Isomer Differentiation by Charge Inversion Tandem Mass Spectrometry: An Investigation into the Structure of the Ionic Products from an $S_N(\text{ANRORC})$ Reaction

Gregory G. Dolnikowski

USDA Human Nutrition Research Center at Tufts University, Boston, Massachusetts, USA

Negative ion fast atom bombardment and negative ion chemical ionization tandem mass spectrometry combined with charge inversion reactions are used to confirm that 2-chloro-5-nitropyridine reacts with the hydroxide ion by an $S_N(\text{ANRORC})$ mechanism in solution to form a ring-opened $\text{C}_5\text{H}_3\text{N}_2\text{O}_3^-$ anion that has the structure of the $(\text{M}-\text{H})^-$ anion of 2-nitro-4-cyano-2-butenal. The addition of excess hydroxide ion forms the expected 2-oxy-5-nitropyridine anion. In the gas phase the same reaction forms only the 2-oxy-5-nitropyridine anion. High energy charge inversion is shown to be an excellent means of differentiating between these isomeric negative ions. (*J Am Soc Mass Spectrom* 1992, 3, 467-470)

In solution the electron deficient nature of the pyridine ring that makes the electrophilic aromatic substitution reactions that are typical of aromatic systems very difficult instead facilitates nucleophilic aromatic substitution reactions. Nucleophilic aromatic substitution is generally thought to occur in solution by three separate mechanisms [1]: the addition-elimination mechanism, the benzyne mechanism, and the aromatic nucleophilic substitution by means of ring opening and ring closing mechanism ($S_N(\text{ANRORC})$). The $S_N(\text{ANRORC})$ mechanism has received less attention [2-4] than the other two mechanisms and it has not been much explored by means of mass spectrometry, even though aromatic nucleophilic substitutions are known to occur in the gas phase [5, 6].

The particular $S_N(\text{ANRORC})$ reaction of interest in this study is the reaction of hydroxide ion with 2-chloro-5-nitropyridine [1] [7, 8]. This reaction has previously been investigated by proton and ^{13}C -nuclear magnetic resonance (NMR) spectrometry and by infrared spectroscopy. In a solution of dimethylsulfoxide and water this reaction forms a stable, isolatable, ring-opened intermediate [2] with the structure of the $(\text{M}-\text{H})^-$ anion of 2-nitro-4-cyano-2-butenal when the molar ratio of 1 to the hydroxide ion is 2:1. Addition of excess hydroxide ion closes the ring again and

leads to the expected nucleophilic aromatic substitution product, the 2-oxy-5-nitropyridine anion [3]. Structures of 1, 2 and 3 are found in Scheme I.

Because 2 and 3 are isomers they are difficult to differentiate by mass spectrometry. When the first research into this reaction was published in 1980, mass spectrometry was not even considered for helping to solve the structure of these compounds, but with the advent of fast atom bombardment (FAB) ionization, these compounds have become amenable to mass spectrometry analysis. This article describes a FAB combined with tandem mass spectrometry (MS/MS) isomer differentiation strategy developed for the products of the solution-phase reaction and compares those FAB tandem mass spectra with the negative ion chemical ionization tandem mass spectra of the product ions generated by the gas-phase reaction of the hydroxide ion and 1.

Experimental

Chemicals

2-Chloro-5-nitropyridine and 2-hydroxy-5-nitropyridine were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. The $S_N(\text{ANRORC})$ intermediate was prepared as in Reinheimer et al. [7], except that it was not isolated by lyophilization. It remained in the dimethylsulfoxide/water solution, and 1 μL of the

Address reprint requests to Gregory G. Dolnikowski, USDA Human Nutrition Research Center at Tufts University, 711 Washington Street, Boston, MA 02111.

Scheme 1

solution was applied to 1 μ L of glycerol on the FAB probe tip for analysis. Several crystals of 2-hydroxy-5-nitropyridine were dissolved in triethanolamine on the FAB probe tip for analysis.

Instrumentation

Two tandem mass spectrometers were employed in this research: a prototype Extrel triple quadrupole mass spectrometer (Extrel ANS, Madison, WI) for low energy collision-induced dissociation (CID) experiments, and a Kratos MS-50 E₁B₁E₂ triple analyzer mass spectrometer (Kratos Analytical, Ramsey, NJ) for high energy CID, and for high energy charge inversion experiments. Argon was the collision gas in the Extrel instrument, helium in the Kratos instrument. Both tandem mass spectrometers were equipped with chemical ionization, FAB, and conversion dynode electron multipliers for negative ion detection. All collision experiments on the Kratos instrument were conducted in a cell located in the third field-free region, before the second electrostatic analyzer. In the negative ion CID experiments the E₂ was scanned to collect the negative product ions of the negative precursor ion. In the charge inversion experiments the E₁B₂ remained the same as in the negative ion experiments, but the polarity of the second electric field was reversed so that the positive product ions of the negative precursor ion were detected when E₂ was scanned.

Ion Source Conditions

In the FAB experiments, 6 keV argon atoms were used to desorb the sample ions. The FAB ion source

was operated at room temperature. In the negative ion chemical ionization (NICI) experiments, distilled water was admitted into the ion source through a needle valve from a heated reservoir, and 2-chloro-5-nitropyridine was introduced as crystals into the ion source by an unheated direct probe. The NICI source was not heated directly, but some heating (approximately to 40 °C) occurred because of indirect heating from the CI filament. The kinetic energy of the NICI electrons was 38 eV.

Accurate Mass Measurements

The accurate mass measurements were made in the NICI mode on the EBE instrument. 2-Chloro-5-nitropyridine was employed as the mass standard (m/z 157.988304) and the unknown anion at nominal m/z 139 was peak-matched against it. Both the standard and the unknown peak shapes were acquired with a multichannel averager to improve the signal-to-noise ratios. The EBE instrument was operated with a resolving power of 10,000 (10% valley definition).

Results

FAB of 2 and 3 produced negative ions at m/z 139. The low energy negative ion CID mass spectra of the M⁻ ions produced by FAB of 2 and 3 yielded product ions of m/z 109 (M - NO₂)⁻, m/z 93 (M - NO₂)⁻, and m/z 46 (NO₂)⁻. The high energy negative ion CID mass spectra of the same M⁻ ions produced the same product ions listed above and one other product ion, m/z 123 (M - O)⁻. The M⁻ ions of 2 and 3 were indistinguishable by either high or low energy CID. This is primarily due to the fact that the nitro group directs the fragmentation of both M⁻ ions.

One possible solution to the problem of differentiating 2 and 3 is to examine the mass spectra of the positive product ions formed by charge inversion of the negative precursor ions. According to a recent review [9], charge inversion is usually a high energy Franck-Condon process that generates mainly direct bond cleavages, not rearrangements. Bowie and Blumenthal [10] have studied nitroaromatic ions by charge inversion mass spectrometry and have proposed charge inversion as a method of investigating the structures of negative ions like these, which do not fragment easily. They have also determined that for dinitrobenzenes at least, charge inversion differentiates between isomers more readily than either negative or positive ion CID.

Two charge inversion mass spectra of the m/z 139 M⁻ ions formed by FAB of 2 and 3 are shown in Figure 1. Even though the three sector tandem mass spectrometer used here did not achieve unit resolution of the product ions, the charge inversion mass spectra clearly distinguished between the two isomeric precursor ions.

Figure 1. FAB-charge inversion product ion spectra of (a) compound 2 and (b) compound 3 produced by a solution-phase reaction of the hydroxide ion with 2-chloro-5-nitropyridine.

Charge inversion creates unstable positive ions from the stable negative ion precursors 2 and 3. Scheme II shows an unstable oxenium ion that is produced from 3 and three unstable resonance forms of a positive ion that is produced from 2. These high energy intermediates are the driving force for the extensive fragmentation observed in Figure 1.

The product ions formed by charge inversion of the M^- ion of 2 indicate that the structure of 2 is ring-opened because there is a product ion (m/z 29) characteristic of the aldehyde functional group. This same ion does not appear in the charge inversion spectra of the M^- ion of 3. The charge inversion mass spectra therefore confirmed the interpretation that 2 and 3 have different but isomeric structures, and that

Scheme II

the solution reaction proceeds by an $S_N(ANRORC)$ mechanism.

In the gas phase one would not necessarily expect to observe a similar reaction to the solution reaction. However, when 1 reacts in an NICI source with OH^- ions derived from NICI of water, an ion of m/z 139 is formed that is not produced in isobutane NICI of 1. Figure 2 shows that the charge inversion mass spectrum of the ion produced by OH^- NICI of 1 is very similar to that of the M^- ion of 3. The measured exact mass of the ion-molecule reaction product was 139.0155, which is 8 ppm less than the calculated exact mass of $C_5H_3N_2O_3^-$ (139.0144). This exact mass measurement confirmed that the product ion has the formula $C_5H_3N_2O_3^-$.

Clearly, a nucleophilic substitution reaction of 1 with OH^- occurs in the gas phase to produce 3 or a closed-ring isomer of 3. Because all known gas-phase and solution phase nucleophiles substitute at the ring position vacated by the leaving group or open the ring, the most likely structure for the ion-molecule product ion is that of the 2-oxy-5-nitropyridine anion.

Because there is no indication of any product ions related to the ring-opened intermediate in Figure 2, the gas-phase reaction may not occur by the $S_N(ANRORC)$ mechanism. It is possible that the gas-phase reaction does proceed by the ring-opening process but that it is so rapid that only the end product 3 is detected. Another possibility is that the gas-phase reaction follows an $S_N(AE)$ process similar to that proposed by van der Plas et al. [8] for reaction of NH_2^- with 1 in solution. Scheme I shows both the $S_N(ANRORC)$ and $S_N(AE)$ mechanisms for the reaction of OH^- with 2-chloro-5-nitropyridine.

Conclusion

These results demonstrate the utility of FAB and NICI combined with charge inversion MS/MS for differen-

Figure 2. NICI charge inversion product ion spectrum of compound 3 produced by a gas-phase reaction of the hydroxide ion with 2-chloro-5-nitropyridine.

tiating a heterocyclic and a ring-opened isomer, and for elucidating the aromatic nucleophilic substitution ion chemistry of an interesting reaction. The MS/MS data agree with earlier NMR studies that the hydroxide ion reacts with 2-chloro-5-nitropyridine in a DMSO/H₂O solution by an S_N(ANRORC) mechanism. The hydroxide ion also reacts in the gas phase with 2-chloro-5-nitropyridine, possibly by an S_N(AE) mechanism, to form the same 2-oxy-5-nitropyridine anion end product as the solution reaction.

Acknowledgments

I thank J. Throck Watson (Michigan State University Mass Spectrometry Facility) and Michael L. Gross (University of Nebraska, Midwest Center for Mass Spectrometry) for allowing me to make use of the TQMS and EBE instruments. I also thank William Reusch of Michigan State University, Roger Hayes of the University of Nebraska, and LeRoy Haynes of the College of Wooster for helpful discussions.

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