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The fragmentation mechanism of β-(*N*-alkyl/arylamino)-α,β-unsaturated carboxylates under electrospray ionization conditions

Short Communication

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Summary. The positive ion mass spectrometric behavior of six β-(*N*-alkyl/arylamino)-α,β-unsaturated carboxylates, α-(1-alkyl/arylaminoethylidene)-γ-lactones, has been studied under electrospray ionization conditions. Their fragmentation pathways are described and supported by tandem mass spectrometry. The protonated compounds are apt to eliminate a water, and a water plus a oxacyclopent-2-yne molecule. Some of the compounds show a tendency to undergo a four-membered ring contraction rearrangement to lose a carbon dioxide. The fragmentation patterns of these compounds exhibit a strong substituent dependency.

Keywords: β-(*N*-alkyl/arylamino)-α,β-unsaturated carboxylate – Mass spectrometry – β-amino-α,β-unsaturated acid – β-amino acid

Introduction

Electrospray ionization mass spectrometry (ESI-MS) is one of the most important analytical techniques for the analysis of nonvolatile and thermally labile compounds, which produces highly abundant protonated molecules due to the low residual energy of the ionization process. ESI, with collision induced dissociation (CID), with which the parent ions are isolated and allowed to react with a collision gas in the collision cell, is a standard technique for the study of fragmentation pathway. Mass spectrometric fragmentation of *N*-butyloxycarbonyl (Boc)-, *N*-benzyloxycarbonyl (Cbz)-protected amino acids and *N-para*-toluenesulfonyl (Ts)-protected amino acids 2,6-bishydroxylmethyl pyridine mono- and di-esters under fast-atom bombardment (FAB) conditions (Garner et al., 1983; Danieli et al., 1989; Chen et al., 1998) had been reported previously. More recently, mass spectrometric fragmentation of amino acid trimethylsilylethyl esters under ESI conditions (Lejeune et al., 2002), mass spectrometric fragmentations of *N*-Cbz-protected aminoalkylphosphonates under electron impact (EI) and ESI conditions (Yuan et al., 1991; Ma et al., 2003), and mass spectrometric fragmentations of *N*-Cbz-protected β-aminoalkanesulfonic acids and β-aminocycloalkanesulfonic acids under negative-ion ESI conditions were also investigated (Xu et al., 2005a).

β-Amino-α,β-unsaturated acids have been widely used in the synthesis of β-amino acids and peptidyl mimetics (Lee et al., 2001; LePlae et al., 2001). We herein report mass spectral studies on α-(1-alkyl/arylaminoethylidene)-γ-lactones, a class of analogues of β-amino-α,β-unsaturated carboxylic esters, under positive ESI conditions. The structures of the compounds are shown in Fig. 1.

Results and discussions

The characteristic positive fragment ions observed in collision-induced dissociation (CID) product-ion spectra of compounds **1**–**6** under ESI conditions were compiled in Table 1. The proposed fragmentation mechanisms, as suggested by observations from CID product-ion spectra of the compounds are shown in Scheme 1. ESI-MS and ESI-MS/MS spectra of compound **5** are shown in Fig. 2.

Either the oxygen atom of the carbonyl group or the nitrogen atom in the title compounds could be protonated

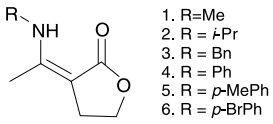


Fig. 1. Structures of α-(1-alkyl/arylaminoethylidene)-γ-lactones

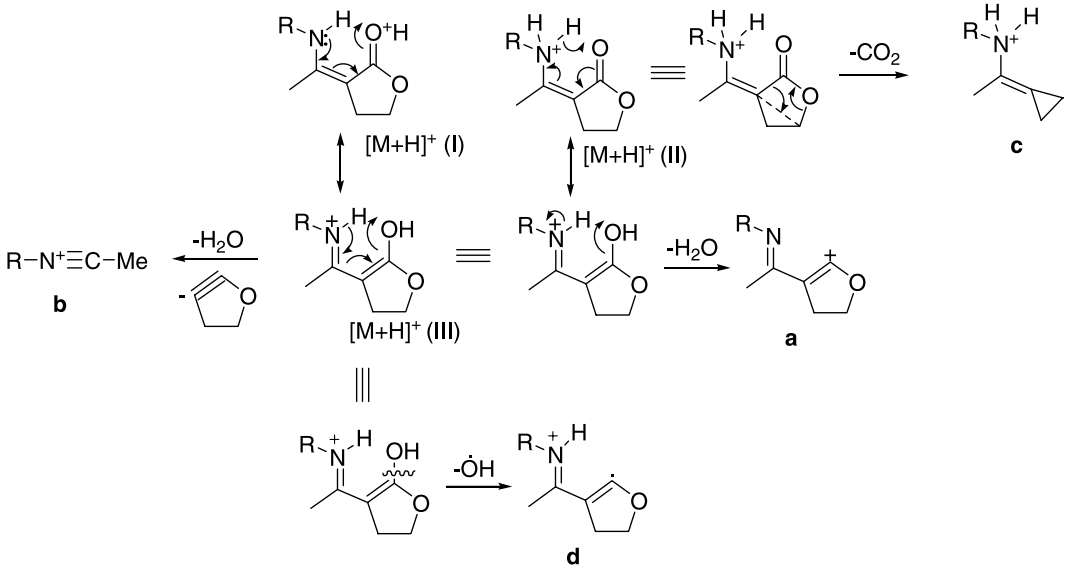
Table 1. Fragment ions observed in MS/MS spectra of compounds **1–6**

Compound	Precursor ions $[M+H]^+$ m/z	Fragment ions m/z (ion)
1	142	124 (**a**), 56 (**b**), 98 (**c**), 125 (**d**)
2	170	84 (**b**), 128 (**e**)
3	218	132 (**b**), 201 (**d**), 91 (**f**)
4	204	186 (**a**), 118 (**b**), 160 (**c**)
5	218	200 (**a**), 132 (**b**), 174 (**c**)
6	282	264 (**a**), 196 (**b**), 203 (**f**), 185 (**g**)

to yield carbonyl *O*-protonated molecular ions **I** or *N*-protonated molecular ions **II**. Both of the protonated molecular ions **I** and **II** could isomerize to the same enolic form $[M+H]^+$ ions **III** via an electron pair or a hydrogen transfer, which had a tendency to eliminate a water molecule to give rise to α-(1-alkyl/aryliminoethyl)-4,5-dihydrofurylium ions (**a**) (Scheme 1). The enolic form $[M+H]^+$ ions **III** could also lose an oxacyclopent-2-yne plus a water simultaneously to produce *N*-substituted acetonitrile ammonium ions (**b**). This is the most common fragmentation path of all these six compounds. The striking feature of the fragmentation is the formation of $[M+H\text{-}44]^+$ ions, which could be observed in the MS/MS spectra of compounds **1**, **4** and **5**. They could be rationalized that the *N*-protonated molecular ions **II** underwent a four-membered ring contraction rearrangement to lose a molecule of carbon dioxide to generate protonated (1-alkyl/arylaminoethylidene)cyclopropane ammonium ions (**c**). The four-membered ring contraction reactions under mass ionization conditions are not very casual phenomenon and have been reported in literatures (Chai et al., 1987; Xu and Zuo, 2003; Xu et al., 2005b).

An obvious substituent effect was observed for the compounds. For compounds **1** and **2**, the protonated molecular ions $[M+H]^+$ (**III**) could undergo an α-cleavage to lose a hydroxy radical to form protonated odd electron 3-(1-alkyl/aryliminoethyl)-4,5-dihydrofuran ammonium ions (**d**) (Scheme 2). For compound **2** with an isopropyl on the nitrogen atom, it could form a $[M+H\text{-}42]^+$ ion, a protonated α-(1-aminoethylidene)-γ-lactone ammonium ion (**e**) via the elimination of a propene from the *N*-subsitituent group of protonated molecular ion. For compound **3**, which has a benzyl on the nitrogen atom, a notable benzyl cation (**f**) at m/z 91 could be observed in its MS/MS spectrum. The fragment ions $[M+H\text{-}79]^+$ (**g**) and $[M+H\text{-}79\text{–}18]^+$ (**h**) were yielded from *N*-*para*-bromophenyl-derived compound **6** by loss of a bromine free radical and a bromine free radical plus a molecule of water from $[M+H]^+$, respectively.

Very recently, the fragmentation mechanism of a series of saturated and unsaturated five-membered lactones (γ-lactones) have been investigated, and it was observed that the main fragment ions are derived from open-chain fragmentation mechanisms followed by neutral loss of carbon monoxide and/or water (Crotti et al., 2004).



Scheme 1. General fragmentation patterns (**a–d**) of α-(1-alkyl/arylaminoethylidene)-γ-lactones **1–6**

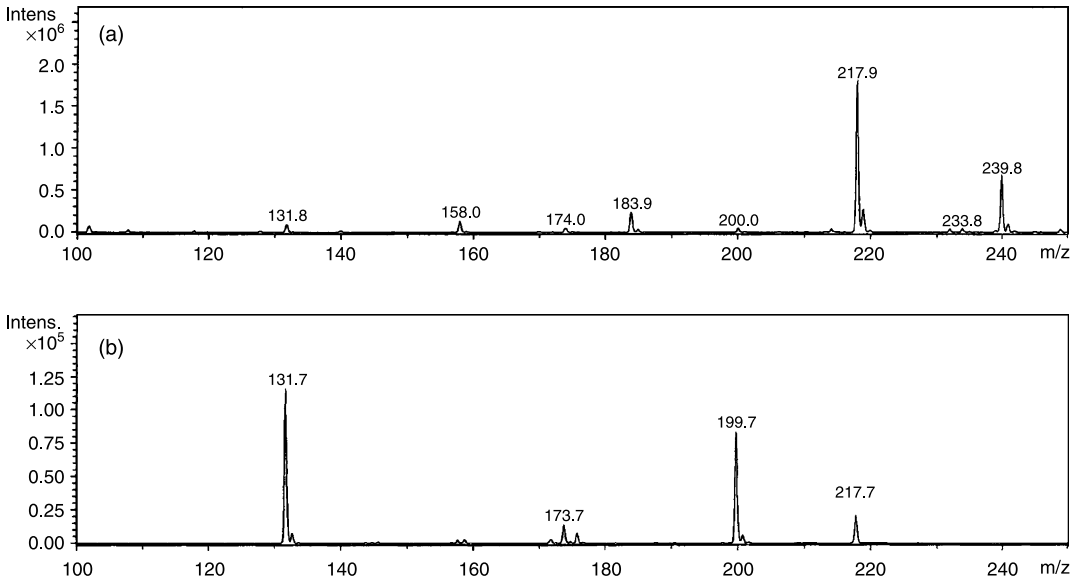
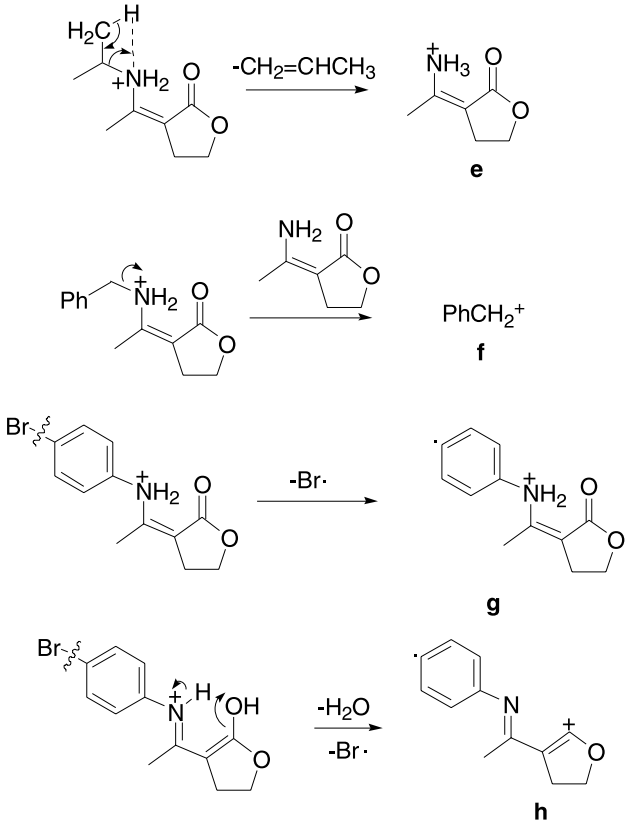


Fig. 2. Positive ion ESI mass spectrum and product-ion spectrum of α-(1-*para*-methylphenylaminoethylidene)-γ-lactone **5**. **a** ESI mass spectrum of protonated **5**; **b** product-ion spectrum of the $[M+H]^+$ion of **5**



Scheme 2. Proposed fragmentation mechanisms for the formation of ions **e**, **f**, **g** and **h**

However, there exists a noticeable difference between the fragmentation patterns of the compounds studied here and those of the γ-lactones under positive-ion ESI conditions reported in the literature. In studies of Crotti et al. (2004), the main fragment ions were derived from elimination of carbon monoxide and/or water from the protonated molecular ions via the lactone ring opening intermediates. While in our investigations, it seemed that ring opening intermediates pathway was not favorable because the neutral loss of carbon monoxide was not founded and all the fragment ions could be explained by Schemes 1 and 2. So the substituente group on the α-carbon of the carbonyl plays an important role for the fragmentation mechanism of γ-lactones.

In conclusion, the mass spectrometric fragmentation of α-(1-alkyl/arylaminoethylidene)-γ-lactones has been investigated. The fragmentation mechanism proposed in Scheme 2 has been supported by CID product-ion spectra. We have discovered that the protonated molecular ions show a tendency to eliminate a water and water plus oxacyclopent-2-yne molecules. The γ-lactone ring of the compounds also shows a tendency to undergo a four-membered ring contraction rearrangement to lose a molecule of carbon dioxide. A good understanding of the fragmentation mechanisms of lactones has significance to determine the structures of larger and more complex natural products with biological activities that contain lactones as part of their structures by the use of mass spectrometric method.

Experimental section

α-(1-Alkyl/arylaminoethylidene)-γ-lactones were prepared by the method described in the literature (Gao et al., 2004). The ESI mass spectra of compounds **1**–**6** were acquired using a Bruker Esquire-LC ESI ion trap

spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to m/z 6000. The experiments were operated in the positive mode as follows: Nitrogen was used as a drying gas with a flow rate of 4 liter/min; nebulizer pressure, 7 lb/in^2 capillary voltage, 4 kV; heated capillary temperature, 300°C. The samples dissolved in methanol were ionized by ESI ionization and continuously infused into the ESI chamber at a flow rate of 0.4 µl/min by a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Company). CID fragmentation was performed using helium collision gas on isolated parent ion. The fragmentation amplitude values were 0.5 –1.0 V and the fragmentation time was 40 ms.

Acknowledgements

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