Neighboring Group Participation in the Electrospray Ionization Tandem Mass Spectra of Polyamine Toxins of Spiders. Part 1: α,ω -Diaminoalkane Compounds

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The fragmentation pattern of *N*-(4-aminobutyl)-3-(4-hydroxyphenyl)prop-2-enamide (**1**) is characterized by an ion formed by neighboring group participation. The phenolic hydrogen is transferred to the primary amino group via a 15-membered ring. This has been proven by H/D exchange reactions and by analyzing homologues and derivatives. (*J Am Soc Mass Spectrom* 1995, 6, 634–637)

Polyamines (e.g., spermidine, spermine) are well known natural products [1]. Many of them have been isolated from plants in which the polyamines are condensed with cinnamic as well as with fatty acids [1]. Because of their thermal lability, structure elucidation of polyamines and polyamine derivatives by means of mass spectral fragmentation (electrospray ionization–mass spectrometry) is possible only if the analytical samples are first converted to their more stable *N*-acetyl derivatives [2]. The mass spectral fragmentation of the acetyl derivatives then allows the deduction of general fragmentation rules for this class of compounds.

Some years ago, toxins that contain a polyamine backbone were isolated from the venom of spiders and wasps. The total number of basic nitrogen atoms in these open chain compounds is between 4 and 11. The number of methylene groups connecting the neighboring nitrogen atoms varies, to our knowledge, from three to five [3]. Because the available amounts of the toxins are very small and they have high polarity, the above-mentioned acetylation method is not useful. In this case, the commonly used method is first purification with reversed-phase high-performance liquid chromatography (HPLC) followed by structure elucidation via fast-atom bombardment, NMR, and Edman degradation [3].

Electrospray ionization mass spectrometry (ESI-MS) is an analytical method that is becoming increasingly important for the study of, for example, water-soluble highly functionalized compounds [4, 5]. To obtain structural information as well as an indication of

molecular weight (from $[M + H]^+$, $[M + nH]^{n+}$, $[M + Na]^+$, etc.), low energy collision-activated decomposition (CAD) with argon is the method of choice [6, 7]. In fact, polyamines such as spermine are so reactive that they even decompose in the ESI ion source if the lens voltages are not very well optimized; they then give similar spectra to those obtained from CAD experiments (Bigler, L.; Hesse, M. Unpublished results).

We have therefore investigated the ESI tandem mass spectrometry fragmentation behavior of cinnamic acid derivatives of diamines that can be used as model compounds for natural polyamine derivatives [1, 3, 8, 9]. In particular, we have analyzed *N*-(4-aminobutyl)-3-(4-hydroxyphenyl)prop-2-enamide (**1**). Compound **1** shows by ESI-MS only the $[M + H]^+$ signal and no fragment ion peaks at all.

Experimental

Materials

All samples (Scheme I) were synthesized in our laboratory (Bigler, L.; Guggisberg, A.; Hu, W. To be published). For ESI-MS, all compounds were dissolved in HPLC grade methanol (3–5 nmol mL⁻¹) purchased from Scharlau (Barcelona, Spain). These concentrations were considerably higher than the minimum required to obtain tandem mass spectra, but were used to ensure that the spectra, had the best possible signal-to-noise ratio. Deuterated compounds were obtained by dissolving the samples in 99.8% *d*₄-methanol (3–5 nmol mL⁻¹) purchased from Cambridge Isotope Laboratories (Woburn, MA) and waiting at least 10 min to achieve a complete substitution of the exchangeable hydrogen atoms.

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Scheme I. Investigated α,ω -diaminoalkane compounds.

Mass Spectrometry

ESI tandem mass spectra were obtained on a Finnigan TSQ 700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA). Samples were introduced continuously through the electrospray interface at a rate of $2 \mu\text{L min}^{-1}$ and with the capillary held at 3.2–3.3 kV. For all CAD experiments, only the $[M + H]^+$ ions were selected in the first quadrupole (Q1) and passed through the collision cell. In this cell, the argon pressure was 3–3.5 mtorr and the collision activation energy (E_{coll}) was varied from 10 to 35 eV, as indicated. The product ions were detected by scanning the third quadrupole (Q3). The product-ion scans were monitored in the range m/z 40–300 over 3 s and 20 scans were averaged to obtain representative spectra. The abundance of the fragment ions in the tandem mass spectra are reported as a percentage relative to the base peak (relative intensity 100%).

To maximize the sensitivity, the resolution on Q1 was typically opened up to produce a parent ion envelope 1.5–2 u wide at half peak height. The resolution on Q3 was set at 0.7–0.8 u wide at half peak height so as to detect low intensity fragment ions.

Results and Discussion

In electrospray tandem mass spectrometry experiments at low energy and with argon as the collision gas, the abundant fragments are generally the result of nonradical reactions that lead to the formation of stable ions like carbonyl carbocations ($\rightarrow c$, m/z 147, Scheme II). This is the result of the loss of stable neutral fragments [10–12] such as NH_3 ($\rightarrow d$, m/z 218) or of $\text{S}_{\text{N}}\text{i}$ -type reactions ($\rightarrow a$, m/z 72).

The presence of the peak at m/z 89 in the spectrum of N-(4-aminobutyl)-3-(4-hydroxyphenyl)prop-2-enamide (1) corresponds to protonated 1,4-diaminobutane (Figure 1). That means, compared with neutral 1, the basic part of the molecule contains two additional hydrogen atoms: one obtained by protonation during ionization and the other transferred from the 3-(4-hydroxyphenyl)prop-2-enamide residue during the fragmentation reaction. Because of the planar structure of this enamide group with 10 sp^2 hybridized atoms, two possible mechanistic pathways for the hydrogen transfer are proposed (see Scheme III).

Scheme II. The fragmentation pathway of N-(4-aminobutyl)-3-(4-hydroxyphenyl)prop-2-enamide (1) by ESI tandem mass spectrometry.

Figure 1. ESI tandem mass spectrometry of $[M + H]^+$ ion of 1. $E_{\text{coll}} = 15 \text{ eV}$.

Mechanism A, the neighboring group participation mechanism. First a proton is transferred from the protonated primary amine to the amide nitrogen atom [13]. Furthermore, to put the primary amine in the vicinity of the *p*-hydroxy group, the $\text{C}=\text{C}$ double bond of the propenamide part must have the (*Z*) configuration. The isomerization reaction at the double bond has to occur in the collision cell or in the skimmer region of

Scheme III. The two fragmentation mechanisms involved in the formation of the protonated 1,4-diaminobutane from compound 1.

the electrospray interface because the synthesized material has the (*E*) configuration. During the essential step, the acidic phenolic proton is transferred to the basic primary amino group. Due to conjugation of the resultant phenolate with the enamide, the amide bond is cleaved and ion *b* (m/z 89, Scheme II) is formed as well as the neutral quinomethane ketene fragment (Scheme IV).

Similar reaction mechanisms via macrocyclic intermediates in the gas phase have been postulated previously [14-18].

Mechanism B, the four-center cleavage reaction. This reaction, well known in mass spectral fragmentation pathways [19], can easily be depicted as in Scheme III.

To differentiate between the two mechanisms, all exchangeable hydrogen atoms (NH, OH) in compound **1** were replaced by deuterium atoms (see Scheme IV) by treatment with d_4 -methanol (Figure 2). In the mass spectrum of the labeled analog, the parent peak is shifted by 5 u. The 1,4-diaminobutane fragments also are substituted preferentially with five deuterium atoms (shift of m/z 89 to 94, $I_{\max} = 13\%$ at 15 eV), which favors the fragmentation reaction by neighboring group participation (mechanism A, Scheme III). As a result, the neutral highly conjugated quinomethane ketene fragment should not contain any deuterium atoms.

On the other hand, the signal from the 1,4-diaminobutane ions with only four deuterium atoms (m/z 93) and 9% maximum relative intensity ($E_{\text{coff}} = 15$ eV) can be explained in terms of mechanism B.

Mechanism A presumes an electron delocalization from the phenolic hydroxy group in the para position through the phenyl ring and the C=C double bond to the amide nitrogen. Another approach to the elucidation of this mechanism is to prevent the electron delocalization by using a compound substituted at the meta position. The spectrum of N-(4-aminobutyl)-3-(3-hydroxyphenyl)prop-2-enamide (**2**) shows a much lower signal intensity at m/z 89 ($I_{\max} = 3\%$ at 20 eV) than that of the 4-hydroxy compound **1**. This small signal is the result of decomposition by mechanism B. This assertion is confirmed by the spectrum of the

Scheme IV. Differentiation between mechanisms A and B by deuterated derivatives.

Figure 2. ESI tandem mass spectrometry of $[M + D]^+$ ion of d_4 -**1**. $E_{\text{coff}} = 15$ eV.

deuterated compound **2** (Figure 3), which clearly shows that no signal is present at m/z 94 and only a small signal is present at m/z 93 ($I_{\max} = 3\%$ at 20 eV).

Figure 4 also illustrates that the fragment ions at m/z 89 result from the sum of the two mechanisms: the maxima are at the same collision energy and the sum of the relative intensities of the signal of the two deuterated fragments m/z 94 and 93 is roughly equal to the relative intensity of the signal at m/z 89 of the unlabeled fragments. Consequently, for substance **2**, the *b*-ion (m/z 89) and the d_4 -*b*-ion (m/z 93) curves are very similar.

The effects of varying the length of the α,ω -diaminoalkane chain on the decomposition also have been studied for the 3-(4-hydroxyphenyl)prop-2-enamide derivatives **3** and **4**. Measurements on non-deuterated and deuterated compounds have shown clearly that both mechanisms are involved. The spectra of the compounds with three or seven methylene groups have a lower fragment ion signal intensity that results from neighboring group participation, because of higher ring tension for the proton transfer.

Figure 5 demonstrates clearly the differences in the abundance of the protonated α,ω -diaminoalkane fragments that result from neighboring group participation. The fact that the length of the aliphatic chain influences the abundance of the ions that have been studied is in agreement with the mechanisms dis-

Figure 3. ESI tandem mass spectrometry of $[M + D]^+$ ion of d_4 -**2**. $E_{\text{coff}} = 15$ eV.

Figure 4. Fragmentation of 1,4-diaminobutane derivatives as a function of collision energy.

cussed in the foregoing text. An alternative fragmentation pathway, for example, the transfer of the phenolic hydrogen (deuterium) atom via the aromatic ring to the carboxyl oxygen, can be excluded because of the less intense signals of the *b* ion analogs in the spectra of compounds 3 and 4.

Conclusion

The transfer of one hydrogen atom via a 15-membered ring transition state depends very much on the geometry of the system. After *E/Z* isomerization of the cinnamoyl part, all 10 sp^2 hybridized atoms keep their planarity. The driving force for the reaction seems to be the formation of the neutral quinomethane ketene

Figure 5. Fragmentation of α,ω -diaminoalkane derivatives 1-4 by neighboring group participation as a function of collision energy.

fragment. This fragment apparently is not produced by the *m*-hydroxy compound 2, as evidenced by a decrease of the signal intensity. The corresponding reaction is, because of the ring strain, less preferred when the hydrogen atom transfer occurs via 14- and 18-membered ring transition states, respectively.

A corresponding mass spectral fragmentation was observed under similar conditions in the spectrum of N^1,N^4 -di(*p*-cumaroyl)-spermidine. (This compound was synthesized by W. Hu and M. Hesse). The ion m/z 292, the analog of ion *b*, represents the base peak (100% relative intensity, 30-eV E_{coff}).

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