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Fragmentation patterns of novel dispirocyclopiperazinium dibromides with strong analgesic activity under electrospray ionization tandem mass spectrometry conditions

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Abstract

The fragmentation patterns of a series of dispirocyclopiperazinium dibromides with strong analgesic activity were analyzed by positive ion electrospray ionization mass spectrometry in conjunction with tandem mass spectrometry (ESI-MS^n). The $[C^{2+}Br^-]^+$ ions showed the characteristic isotopic peaks with high intensity. In each of their MS^2 spectra, only the $[C^{2+}Br^-–HBr]^+$ ion peak was observable. Further analysis indicated that a selective rearrangement occurred in the unsaturated spirocyclopiperazine ring to achieve dihydropyrrole moiety. Meanwhile, the $[C]^{2+}$ ions were unique and always the base peaks. The ions $[C^{2+}Br^-]^+$ and $[C]^{2+}$ were formed from the equilibrium of precursor molecules 1 in solution, and the latter ions could not be observed in the MS^2 spectra of ions $[C^{2+}Br^-]^+$. The related fragmentation mechanisms were proposed.

Keywords: Mass spectrometry; Dispirocyclopiperazinium dibromides; Fragmentation pathway

1. Introduction

The discovery of highly efficient analgesics without the side effects of drug dependency is highly desirable in pain management. Recently, N^1,N^1-dimethyl-N^4-phenylpiperazinium iodide (DMPP), a well-known nicotinic agonist [1–3], has received interest as a unique ligand among the hundreds of nicotinic agonists. This quaternary ammonium salt does not cross the blood brain barrier (BBB), however, it presents a $K_i = 250$ nM as a nicotinic receptor of the rat brain labeled by [^{3}H]-cytisine [4]. In our previous papers, several classes of piperazinium compounds with significant analgesic activity have been synthesized [5–8], and their structures are similar to DMPP. As an extension of our research, a series of biologically active dispirocyclopiperazinium salts have been reported more recently [9,10], and these results are thought to be useful for elucidating the factors that influence the interactions between non-traditional ligands and neuronal nicotinic acetylcholine receptors [11].

As a part of our work, we need to develop an efficient analytic method for this kind of novel analgesic quaternary ammonium salts. Mass spectrometry (MS) has been used in the analysis of other quaternary ammonium salts owing to its high specificity and sensitivity. For instance, quaternary ammonium pesticides (QUATS) have been analyzed through thermospray [12], fast atom bombardment (FAB) [13], and atmospheric pressure ionization (API) [14–19] mass spectrometric approaches. Generally, previous reports focus on either detection of the quaternary cation (M_q^+) or selected reaction monitoring (SRM) of a characteristic transition by using tandem mass spectrometry (MS/MS). To date, MS/MS studies have concentrated only on the major product ions and many of the weaker transitions remaining unassigned.

In this work, we wish to report the fragmentation patterns of the unique dispirocyclopiperaziniums with general formula (**1**) under electrospray ionization tandem mass

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spectrometry (ESI-MS/MS) and assignment of some weaker transition ions

2. Experimental

2.1. Preparation of samples

The dispirocyclopiperaziniums **1** were synthesized in our laboratory. The structures were confirmed using ^{1}H NMR, ^{13}C NMR, ESI-MS, and elemental analysis. The detailed experimental procedures and biological activities were reported elsewhere [9,10].

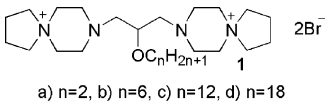
2.2. Mass spectrometers

The ESI mass spectra were recorded on Bruker ESQUIRE-LC^{TM} ESI ion trap spectrometer equipped with a gas nebulizer probe, and capable of analyzing ions up to *m/z* 6000. The MS^{n} spectra were obtained by collision-induced dissociation (CID) with helium after isolation of the interested precursor ions. The experiments were performed in positive mode using the following experimental conditions: drying gas- nitrogen, at a flow rate of 4 L/min; nebulizer pressure—7 psi; capillary voltage—4 kV; heated capillary temperature—300 °C. The samples, dissolved in methanol, were ionized by ESI and continuously infused into the ESI chamber at a flow rate of 3 μL/min using a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Company).

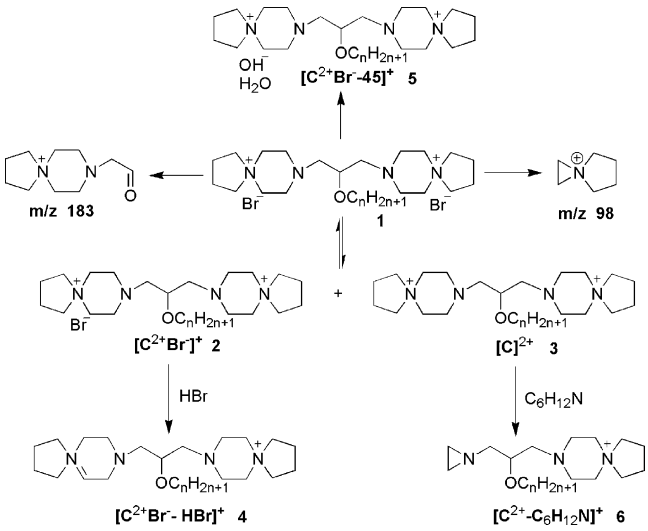
3. Results and discussions

3.1. Ion peaks shown in the full scan ESI-MS spectra

All compounds **1a**–**d** showed similar fragmentation pathways under positive ion ESI conditions, which are summarized in Schemes 1 and 2. There were seven main ions in the ESI-MS spectra for each compound (Table 1). In general, dication (C^{2+}) and its two bromide anions were equilibrated in solution. The loss of one Br^{-} from compounds **1** afforded mono-cations $[C^{2+}Br^{-}]^{+}$ **2**, which were characterized by the double isotope ion peaks. By loss of two bromine anions, the ions $[C]^{2+}$ **3** were formed as the base peaks. When $[C^{2+}Br^{-}]^{+}$ ions **2** were isolated, none on $[C]^{2+}$ **3** peaks could be observed in the MS^{2} spectra. Meanwhile, the ions $[C^{2+}Br^{-}–45]^{+}$ **5** were possibly the hydrolyzed products of **1** since the dispirocyclopiperazinium dibromides are deliquescent. Moreover, there were two fragment ions $[C^{2+}Br^{-}–HBr]^{+}$ **4** and $[C^{2+}–C_6H_{12}N]^{+}$ **6**. Both fragment ions at *m/z* 183 and 98 were observed for all of the four compounds **1a**–**d**. However, no peak could be found at the position corresponding to the molecule **1** on the ESI-MS spectra. The ESI mass spectrum of compound **1b** is shown in Fig. 1a as a representative spectrum for this class of compounds.



Scheme 1. General formula of compound **1**.



Scheme 2. Ions commonly observed in the full scan ESI-MS spectra.

3.2. Fragmentation patterns of ions $[C^{2+}Br^{-}]^{+}$

In order to identify and characterize the detailed fragmentation patterns, the ESI-MS^{2} spectra of ions $[C^{2+}Br^{-}]^{+}$ **2** were recorded. The $[C^{2+}Br^{-}–HBr]^{+}$ ions **4** were observed as the only observable fragment ions in the MS^{2} spectra of $[C^{2+}Br^{-}]^{+}$ **2** (Fig. 1b for **1b**), and they were formed by the elimination of one hydrogen bromide from their precursor ions. Interestingly, these ions could not be detected in the MS^{2} spectra of $[C]^{2+}$ (**3**). The results verified an unexpected behavior that $[C^{2+}Br^{-}–HBr]^{+}$ ions **4** were originated

Table 1
The main ESI-MS spectra data of compounds **1**

Compound	$[C^{2+}Br^{-}]^{+}$ **2**	$[C]^{2+}$ **3**	$[C^{2+}Br^{-}HBr]^{+}$ **4**	$[C^{2+}Br^{-}–45]^{+}$ **5**	$[C^{2+}–C_6H_{12}N]^{+}$ **6**	Common ions
1a	447(95), 445(89)	183(100)	365(12)	401(25)	268(8)	183, 98
1b	503(19), 501(18)	211(100)	421(2)	457(16)	324(5)	
1c	587(48), 585(33)	253(100)	505(2)	541(13)	408(4)	
1d	671(3), 669(3)	295(100)	589(2)	625(5)	492(2)	

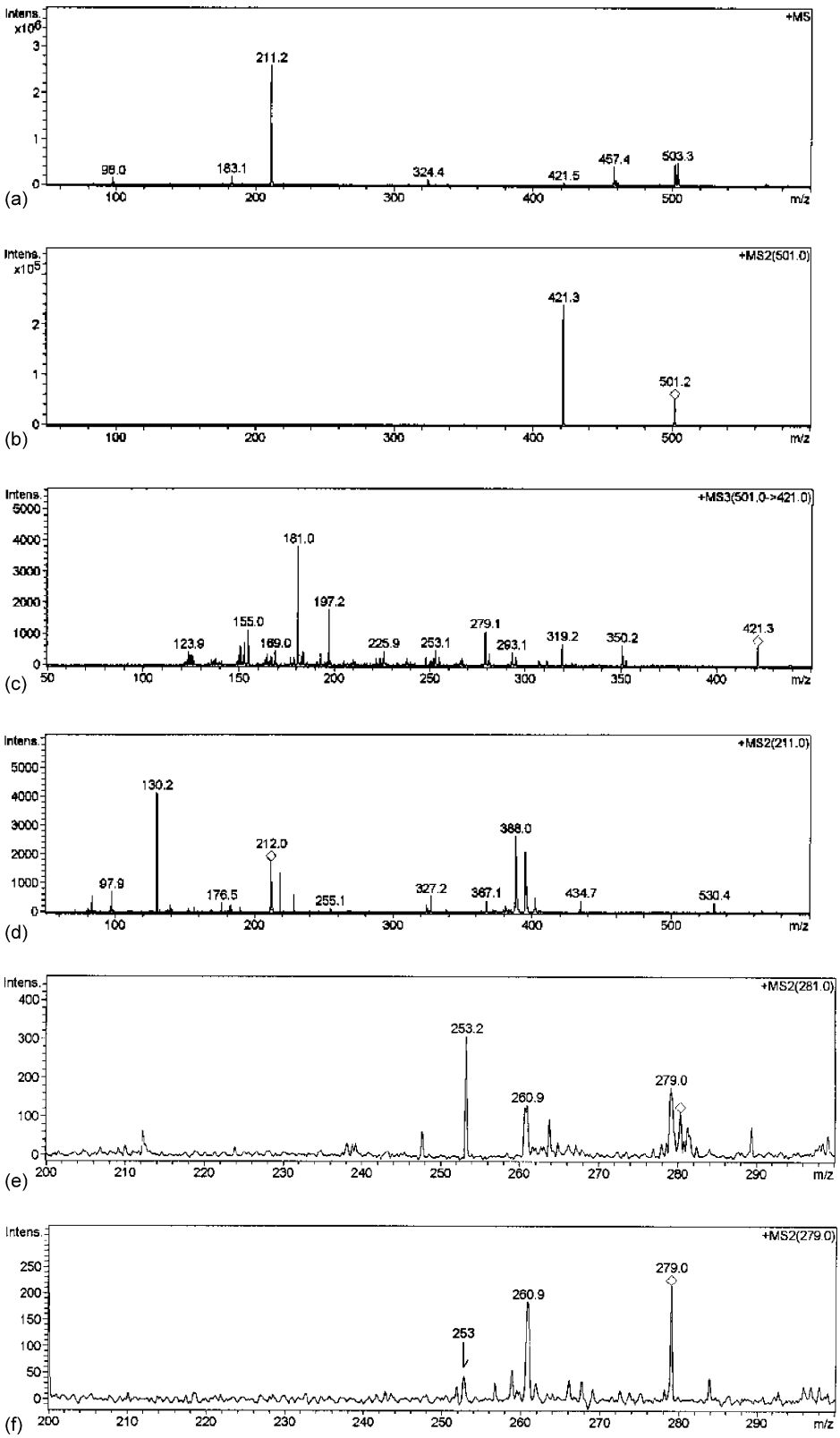


Fig. 1. ESI-MS^n spectra of compound **1b**.

Table 2
ESI-MS^3 spectra of fragment ion $[C^{2+}Br^{-}–HBr]^{+}$ **4**

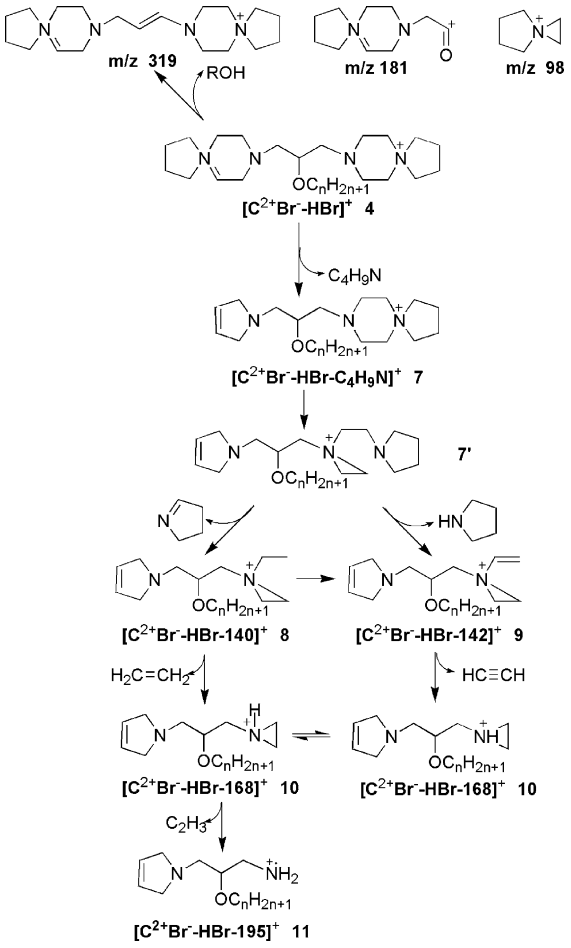
Precursor ions **4** $[C^{2+}Br^{-}$-HBr$]^{+}$	Fragment ions (relative intensity percentage)				
	$[C^{2+}Br^{-}–HBrC_4H_9N]^{+}$ **7**	$[C^{2+}Br^{-}–HBr140]^{+}$ **8**	$[C^{2+}Br^{-}–$ HBr142$]^{+}$ **9**	$[C^{2+}Br^{-}–$ HBr168$]^{+}$ **10**	$[C^{2+}Br^{-}–$ HBr195$]^{+}$ **11**
4a	294(26)	225(10)	223(32)	197(15)	170(7)
4b	350(17)	281(10)	279(29)	253(12)	226(12)
4c	434(21)	365(20)	363(43)	337(16)	310(26)
4d	518(30)	449(7)	447(52)	421(50)	394(40)

from $[C^{2+}Br^{-}]^{+}$ ions. In other published reports, similar ions of diquaternary ammonium salts have been assigned as $[C^{2+}–H^{+}]^{+}$ [15–18], $[M_q–H]^{+}$ or $[M^{\bullet+}–H^{\bullet}]^{+}$ [20]. However, these ions were not observed by MS^n spectra or showed low abundance.

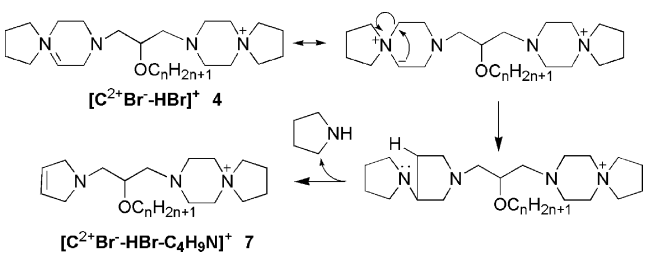
In order to further understand the fragmentation patterns, the ESI-MS^3 spectra of the precursor ions $[C^{2+}Br^{-}–HBr]^{+}$ **4** were recorded (Fig. 1c for **1b**), and the results were listed in Table 2. Various fragmentation pathways are summarized in Scheme 3. There were several dominant fragmentation patterns in the ESI-MS^3 spectra of the precursor ions **4**. The precursor ions $[C^{2+}Br^{-}–HBr]^{+}$ **4** rearranged during the loss of tetrahydropyrrole to afford the fragment ions $[C^{2+}Br^{-}–HBr–C_4H_9N]^{+}$ **7**. Interestingly, the selective cleavage occurred in the spirocyclodehydropiperazine moiety of **4** rather than the spirocyclopiperazinium moiety that could be confirmed by further MS^n analysis. The proposed mechanism is shown in Scheme 4. In the Ylide resonance structure of **4**, the carbon anion attacks the α-carbon of ammonium cation and leads to the spiro-structure that is further transformed to two connected tetrahydropyrrole rings. The ions **7** were then formed by the removal of the terminal tetrahydropyrrole ring.

By subsequent rearrangements and expulsions of the species, the fragmentation ions **7** gave a series of ions at *m/z* $[C^{2+}Br^{-}–HBr–140]^{+}$ **8**, $[C^{2+}Br^{-}–HBr–142]^{+}$ **9**, $[C^{2+}Br^{-}–HBr–168]^{+}$ **10**, and $[C^{2+}Br^{-}–HBr–195]^{+}$ **11**. Differing from the rearrangement of unsaturated spirocyclopiperazine moiety (**4**–**7**), another rearrangement mechanism was proposed to explain the fragmentations of saturated spirocyclopiperazine moiety (Scheme 3). The lone electron pair on nitrogen attacks the α-carbon of ammonium nitrogen and transforms the piperazine ring to azacyclopropane derivatives **7′**. After losing dihydropyrrole or tetrahydropyrrole from **7′**, the ions of $[C^{2+}Br^{-}–HBr–140]^{+}$ **8** and $[C^{2+}Br^{-}–HBr–142]^{+}$ **9** are obtained respectively. Subsequent expulsion of ethylene or acetylene moiety from the corresponding fragment ions **8** or **9** yielded the fragment ions **10**, which were proved by the following MS/MS spectra of ions **8** and **9** (Fig. 1e and 1f for **1b**). Meantime, the fragment ions **9** could come from the fragment ions **8** by losses of two hydrogens as well. Afterwards, elimination of C_2H_3 from ions **10** affords fragment ions **11** which were supported by the MS/MS spectra of ions **10**.

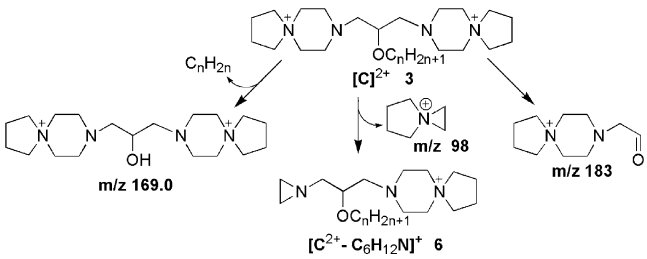
Other fragmentation patterns include that the characteristic ions $[C^{2+}Br^{-}–HBr]^{+}$ **4** lost ROH to produce ion at *m/z* 319 and lost RH and $C_9H_{18}N_2$ simultaneously to form ion



Scheme 3. Fragmentation patterns of ions **4** by tandem mass spectrometry.



Scheme 4. Supposed rearrangement mechanism for the formation of ions **7** from precursor ions **4**.



Scheme 5. Fragmentation patterns of ions $[C]^{2+}$ **3** by tandem mass spectrometry.

at *m/z* 181. 3-Azadispiro[2.3]heptane cation at *m/z* 98 was also detected in MS^3 spectra. Based on the ESI-MS^3 analysis for this kind of quaternary ammonium salts, we have verified our proposed fragmentation pathways that are different from others.

3.3. Fragmentation patterns of $[C]^{2+}$ 3

The ESI-MS^2 spectra of fragment ions $[C]^{2+}$ **3** were recorded in order to further confirm the fragmentation pathways (Fig. 1d for compound **1b**). Comparing with the exclusive fragmentation patterns of $[C^{2+}Br^-]^+$ **2**, the fragment ions $[C]^{2+}$ **3** showed a striking difference in MS^2 spectra. There were several dominant fragmentation ions as shown in Scheme 5. Through the electronic transfer and rearrangement, $[C]^{2+}$ species **3** generated a pair of complementary ions, 3-azadispiro[2.3]heptane cation at *m/z* 98 and monospirocyclopiperazinium **6** at *m/z* $[C^{2+}–98]^+$. Notably, $[C^{2+}–C_6H_{12}N]^+$ ions were also appeared in full scan ESI mass spectra (Scheme 2). Other interesting ions at *m/z* 183 were also observed in the MS/MS spectra.

By comparing the spectra of full scan ESI-MS of **1** with MS^n spectra of $[C^{2+}Br^-]^+$ **2** and $[C]^{2+}$ **3**, it can be concluded that the fragment ion at *m/z* 98 is attributed to both $[C]^{2+}$ **3** and $[C^{2+}Br^-]^+$ **2**, the fragment ions at *m/z* 183 and ions $[C^{2+}–C_6H_{12}N]^+$ **6** in the ESI-MS spectra are due to $[C]^{2+}$ **3**, and other dominant ions are due to $[C^{2+}Br^-]^+$ **2**.

4. Conclusions

Electrospray ionization mass spectra of four novel dispirocyclopiperazinium dibromides **1a**–**d** with strong analgesic activity have been studied and their representative fragmentation pathways were rationalized and supported by tandem mass spectrometry. From our results, Both of the ion $[C^{2+}Br^-]^+$ **2** and $[C]^{2+}$ **3**, which come from the equilibrium of **1** in solution, are the precursor ions for all other fragmentation ions. In the full scan ESI-MS spectra, the fragment ion at *m/z* 98 is attributed to both $[C]^{2+}$ **3** and $[C^{2+}Br^-]^+$ **2**, the fragment ions at *m/z* 183 and ions $[C^{2+}–C_6H_{12}N]^+$ **6** in the ESI-MS spectra are due to $[C]^{2+}$ **3**, and other dominant ions are attributed to $[C^{2+}Br^-]^+$ **2**. Through the consecutive losses of some species, the ions of $[C]^{2+}$ **3** and $[C^{2+}Br^-]^+$ **2** produced some unique fragmentations, which will be helpful for the study of fragmentation pathways in quaternary ammonium salts under different MS conditions. The results allow us to easily identify specific dispirocyclopiperazinium dibromides during synthesis. In addition, the fragmentation patterns will be helpful to further study the metabolic pathways and pharmacokinetic properties of these compounds as prodrugs.

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