present work was to obtain structural information on such compounds by applying tandem mass spectrometry [7] to fragment ions formed by CAD of ions derived from the pre-existing cation. The electrospray ionization (ESI) technique [8] was the selected method since it is much more sensitive than the FAB technique [9] and more suitable than matrix assisted laser desorption (MALDI) [10] for the detection of low molecular weight ions (*m*/*z* lower than 500 Th). Another advantage of the proposed approach is the facile coupling of liquid chromatography to ESI mass spectrometer. Unfortunately, azolium salts are highly polar compounds that failed to be retained on the reversed-phase stationary phase. So, quality control of this class of compounds by LC/MS/MS is not feasible because the different molecules will have very similar retention times. Only MS/MS experiments are relevant.

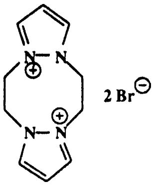
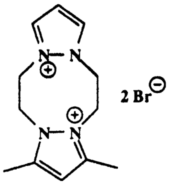
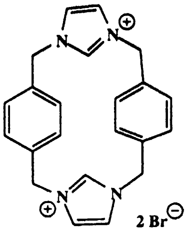
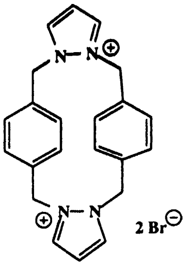
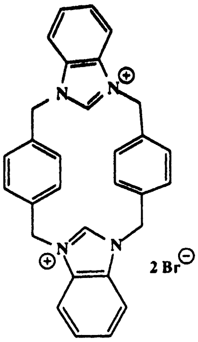
2. Experimental results and discussion

Compounds **1**, **2** and **4** (bispyrazolium salts), **3** (a bisimidazolium salt) and **5** (a bisbenzimidazolium salt) have been studied. Their structures are shown in Table 1. All analytes were introduced as dibromides.

Generally, the ESI mass spectra of compounds $C^{++}2Br^-$ have been found to contain three ions [4,5]: C^{++} detected at C/2 (half nominal mass), and the two ions $[C–H]^+$ and $[CBr]^+$. In this study, the mass spectra also contained fragment and adduct ions, the relative abundances of which depend on the cone voltage value. For compounds **1** and **2**, $[C–H]^+$ and $[CBr]^+$ ions were the major ions, the doubly charged ion at C/2 was not observed. The mass spectrum of compound **1** is displayed in Fig. 1 ($[C–H]^+$: *m*/*z* 189, $[CBr]^+$: *m*/*z* 269/271). The absence of the doubly charged cation in the spectrum of such compounds could be explained assuming that reactions occur between this cation and the bromide anion as observed for 2-pyrazolinium salts [11].

The nature of the $[CBr]^+$ ion was questioned: ion-pair species or covalent entity? Cationic analytes were reported in the literature [12] to exhibit ionic clusters of the type $[nC^+(n-1)X^-]^+$ and such ion-pairs were found sufficiently tight to survive energetic

Table 1
Studied molecules

	Compound no.				
	1	**2**	**3**	**4**	**5**
Structure ($C^{++}2Br^-$)					
Cation weight C^{++} (Da)	190	218	342	342	442

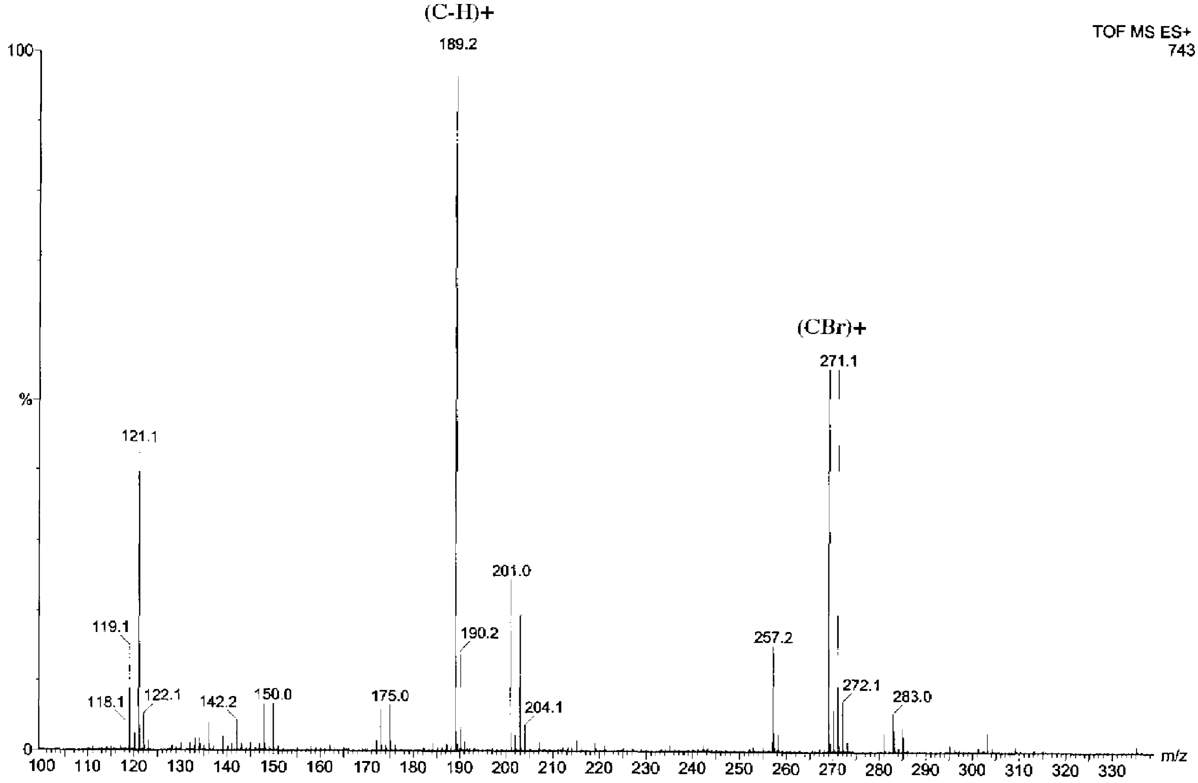
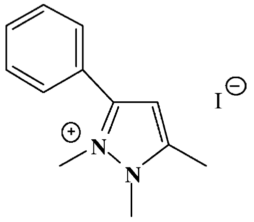


Fig. 1. Mass spectrum of compound **1**.

collisions such as those involved in in-source collision. The fact that high mass adduct ions were observed in the recorded $C^{++}2Br^{-}$ mass spectra indicated that ion-pairing was occurring to some extend. The ion $[CBr]^{+}$ could thus be described as $[C^{++}Br^{-}]^{+}$. Nevertheless, the presence of some covalent species, actually written $[CBr]^{+}$, was searched. Two experiments were designed to distinguish between ion-pair and covalent species. A monocation compound bearing an iodide as counterion (compound **6** shown in Scheme 1) was analyzed at a low cone voltage to diminish in-source collision and subsequently favor the formation of clusters. The first ion-pair $[2C^{+}I^{-}]^{+}$ was selected for MS/MS experiments undertaken at four different collision energies (5, 10, 15, and 50 eV). Under all conditions, the CAD mass spectra exhibited with the highest abundance the monocation C^{+} demonstrating that the weak non-covalent interaction was easily broken. The second experiment involved mixing a solution of compound **2** ($C^{++}2Br^{-}$) with a solution of NaI. The mass spectrum was recorded at a low cone voltage to visualize ion-pairs of the type $[nC^{++}nI^{-}]^{+}$. As expected, the $[CBr]^{+}$ ion has been replaced by the $[CI]^{+}$ ion. The dual nature of such $[CI]^{+}$ ion was envisaged: the CAD mass spectra of the two isobaric conceived ions, $[CI]^{+}$ by formation of a covalent bond between the dication and the iodide and ion-pair $[C^{++}I^{-}]^{+}$, should



Scheme 1. Structure of the monocation (compound **6**).

be different since the weaker non-covalent interactions were expected to be broken prior to covalent bonds. To perform a direct comparison with the previously studied ion-pair $[2C^{+}I^{-}]^{+}$, the same experimental parameters were set-up to conduct the dissociation experiments (collision energies at 5, 10, 15, and 50 eV). In this case, no specific bond were preferentially broken which was in favor of the covalent structure. Besides, the same fragmentation pattern was observed for $[CI]^{+}$ and $[CBr]^{+}$ ions dissociation: the ions containing the halogen atom were shifted by 48 Da whereas the others remained unchanged. To illustrate the results, the CAD mass spectra recorded in the first and second experiments at a cone voltage of 20 V and a collision energy of 15 eV are displayed in Fig. 2.

In the mass spectra of compounds **1–6**, the presence of the bromide anion splits each signal in the mass spectrum which contained this atom into a doublet of two peaks of the same intensity and separated by a mass difference of 2 Da (Fig. 1, $[CBr]^{+}$: *m*/*z* 269/271). This feature has been used to analyze the CAD spectra using bromine as a label. The two sets of CAD spectra have been systematically recorded by fragmenting specifically one ion of the isotopic cluster that is ion containing either ^{79}Br or ^{81}Br isotope. The comparison of both sets of spectra allowed identification of the ions containing a bromine atom. A signal shifted by a mass difference of 2 Da between the two spectra featured a bromine-containing ion whereas unaffected ions were bromine-free. This approach, already described by other authors [13–17], requires sufficient resolution in the parent ion selection to discriminate ions in the isotopic cluster. As an illustration, the structure of the $[CBr]^{+}$ ion (*m*/*z* 269/271 in Fig. 1) was

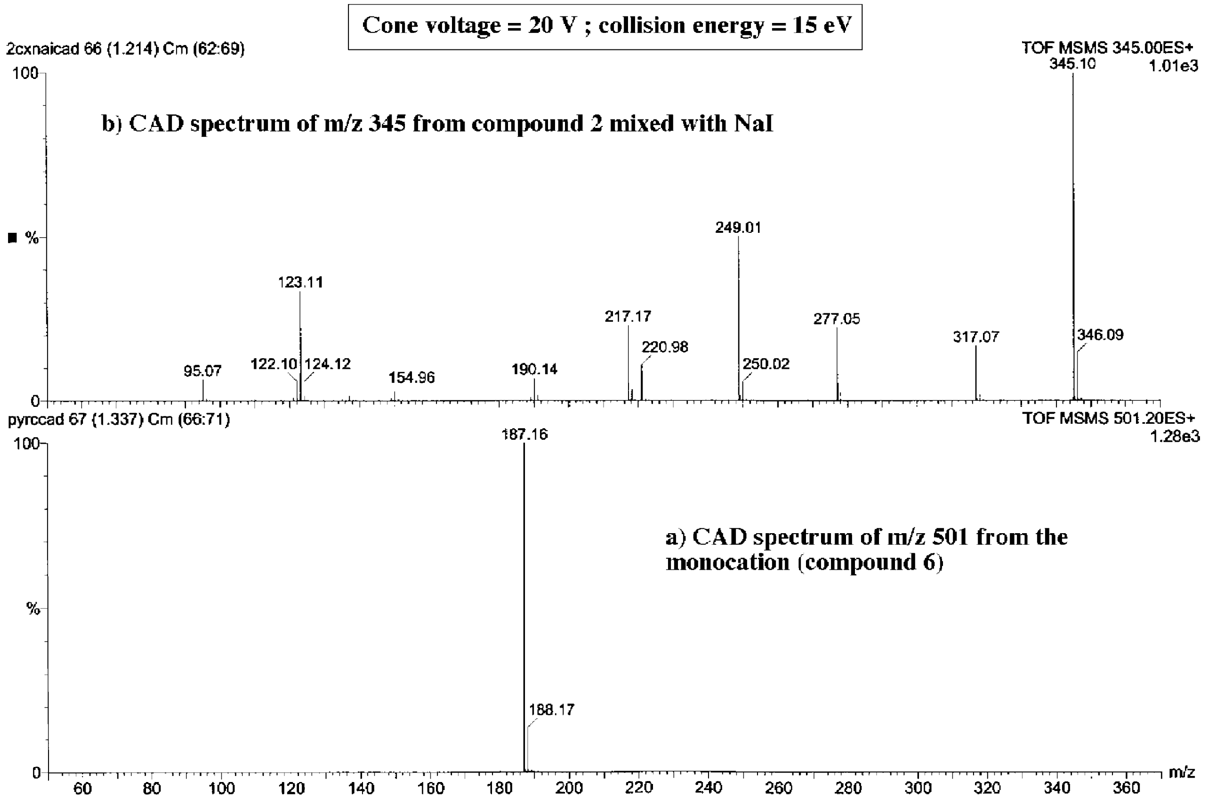


Fig. 2. (a) CAD spectrum of *m*/*z* 501 from the monocation (compound **6**); (b) CAD spectrum of *m*/*z* 345 from compound **2** mixed with NaI.

established unambiguously by comparing the CAD spectra of each isotope of the $[CBr]^+$ cluster. Indeed, some ions contained the bromine atom (CAD of *m*/*z* 269 gave *m*/*z* 201 and CAD of *m*/*z* 271 gave *m*/*z* 203) whereas others were bromine-free (CAD of *m*/*z* 269 and *m*/*z* 271 gave *m*/*z* 95). This was another proof that the bromine atom was necessarily covalently linked to the skeleton and this new bond has been formed in the mass spectrometer. Such reactivity required a doubly charged cation to be evidenced since the same behavior on the singly charged species would have led to a neutral molecule, not detected in mass spectrometry. This is the first example of charge labeling.

In order to rationalize the dissociation processes, CAD mass spectra of the $[CBr]^+$ ion from compounds **1** and **4** were recorded under several cone voltages and collision energies. Increasing the cone voltage favored the opportunity for ion–molecule reaction and enhanced energy deposition in the precursor ion. The influence of the cone voltage (20, 50 and 80 V) was assessed by comparing the CAD mass spectra recorded at a fixed collision energy (15 and 25 eV) (Fig. 3). The mass spectra were identical whatever the cone voltage value except that the $[C–H]^+$ at *m*/*z* 189 obtained by the loss of HBr was only detected at low cone voltage (20 V), higher values providing further fragmentation to the ion at *m*/*z* 95 (path a in Scheme 2). At a fixed cone voltage (20, 50 and 80 V), increasing the collision energy from 15 to 25 eV led to CAD mass spectra containing more fragment ions that were produced from multiple bond ruptures. The observed product ions are gathered in Table 2.

Regarding the acquisition of primary ESI mass spectra, the value of the cone voltage influenced greatly the nature and the relative abundances of the recorded ions. This experimental parameter was first optimized prior to MS/MS experiments. The presence of fragment ions in the primary mass spectra obtained

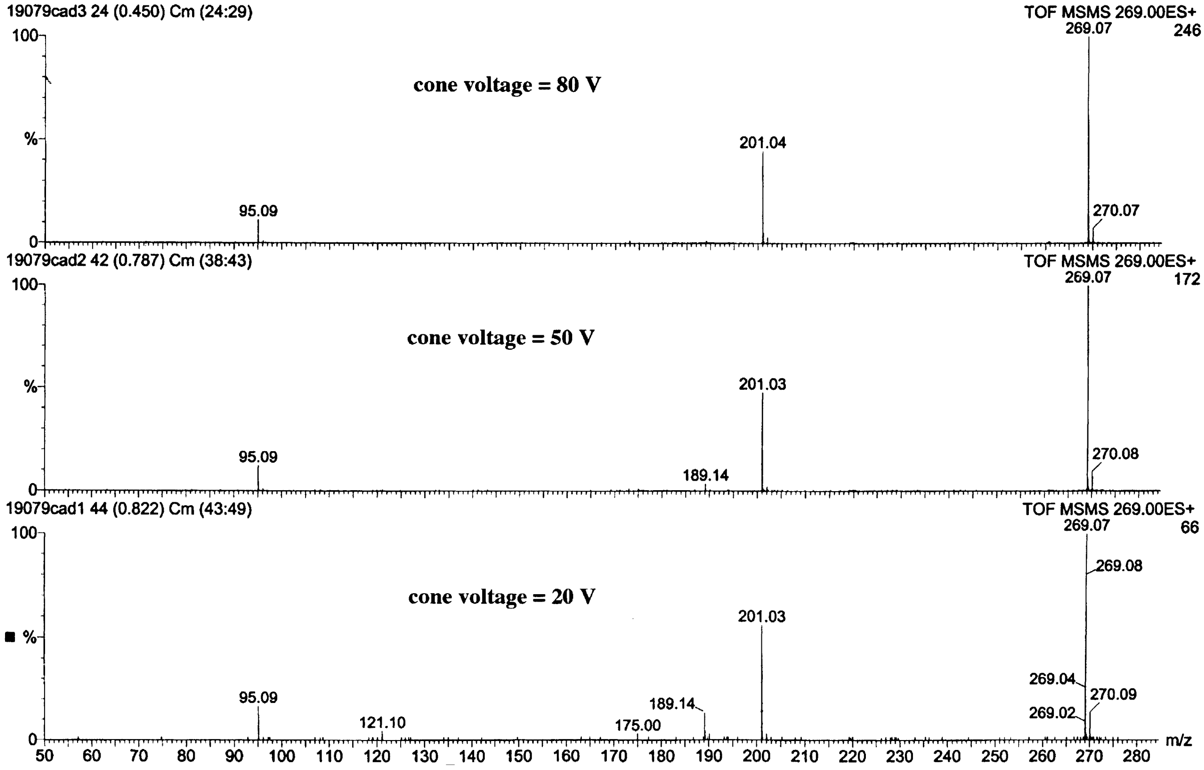
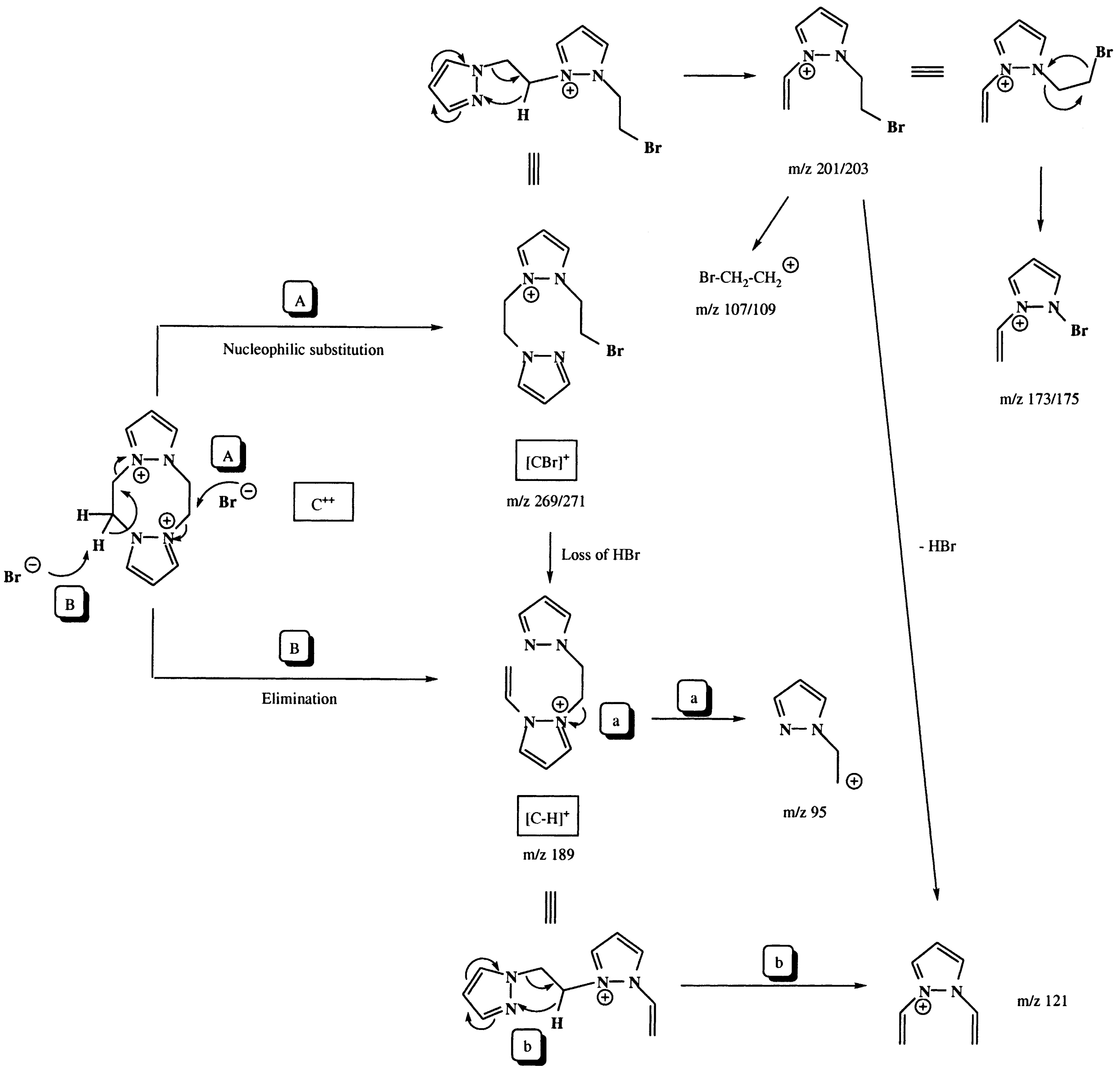


Fig. 3. CAD spectra of *m*/*z* 269 from compound **1** at different cone voltages and at the same collision energy (15 eV).



Scheme 2. Fragmentation mechanisms of compound **1**.

with high cone voltages revealed in-source dissociations of the two main ions [CBr]$^+$ and [C–H]$^+$. Fragmentation pathways were deduced from the primary mass spectra and confirmed by appropriate CAD experiments of [C–H]$^+$ and [CBr]$^+$ ions. As an illustration, the fragmentation mechanisms of compound **1** are reported in Scheme 2. MS/MS experiments were then conducted to dissociate fragment ions to gain further insight in the fragmentation pathways. This strategy involving the production of fragment ions in the ionization source followed by the selective sampling of one fragment ion and its subsequent CAD could be compared to MS^n experiments conducted in ion trap analyzers. As shown in Fig. 4a

Table 2
Relative abundances of ions recorded in CAD spectra of $[CBr]^+$ ion for compound **1**

Collision energy (eV)	Cone voltage (V)	Precursor	Fragment ions					
		$[CBr]^+$ *m/z* 269 (%)	$[C–H]^+$ *m/z* 189 (%)	Path a: *m/z* 95 (%)	Path b: *m/z* 121 (%)	*m/z* 201 (%)	*m/z* 173 (%)	*m/z* 107 (%)
15	20	100	10	10	–	50	–	–
	50	100	1	10	–	50	–	–
	80	100	1	10	–	50	–	–
25	20	20	10	50	15	100	15	20
	50	20	1	30	5	100	20	20
	80	20	–	30	5	100	20	20

and b, dissociation of each isotope of a fragment ion issued from the $[CBr]^+$ ion allowed completion of the fragmentation pathways depicted in Scheme 2. Bromine-containing structures were evidenced according to the mass labeling method (for instance, *m/z* 107 from the CAD of 201 (Fig. 4a), *m/z* 109 from the CAD of 203 (Fig. 4b)). The recorded ions from MS or MS/MS data enabled full characterization of the studied molecules.

The results concerning compounds **3–5** are described below. Close inspection of the doubly charged cation signal showed the contribution of two different

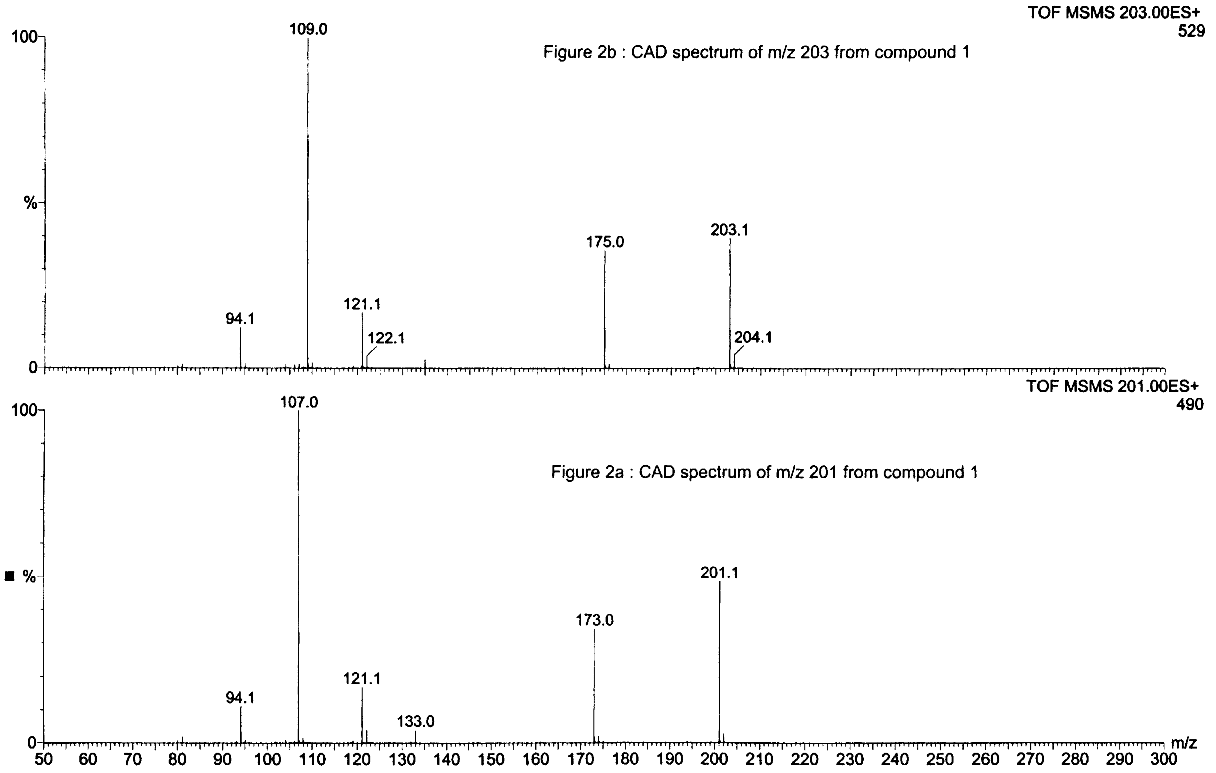


Fig. 4. (a) CAD spectrum of *m/z* 201 from compound **1**; (b) CAD spectrum of *m/z* 203 from compound **1**.

structures: the expected doubly charged ion at *m*/*z* C/2 and a singly charged ion corresponding to half of the studied molecule detected also at C/2. Distinction between the two ions was performed by charge labeling. In the isotopic cluster, the ^{13}C-containing ion was separated by 0.5 and 1 Th for the doubly charged molecule and the singly charged molecule, respectively. The relative abundances of these ions were cone voltage dependent as seen in Fig. 5. At low cone voltage, where the ion internal energy was rather low, the doubly charged molecule dominated. On the contrary, a high cone voltage favored the production of the singly charged species. This is the second example of charge labeling.

The CAD spectra of [CBr]$^+$ ions were recorded systematically using bromine mass labeling. The major ions detected are gathered in Table 3. Two fragmentations occurred by the losses of the neutral molecules HBr and the heterocycle. The nature of the latter influenced the order of the neutral loss. With imidazole containing molecules (compounds **3** and **5**), HBr was lost first followed by the heterocycle. In the case of the pyrazole containing structure (compound **4**), simultaneous loss of HBr and the heterocycle was observed. Such behavior was particularly useful to distinguish between the two isomers compounds **3** and **4**, which exhibited the same primary mass spectrum. The CAD spectra of the parent ion at *m*/*z* 423 from compounds **3** and **4** are reproduced in Fig. 6a and b, respectively. The former contained only the fragment ions at *m*/*z* 341 (loss of HBr) and 273 (loss of both HBr and heterocycle) whereas the latter exhibited an additional fragment ion at *m*/*z* 355 (loss of the heterocycle). Obviously, the loss of HBr from each selected parent ion in the [CBr]$^+$ cluster provided the same ion at *m*/*z* 341 (Table 3) whereas the loss of

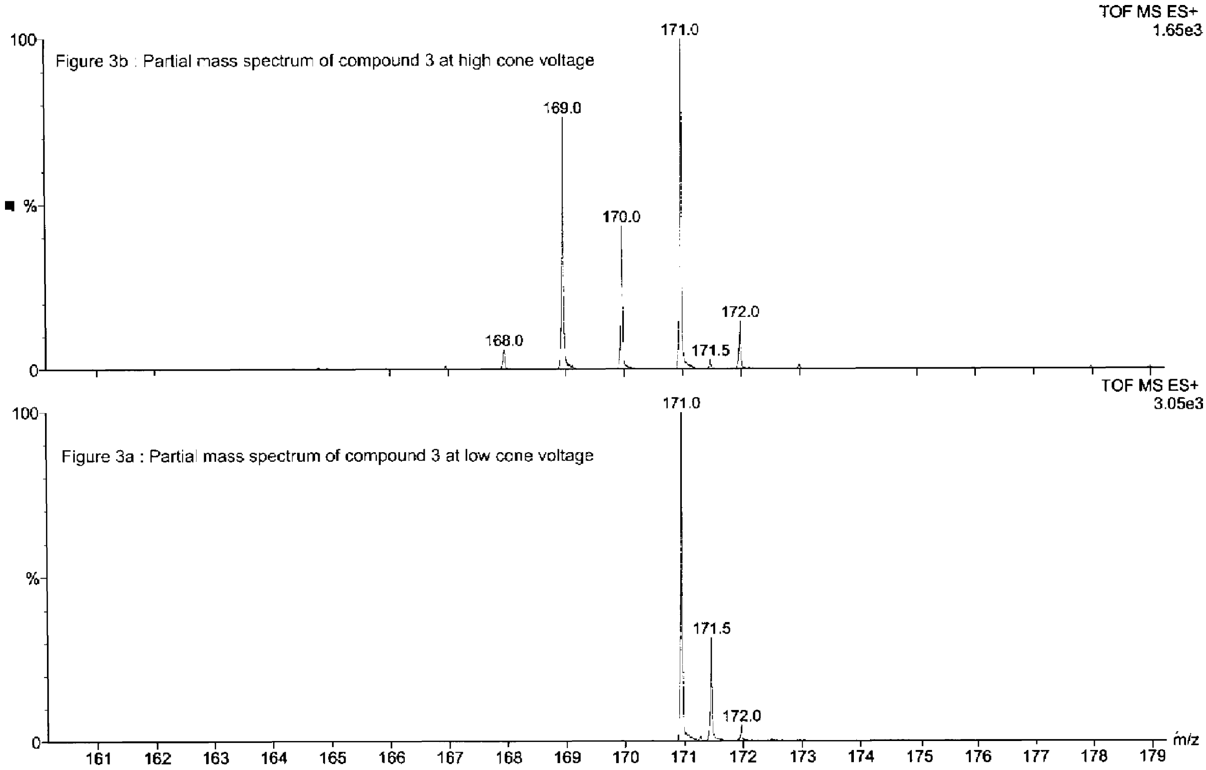


Fig. 5. (a) Partial mass spectrum of compound **3** at low cone voltage; (b) partial mass spectrum of compound **3** at high cone voltage.

Table 3
Ions recorded in CAD spectra of $[CBr]^+$ ion for compounds **3**–**5**

Compound no.	$[CBr]^+$	Loss of heterocycle	Loss of HBr	Loss of (heterocycle + HBr)	$[C/2]^+$	$[C/2–2]^+$
3	421	–	341	273	171	169
	423	–	341	273	171	169
4	421	353	341	273	171	169
	423	355	341	273	171	169
5	521	–	441	323	221	219
	523	–	441	323	221	219

the heterocycle gave ions still mass labeled by the remaining bromine atom (Table 3). The fragmentation pathways of the $[CBr]^+$ ion were presented in Scheme 3. The loss of the heterocycle, the formation of the singly charged C/2 ion corresponding to half of the studied molecule and the species $[C/2–2]^+$ were observed.

3. Experimental

Syntheses of compound have been described elsewhere [18]. ESI mass spectra were recorded on a Q-Tof I mass spectrometer (Micromass, Manchester, UK) fitted with an electrospray ion source. The mass spectrometer was calibrated in the positive ion mode

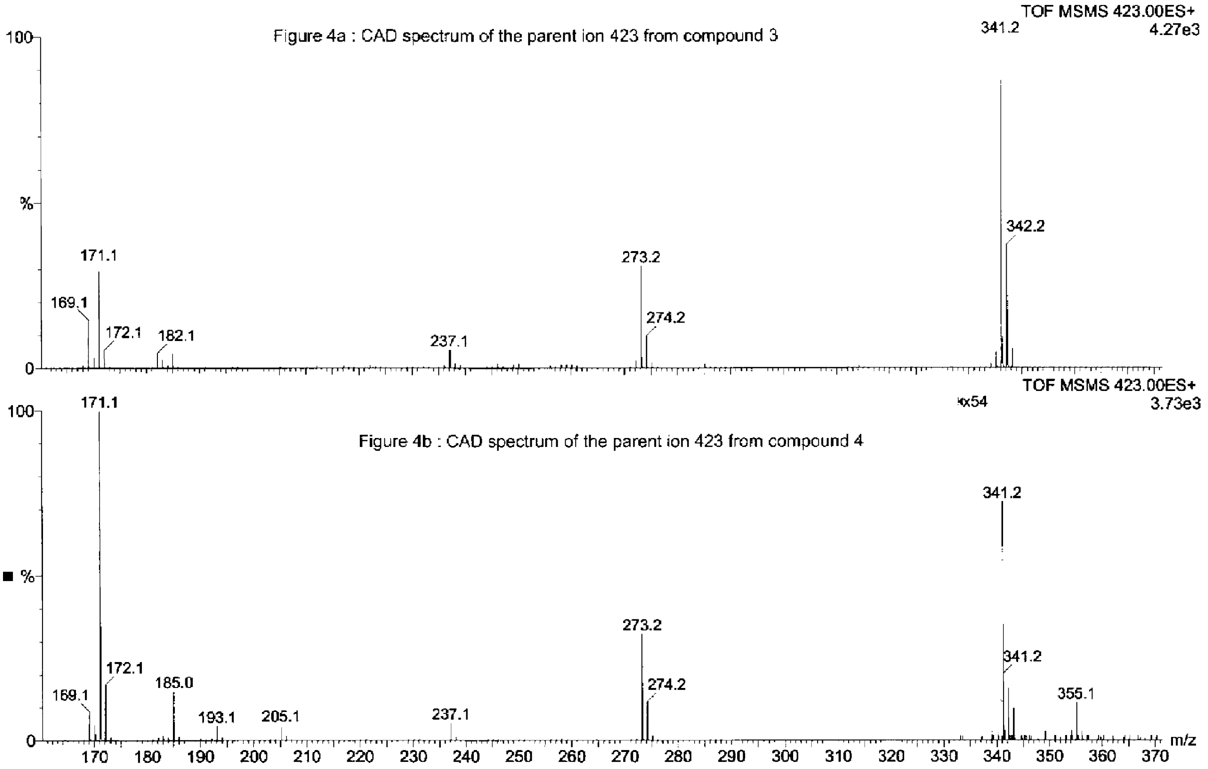
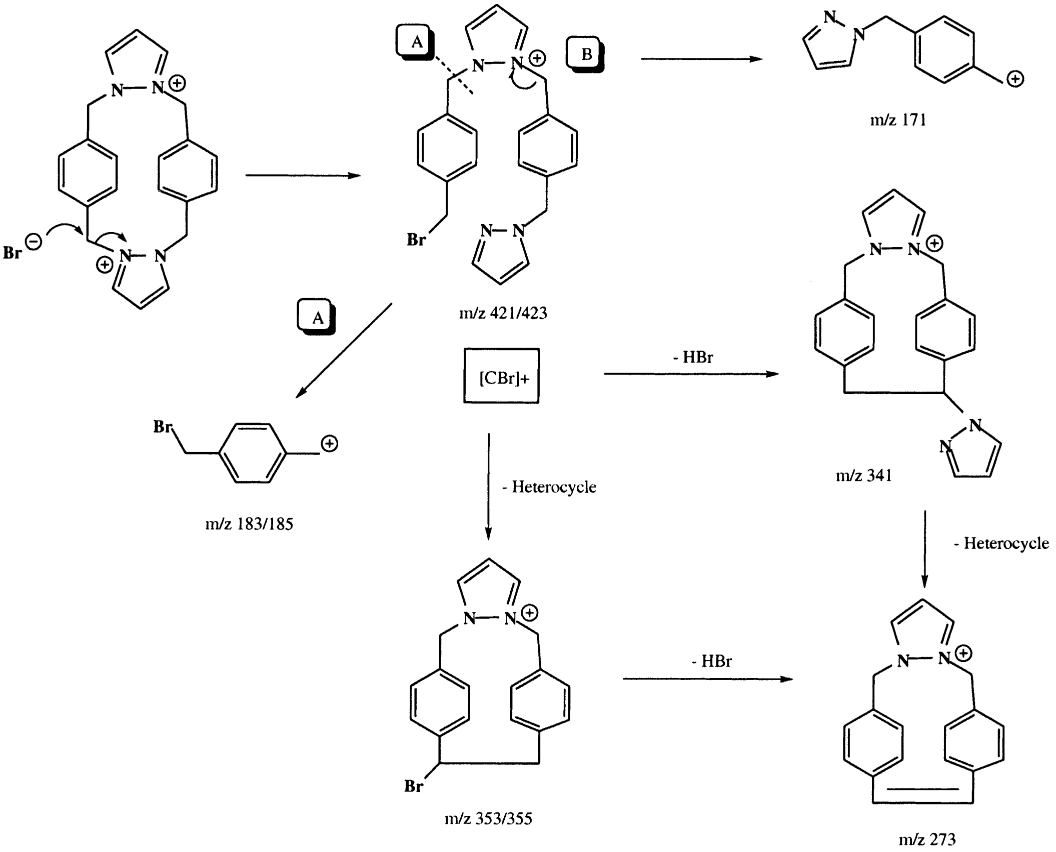


Fig. 6. (a) CAD spectrum of the parent ion $[CBr]^+$ at m/z 423 from compound **3**; (b) CAD spectrum of the parent ion $[CBr]^+$ at m/z 423 from compound **4**.



Scheme 3. Fragmentation mechanisms of $[CBr]^+$ ion of compound **4**.

using a mixture of NaI and CsI. Data were acquired by the Tof analyzer at 1 acquisition/s from *m/z* 50 to 1000 with a resolution of 5000. Ten acquisitions were summed to produce the final spectrum. Samples were dissolved in the mixture H_2O/CH_3CN (50:50, v/v) and infused into the ESI source at a flow rate of 10 μL/min. Special care must be taken in the sample handling to avoid counterion exchange otherwise there will be no bromine left to act as a mass label. Voltages were set at +3.0 kV for the capillary and adjusted for the sampling cone. The source was heated at 100 °C. Nitrogen constituted both nebulizing and desolvatation gas. The latter was heated at 100 °C. In MS/MS experiments, the quadrupole analyzer was set in the rf mode using argon as collision gas and a collision energy varying from 15 to 30 eV depending on the samples.

4. Conclusion

The studied heterocyclic salts were difficult to analyze by NMR spectroscopy due to the presence of symmetry elements. FAB and ESI mass spectrometry allowed identification of the molecular weight but distinction between isomers was not feasible. The use of the hyphenated LC/MS technology failed to answer the question since the compounds were not chromatographically resolved. We have shown that MS/MS experiments were of particular benefit in complete structural identification of this class of molecules. Two fundamental data in mass spectrometry, that is mass and charge, were used as labels, first to determine ions containing a bromine atom and second to distinguish between singly and doubly charged ions detected at the same *m*/*z* value.

Acknowledgements

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