JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2008; **43**: 1636–1640
Published online 19 June 2008 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/jms.1442

JMS

Recognizing α-, β- or γ-substitution in pyridines by mass spectrometry

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Received 29 January 2008; Accepted 6 May 2008

A general mass spectrometric method able to recognize the site of substitution of monosubstituted pyridines is described. The method requires that the molecule under investigation forms, upon ionization and dissociation, the respective α-, β- or γ- pyridinium ion of *m/z* 78. Pyridinium ions are stable and common fragments of ionized and protonated pyridines and are found to function as appropriate structurally diagnostic fragment ions. They can be identified by their characteristic and nearly identical collision-induced dissociation behavior and distinguished by the combined use of two structurally diagnostic ion/molecule reactions with acetonitrile and 2-methyl-1,3-dioxolane. α-, β- or γ-substitution in pyridines can, therefore, be securely recognized via an MS-only method based on structurally diagnostic ions and by the inspection of a single molecule (no need for intracomparisons within the whole set of isomers).

KEYWORDS: ion/molecule reactions; isomer distinction; pyridines; pentaquadrupole mass spectrometry; structurally diagnostic ions

INTRODUCTION

Mass spectrometry primarily measures mass, but isomers have exactly the same mass. The distinction of isomers has therefore been one of the greatest challenges of and a central issue in mass spectrometry.[1–10] General MS methods for isomer recognition are unavailable, and isomers have been treated therefore in a set-by-set intracomparative manner involving all members of the set. This limitation is in contrast to, for instance, the ability of NMR to perform absolute recognition of a single isomer via characteristic and general patterns of peaks and coupling constants such as those observed for molecules bearing *ortho-*, *meta-* or *para*-substituted benzene rings.[11] Looking for MS-only methods to recognize isomers that would benefit from the high speed and sensitivity of mass spectrometry, we have proposed a methodology for absolute (a non set-by-set intracomparative approach) and general recognition of constitutional isomers based on structurally diagnostic fragment ions (SDFI).[12] This method requires selection of suitable SDFI shared by the greatest possible number (ideally all) of the members of the isomeric family. The main requisites for SDFI are: (1) they should be a common fragment ion for the isomeric family; (2) they should contain and retain the structural information of the precursor molecule; (3) they should be a stable gaseous species with no easy structural rearrangements and (4) they should display contrasting collision-induced dissociation (CID) or bimolecular reactivity, or both.

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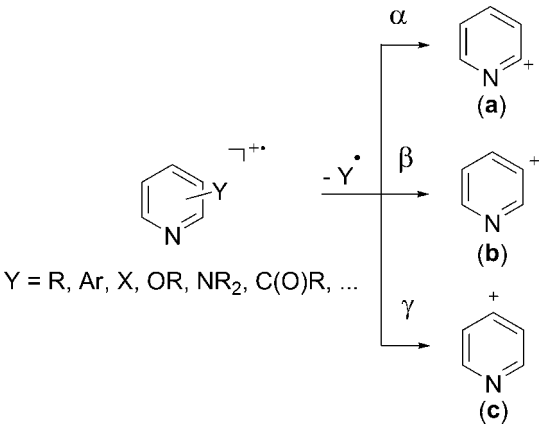
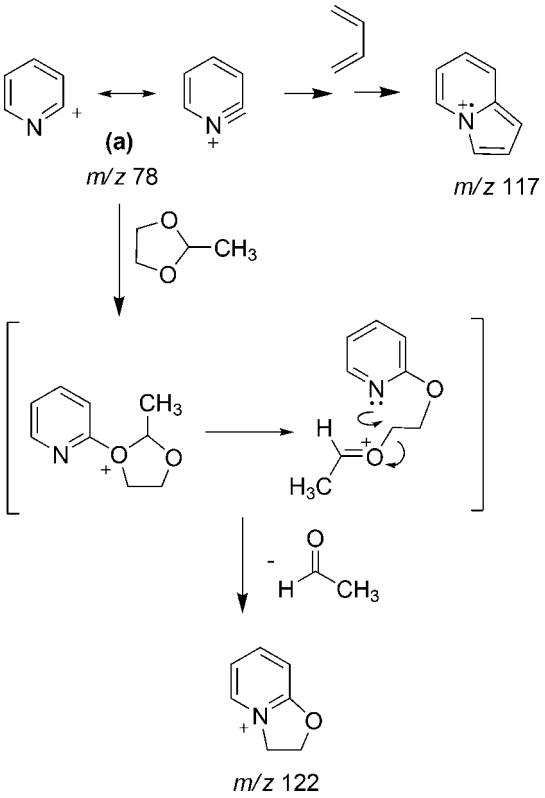
We have so far succeeded in finding SDFI for several different families of constitutional isomers. For instance, α-, β- and γ-pyrimidinium ions function as proper SDFI for isomeric monosubstituted pyrimidines,[12] *ortho-*, *meta-* and *para*-hydroxy and aminobenzoyl cations for isomeric acylanilines and acylphenols,[13] nitrobenzoyl cations for acyl nitrobenzenes,[14] pyrrolyl cations for isomeric monosubstituted pyrroles,[15] distonic dehydrobenzoyl cations for acylbenzene derivatives,[16] and naphthyl acylium ions for isomeric α- and β-acyl naphatalenes.[17] A similar approach has been used recently to identify hexoses via CID of isobaric oxocarbenium fragment ions from aldohexoses and ketohexoses.[18] For monosubstituted pyridines,[12] we have tested the isomeric α-, β–and γ-pyridinium ions (**a–c**) as SDFI (Scheme 1). These stable aromatic ions lie in deep potential wells, are connected by relatively high-energy barriers (higher than 60 kcal mol^{-1})[19] and their charges are placed in localized sp^2 σ orbitals. The information on the ring position of the original substituent is therefore held tightly in their stable gaseous structures.

The position of the formal charge site in the pyridine ring has also been found to influence considerably the electronic structure and hence the reactivity of the isomeric pyridinium ions **a–c**.[19] For **a**, effective overlap occurs between the fully occupied sp^2 nitrogen orbital and the adjacent and coplanar empty sp^2 orbital of C^+, and this overlap leads to the prevalence of a heteroaryne ion structure (Scheme 2). The α-pyridinium ion **a** is therefore readily characterized since it reacts promptly with conjugated dienes to form ionized indolizines[20] and by ionic transacetalization[21–23] with cyclic acetals to afford a bicyclic dihydrooxazolopyridinium ion (Scheme 2). Thus, α-substitution in pyridines could be readily



Scheme 1. SDFI for mono-substituted pyridines.



Scheme 2. Structurally diagnostic ion/molecule reactions of the α-ion **a**.

recognized as long as a single analyte molecule forms, upon ionization and dissociation, the respective SDFI (**a**) of m/z 78. However, β- and γ-substitution could not be recognized since both **b** and **c** were found to react with cyclic acetals predominantly by proton transfer.

Since the publication of our first results on **a–c** as SDFI,[12] we have been searching for a structurally diagnostic ion/molecule reaction[24–28] that would distinguish **b** and **c**. Herein we report our findings about this long-hoped-for reaction and summarize the combined use of two gas-phase reactions to distinguish the whole set of isomers **a–c**. Consequently, we show that as long as the analyte molecule (a single monosubstituted pyridine with an unknown site of substitution: α, β or γ) forms, upon ionization and dissociation, one of the isomeric fragment ions **a–c** of m/z 78 (and this requisite is expected to be met by many such molecules), its substitution site can be recognized by this method.

EXPERIMENTAL SECTION

The MS^2 experiments were performed with an Extrel pentaquadrupole (QqQqQ) mass spectrometer, described in detail elsewhere.[29] Basically the QqQqQ consists of three mass-analyzing quadrupoles (Q1, Q3, Q5), in which ions are selected and measured, and two reaction quadrupoles (q2, q4), which are used to perform either low-energy ion/molecule reactions or CID.[30] For the two-stage MS^2 experiments, the ion of interest was generated by dissociative 70-eV electron ionization (EI) of the following precursors: **a** (2-ethylpyridine), **b** (3-ethylpyridine) and **c** (4-ethylpyridine). After selection by Q1 and 15-eV CID with argon or low-energy (*ca.* 1 eV) ion/molecule reactions with 2-methyl-1,3-dioxolane or acetonitrile in q2, Q5 was scanned to record the product ion mass spectrum, whereas Q3 was operated in the rf-only mode. The target gas pressures in q2 corresponded to a typical beam attenuation of 40–60%, that is, multiple-collision conditions were used.

RESULTS AND DISCUSSION

Upon 15-eV CID with argon, the three isomers **a–c** are indistinguishable since they display practically identical product ion mass spectra (Fig. 1 shows, for illustration, the spectrum for **c**). They dissociate by consecutive losses of HCN and H to form the fragment ions of m/z 51 and 50 in similar abundance ratios. These ions have also been shown to display similar dissociation behaviors under high-energy CID and neutralization–reionization, with no SDFI.[31]

Figure 2 shows the product ion mass spectra for the ion/molecule reactions of **a–c** of m/z 78 with 2-methyl-1,3-dioxolane. These reactions were performed under the same conditions (collision energy, instrument settings and neutral gas pressure) that were tuned to maximize the yield of bimolecular products for the reactions with acetonitrile (see below). Note the unique transacetalization reactivity of the α isomer **a** (Fig. 2(a)), which yields an abundant product ion of m/z 122. Isomers **b** (Fig. 2(b)) and **c** (Fig. 2(c)) react distinctively from **a,** but very similar to each other,

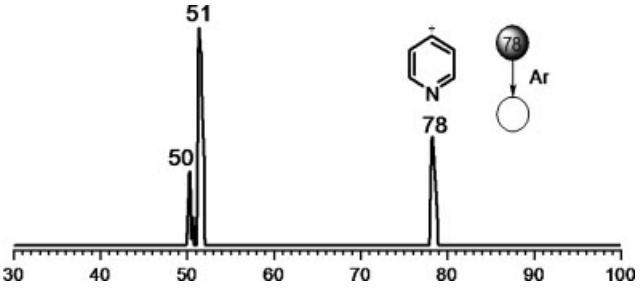


Figure 1. Double-stage (MS^2) product ion mass spectrum for CID with argon of the γ-pyridinium ion (**c**) of *m/z* 78. The corresponding spectra for the isomeric α (**a**)[12] and β (**b**) ions are practically identical to that of **c** and are not shown.

DOI: 10.1002/jms

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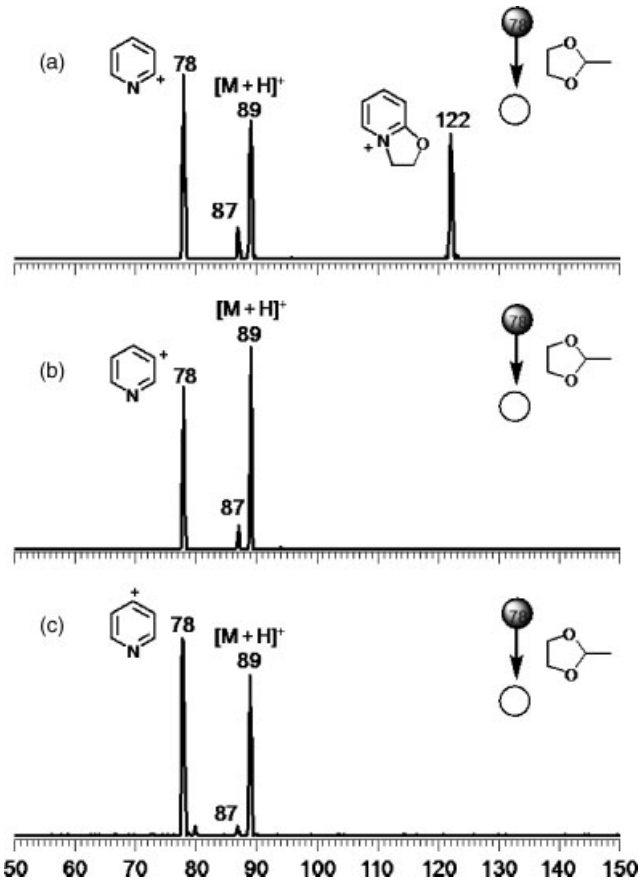


Figure 2. Double-stage (MS^2) product ion mass spectra for the reaction with 2-methyl-1,3-dioxolane of the isomeric (a) α- (**a**); (b) β- (**b**) and (c) γ-pyridinium (**c**) ions. The minor product ion of *m/z* 87 arises from net hydride abstraction.

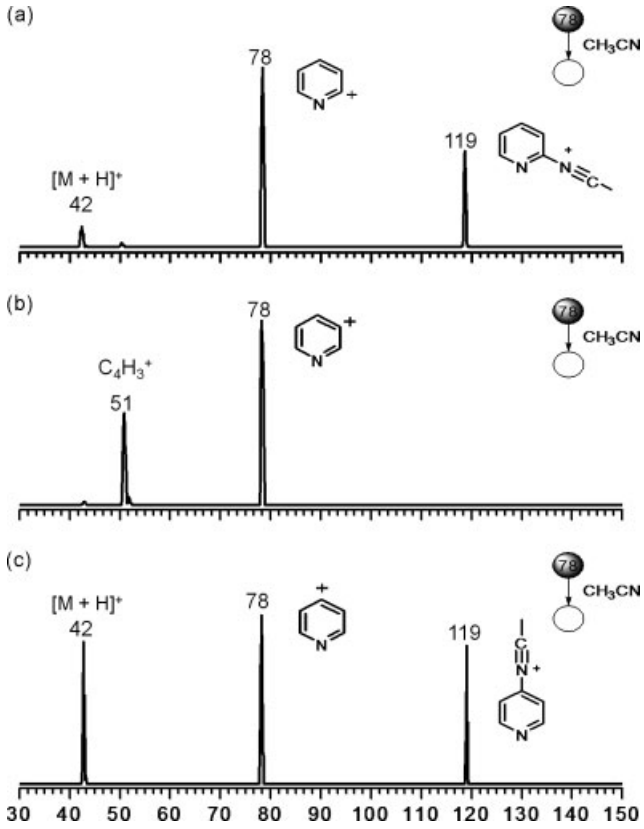


Figure 3. Double-stage (MS^2) product ion mass spectra for the reaction with acetonitrile of the isomeric (a) α- (**a**); (b) β- (**b**) and (c) γ-pyridinium (**c**) ions. For an investigation of the most favorable gaseous structure for the $C_4H_3^+$ ion the reader is referred to.[34]

predominantly by proton transfer to form protonated 2-methyl dioxolane of m/z 89. (Under these optimized conditions and with great care to avoid cross-contamination of samples, **b** and **c** were observed to form no product ion of m/z 122. Previously[12] **b** and **c** were observed to form the ion of m/z 122 to very minor extents.)

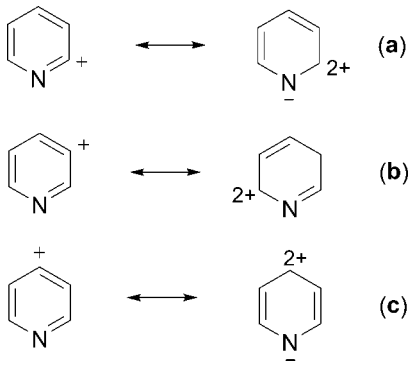
We have tested many neutral reactants to distinguish between **b** and **c**. Among them, acetonitrile[32,33] was the only one found so far to function properly (Fig. 3). Both **a** (Fig. 3(a)) and **c** (Fig. 3(c)) react with acetonitrile by proton transfer (to form protonated acetonitrile of m/z 42) and by nucleophilic addition (to form the intact adduct of m/z 119), but **b** is unreactive (unsuccessful attempts were made to optimize collision conditions so as to favor bimolecular products for **b**). Upon *ca*. 1-eV energy collisions with acetonitrile, **b** forms no adduct, dissociating instead by the loss of HCN to form the fragment ion of m/z 51. This loss has been found to dominate the dissociation of metastable **a**–**c**.[31]

Theoretical calculations at the CBS-Q level have indicated the following order of stability for the isomeric pyridinium ions: **a** (zero) > **b** (17.9 kcal mol^{-1}) > **c** (22.9 kcal mol^{-1}).[19] Therefore, the lack of bimolecular reactivity of **b** towards acetonitrile and its prompt dissociation cannot be directly related to the stability order. Nucleophilic substitution in neutral pyridine is known to take place preferentially in positions α and γ due to the ability of N to accommodate the negative charge of the key intermediate.[35] An analogy can therefore be made for acetonitrile addition by means of the canonic forms shown in Scheme 3. The positive charge density at the site charge of **a** and **c** may be enhanced by resonance and the ability of N to accommodate the negative charge whereas **b** lacks such effect. As a result, nucleophilic addition to **a** and **c** would be favored. Indeed, B3LYP/6-31G(d,p) calculations show the following charge density for the ions at the formal charge site: **a** (0.39), **b** (0.13) and **c** (0.26). The greater density for **a** as compared with **c** could then be rationalized in terms of the additional inductive effect of the electronegative N atom.

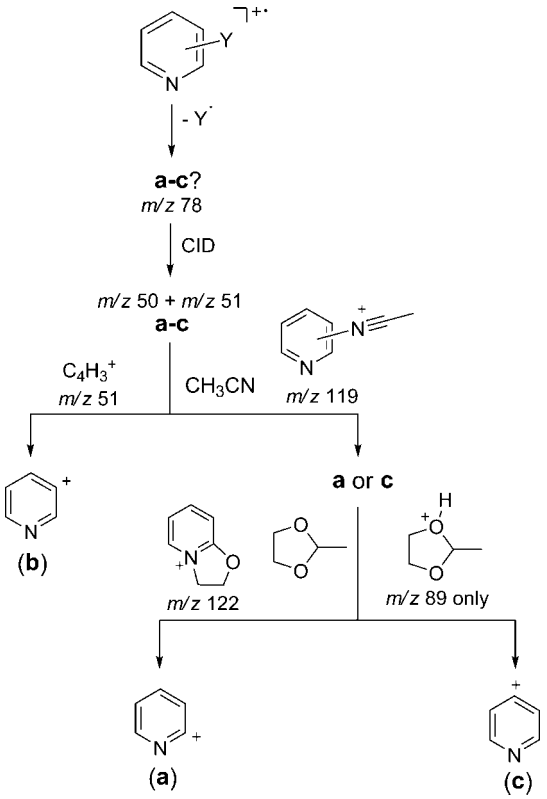
Scheme 4 summarizes the complete method. The molecule under investigation should first be ionized and dissociated to form the respective SDFI of m/z 78. Pyridinium ions are common fragments in 70-eV EI of pyridines, but the use of atmospheric pressure ionization techniques such as ESI and APCI should form mainly the intact protonated molecule $[M + H]^+$ with little or no dissociation. The SDFI of m/z 78 should then be (hopefully) formed by CID of $[M + H]^+$ via MS/MS experiments. Although the success in forming the SDFI from protonated pyridines is not so easily predicted, the protonated test molecule **1** (see below) was found to form **a** via CID.

The diagnostic ion of m/z 78 should then be selected and subjected first to CID with argon (Fig. 1) to verify whether one of the isomeric pyridinium ions **a**–**c** has been formed.

DOI: 10.1002/jms



Scheme 3. Canonic forms for the isomeric pyridinium ions.

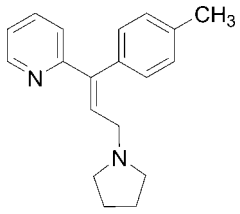


Scheme 4. Complete method for recognizing the site of substitution in pyridines via the SDFI **a**–**c**.

If a pyridinium ion is indeed identified, it should then be characterized via reactions with acetonitrile. If no adduct is formed and the only product observed is the fragment ion of m/z 51, **b** has been sampled from a β-substituted pyridine. If the acetonitrile adduct of m/z 119 is formed, the ion should then be reacted with a cyclic acetal (2-methyl-1,3-dioxolane, for instance). If the transacetalization product (m/z 122) is formed abundantly, then **a** has been formed from an α-substituted pyridine. If proton transfer occurs exclusively, then **c** has been formed from a γ-substituted pyridine.

Test molecule

The monosubstituted pyridine **1** (tripolidine) was selected as an illustrative example of the application of the method. Its hydrochloride form is an antihistaminic drug used to combat the symptoms associated with allergies and is sometimes combined with other cold medications designed to provide



1

Tripolidine Structure

general relief for flu-like symptoms. To recognize the correct site of substitution on the pyridine ring (α, β or γ) via a non set-by-set intracomparative approach, its SDFI of m/z 78 was formed upon dissociative 70 EI and investigated following Scheme 4. The ion was found to dissociate upon 15-eV CID to form the expected pair of fragments of m/z 51 and 50 and to react with acetonitrile and 2-methyl 1,3-dioxolane to form the product ions of m/z 119 and m/z 122, respectively (spectra not shown). Thus, according to Scheme 4, the SDFI is recognized as **a** and the analyte molecule is correctly recognized as an α-substituted pyridine.

CONCLUSION

Using the isomeric α-, β- and γ-pyridinium ions **a**–**c** as SDFI and CID for their identification and a combination of two structurally diagnostic ion/molecule reactions for their distinction, an absolute (non set-by-set intracomparative), general and MS-only method able to recognize all three sites of substitution (α, β or γ) in monosubstituted pyridines is now available.

Acknowledgements

We thank the Brazilian Science Foundations FAPESP and CNPq for financial support.

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DOI: 10.1002/jms