Reduction of a Quaternary Phenanthridinium Salt in a Chemical Ionization Source when Introduced by Direct Injection in Acetonitrile

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Reduction of phenanthridinium methiodide to its dihydroderivative was observed when a solution of the sample in acetonitrile was injected in the chemical ionization source of a combined liquid chromatograph/mass spectrometer equipped with a direct liquid introduction interface and a polarized desolvation chamber.

INTRODUCTION

It has been frequently observed that some substances introduced in the mass spectrometer ion source as a liquid solution, then submitted to ion or atom bombardment under secondary ion mass spectrometry (SIMS) or fast atom bombardment (FAB) conditions,^{1,2} or to an inductively coupled plasma,³ often produce ions derived from the intact molecule by classical chemical reactions such as proton or cation addition, oxidation and reduction. Whether these reactions occur predominantly in the gas-phase after desorption of the neutral precursor, similarly to the redox reactions observed under the conditions of conventional chemical ionization (CI),⁴ or in the liquid matrix is still controversial.

Mass spectrometric techniques combining liquid chromatography with mass spectrometry (LC/MS), especially the methods that nebulize liquid solutions directly inside the ion source of a CI mass spectrometer,⁵ offer another possibility to investigate chemical reactions occurring during ionization and fragmentation of organic substances introduced in solution. For instance, the reduction of various nitroaromatic molecules introduced in aqueous solutions have been reported.⁶⁻⁸

Chemical reactions in liquid solutions directly injected into a mass spectrometer are more conveniently observed in the absence of a chromatographic column that would only act as a diluting and a retarding unit with no particular advantage. The recording of the mass spectrum follows 10-30 s after the injection of a sample pulse. Repeated injections, changes of the sample or additive concentrations, setting the injection pulse width or the solution pH is easily achieved. For example, an LC/MS instrument fitted with a direct liquid introduction (DLI) interface can record mass spectra of molecules placed in different chemical environments. This long-existing feature of LC/MS with direct coupling (also recently available to FAB with continuous solution introduction,⁹) has not gained a wide acceptance in laboratories, probably because analytical appli-

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0030-493X/87/110736-04\$05.00 © 1987 by John Wiley & Sons, Ltd. cations had a higher priority. In this paper, we report on a reduction reaction observed during the investigation of phenanthridine methiodide in solution in acetonitrile, using an LC/MS instrument with a diaphragm DLI interface.

RESULTS AND DISCUSSION

N-Methyl phenanthridinium iodide melts at 202 °C. This salt has no measurable boiling point as it decomposes spontaneously by eliminating methyl iodide. The resulting free base is more volatile and thermally stable (m.p. 106-107 °C, b.p. 349 °C).

Solution mass spectra of the sample were consistent with its chemical properties. With the desolvation chamber and the CI source block at 200 °C, the peak for m/z 180 corresponding to the protonated free base was almost the only ion observed (Fig. 1). In contrast to this, a peak at m/z 194, corresponding to the intact cation, Γ^+ , was recorded when the desolvation chamber and the ion source were at 90 °C. Ions derived from the free base were minor or absent. However, the intact cation was accompanied by a ΓH_2^+ ion at m/z 196. Ions corresponding to the intact iodide salt were absent. The deuterium-labelled analogue N-trideuteromethyl phenanthridinium iodide showed similar mass spectra and elution profiles, the m/z 194 and 196 ions being shifted to 197 and 199, respectively.

The abundance ratio of the Γ^+ over ΓH_2^+ ions was not constant, but varied erratically from 30% to 80%. There is no reason to believe that, for the unlabelled sample, the ion at m/z 194 could arise from the ion at m/z 196 by the loss of a hydrogen molecule. We rather assume that phenanthridinium iodide was partly reduced during its transfer through the interface. The mass spectrum was thus produced by the mixture of two quaternary ammonium molecules.

Specific ion current profiles (Fig. 2) show distorted elution peaks with tailing rear edges. This indicates pronounced sample adsorption on the metallic parts of the desolvation chamber and the ion source (such distortions would severely alter chromatographic separations during an LC/MS analysis). Adsorption occurred because metal parts on the flight path for the liquid

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Figure 1. Mass spectra of *N*-methyl phenanthridinium iodide at (a) high and (b) low source temperature, (c) its deuterated analogue and (d) CI reagent ions from acetonitrile $(I_{42}/I_{\Gamma H_2} \approx 2000)$ (d).

droplets were at 110 °C below the melting temperature of the sample. Contact between liquid droplets and these cold surfaces could not be avoided. Variation of the voltage applied to the desolvation chamber in the interval ± 200 V showed no influence on elution profiles.

Intact gas-phase quaternary ammonium ions, including quaternarized primary or secondary amines, and pyridinium derivatives, have been produced in abundance under a wide variety of mass spectrometric conditions, e.g. thermal desorption,^{10,11} thermospray ionization,¹² FAB,¹³ SIMS,¹⁴ CI¹⁵ or electron impact (EI).¹⁶ None of these experiments appeared to induce important reduction reactions. The intact cation Γ^+ was generally abundant but a strong ΓH^+ or ΓH_2^+ ion was not observed. On the other hand, phenanthridine methiodide in solution is known to be easily reduced



Figure 2. Ion current profiles after the injection in the CI source at 90 °C of N-methyl phenanthridinium iodide in a stream of acetonitrile.

by metal hydrides or solvated electrons¹⁷ to give Nmethyl-5,6-dihydrophenanthridine. A similar reaction during our experiments would not be surprising, yet it is interesting to address the question of the nature of the reduction mechanism and of the site of the reaction.

Reduction reactions during mass spectrometric investigation of liquid solutions have been observed under FAB conditions. For example, a two-electron reduction of a triple-charged ion, resulting from multiple protonation of the sample in solution, has been suggested by Cerny and Gross² to explain the presence of a MH_3^+ in the mass spectrum of guanosine in glycerol. However, a one-electron reduction process appears to be more frequently observed and it generally predominates over multiple-electron reduction mechanisms. Although the distinction between the isotopic peak of m/z 194 and a hypothetical ΓH^{++} ion at m/z 195 was beyond the capabilities of our quadrupole, there is no reason to assume that a double protonation and a twoelectron reduction would be more favoured in our case than a single protonation and a one-electron reduction; thus it is doubtful that the above mechanism occurred significantly.

Field has suggested that FAB of glycerol liberates hydrogen atoms,¹⁸ and Pelzers et al.¹ believe that these fragments are responsible for redox equilibrium with oxidized species present in the solution. A comparable mechanism could be invoked under our DLI conditions with, however, the major difference that hydrogen molecules, and not hydrogen atoms, would be generated. Abundant acetonitrile-derived ions present at the lowmass end at m/z 42, $[CH_3CNH]^+$; m/z 56, $[CH_3CNCH_3^+]$; and m/z 83, $[(CH_3CN)_2H]^+$ are accommass end at m/z 42, [CH₃CNH]⁺ m/z 56, panied by satellite peaks at two mass units lower (Fig. 1), indicating a probable loss of hydrogen from each precursor ion. This could be the source of hydrogen that reduced the 5,6-double bond of the Γ^+ cation. These H₂ losses have not been observed under the experimental conditions of Voyksner et al.,¹⁹ who analysed acetonitrile ions under DLI conditions, using a liquid chromato-



Figure 3. Schematic representation of the experimental set-up.

graphic/tandem mass spectrometric instrument, but in the absence of a polarized desolvation chamber, which could explain the different result.

The remaining question is the site of the reaction. It could take place on the walls of the desolvation chamber held at 100 V, during sample adsorption, in a manner similar to a classical anodic reduction process. Alternatively, reduction could occur in the gas-phase. A highpressure CI source generally contains a significant concentration of thermal electrons that could be particularly abundant in an acetonitrile plasma, as evidenced by the intense and often exclusive M-+ ions observed in the negative ion mass spectra of samples analysed under DLI conditions with this solvent.²

EXPERIMENTAL

The modified Nermag quadrupole mass spectrometer and the DLI interface were as described previously.^{21,22}

The ion source with a polarized desolvation chamber is shown in Fig. 3. A constant flowrate of 30 μ l min⁻¹ (0.03 $cm^3 min^{-1}$) of acetonitrile was delivered by a Gilson module, Model 302/5S. Sample solutions were introduced by a Model 7125 Rheodyne injector fitted with a 10-µl loop. The injector was directly connected to the diaphragm in the DLI probe by 50 cm of a 0.2 mm $o.d. \times 0.1$. mm i.d. Monel capillary tube.

The ion-source block, the repeller block and the first lens were at 0 V. The desolvation chamber was at 100 V (the optimum voltage maximizing solvent ion intensities), which was below the breakdown voltage for a discharge;¹² ionizing electron energy was 100 eV. Mass spectra were acquired every 1.5 s, using a Nermag Model Sidar 5A data system.

Phenanthridine from Aldrich was reacted at 50 °C for 30 min with a tenfold molar excess of CH₃I or CD₃I.²³ The resulting salt was washed with chloroform. Solutions at 0.5 mg ml^{-1} (mg cm⁻³) were prepared in acetonitrile containing 1% water.

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