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REVIEW

Mass Spectrometry as a Tool for Studying Tautomerism*

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Abstract—The review contains new data on tautomerism of organic compounds belonging to different classes, which were obtained by mass spectrometry and confirmed by quantum-chemical calculations.

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1. INTRODUCTION

Tautomerism of organic compounds in condensed phase was extensively studied by spectrometric methods, mainly by IR and NMR techniques. Mass spectrometry studies started 40 years ago, but just recently the importance of mass spectral data for studying tautomeric properties in the gas phase has been recognized. Mass spectrometry can provide valuable information on tautomeric equilibria while studying mass spectra of organic compounds belonging to different classes. The relevance of mass spectral data relies upon several facts, two of which are of key importance:

* The text was submitted by the authors in English.




From left to right: Jorge J.P. Furlong, M. Mercedes Schiavoni, Eduardo A. Castro, and Patricia E. Allegretti.

(1) Mass spectral fragmentation pattern should be tautomer-specific since the corresponding abundance ratios are supposed to correlate with the concentrations of, e.g., ketone and enol tautomers;

(2) Ionization in the ion source is assumed to have no effect on the position of tautomeric equilibrium, so that the results reflect the tautomer ratio in the gas phase prior to ionization.

Some carbonyl compounds do not exhibit noticeable tautomerism, so that abundances of fragment ions assignable to the enol form are very low or cannot be measured. Enolization of thiocarbonyl compounds is more appreciable (which correlates adequately with the oxygenated analogues); therefore, study on their mass spectra is interesting from the viewpoint of reaching some degree of generalization.

In addition, experimental findings are supported by semiempirical theoretical calculations, which proved to be useful not only for revealing correlations within a family of compounds but also for calculating heats of tautomerization in the gas phase.

Reports on the use of mass spectrometry for studying tautomerism become less common. One reason is that interpretation of the MS results is not as straightforward as it was once believed, even though Terent'ev and Kalandarishvili noted in the recent review that "Mass spectrometry is the most informative and practical method for studying and identifying tautomers in the gas phase" [1]. In fact, mass spectrometry seems to be very informative for studying and identifying tautomers, because in this case external factors like solvents, intermolecular interactions, etc., can be excluded by transferring the tautomeric system into gas phase, where the process becomes truly unimolecular [1].

There present review covers data published in the last four decades on the use of mass spectrometry for studying tautomerism of organic compounds.

2. TAUTOMERISM BEFORE OR AFTER IONIZATION

A critical aspect is to understand where the equilibrium takes place in the instrumental setup. It has been shown that exchange of enolizable hydrogen atoms for deuterium atoms takes place in the inlet system of a mass spectrometer [2, 3], indicating that keto–enol equilibrium might be established in the inlet system. MacLeod studied possible operation of tautomerism before and after electron-impact induced fragmentation of molecular ions [4]. A study of the double McLafferty transfer process in the mass spectra of two cyclic ketones and a substituted malonic ester, at both high and low electron energies, has shown that little tautomerism of the intermediate single McLafferty enol ion occurs in these cases. In addition, tautomerism of the molecular ion in a number of carbonyl compounds appears not to be a prerequisite for γ-cleavage in their mass spectra.

Larsen and co-workers [5] are to be credited with initiating a new application of mass spectrometry, which consists of studying the effect of the inlet system temperature on the mass spectra and estimating the corresponding heats of tautomerization. They met several difficulties in their study, mainly due to the very narrow temperature range (26°C) available. Keto–enol tautomerism in three β-diketones has been studied by means of the effect of variation of the inlet temperature on the mass spectra. However, according to the authors, comparison of differently substituted β-diketones has the disadvantage that the relative abundances in the mass spectra would depend not only on the keto-enol tautomerism, but also on differences in the bond strength. Notwithstanding, the changes in the mass spectra of acetylacetone, 3-methylacetylacetone and 3-allylacetylacetone with the temperature of the inlet system could be correlated with the expected changes in their keto–enol equilibria.

It was concluded that consistent conclusions regarding keto–enol equilibria of β-diketones in the gas phase can be drawn by studying the effect of the inlet temperature on their mass spectra. Obviously, identification of peaks as being formed exclusively from the ketone or enol form is absolutely necessary, though it may be difficult to determine whether a mass peak is "pure." In the present review we describe a quantitative approach which allows determination of ΔH values in reasonable agreement with the data determined independently and supports the belief that some peaks arise almost exclusively from electron impact on either ketone or enol form with only minor contribution from the other form.

By introduction of the well-known van't Hoff equation: $\ln K = -\Delta H/RT + C$, the following equation is derived (assuming that the response factors for both tautomers are similar):

$$\ln(I_{enol}/I_{ketone}) = \ln K + a = -\Delta H/RT + C + a,$$

where I is the peak abundance in the mass spectrum, and a is a proportionality coefficient. The plot of

$\ln(I_{enol}/I_{ketone})$ versus $1/T$, where T is the absolute temperature, should therefore give a straight line from which ΔH can be estimated (provided that the above assumptions are valid).

The mass spectra of other β-diketones were studied [6–14], and comparison of the data for differently substituted compounds showed that the fragmentation pattern depends on the ketone–enol ratio (i.e., prior to ionization). The diketone and ketone–enol tautomers of aliphatic 1,3-diketones could be easily separated by gas chromatography [13], which is not a usual case for tautomers mixtures. The mass spectra of the tautomers are quite different, and the main fragmentation pathways can be easily assigned to non-interconvertible tautomeric molecular ions. Furthermore, isomers differing by the position of substituents could also be identified by their mass spectra.

Orlov and coworkers [14] stated that the ratio ketone–enol in the initial diketone cannot be found from peak intensities of the fragment ions. They used the heats of formation of charged fragments and neutral 1,3-diketones to calculate the heats of gas-phase reactions.

3. STUDIES ON DIFFERENT COMPOUND SERIES

Similar observations concerning tautomerism of neutral species were reported for other compound series. Larson and co-workers carried out mass spectrometric studies on amides and thioamides and revealed displacement of the tautomeric equilibrium toward the imide form in the case of thioamides [15]. The results of mass spectrometric studies on gas-phase tautomerism of oxazolidines and β-diketones [16] suggested that tautomeric transformations of acetylacetone involve wall collisions and/or follow intramolecular (four-center) mechanism. Since this process occurs in the ion source it should override any preliminary tautomerization that may occur in the heated inlet system. A good correlation between ΔH values and substituent effects on the ring–chain tautomerism of oxazolidines in the gas phase and in nonpolar solution in no way requires that the detailed mechanism involved be similar. On the basis of the data for two completely different tautomeric processes some comments were made on the merits of the mass spectrometric method, in particular *vis-a-vis* nuclear magnetic resonance. From the viewpoint of determination of thermodynamic quantities, mass spectrometry seems to be very restricted: equilibrium constants cannot be obtained, and enthalpy differences are only approximate. An important advantage of this approach, however, is the fact that some insight into the mechanism (molecularity) of the tautomerization process can be obtained. In most systems assignment of tautomer-specific fragment ions is possible, though labeled analogs may be required.

The fragmentation patterns of thioacyl derivatives of 2-aminothiazole and 2-aminobenzothiazole and their fixed imino and amino tautomeric forms indicated prevalence of the imino tautomer in the molecular ions of their trifluorothioacetyl derivatives [17]. On the other hand, the molecular ions of thioacetyl and thiobenzoyl derivatives were mainly amino tautomers. Rearrangement with elimination of RCN and formation of thiazole-2-thione or benzothiazole-2-thione ion was characteristic for all the examined compounds. In addition, the given interpretation of the main fragmentation paths was supported by the mass spectra of *N*-trideuteromethyl and *N*-deuterated derivatives, and high-resolution mass spectrometry was used to determine the elemental composition of some selected peaks.

Mass spectrometric identification and differentiation of pyrimidin-4(3*H*)-ones and pyrimidin-2(1*H*)-ones was carried out. Substitution at the nitrogen atom in position *1* or *3* made distinction of the two sets of compounds very easy because of their characteristic fragmentation pathways [18]. The most interesting were the spectra of N-unsubstituted derivatives, which illustrated prevalence of two possible NH tautomers over the 4-hydroxy structure.

Partial gas-phase amino–imino tautomerism of 2-allylamino-4,5-dihydrothiazol-4-one was established on the basis of comparison of mass-spectrometric fragmentation patterns of fixed amino and imino tautomers [19]. Linear relations between the relative intensities of specific fragment ions of azo and hydrazone tautomers of 2,3-dioxobutyranilide 2-phenylhydrazones, pK_b values, and Hammett constants σ were discussed, and it was concluded that electron-donating substituents favor formation of the hydrazone tautomers [20]. Mass spectral study on ring–chain tautomerism of 3-amino-(or oxo)pyrazolidines indicated that most of them exist partly in the open-chain form [21]. Gas-phase ring–chain tautomeric equilibria of fourteen 1,3-oxazolidines derived from norephedrine and norpseudoephedrine were studied by mass spectrometry. Using electron-impact ionization (14 eV), these equilibria were shown to be comparable with those observed in nonpolar solvent, and the following single equation

was fulfilled: $\log Kx = \rho\sigma^{+} + C$, where $\rho = 0.58\pm0.06$ and $0.55+0.03$ and $C = 0.14+0.05$ and 0.30 ± 0.03 for norephedrine and norpseudoephedrine derivatives, respectively. Approximate values of enthalpy differences were also determined [22].

A leading contribution to studies on tautomerism by mass spectrometry was made by Maquestiau and Flammang (for two comprehensive reviews of their work, see [1, 23]). Using various techniques, such as primary isotope effects upon metastable ions [24, 25], they concluded that OH tautomers predominate for 3- and 4-hydroxypyridines, SH tautomers predominate for 3- and 4-sulfanylpyridines, and NH tautomers predominate for 2- and 4-oxoquinolines.

The case of 2-hydroxypyridines was less clear; these compounds were assumed to exist as mixtures of OH and NH tautomers, the former prevailing. Baldwin and Langley later confirmed these results by using differences in the kinetic energy release (KER) associated with mass-spectrometric decomposition of metastable molecular ions [26]. Kelley and Bernstein [27] studied tautomerism of the pyridin-2(1*H*)-one–2-hydroxypyridine system in a supersonic jet expansion. They used time-of-flight mass spectrometry to characterize each tautomer and their clusters with water and ammonia. A surprising conclusion of this study is that pyridin-2(1*H*)-one molecule is not planar and that it exists in two conformations.

Ion cyclotron resonance (ICR) provides quantitative information about tautomeric equilibria in the gas phase. The results are often complementary to those obtained by mass spectrometry. In principle, gas-phase proton affinities, as determined by ICR, should provide quantitative data on tautomeric equilibria. The problem is the necessity of correcting the measured values for the model compounds, generally methyl derivatives, by the so-called N-, O-, or S-methylation effect. Since the difference in stability between tautomers is generally not too large (otherwise determination of the most stable tautomer is trivial) and since the methylation effects are difficult to calculate, the result is that proton affinity measurements allow only semiquantitative estimates of individual tautomer stabilities [28]. It was proved that OH and SH tautomers predominate for pyridinones and pyridinethiones [29–31]. For the complicated case of 2-thiouracil (six aromatic tautomers) Katritzky and Eyler [32] concluded that the oxo-thioxo tautomer is the most stable one. Similar studies were carried out on azoles [33–35].

The mass spectra of oxazepam and *N*-hydroxy-2-fluorenylacetamide could be explained only if all ring–chain tautomeric forms are considered [36]; it was concluded that certain tautomeric equilibria are relevant in the interpretation of mass spectra. Facile loss of CO in the mass spectra of anthrapyridone derivatives suggested that in the excited state the decomposition proceeds from the lactam tautomer [37].

The mass spectra of 4-$H_2NC_4H_4SO_2NHR$ (R = pyridin-2-yl, pyrimidin-2-yl, 5,6-dimethoxypyrimidin-4-yl, 6-methoxypyrazyn-2-yl, 3-methoxypyrazin-2-yl, thiazol-2-yl, 1,3,4-thiadiazol-2-yl) were compared with those of their N-methylated analogs fixed in the amino or imino form [38]. The results indicated that the examined compounds exist mainly in the amino form, but some amount of the imino form was also present in the case of thiadiazolyl derivatives.

Kostyuchenko and Stepanov [39] studied the behavior of some azonaphtols and related Schiff bases under electron impact and found that the keto form prevails in the gas phase. These findings strongly contradict the recent data on the thermodynamics of tautomeric transformations of this compound in methylcyclohexane–toluene mixture [40, 41], as well as the results of *ab initio* quantum-chemical calculations [42, 43].

Tautomeric equilibria of substituted triazin-5-ones were studied by comparing their mass spectra with those of their methylated derivatives [44]. The results for 6-methyltriazin-5-one were confirmed by comparison with the kinetic energy spectra of ions generated from *N*-ethyl derivatives. Their tautomeric equilibria were shown to depend on the nature of substituents in the 3- and 6-positions.

Mass spectra were recorded for 5-methoxy- and 4-dimethylamino-2-nitrosophenol and 1- and 2-nitrosonaphtols [45]. The spectra were independent of the inlet temperature, indicating that either these compounds do not exhibit tautomerism in the vapor phase or the heat of isomerization is quite low. However, the fragmentation patterns implied tautomerism of the molecular ion.

The course of dissociative ionization under electron impact of hetarylformazans of the pyrimidine and quinazoline series was investigated in comparison with triarylformazans. A quantitative assessment of the tautomeric forms according to the contributions of particular fragmentation processes to the total ion current was given. It was established that the form of diazinyl-

formazans in which the heterocycle occupies the 5-position (i.e., the hydrazone fragment) predominates [46].

Tautomerism of some 2-aroylbenzamides was examined by mass spectrometry and thermal tautomerization was demonstrated [47]. The mass spectra of substituted 4-hydroxyhexahydropyrimidine-2-thiones were analyzed, and the results indicated that ring–chain tautomerism exists [48].

The results of gas chromatographic and spectroscopic studies on bidentate β-enimino ketones were discussed with reference to the NMR and mass spectrometric data. Isomerism, steric effects, and structural features were confirmed by gas chromatography–mass spectrometry [49]. The mass spectra of phenolic imines could be easily distinguished from their chromene tautomers by the ion peaks $[M - NHR]^+$ and $[M - OH]^+$, respectively. *o*-Quinoid tautomers could be excluded by measuring ionization energies [50]. Continuing with other compound series, a comparative study on mass spectrometric behavior of *ortho*-hydroxy nitroso quinoline, isoquinoline, and coumarin derivatives was carried out [51].

The mass spectra of some 2-amino-4*H*-3,1-benzothiazine and 2-amino-4*H*-pyrido[4,3-*d*][1,3]thiazine derivatives may be used to identify different tautomers even when the corresponding imino compounds are not available for comparison [52].

Klyuev and coworkers [53] published a large review (146 references) where approaches to evaluation of prototropic tautomerism by mass spectrometry were formulated, and theoretical aspects of studies on tautomerism by mass spectrometry were considered.

The UV, NMR, and IR data indicated that 2,3-dihydro-1*H*-1,5-benzodiazepine-2-thiones in solution exist in the thione form [54]. Comparison of the fragmentation patterns of these compounds suggested that thione–enaminothiol tautomeric equilibrium exists in the gas phase.

The electron impact (EI) mass spectra of 4-amino-substituted 1,2-dihydro-1-methylpyrimidin-2(1*H*)-ones were reported in [55]. Decomposition of their molecular ions significantly depends on the substituent nature. The fragmentation paths suggest the presence of particular tautomeric forms in the gas phase.

Mass spectrometry represents a very sensitive method for studying tautomeric equilibria, for it is capable of detecting forms which make only minor contributions and could not be detected using other techniques.

4. TAUTOMERIZATION OF MOLECULAR IONS

In some cases interconversion of molecular ions derived from different tautomers was observed. The gas-phase ion chemistry of sterically crowded ketone–enol pairs was studied using several experimental techniques to shed light on some remarkable mechanistic features of these systems [56]. Labeling experiments proved that elimination of propene from ionized isopropyl trimesitylvinyl ether is a site-specific process which generates ionized enol (but not tautomeric ketone) via a four-membered transition state,.

The keto–enol tautomerism of phenol and cyclohexa-1,3-dien-5-one radical cations in the gas phase was postulated to explain the behavior of $C_6H_6O^{+\cdot}$ radical cations [57]. Contrary to previous reports, measurement of metastable kinetic energy release for the reaction $C_6H_6O^{+\cdot} \rightarrow C_5H_6^{+\cdot} + CO$ showed that both phenol and cyclohexadiene ions are interconvertible if they are sufficiently activated to decompose via loss of CO. The phenol ions undergo isomerization to ketone form via a high-energy sigmatropic [1,3]-hydrogen shift, which is the rate-determining step for CO loss. Because of a large kinetic barrier for the ketonization, a large fraction (~20%) of excess energy in the transition state is released as kinetic energy in the carbonylation of metastable ions.

The stability and interconvertibility of ketone and enol forms of methyl acetate molecular ion prior to fragmentation in the gas phase was also studied [58]. The heats of formation of these tautomers were determined. Fragmentation of both isomers via loss of $MeO^{\cdot}$ occurred at the thermochemical threshold for the formation of $[MeCO]^+$ ion, so that these isomers may freely interconvert at internal energies corresponding to the decomposition threshold.

Fragmentation of the protonated molecular ions of Bupropion [(±)-2-(*tert*-butylamino)-1-(3-chlorophenyl)-propan-1-one] produced by collision-induced decomposition was shown to depend on the ionization method. The daughter ion products did not depend on the energy of decomposition, i.e., high- or low-energy collisions, but depended on the ketone–enol ratio that could be influenced by the ionization process. However, the $[M + H]^+$ ions formed under electron impact arose from the ketone tautomer. This means that the molecular ions produced in the fast atom bombardment process are strongly associated with solvent ions during their formation [59].

5. SOFT IONIZATION OF POLAR TAUTOMERIZABLE COMPOUNDS

The formation and collision-induced dissociation (CID) behavior of a series of complexes containing cyclic or linear diketone ligands and alkaline, alkaline earth, or transition metal ions were investigated [60]. Negative quasimolecular ions of aromatic carboxylic acid amides were observed unexpectedly under electrospray ionization conditions [61]. Hypothetically, deprotonation of either carboxamide or carboximidic acid tautomers can produce anions with equivalent resonance structures whose stability is affected by conjugated aromatic substituents. A series of *meta*- and *para*-substituted benzamides were analyzed using electrospray ionization mass spectrometry in aqueous–methanolic solutions. The degree of ionization was found to be pH-dependent and was enhanced by electron-withdrawing substituents and suppressed by electron-donating groups. The observed effect on the apparent acidity could be accounted for by resonance stabilization.

Aryl-substituted 4-hydroxycoumarins [2, 62] were studied by electrospray ionization (ESI) mass spectrometry. Their fragmentation in the ion source or in the collision cell of a triple quadrupole mass spectrometer was investigated. The effect of substitution and tautomerism on the formation of quasimolecular ions and mass spectral fragmentation was rationalized.

6. MASS SPECTROMETRY AND THEORETICAL CALCULATIONS

There are many reports that involve theoretical calculations as means to support the mass spectral data. Tautomerism of tetrazole, 5-methyltetrazole, and its isotopically substituted derivatives was discussed on the basis of their fragmentation patterns and quantum-chemical calculations by the LCAO MO method in the CNDO/2 approximation [63]. Tautomeric equilibria of these compounds in the gas phase were found to be displaced toward the 2*H*-tautomer.

Tautomerism of 2,4-dihydroxyquinoline in the gas phase was discussed in terms of its fragmentation pattern under electron impact and of quantum-chemical calculations by the LCAO MO method in the CNDO/2 approximation [64]. Comparison of the mass spectra of 2-aryl-4,6-dioxo-1,3-thiazines with those of model compounds indicated that they exist in the gas phase primarily in the hydroxycarbonyl form [65]. A systematic comparison of structural effects on the intrinsic reactivities of carbonyl and thiocarbonyl compounds was carried out [66]. The same set enlarged by inclusion of very large systems such as di-*tert*-butyl and bis(1-adamantyl) thioketones was also studied at the AM1 semiempirical level in order to get a more complete view of structural effects. The agreement between the calculated and experimental changes in thermodynamic functions was good in all cases.

The mass spectral behavior of N-unsubstituted pyrimidin-4-ones with RCH_2-type substitution at C^2 was found to differ from that of N-substituted and/or 2-aryl- or 2-methyl-substituted analogs [67]. The predominant intramolecular cyclization seems to involve N^3 (in agreement with the prevalence of the 3-NH tautomer) and the *ortho* position in the aryl moiety in compounds having an $ArCH_2$ substituent at C^2. The AM1 SCRF calculations on 2- and 6-substituted and 2,6-disubstituted pyrimidin-4-ones supported the mass spectral observations. H-Bonded pairs of deoxyribonucleosides were investigated by FAB MS and MNDO/H quantum-chemical calculations [68].

The position of the amino-oxo–imino-oxo tautomeric equilibrium of gaseous deoxycytidines was found to be affected by acylation of the exocyclic atom of the nucleobase. AM1 quantum-mechanical calculations showed that the imino-oxo tautomers are thermodynamically more stable and that their lowest unoccupied molecular orbitals have lower energy as compared to the corresponding amino-oxo isomers [69].

The unimolecular gas-phase chemistry of ethylene phosphonate ions $(CH_2O)_2P(H)=O^{+\cdot}$ and tautomeric ethylene phosphite $(CH_2O)_2POH^{+\cdot}$ was studied using mass spectrometry-based experiments in combination with isotopic labeling and computational quantum chemistry at the CBS-QB3 level of theory [70]. Proton affinities of 32 NH- and N-methylpyrazoles were determined by FTICR. These measurements coupled with *ab initio* calculations made it possible to determine the effect of substituents at position *3*(*5*) (e.g. methyl, ethyl, *tert*-butyl, phenyl, nitro, amino, and ethoxycarbonyl groups) on K_T. Tautomerism and the site of protonation in the ring of histamine were also studied by FTICR [71, 72].

7. AUTHORS' STUDIES

7.1. Gas Chromatography–Mass Spectrometry Determinations

As was demonstrated for keto–enol tautomerism of a series of 1- and 3-substituted acetylacetones [73] and

a variety of carbonyl and thiocarbonyl compounds [74–83], there is no significant interconversion of tautomeric forms in the gas phase following electron impact ionization in the mass spectrometer, i.e., molecular ions $M^{+\cdot}$ do not undergo unimolecular tautomerization. It is even more surprising that, once the solvent is separated after injection in GC–MS experiments, neither intermolecular nor unimolecular mechanism of tautomeric transformation is operative even with no GC separation. These conclusions were supported by studies on the effects of ion source temperature (negligible effect) and injector temperature, and shift of tautomeric equilibrium was in agreement with the corresponding heats of tautomerization [79]. In fact, these equilibria are likely to establish very rapidly under the GC conditions.

Separation of tautomers in an analytical column is usually very difficult; consequently, different pathways of fragmentation of tautomeric forms are to be used for identification of particular tautomers [79]. Taking into account the above stated, as well as strong similarity between the mass spectra included in commercial databases and those obtained by GC–MS spectra, analytical separation was not considered to be critical for our studies. It was believed that most of the above conclusions could be useful to analyze spectra recorded with mass spectrometers equipped with a direct inlet probe.

Correlation of the results obtained by GC–MS with those of semiempirical molecular orbitals calculations for amides, ureas, hydantoins, isoquinolinones, ketones, diketones, and lactones demonstrated that mass spectrometry provides an adequate tool for predicting tautomeric equilibrium shifts within a series of organic compounds [74–83].

7.2. Theoretical Methods

The Hartree–Fock (HF) model [84] is a kind of branching point where additional approximations may be invoked, leading to semiempirical methods, or it can be improved by adding additional determinants generating solutions which can be made to converge toward the exact solution of the electronic Schrödinger equation [85].

The cost of performing HF calculations scales normally as the fourth power of the number of basis functions. This arises from the number of two-electron integrals necessary for constructing the Fock matrix. Semiempirical methods reduce the computational cost by diminishing the number of these integrals [86]. The most obvious way is to neglect all or some of them. Semiempirical methods achieve this in part by explicitly resorting to the valence shell approximation, i.e., by considering only valence electrons of a system; the core electrons are subsumed into the nuclear core [87]. The rationale behind this approximation is that electrons involved in chemical bonding and other chemical phenomena are those located in the valence shell. Semiempirical calculations invariably use basis sets comprising Slater-type *s*, *p*, and sometimes *d* orbitals. The orthogonality of such orbitals enables further simplifications to be made to the Roothaan–Hall equations [88].

The Austin Model 1 (AM1) method was designed to eliminate the problems with the Modified Neglect of Diatomic Overlap (MNDO) method [89], which were considered to arise from the tendency to overestimate repulsions between atoms separated by a distance approximately equal to the sum of their van der Waals radii. The strategy adopted was to modify the core–core term using Gaussian functions. Both attractive and repulsive Gaussian functions were used; attractive Gaussian functions were designed to overcome the repulsion directly and were centered in the region where the repulsion was too large. Repulsive Gaussian functions were centered at smaller internuclear separations. With this modification AM1 was a significant improvement over other similar semiempirical methods.

The main advantage of semiempirical molecular orbital programs over *ab initio* molecular orbital programs is their speed. The result of integral simplification was that the time required for the calculation increased only by a factor of N^3, where N is the number of basis functions; therefore, fairly large molecules can be studied directly. There is also a helpful additional advantage. Since the calculation procedures are parametrized against experimental results and these include effects of electron correlation, some allowance for this effect is implicit [90].

Calculations were run using the HYPERCHEM package [91]. The authors have chosen the Polak–Ribiére first-order minimization algorithm which is frequently employed in molecular modeling. This method gradually changes coordinates of atoms as they move closer and closer to the minimum point. The starting point for each interaction is the configuration obtained from the previous step. For the first interaction the starting point is the initial configuration of the system provided by the user through, for example, the Model Building option. The conjugate gradient implies

that both the gradients and the direction of successive steps are orthogonal but the directions are conjugate (indeed, the method is more properly called the conjugate directions method). A set of conjugate directions has the property that the minimum is reached in *m* steps for a quadratic function of *m* variables. The root mean square gradient was chosen equal to 0.012 kcal/mol.

7.3. Studies on Different Compound Series

This section covers authors' contribution to mass spectrometric studies on tautomerism of several series of organic compounds, which was supported in many cases by semiempirical molecular orbital calculations.

7.3.1. Carbonyl compounds and their thio analogs. Enolization of ketones is generally favored by increase in steric load exerted by substituents in the α-position with respect to the carbonyl group. In general, elimination of OH from the molecular ion can be assigned to the enol form, while loss of R, to the ketone form. The ratio of the ketone and enol tautomers may be estimated by the relative abundances of the $[M - R^+]$ and $[M - OH]^+$ ions. Analysis of the mass spectra of selected ketones allowed the authors to establish an acceptable correlation between the abundance ratio and approximate enolization equilibrium constants for the neutral species, calculated by the semiempirical AM1 method [74].

Unlike oxygen analogs, thioketones yield a higher proportion of molecular ions, indicating greater ability of sulfur atom to stabilize radical cation. In addition, thioketones attract particular interest due to their tendency to shift tautomeric equilibrium toward the enethiol form. Elimination of SH was found to be characteristic fragmentation pattern. Furthermore, $[M - SH_2]^{+\cdot}$ ions can also be assigned to the enethiol form, while the fragment $[M - CHR^2R^3]^+$ can be assigned to the thioketone tautomer [83]. These results are consistent with molecular orbital theory calculations. Taking into account that the calculations correspond to tautomeric equilibria involving neutral species, it may be concluded that subsequent ionization does not affect the position of these equilibria.

The predictive value of this methodology was supported not only by the effect of the nature and size of substituents on the position of tautomeric equilibria but also by good correlation between the ratio of selected ion abundances and calculated (AM1) heats of tautomerization for the neutral species.

Mass spectra of some β-diketones were analyzed with a view to predict the occurrence of tautomeric forms and correlate the results with theoretical reactivity studies [78]. The influence of substitution was discussed and found to be consistent with the expected electronic and steric effects. Acceptable correlation between the experimental data and theoretical results (AM1) was found for the neutrals molecules. Both methods provide a useful tool to study enolization phenomena.

7.3.2. β-Keto esters, their thio analogs, and lactones. A series of β-keto esters: ethylacetoacetate, methylacetoacetate, α-chloromethylacetoacetate, and α-chloromethylacetoacetate, was selected for chromatographic and mass spectrometric determinations. Additionally, the feasibility of gas chromatographic separation of different tautomers was examined and confirmed by analysis of the corresponding mass spectra. Mass spectrometry allowed identification of both keto and enol forms and estimation of their relative amounts assuming that they are characterized by similar response factors [92]. Separation of tautomers by GC–MS is possible when usually unstable enols exhibit structural features like intramolecular H-bonding which favors enolization. Electronic and steric effects are consistent with the above findings.

Mass spectrometry was used to examine tautomerization of some esters and their sulfur analogs [81]. It was shown that mass spectrometry data are helpful in demonstrating the occurrence of enol structure for thioesters and especially for dithio analogs. To estimate the occurrence of tautomers in equilibrium, appropriate fragmentation patterns were assigned to the ketone and enol forms. Elimination of XH from the molecular ion could be assigned to the enol form, and elimination of CH_2R, to the ketone form. Despite the fact that the analyses were performed by GC–MS, no chromatographic separation was observed, so that the mass spectra resulted from a mixture of both tautomers. Contrarily, chromatographic separation of tautomeric forms of β-keto esters was reported previously [92].

Esters are rather unique in regard to their mass spectra and the use of mass spectrometry to study their tautomeric equilibria. Ion assignments may not be specific to particular tautomers; however, oxygen-containing esters give no enol form, while their fraction for thio esters and especially dithio esters may be significant. When the heteroatom is selenium, the equilibrium strongly shifts toward the enol tautomer. The ease of enol formation increases in the series thio

esters < dithio esters < seleno esters and is consistent with the size of R^1. AM1 semiempirical molecular orbital calculations for neutral molecules were also in agreement with the experimental results [81]. Additionally, information about the stability of *Z*/*E*-enol isomers is provided.

There is a remarkable tendency to enolization upon replacement of oxygen by sulfur in esters. This behavior is evidenced not only by mass spectra but also by theoretical calculations and their mutual correlation. Once again, these results indicate the possibility of evaluating tautomerism by mass spectrometry since it reflects adequately tautomeric equilibria between neutral species with negligible influence of tautomeric equilibria between ionic species in the gas phase. It is important to take into consideration that reactivity of radical ion species may differ appreciably from the behavior of the corresponding neutral molecules.

Likewise, enolization of lactones is favored by oxygen–sulfur exchange in the functional groups [80]. Analysis of the corresponding mass spectra allowed unambiguous assignment of some fragment ions to specific tautomers. Effects of the heteroatom and ring size were also observed. AM1 theoretical calculations were consistent with the mass spectral data, and they supported the fact that the equilibria being measured involve neutral molecules with a minimum impact (if any) of tautomerization between ionized species (radical cations) [80].

7.3.3. Nitrogen-containing heterocycles. Analysis of the mass spectra of some substituted hydantoins was carried out to demonstrate that substitution is relevant to tautomerism. It was shown that the C^2-enol is the predominant tautomer. Enolization of all N^1-unsubstituted hydantoins involves the N^1H hydrogen atom, otherwise N^3H is involved. Furthermore, molecular orbital calculations were performed using the semiempirical AM1 method [89] with a view to obtain additional support for the tautomerism of hydantoins. Theoretical calculations confirmed the C^2-enol structure and disregarded tautomerization between ionic species inside the mass spectrometer.

Analysis of the mass spectral data of some isoquinolinones allowed us to assign loss of 28 a.m.u ($[M - CO]^{+\cdot}$) to the lactam structure (except for the mass spectrum of ethylisoquinolinone which can generate the $[M - C_2H_4]^{+\cdot}$ ion after the McLafferty rearrangement) and loss of 19 a.m.u to the lactim structure. Differences in the heats of formation between the lactam and lactim forms of the neutral molecules and the corresponding radical cations were calculated. A reasonably good correlation with the mass spectral data was achieved only for the neutral molecules. Thus application of mass spectrometry techniques together with semiempirical theoretical calculations gave a suitable way to analyze tautomerism of isoquinolinones.

7.3.4. Amides and thioamides. Likewise, a combination of mass spectrometry and semiempirical quantum-chemical calculations provided a useful tool for analysis of tautomerism of some amides and thioamides [77]. Temperature effect on equilibria can be used as a methodology for experimental determination of heats of reaction. In order to ponderate the use of mass spectrometry as an adequate analytical tool for studying rapid equilibria, as is the case of tautomerization, the effect of the sample admission system temperature in experimental setup was studied to estimate the heat of tautomerization and prove that enolization takes place before ionization (i.e., almost no tautomerization occurs between ionic species in the ion source) [5]. To determine the heat of tautomerization from mass spectral data it is necessary to (1) assign fragment ions to particular tautomers, (2) calculate imide/amide ion abundance ratios for different fragment ion pairs for the same thioamide (for validation purposes), (3) repeat these calculations for different sample admission temperatures.

The use of different ionizing electron energies provides additional support for this methodology. Variation of electron energy could affect the relative abundances of fragment ions, but it should not change the heat of reaction [89]. Finally, since the enthalpy (or heat) of formation can be estimated from semiempirical theoretical calculations, the existence of reasonably good correlations with the experimental data provides an additional support for using mass spectrometry as a valuable tool for studying tautomeric equilibria.

Evidence that transformations after ionization do not occur or are insignificant was found not only by experimental spectrometric strategy but also by the theoretical methodology employed. A reasonable correlation was found between the mass spectral data and AM1 calculations of neutral molecules while no correlation could be observed for the corresponding molecular ions.

7.3.5. Ureas and thioureas. Mass spectra of some ureas and thioureas were studied to analyze the predictive power of this technique with additional support of molecular orbital calculations [82]. Since GC–MS

instrumentation was employed, chromatographic separation of amide tautomers was not attained. In all these cases it is not possible to assign unambiguously a fragment ion to the keto form since ureas have two amine moieties. The use of labeled ureas was helpful in this sense.

The difference in the relative heats of formation can be explained in terms of stability of the *E* and *Z* isomers. The *Z* isomers are favorable for ureas with large substituents, while the *E* isomers of small ureas seem to involve a lower tautomerization energy. It was not surprising to find similar values for both *E* and *Z* isomers of the isothiourea tautomer for asymmetric allylthiourea.

Like other tautomerizable compounds, shift of tautomeric equilibrium of ureas toward isourea structure becomes appreciable in going from oxygen to sulfur and then to selenium analogs. This can be easily demonstrated by mass spectrometry and theoretical calculations. Loss of 81 a.m.u. ($[M - SeH]^+$) is observed for seleno derivative. This fact is consistent with the higher relative amount of isourea form in equilibrium mixture (Se > S > O), and such behavior is easily explained by lower tendency of Se to form double bonds (Se < S < O). The $[M - XH]^+/[M - XH_2]^+$ relative abundance ratio strongly increases for the HN=C–SeH structure due to lower thermodynamic stability of H_2Se compared to H_2S and H_2O. The results for the neutral molecules correlate adequately with the mass spectral data. As noted above, the *Z* isomers require lower energy for the tautomerization. It was demonstrated once again that mass spectrometry is useful for studying tautomerism of urea derivatives. AM1 semiempirical calculations not only support the spectrometric data but also indicate that ionization in the mass spectrometer does not affect tautomeric equilibrium established between neutral species (at least for monocarbonyl compounds) [74–83]. The results for radical cations do not show a rationalizable trend.

8. CONCLUSION

Thus the application of mass spectrometry together with semiempirical theoretical calculations provides a suitable way to analyze tautomerism of organic compounds in the gas phase. Reasonable employment of these two methods enables one to achieve a predictive capability in studying tautomeric transformations of several series of structurally related compounds.

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