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Mass spectral study of the occurrence of tautomeric forms of selected enaminones

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ABSTRACT

The mass spectra of enaminones can provide valuable information with regards to tautomeric equilibria in the gas phase. Mass spectra of selected enaminones have been analyzed and specific fragmentation assignments have been done to characterized and weigh co-existing all possible tautomers of enaminones. Thioenaminones are of particular interest due to their tendency to shift the tautomeric equilibrium towards the thioenolimine or thioenolenamine form. Mass spectra of differently substituted enaminones are examined looking for common mass spectral behaviors. Ion fragmentations from specific tautomers allow predicting the most stable structure for the selected compounds. Acceptable correlation between the experimental data and theoretical results are found only with the neutral species, indicating that mass spectrometry could be resourced as a tool for the investigation of tautomerism of neutral species in the gas phase.

1. Introduction

Enaminones are a group of organic compounds derived from β-diketones, β-keto esters or other compounds containing a β-dicarbonyl moiety and the conjugated system C=C–C=O. The chemistry of enaminones is a developing field in organic synthesis [1,2]. These compounds are versatile synthetic intermediates that combine the ambident nucleophilicity of enamines with the ambident electrophilicity of enones [3]. There are many reports on functionalization of enaminone in the literature by the introduction of different substituents on the nitrogen, the α-carbon and the β-carbonylic carbon atoms. These derivatives have been extensively used for the preparation of a variety of heterocyclic systems some natural products and their analogues [4–6]. The enaminones are very useful synthetic intermediates in the preparation of biologically active heterocycles [7–9], including anticonvulsants [10], and anti-inflammatory [11] and anticancerous [12].

The open-chain enaminones have proven to be excellent prodrug of primary amines primarily by its ability to transport across biological membranes, while some cyclical enaminones are effective antiepileptic agents that act as blockers of the sodium channel conduction in nerve cells [13].

Understanding the mechanism of many reactions and biochemical processes, including those involving specific interactions with proteins, enzymes, and receptors (in which a substrate or active intermediary tautomerizes), requires a comprehensive understanding of the tautomerization process.

Tautomerism has an important role in biological system and has been actively investigated by many researchers. For example, the origin of serious DNA mutation is regarded as keto-enol and/or amine–imine tautomerisms [14–17].

It has been demonstrated in the case of keto-enol tautomerism of a variety of carbonylic and thiocarbonylic compounds [18–24], that there is no significant interconversion of the tautomeric forms in the gas phase following electron impact ionization in the mass spectrometer (molecular ions, $M^{+\bullet}$, do not seem to undergo unimolecular tautomerization) and, even more surprising, for GC/MS experiments, once the solvent is separated after injection in the injection port of the gas chromatograph, tautomerism mechanisms would not seem to take place even with no GC separation (under the selected experimental conditions). These conclusions are supported by temperature studies at the ion source (negligible effect) and at the injection port of the gas chromatograph with a shifting effect in agreement with the corresponding heats of tautomerization [18,23,24]. In fact, this process would take place very fast under the working conditions in the GC.

Separation of tautomers in the analytical column are frequently very difficult, consequently the different pathways of

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fragmentation of the tautomeric forms have to be used for identification of individual tautomers. For this reason and because of the high similarity between MS (commercial databases) and GC/MS spectra, analytical separation has not been considered critical for the present work. Analogously, it is thought that most of the conclusions could be useful to analyze spectra registered with mass spectrometers equipped with direct insertion probes.

This work, the study tautomeric equilibria spectrometric present in selected enaminones has been carried out, using mass spectrometry as a predictive tool.

2. Materials and methods

2.1. Synthesis of enaminones

Not commercially available enaminones were synthesized and purified according to literature procedures, by condensation of β-dicarbonyl compounds with primary or secondary amines in refluxing toluene followed by azeotropic removal of water [25,26].

The compounds under study were identified by NMR (^{1}H and ^{13}C) (Table 1, Supplementary information).

2.2. Gas chromatography–mass spectrometry—Single quadrupole

These determinations were performed by injection of methanol solutions (1 μl, 0.1%) in an HP 6890 Chromatograph coupled to an HP 5972A mass selective detector. The analytical column was a HP5-MS (30 m × 0.25 mm × 5 μm) using Helium as carrier gas (0.6 ml/min). The temperatures set points were: 200 °C at the injector, 300 °C at the interface, 185 °C at the ion source and the oven ramp was 40 °C (5 min), 20 °C/min, 290 °C. The electron energy was 70 eV and the pressure in the mass spectrometer was low enough (<10^{-5} torr) as to preclude ion-molecule reactions (no autoprotonation observed).

2.3. Gas chromatography–mass spectrometry—Ion trap

These determinations were performed by injection of methanol solutions (1 μl) in a Thermo Quest Trace 2000 coupled to Finnigan Polaris ion trap detector (unit mass resolution) under the same experimental conditions already mentioned for the single quadrupole GC/MS system. This instrumentation was utilized to confirm proposed fragmentation pathways by CID (collision induced dissociation) using Helium as the damping gas, a CID voltage of 5–7 eV and an excitation energy of 0.35–0.45 eV (values were optimized for each ion transition). These experiments were done by selecting a precursor ion from the full-scan spectrum and carrying out the corresponding MS/MS product ion scan (Tables 1–4).

Table 1
MS2 data for 4-amino-2-penten-2-one (**I**).

Precursor ion (*m*/*z*)	Relevant product ions (*m*/*z*)
99	81, 82, 84
84	56, 57, 66
82	67, 40

Table 2
MS2 data for 3-amino-1-phenyl-2-butenone (**II**).

Precursor ion (*m*/*z*)	Relevant product ions (*m*/*z*)
161	160, 145, 144, 143, 120, 105, 84
120	102
105	77

Table 3
MS2 data for 4-phenylamine-3-penten-2-thione (**VIII**).

Precursor ion (*m*/*z*)	Relevant product ions (*m*/*z*)
191	176, 158, 157, 130, 118, 114, 99, 93, 59
158	143
157	142

Table 4
MS2 data for 4-dimethylamino-3-buten-2-one **(IX)**.

Precursor ion (*m*/*z*)	Relevant product ions (*m*/*z*)
113	98, 96, 43
70	55

2.4. Magnetic nuclear resonance determinations

^{1}H NMR spectra in $CDCl_3$, were recorded with a Varian Mercury Plus spectrometer operating at 4.7 T. The typical spectral conditions were as follows: spectral width 3201 Hz, acquisition time 4.09 s and 16 scans per spectrum. Digital resolution was 0.39 Hz per point. Deuterium from the solvent was used as the lock and TMS as the internal standard. Sample concentration was 20 mg/ml in deuterated chloroform. Measurements were performed at 25 °C.

^{13}C proton decoupled and gated decoupled spectra were recorded with the same spectrometer from $CDCl_3$ solutions at 25 °C. The spectral conditions were as follows: spectral width 10,559 Hz, acquisition times 1.303 s and 1000 scans per spectrum. Sample concentration was 40 mg/ml in deuterated chloroform and digital resolution was 1.29 Hz per point.

A standard one-dimensional (1D) proton NMR spectrum and a carbon spectrum with broad-band proton decoupling were run of each sample, supplemented by 2D gradient-selected COSY and multiplicity-edited HSQC experiments to help with the assignment of signals. All 2D spectra were recorded with the same spectrometer.

Vendor provided pulse sequences were used throughout the work.

2.5. Theoretical calculations

There are several computational procedures for treating tautomeric equilibria, being density functional theory (DFT) methods [27] dominant over the last few decades. Given the enormous number of available functionals, the prediction of the tautomerism by quantum chemistry depends strongly on the DFT functional and basis set used [28].

All tautomers of compounds under study were subjected to geometry optimizations using the DFT. In order to aim this, B3LYP hybrid exchange-correlation functional [29] together with the 6-31G(d,p) basis set as implemented in the Gaussian 03 package [30] was used. Numerous conformations were computed in order to ensure that the lowest energy conformation was obtained for each molecular system. All geometrical parameters were optimized without constraints.

3. Results and discussion

The relevance of spectrometric data as a predictive tool in regard to tautomeric equilibria depends mainly on the fact that the contribution due to tautomerization of molecular ions in the gas phase does not take place or can be ignored. The importance of this point comes from the physicochemical properties of radical ions that can be quite different from the neutral species. This could be the reason of possible distortion of results and loss of the desirable predictive power of the methodology. In fact, based on previous success