World Journal of Chemistry 2 (2): 25-62, 2007
ISSN 1817-3128

Tautomeric Equilibria Studies by Mass Spectrometry

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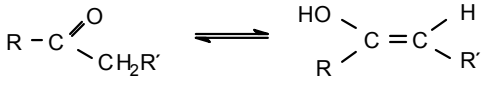
Abstract: Tautomerism in organic chemistry has been extensively studied in condensed phase by spectrometric methods, mainly by IR and NMR techniques. Mass spectrometry studies start 40 years ago but just recently it has been recognized the importance of the mass spectral data for the study of tautomerism in the gas phase. Mass spectrometry can provide valuable information in regard to tautomeric equilibria when studying mass spectra among the members of different families of organic compounds. The relevance of the mass spectral data resides on several facts but there are two that are of key importance: Mass spectral fragmentation assignments should be tautomer specific since the corresponding abundances ratios are supposed to be correlated to the keto/enol contents. Ionization in the ion source it is supposed to have no effect on the position of the equilibrium so that the results reflect the tautomers content in the gas phase previous to ionization. Some of the carbonylic compounds do not exhibit noticeable tautomerism so the fragment abundances assigned to the enol form is very low or not measurable. Since enolization is more noticeable in the case of thio-derivatives (which correlates adequately with the oxygenated analogues), the study of their mass spectra is an interesting choice to reach some degree of generalization. In addition, experimental findings are supported by semiempirical theoretical calculations, which probed to be adequate not only for supporting tendency correlations among the members of a compound family but also to calculate heats of tautomerization in gas phase. Reports using mass spectrometry for tautomerism are becoming less common. One of the reasons is that now it would appear that the interpretation of MS results is not as straightforward as it was once believed, even though in a recent review it was written 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]. This review covers the study of Tautomerism by Mass Spectrometry in the last four decades.

Key words: Mass spectrometry . tautomerism . carbonylic compounds thiocarbonylic compounds . abundance ratios . keto-enol equilibrium . theoretical calculations

TAUTOMERISM BEFORE/AFTER IONIZATION

A critical aspect in the study of chemical reactions is to understand where the equilibrium takes place in the instrumental setup.

It has been shown that the exchange of enolizable hydrogen atoms for deuterium atoms takes place in the inlet system of a mass spectrometer [2, 3], which indicates that the keto-enol equilibrium might be established in the inlet system.



(1)

MacLeod studied the 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

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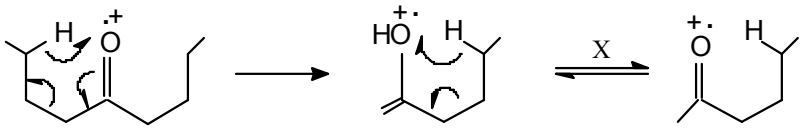


Scheme 1

energies, has shown that little tautomerism of the intermediate single McLafferty enol ion occurs in these cases (Scheme 1). 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 type of application of mass spectrometry, which consists in the study of the effect of the inlet system temperature on mass spectra and the estimation of the corresponding heats of tautomerization. They met several difficulties in their study, mainly due to the very small temperature range (26ºC) available to them. The 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, the 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 the differences in bond strength. Notwithstanding, the changes in the mass spectra of acetylacetone, 3-methylacetylacetone and 3-allylacetyl acetone with the temperature of the inlet system could be correlated with the expected changes in the keto-enol equilibria for the three compounds.

So, it was concluded that it seems possible to derive consistent conclusions regarding keto-enol equilibria of β-diketones in the gas phase by a study of the effect of the inlet temperature on mass spectra. Certainly to identify peaks as being formed almost exclusively from either the keto or the enol form of the molecules is absolutely necessary although it is not simple to determine whether a mass peak is "pure". The quantitative approach described in the text, which allows the determination of ΔH values in reasonable agreement with data determined independently, supports the belief that some peaks arise almost exclusively from electron-impact on either the keto or the enol form with only minor contributions from the other form.

By introduction of the well-known van´t Hoff equation: ln K =-ΔH/RT+ C, the following equation is derived (respond factors for both tautomers should be similar):

$$\ln \frac{I\ enol}{I\ keto} = \frac{\ln [enol]}{\ln [keto]} + a = \ln K + a = -\frac{\Delta H}{RT} + C + a \qquad (2)$$

where "I" is peak abundance in the mass spectrum and "a" is a constant of proportionality.

A plot of ln (I enol/ I keto) versus 1/T, where T is the absolute temperature at which the ratio I enol/ I keto has been determined should therefore give a straight line from which ΔH can be estimated provided the given assumptions are valid.

The mass spectra of other β-diketones have been studied [6-14] and by comparison of differently substituted compounds it has been found that the fragmentation is influenced by the keto-enol content of the compounds (this means previous to ionization).

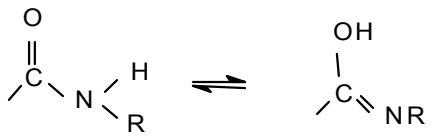
The diketo and keto-enol tautomers of aliphatic 1,3-diketones could be easily separated by gas chromatography [13], which is not the usual case for tautomers mixtures. The mass spectra of the tautomers are quite different and the main fragmentation pathways can be easily linked to the structures of the (non-interconverting) tautomeric molecular ion. Furthermore, isomers differing by the position of the substituent could also be identified by their mass spectra.

Orlov and coworkers [14] stated that the keto-enol ratio of the starting diketones cannot be found from peak intensities of the fragment ions. They resort to the heats of formation of the charged fragments and neutral 1,3-diketones for the calculations of the heats of gas-phase reactions.

STUDIES ON DIFFERENT COMPOUNDS FAMILIES

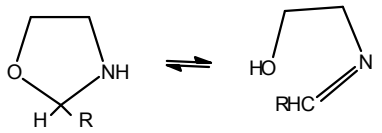
Similar observation respect to tautomerism involving neutral species has been reported for other compound families.

Larson and coworkers carried out mass spectrometric studies for amides and thioamides and pointed out the tautomeric equilibrium displacement towards the imidol form in the case of thioamides [15].



(3)

The results of an investigation of gas-phase tautomerism of oxazolidines and β-diketones [16] using mass spectrometry suggested that keto-enol tautomerism in acetylacetone involves wall collisions and /or occurs by an intramolecular (four centered) mechanism. Since this process occurs in the source it would override any prior tautomerization that may occur in the heated inlet system. The good correlation between ΔH values and substituent effects on the oxazolidine ring-chain tautomerism in the gas phase and those in non-polar solution in no way require that the detailed mechanism involved be similar (Eq. 4). With data on two completely differently tautomeric processes some comments were made on the merits of the mass spectrometric method, particularly *vis a vis* nuclear magnetic resonance. In terms of providing 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 should be possible, although labeled analogues may be required.



(4)

The fragmentation patterns of the thioacyl derivatives of 2-aminothiazole and 2-aminobenzothiazole and their fixed structure imino and amino tautomeric forms gave evidence of the predominance of the imino tautomer in the molecular ions of the trifluorothioacetyl compounds [17] (Fig. 1, 2). On the other hand, the molecular ions of the thioacetyl and thiobenzoyl derivatives are mainly the amino tautomers. A rearrangement with elimination of RCN and formation of the 2-thiazolothione (or 2-benzothiazolotione) ion was characteristic for all the compounds investigated (Scheme 2). Additionally, evidence for the interpretation of the main fragmentation paths was provided by the mass spectra of the N-trideuteromethyl and N-deuterated derivatives, high-resolution mass spectrometry being used to determined the elemental composition of some selected peaks.

A mass spectrometric identification and differentiation of pyrimidin-4(3H)-and-2(1H)-ones (Fig. 3) was carried out. N-substitution at position 1 or 3 made the distinction of the two sets of compounds very easy because of their characteristic fragmentation pathways [18]. Most interesting were the spectra of the N-unsubstituted derivatives, which illustrated a predominance of the two possible NH tautomers in relation to the 4-hydroxy structure.

Partial gas-phase amino-imino tautomerism of 2-(allylamino)thiazolin-4-one (Fig. 4) was established on the basis of comparison of mass-spectrometric fragmentation patterns of fixed amino and imino tautomers [19].

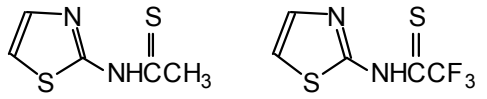


Fig. 1: Thioacyl derivative of 2-aminothiazole and its corresponding trifluorothioacetyl analogue

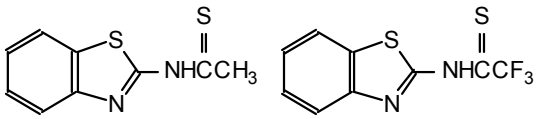
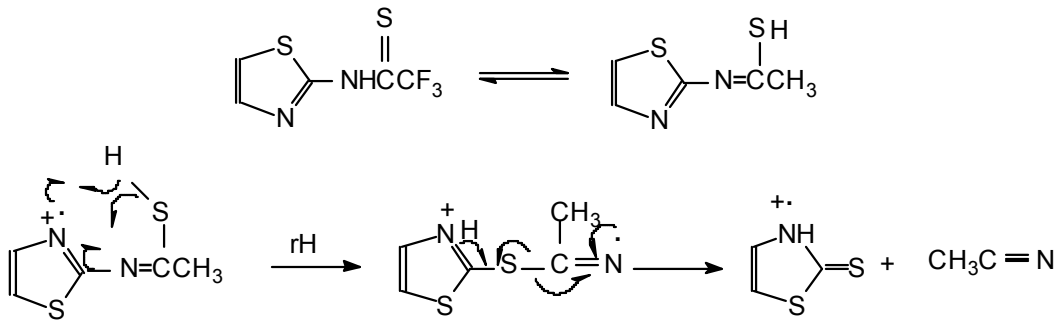


Fig. 2: Thioacyl derivative of 2-aminobenzothiazole and its corresponding trifluoro-thioacetyl analogue



Scheme 2

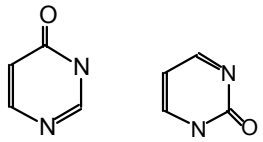


Fig. 3: Structures of pyrimidin-4(3H)-and-2(1H)-ones

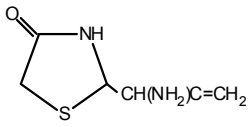


Fig. 4: Structure of 2-(allylamino)thiazolin-4-one

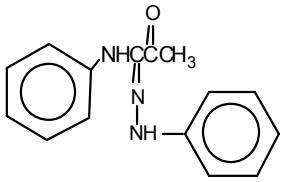


Fig. 5: Structure of 2,3-dioxobutyranilide-2-phenylhydrazone

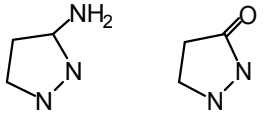


Fig. 6: Structures of 3-aminopyrazolidine and 3-oxopyrazolidine

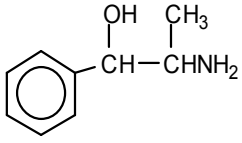
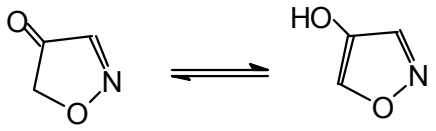


Fig. 7: Structure of norephedrine (1S,2R) and norpseudoephedrine (1R,2R)

A linear relation between the relative intensities of specific fragments of azo and hydrazone tautomers of 2,3-dioxobutyranilide-2-phenylhydrazones (Fig. 5) in mass spectra, the pkB and Hammett σ-values were discussed concluding that electron donating substituents lead to the formation of hydrazone tautomers [20].

Comparison on the electron-impact-induced mass spectra of isoxazol-5-ones with those of the 5-methoxy, 2-methyl and 4,4-dimethyl derivatives, indicated that in the vapor phase and prior to fragmentation, isoxazol-5-ones exist in the CH tautomeric form [21] (Eq. 5). For 5-methoxyisoxazoles a thermal isoxazole → azirine → oxazole rearrangement occurs (Scheme 3) leading to variations in ion intensities in the spectra depending on experimental conditions.

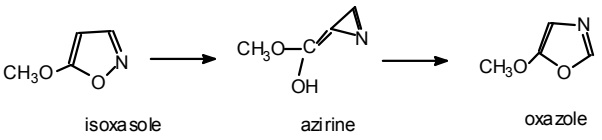


(5)

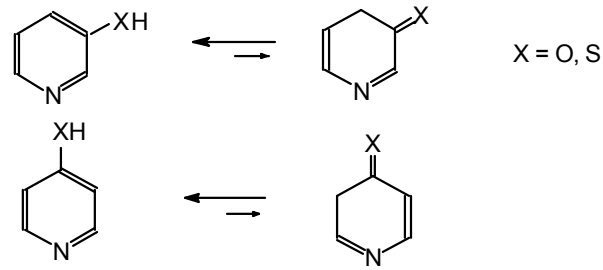
The mass spectral study of the ring-chain tautomeric forms of 3-amino (or oxo) pyrazolidines (Fig. 6) indicated that most of them exist partly in the open form [22].

Gas-phase ring-chain tautomeric equilibria with fourteen 1,3-oxazolidines derived from norephedrine and norpseudoephedrine (Fig. 7) were studied by means of mass spectrometry. Using 14 eV ionizing electrons these equilibria were comparable to those in non-polar solvent and obeyed the simple equation: $\log Kx = \rho\sigma^{+} + C$ 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 observed [23].

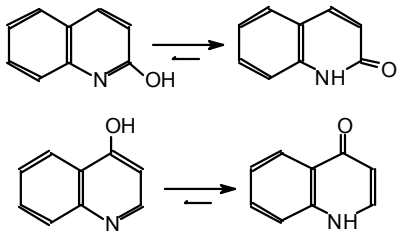
A leading contribution to tautomerism studies by mass spectrometry was made by Maquestiau and Flammang (for two comprehensive reviews of their work [1, 24]). Using various techniques such as primary isotope effects upon metastable ions [25, 26] they concluded that OH tautomers predominate for 3-and 4-hydroxypyridines, that SH tautomers predominate for 3-and 4-mercaptopyridines and NH tautomers predominate for 2-and 4-quinolones (Scheme 4 and 5 respectively).



Scheme 3

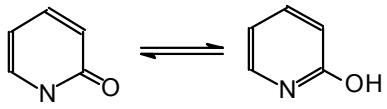


Scheme 4



Scheme 5

The case of 2-hydroxypyridines was less clear, they concluded the probable existence of only OH tautomer, but this conclusion should be changed to reflect the predominance of this tautomer with the presence of significant quantities of the NH tautomer. Baldwing and Langley confirmed these results in subsequent work by using differences in the kinetic energy release (KER) associated with the mass spectrometric decomposition of metastable molecular ions [27]. Kellery and Bernstein [28] have studied the tautomerism of the 2-pyridone/2-hydroxypyridine system (Eq. 6) 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 2-pyridone is not planar and exists in two conformations.



(6)

Ion Cyclotron Resonance 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 need to correct 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

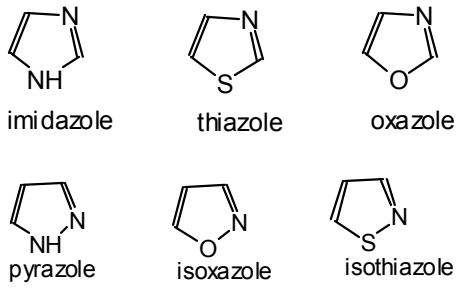


Fig. 8: Structures of 1,3-and 1,2-azoles

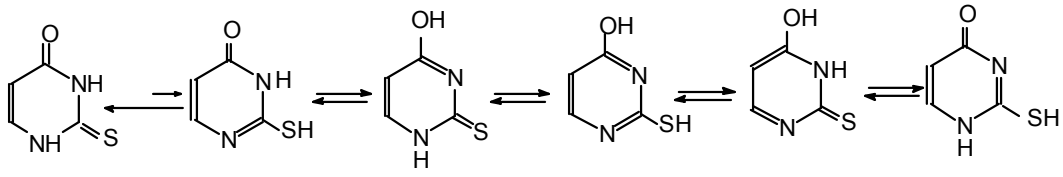
estimates of individual tautomer stabilities [29]. For pyridones and thiopyridones it was proved that OH and SH tautomers predominate [30-32].

For the complicated case of 2-thiouracil (six aromatic tautomers, Scheme 6) Katritzky and Eyler [33] concluded that the oxo-thioxo tautomer is the most stable tautomer. Similar studies were carried out for azoles [34-36] (Fig. 8).

Fourier transform ion cyclotron resonance experiments showed that a variety of molecules catalyze the hydrogen transfer which converts ionized acetaldehyde $CH_3CHO^{+\cdot}$ to its vinyl alcohol counterpart $CH_2CHOH^{+\cdot}$. Each of these ions could be characterized by its specific bimolecular reactions with selected reactants. Calculations showed that two pathways, for which the rate determining barriers have almost the same energy, are feasible. The first transition state involves a direct catalyzed 1,3-H transfer, while the second involves two successive 1,2-H transfers. A detailed experimental study, using methanol as a catalyst as well as labeled reactants, indicated that only the first pathway operates in the isomerization process. The different steps of these two independent pathways were elucidated [37].

The mass spectra of oxazepam and N-hydroxy-2-fluorenylacetamide (Fig. 9) could be explained only if all ring-chain tautomeric forms are considered [38], concluding that certain tautomeric equilibria are relevant in the interpretation of mass spectra.

The facile loss of CO in the mass spectra of anthrapyridone derivatives suggested that in the excited state the decomposition proceeds from the lactam tautomer [39].



Scheme 6

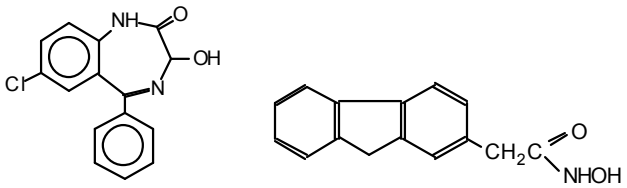


Fig. 9: Structures of oxazepam and N-hydroxy-2-fluorenylacetamide

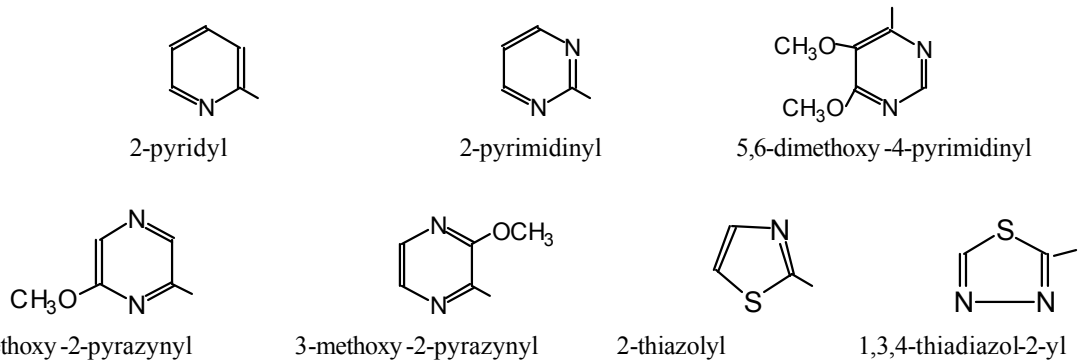


Fig. 10: Structures of the substituents of 4-$H_2NC_4H_4SO_2NHR$

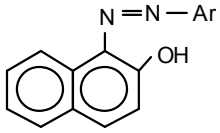


Fig. 11: Structure of azonaphtols

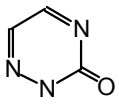


Fig. 12: Structure of triazininone

The mass spectra of 4$H_2NC_4H_4SO_2NHR$ (R= 2-pyridyl, 2-pyrimidinyl, 5,6-dimethoxy-4-pyrimidinyl, 6-methoxy-2-pyrazynyl, 3-methoxy-2-pyrazinyl, 2-thiazolyl, 1,3,4-thiadiazol-2-yl, Fig. 10) were compared with those of N-methylated analogues fixed in the amino or imino form [40]. The results indicated that the compounds studied existed mainly in the amino form, but some of the imino form was also present in the case of the thiadiazolyl derivatives.

Kostyuchenco and coworkers [41] studied the mass spectral behavior of some azo-naphtols (Fig. 11) and related Schiff bases and indicated that the keto form prevails in gas phase. Such finding contradicts strongly with recent published results about the tautomeric thermodynamics of this compound in methyl cyclohexane/toluene mixture [42, 43] as well as the published *ab-initio* quantum-chemical calculation [44, 45].

The tautomeric equilibria of substituted 5-triazininones (Fig. 12) were studied by comparing their mass spectra with those of their methylated derivatives [46]. The results for 6-methyl-5-triazoninone were confirmed by comparison with the mass analyzed ion kinetic energy spectra of ions generated from N-ethyl derivatives. The tautomeric equilibria are dependent upon the nature of the substituents in the 3-and 6-positions.

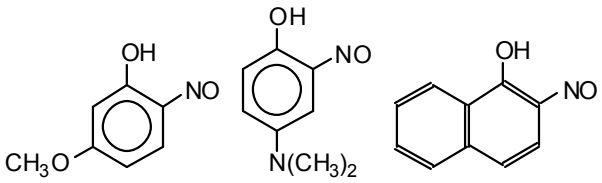
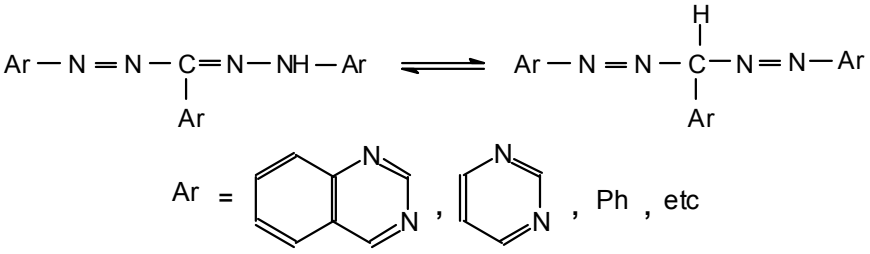


Fig. 13: Structure of 5-methoxy-and 4-(dimethylamino)-2-nitrosophenol and 1-nitroso-α-naphtol

Mass spectra were recorded for α-and β-5-methoxy-and 4-(dimethylamino)-2-nitrosophenol and 1-nitroso and 2-nitroso-α-and β-naphtol [47] (Fig. 13). The spectra were independent of the insertion temperature, suggesting that either the compounds do not exhibit tautomerism in the vapor phase or that the heat of isomerization is quite low. However, according to the authors the fragmentation patterns suggest tautomerism of the molecular ion.

The course of the dissociative ionization under electron impact in "heteroarylformazans" of the pyrimidine and quinazoline series was investigated in comparison with "triarylformazans" (Scheme 7). A quantitative assessment of the tautomeric forms



Scheme 7

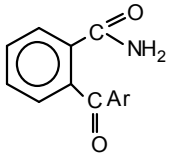


Fig. 14: Structure of 2-aroylbenzamide

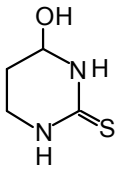


Fig. 15: Structure of 4-hydroxyhexahydropyrimidine-2-thione

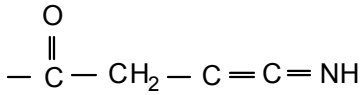


Fig. 16: Structure of β-ketoenemines

according to the contributions from the individual fragmentation processes to the total ion current was given. It was established that in diazinylformazans the form in which the heterocycle occupies the fifth position (enters the hydrazone fragment of the formazone molecule) predominates [48].

The tautomerism of some 2-aroylbenzamides (Fig. 14) was examined by mass spectrometry and thermal tautomerization was demonstrated [49].

The mass spectra of substituted 4-hydroxyhexahydropyrimidine-2-thiones (Fig. 15) were analyzed and the results indicated that ring-chain tautomerism exists [50].

Gas chromatographic and spectroscopic studies of bidentate β-ketoenemines (Fig. 16) were discussed with reference to NMR and mass spectrometry. Tautomerism, steric effects and structural studies were confirmed by gas chromatography-mass spectrometry [51].

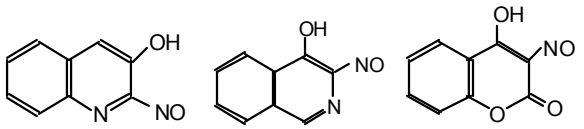


Fig. 17: Structures of *o*-hydroxy nitroso derivatives of quinoline, isoquinoline and coumarin

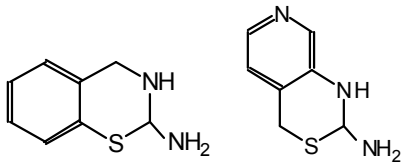
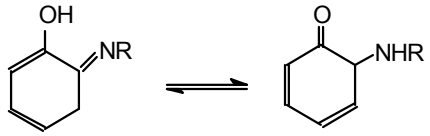


Fig. 18: Structures of 2-amino-4H-3,1-benzothiazine and 2-amino-4H-pyrido [4,3-d] [13] thiazine

The mass spectra of phenolic imines could be easily distinguished from their chromene tautomers (Eq. 7) by mass spectral peaks $[M\text{-}NHR]^+$ and $[M\text{-}OH]^+$ respectively. *o*-Quinoid tautomers could be excluded by measuring ionization energies [52].



(7)

Continuing with other compounds families, a comparative mass-spectrometric behavior of *o*-hydroxynitroso derivatives in the quinoline, isoquinoline and coumarin series (Fig. 17), was carried out by studying their tautomerism in the gas phase [53].

ass spectra of some 2-amino-4H-3,1-benzothiazine and 2-amino-4H-pyrido[4,3-d][13]thiazine derivatives (Fig. 18) suggested a method for tautomer identification even when the corresponding imino compounds are not available for comparison [54].

A review with 146 references on the formulation of approaches to evaluate prototropic tautomerism by mass spectrometry was carried out by Klyuev and coworkers [55], considering theoretical aspects of the study of tautomerism by mass spectrometry.

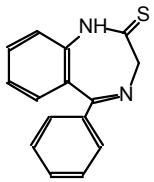


Fig. 19: Structure of 2,3-dihydro-1H-1,5-benzodiazepine-2-thione

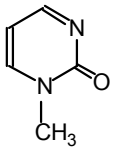


Fig. 20: Structure of 1,2-dihydro-1-methylpyrimidin-2-one

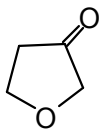


Fig. 21: Structure of 3-oxotetrahydrofuran

UV, NMR and IR spectroscopic evidence suggested that 2,3-dihydro-1H-1,5-benzodiazepine-2-thiones (Fig. 19) exist in the thione tautomeric form in solution [56]. Comparison of mass spectroscopic fragmentation patterns of these compounds suggested that thione-enaminethiol tautomeric equilibrium exists in the gas phase.

Electron impact (EI) mass spectra of 4-amino-substituted and 4-amino-disubstituted 1,2-dihydro-1-methylpyrimidin-2-ones (Fig. 20) were reported [57]. The EI-induced decomposition of their molecular ions is significantly dependent on the chemical nature of the substituents. The fragmentation paths suggested the presence of tautomeric particular forms in the gas phase.

A ^{1}H-NMR study showed extensive enolization in the 3-oxo-tetrahydrofurans (Fig. 21) with branched substitution at C-5 [58]. The 3-oxotetrahydrofuran ring is easily changed conformationally, a fact that is reflected by changes in vicinal and long range coupling values. The mass spectra of the keto and enol molecules showed different fragmentation patterns. The expulsion of an alkane moiety, directly from the molecular ion was observed for the enol form. Mass spectrometry allowed easy characterization of 2-or 5-substituted 3-oxo-tetrahydrofurans.

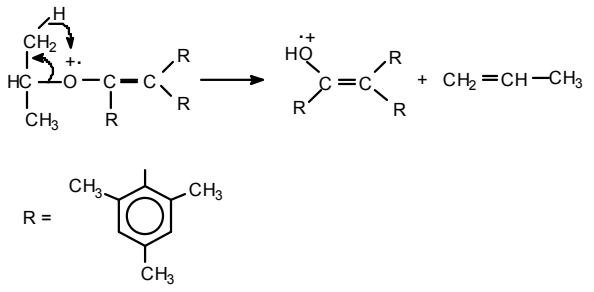
Mass spectrometry represents a very sensitive method for the study of tautomeric equilibria since it is capable of detecting forms which make only minor contributions and which could be undetected using other techniques.

TAUTOMERIZATION OF MOLECULAR IONS

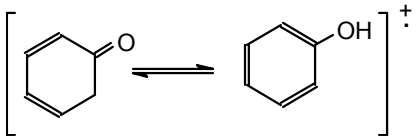
In particular cases, interconversion of the molecular ions of the tautomers has been observed. The gas-phase ion chemistry of sterically crowed keto-enol pairs was probed by using several experimental techniques to shed light on some highly remarkable mechanistic features of these systems [59]. Labeling experiments proved that propene loss from ionized *iso*-propyl trimesitylvinylether is a site-specific process by which, *via* a 4-membered transition state, the ionized enol and not its tautomeric ketone is generated (Scheme 8).

Kinetic energy release measurements support earlier conclusions that enol cation radicals in a rate-determining step isomerize to (excited) ketones from which dissociations of the C(α)-C(β) bond take place. A correlation of the kinetic energy release, associated with this reaction, with the effects of substituents attached to the α-aryl group was not possible. Further, kinetic energy release measurements did not reflect the different behavior of keto-enol tautomers in the reciprocal Me-H migration. Similarly, the analysis of metastable ion spectra was not a means of distinguishing keto-enol forms. In contrast, for most pairs, a straightforward characterization was possible based on the analysis of collision-induced reactions.

The keto-enol tautomerism of the gas-phase phenol and 1,3-cyclohexadien-5-one radical cations was postulated to explain the ion chemistry of C_6H_6O radical cations [60] (Scheme 9). 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 interconvert if they are sufficiently activated to decompose by undergoing CO loss. The phenol ions isomerize to a keto form by a high-energy sigmatropic [1, 3] hydrogen shift, which is the rate-determining step for CO loss.



Scheme 8



Scheme 9

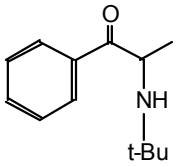


Fig. 22: Structure of bupropion

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 reaction of metastable ions.

The stability and interconvertibility of keto and enol forms of methyl acetate molecular ions prior to fragmentation in the gas phase was also studied [61]. The heats of formation of the keto and enol forms of MeOAc were determined. Fragmentation by loss of $MeO^{\cdot}$ occurred at the thermochemical threshold for $[MeCO]^{+}$formation of both isomers, which thus freely interconvert at internal energies corresponding to this decomposition threshold.

The fragmentation of the protonated molecular ions of "bupropion" (Fig. 22) produced by collision-induced decomposition was shown to depend on the ionization method used to form the $[M+H]^{+}$ ion. The daughter ion products did not depend on the energy of decomposition, i. e., high-or low-energy collisions, but on the ratio of the keto-enol equilibrium that would be influenced by the ionization process. However, the $[M+H]^{+}$ ions formed by EI arises from the keto tautomer. This suggested that the molecular ions produced in the FAB process are strongly associated with solvent ions during their formation [62].

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 [63]. Electrospray ionization (ESI) was utilized for introduction of the metal ion complexes into a quadrupole ion trap mass spectrometer. The proximity of the carbonyl groups was crucial for formation and detection of ion complexes by ESI. For example, no metal ion complexes were observed for 1,4-cyclohexanedione, but they were readily detected for the isomers, 1,2-and 1,3-cyclohexanedione. Although the diketones form stable double charged complexes, the formation of singly charged alkaline earth complexes of the type $(nL + M^{2+}\text{-}H^{+})^{+}$ where L = 1,3-cyclohexanedione or 2,4-pentanedione is the first evidence of charge reduction. CID investigations provided further evidence of charge reduction processes occurring in the gas-phase complexes. The CID studies indicated that an intramolecular proton transfer between two diketone ligands attached to a doubly charged metal ion, followed by elimination of the resulting protonated ligand, produces the charge-reduced complex. For transition metal complexation, the preference for formation of doubly charged *versus* singly charged complexes correlated with the keto-enol distribution of the diketones in solution.

Negative quasimolecular ions of aromatic carboxylic acid amides were observed unexpectedly under electrospray ionization conditions [64]. Hypothetically, deprotonation of either carboxamide or carboximidic acid tautomers can produce anions with equivalent resonance structures, the stability of which is affected by conjugated aromatic substituents. In this study, 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 apparent acidity could be accounted for by resonance stabilization.

Aryl-substituted 4-hydroxycoumarins [2, 65] (Fig. 23) were investigated 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 influence of substitution and tautomerism on the formation of quasimolecular ions and mass spectra fragmentation was explained. Mass spectral studies on some deuterated compounds proved some of the proposed fragmentation pathways. Results obtained are very useful in the process of detection and characterization of hydroxycoumarins, as well as for structural elucidation of their more complex derivatives [66].

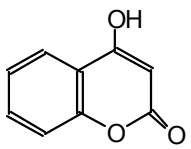


Fig. 23: Structure of 4-hydroxycoumarin

MASS SPECTROMETRY/THEORETICAL CALCULATIONS

They are many reports that involve theoretical calculations as a means to support the mass spectral data.

The tautomerism of tetrazole, 5-methyltetrazole, (Fig. 24) and its isotopically substituted derivatives has been discussed on the basis of their fragmentation patterns and of quantum-chemical calculations by the LCAO MO method in the CNDO/2 approximation [67]. The equilibrium of these compounds in the gas phase was found to be displaced towards the 2H-tautomer.

The tautomerism of 2,4-dihydroxyquinoline (Fig. 25) in the gas phase was discussed in terms of the electron impact fragmentation pattern and of quantum-chemical calculations by the LCAO-MO method in the CNDO/2 approximation [68]. Dihydroxyquinoline methylated at position 4 can only exist in two tautomeric forms. Thus, comparison of mass spectrometric data of this compound with dihydroxyquinoline provided additional evidence for the gas phase equilibrium of the latter. The equilibria of these compounds in the gas phase were found to be displaced towards the hydroxyl tautomers.

Comparison of the mass spectra of 2-aryl-4,5-dioxo-1,3-thiazines (Fig. 26) with those of model compounds indicated that them exist in the gas phase primarily in the hydroxycarbonyl form [69].

A systematic comparison of structural effects on the intrinsic reactivities of carbonyl and thiocarbonyl compounds was carried out [70]. The gas-phase basicities of a wide variety of thiocarbonyl compounds, XCSY (as well as for some carbonyl derivatives) were determined by means of Fourier Transform Ion Cyclotron resonance spectrometry and SCF and MPG *ab-initio* calculations at different levels of accuracy were performed on 27 different neutral compounds and their protonated forms.

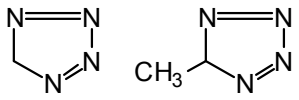


Fig. 24: Structures of tetrazole and 5-methyltetrazole

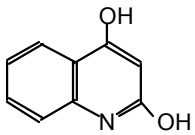


Fig. 25: Structure of 2,4-dihydroxyquinoline

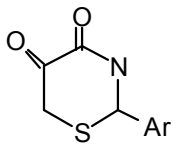


Fig. 26: Structure of 2-aryl-4,5-dioxo-1,3-thiazine

The same set, enlarged by the inclusion of very large systems such as di-*ter*-butyl-and *bis*-(1-adamantyl) thioketones (Fig. 27) was also investigated 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 state functions was good in all instances.

Correlation analysis of the experimental data showed that: substituent effects on the gas-phase basicity of thiocarbonyl compounds are linearly related to those of their carbonyl homologues with a slope of 0.80 and these effects can be quantitatively analyzed in terms of polarizability, field and resonance effects (Taft-Topsom model).

Comparison of the gas-phase basicities of thiocarbonyl and carbonyl compounds with solution basicities and nucleophilicities shed light on different structural and solvation effects. Substituent effects on both neutral and protonated species were explored by means of appropriate isodesmic reactions. These results confirmed that all thiocarbonyl compounds investigated are sulfur bases in the gas phase. The features revealed by correlation analysis can be rationalized in terms of the interactions between the molecular orbitals of the substituent and the parent compound.

The mass spectral behavior of N-unsubstituted pyrimidin-4-ones (Fig. 28) with CH_2-R type substitution at C-2 was found to differ from that of analogues that are N-substituted and/or 2-aryl or 2-methyl substituted [71]. A dominant intramolecular cyclization seems to occur between N-3 (in agreement with the predominance of the 3-NH tautomers) and the *ortho* positions of the aryl moiety in compounds with a CH_2-aryl substitution at C-2. AM1 SCFR calculations on 2-and 6-substituted and 2,6-disubstituted pyrimidin-4-ones supported the mass spectral observations.

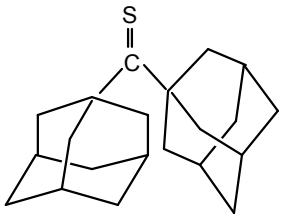


Fig. 27: Structure of *bis*-(1-adamantyl) thioketone

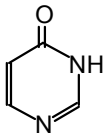


Fig. 28: Structure of pyrimidin-4-one

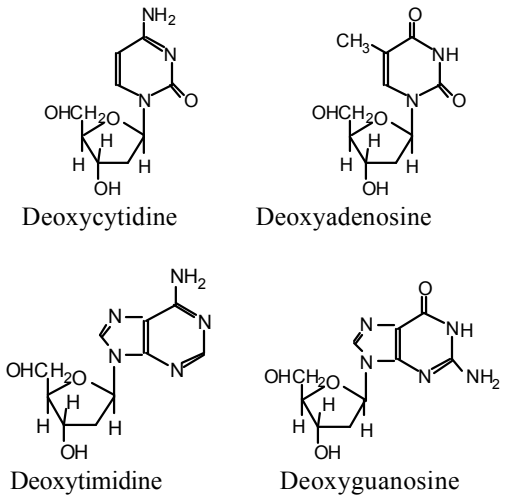


Fig. 29: Structures of deoxyribonucleosides

H-bonded pairs of deoxyribonucleosides (Fig. 29) were investigated by fast atom bombardment mass spectrometry and MNDO/H quantum-chemical calculations [72]. The enol or imine forms of the nitrogen bases could form pairs with energies comparable to canonical base pair energies. Orders of pair stability, measured by different mass spectral techniques, agreed with each other.

The position of the amino-oxo-imino-oxo tautomeric equilibrium of gaseous deoxy-cytidines was found to be affected by the acylation of the exocyclic atom of the nucleobase. AM1 quantum-mechanical calculations showed that the imino-oxo tautomers are thermodynamically more stable and possess lower-lying lowest unoccupied molecular orbitals than the related amino-oxo isomers [73]. Accordingly, ionization for the acylated nucleosides by fast atom bombardment produces stable radical anions. The lowest critical-energy unimolecular dissociation of the $(M-H)^-$ species which corresponds to the elimination of isocyanate units, gave further insights into the existence of the gaseous deoxycytidines in their imino-oxo form.

The unimolecular gas-phase chemistry of the ethylene phosphonate ions, $[\text{-}OCH_2CH_2O\text{-}]\ P(H)=O^{+\cdot}$, $Ia^{+\cdot}$ and its tautomer ethylene phosphite, $[\text{-}OCH_2CH_2O\text{-}]\ P\text{-}OH^{+\cdot}$, $Ib^{+\cdot}$, was investigated using mass spectrometry-based experiments in conjunction with isotopic labeling and computational quantum chemistry, at the CBS-QB3

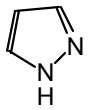


Fig. 30: Structure of pyrazole

level of theory [74]. A facile tautomerization of the "keto" ion $Ia^{+\cdot}$ into its more stable (by 34 kcal/mol) "enol" isomer $Ib^{+\cdot}$ is prevented by a substantial 1,2-H shift barrier (14 kcal/mol relative to $Ia^{+\cdot}$). In line with this, the collision-induced dissociation (CID) and neutralization-reionization (NR) spectra of the two isomers are characteristically different. The 1,2-H shift barrier separating $Ia^{+\cdot}$ and $Ib^{+\cdot}$ is calculated to lie close to the thermochemical threshold for the formation of $C_2H_4^{+\cdot}$ + HO-P(=O)$_2$. This reaction dominates the closely similar metastable ion (MI) spectra of these tautomers. At these elevated energies, the "enol" ion $Ib^{+\cdot}$ can undergo ring-opening by CH_2O or C_2H_4 cleavage, yielding ion-dipole complexes of the type $[C_2H_4]^{+\cdot}$ / HO-P(=O)$_2$ and H-bridged radical cations $CH_2{=}O\ldots[H\ldots O\ldots P\ldots OCH_2]^{+\cdot}$, respectively. Theory and experiment yielded a consistent potential energy profile for the cyclic phosphonate/phosphite system showing that non-dissociating ions $Ia^{+\cdot}$ retain their structure identity in the microsecond time-frame. However, the interaction of $Ia^{+\cdot}$ with benzonitrile in a chemical ionization type experiment readily yielded the more stable "enol" type ion $Ib^{+\cdot}$. Experiments with benzonitrile-d_5 supported the proposal that this interaction does not involve the lowering of the 1,2-H shift barrier between the tautomers, via a proton-transport catalysis type mechanism.

Proton affinities of 32 N-H and N-methylpyrazoles (Fig. 30) were determined by FTICR. From these measurements coupled with *ab-initio* calculations it was possible to determine the effect of many substituents at position 3(5) (e. g. methyl, ethyl, *ter*-butyl, phenyl, nitro, amino, ethoxycarbonyl) on K_T. The tautomerism and protonation site (on the ring) of histamine has also been studied by FTICR [75, 76].

The reactions of ionized pyridazine, aminopyrazine and aminopyridine (Fig. 31) and the corresponding α-distonic ions were examined by a combination of tandem mass spectrometric techniques, including analysis of metastable ions, collision-induced dissociation and neutralization-reionization mass spectra (NRMS) [77]. Further insight into the relative stability and energy barriers towards tautomerism of each ionized heterocycle with its α-distonic isomer was obtained by computational methods. In all these systems, both the conventional radical-cation and the α-distonic tautomer were found to be stable species,

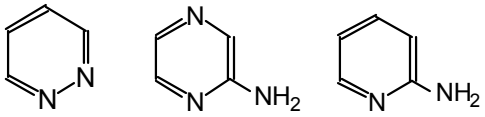


Fig. 31: Structures of pyridazine, aminopyrazine and aminopyridine

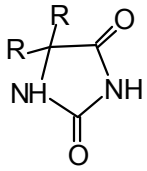


Fig. 32: Structure of 5,5-disubstituted hydantoins

which exist in discrete energy wells, with a significant barrier towards their interconversion. Although each α-distonic ion was sufficiently stable to survive neutralization-reionization, the conventional ionized heterocycle was more stable in each case.

TAUTOMERISM-THEORETICAL CALCULATIONS

Although it is not intended to cover the work done by theoretical calculations in regard to tautomerism, reference is made to some of the recent ones.

Employing the NMR parameters of 5,5-disubstituted hydantoins (Fig. 32) and the results of accompanying semiempirical AM1 and PM3 and *ab-initio* (3-21G and, when sulfur atom is present, 3-21G") quantum-chemical calculations, the tautomerism, the acidity, the stereochemistry and the π-electron density distribution along the hydantoin molecule was studied [78].

Tautomerism and protonation of guanine and cytosine (Fig. 33) in the gas phase and in aqueous solution were examined by theoretical methods [79]. High-level *ab-initio* calculations with conclusion of correlation effects at the Moeller-Plesset level were used to study these processes in the gas phase. The influence of solvent has been examined using self-consistent reaction field and Monte Carlo free energy perturbation simulations. The results provided a complete and accurate picture of tautomerism and protonation of these nucleic acid bases. Comparison with the available experimental data gave confidence in the quality of the results derived from theoretical computations. Inspection of the most stable tautomeric forms for the neutral and protonated nucleic acid bases allowed rationalization of the formation of unusual DNA structures like the triple helix.

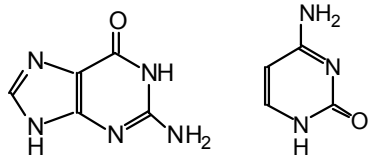
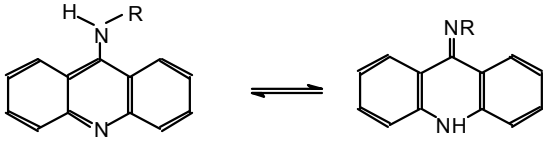


Fig. 33: Structure of guanine and cytosine

AM1 calculations showed 2-nitraminepyridine to contain 97.7% of 1,2-dihydro-2-nitrimino-pyridine form (Eq. 8), which was found to be the most stable in the gas phase [80]. Instead, non-planar nitramine form was found to dominate for 4-nitraminopyridine. The results were related to experimental (X-ray and ^{15}NMR) data.

(8)

Stationary fluorescence excitation spectra of neutral, mono-and diprotonated 9-acridinamine (Eq. 9) in liquid phases were studied and it was observed that they correlate with the relevant electronic absorption spectra only with respect to band position but not their relative intensities [81]. This suggested that neutral and diprotonated 9-acridinamine co-exist in two tautomeric forms whereas nonprotonated appears only in one such form, both in the ground and excited states. This was confirmed by the results of semiempirical calculations carried out at the PM3C1 level. This method was also used to predict the geometries of molecules in the ground and first excited singlet states and subsequently the energies (wavelengths) of adiabatic absorption and emission transitions, which correlated qualitatively with the experimental ones.



(9)

The extent of tautomerism and conformational isomerism in formhydroxamic acids (Fig. 34) was explored using *ab-initio* calculations [82]. Several new conformers were identified, as well as a new tautomeric form that may be viewed as the N-oxide of imidic acid. In most cases, this new isomers were found to be less stable than those previously discussed in the literature. Hydration effects were explored using AM1-SM2 and PM3-SM3 calculations. The new N-oxide tautomers

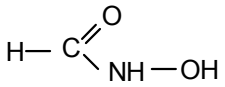


Fig. 34: Structure of formhydroxamic acid

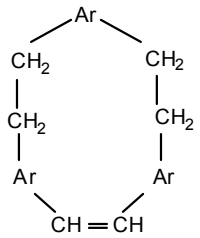


Fig. 35: Structure of selected cyclophane

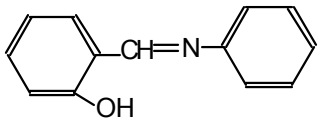


Fig. 36: Structure of salicylidenaniline

were found to be preferentially stabilized compared to the gas phase, but not to the extent of making them competitive with the global minimum.

AM1 semiempirical calculations were carried out to determine both ΔH and ΔS on the enol/enol tautomerism of 25 β-diketones and 8 β-ketoaldehydes [83]. In the first case, $\delta\Delta G_{exp}$ was determined by Hansen [84] and his values correlated reasonably well with $\delta\Delta G_{calc.}$. The calculated differences in energy were found to be linearly related to some geometrical characteristics of the ketoenol, namely the angles about the substituent on the central carbon. To check if this geometrical dependence was related to the Mills-Nixon effect, parallel AM1 calculations on the tautomerism of β-ketoaldehydes and 3(5),4-disubstituted N*H*-pyrazoles were carried out conforming the influence of the Mills-Nixon effect on the enol/enol tautomerism of β-dicarbonyl compounds.

A unique substituted cyclophane system which possesses two ethano and a single vinyl bridges in symmetrical fashion (Fig. 35) was considered for AM1 (RHF) type semiempirical calculations [85]. The phane decks of this system are actually embedded phenol and benzylideneaniline moieties and they are also the constituents of salicylidenaniline structure (Fig. 36), which is the simplest photocromic dye. In this tautomerism and way, it was investigated how cyclophane structure affects the keto-enol UV-VIS spectra when this cromophores exist in a different structure than salicylideneanilene. The enol and keto

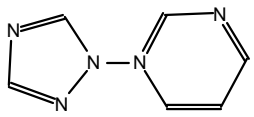


Fig. 37: Structure of triazolopyrimidine

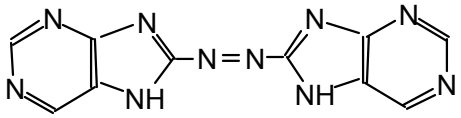


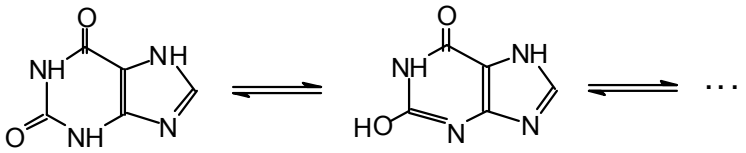
Fig. 38: Structure of 8,8'-azopurine

forms of the resultant cyclophane system were found to be stable but endothermic being the enol form more favorable. The theoretical UV-VIS spectra of the tautomers were obtained and compared with salicylidenealine tautomers.

The tautomerism of triazolopyrimidines (Fig. 37) in the gas phase as well as in DMSO was studied with *ab-initio* and semiempirical methods [86]. The self-consistent reaction field method SCI-PCM (self-consistent isodensity polarized continuum model) was used to represent solvent effects in the *ab-initio* HF/6-31G* calculations. Electron correlation was included at the second-order Möller-Plesset perturbation level (MP2). The calculated equilibria of tautomerism, taking solvent effects into account, were in good agreement with the ^{15}N NMR spectroscopy data.

Ab-initio and semiempirical calculations were carried out for the five tautomeric forms of 8,8'-azopurine (Fig. 38) both in the gas phase and in aqueous solution employing the SM2 solvation model [87]. The gas phase the stability order was different in each phase.

Heats of formation, entropies, Gibbs free energies, relative tautomerization energies, tautomeric equilibrium constants, relative proton affinities, dipole moments and ionization potentials for the fourteen possible tautomers of xanthine (Scheme 10) were studied using semiempirical AM1 and PM3 quantum-chemical calculations at the SCF level in the gas and aqueous phases, with full geometry optimization [88]. The COSMO solvation model was employed for aqueous solution calculations. The calculations showed that the two diketo-tautomers are the predominant species at room temperature in the gas and aqueous phase. But, the first more stable tautomer is the dioxo-7H tautomer. Comparison with available experimental data provided support for quality of results derived from theoretical computations. The entropy effect on the Gibbs free energy of the xanthine is very small and



Scheme 10

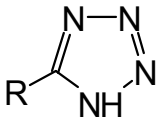


Fig. 39: Structure of 5R-tetrazols

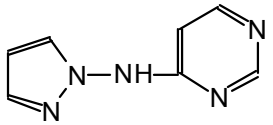


Fig. 40: Structure of 6-aminopyrazolopyrimidine

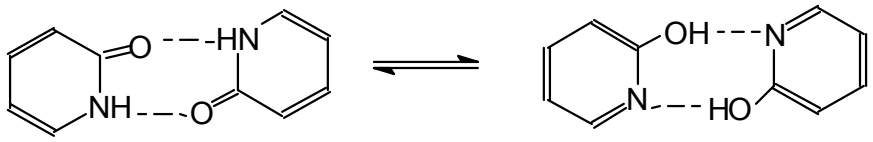
there is little significance for the tautomeric equilibria of the base. The enthalpic term is found to be dominant also in the determination of the equilibrium constant.

The energies, geometries and charge distributions of five stable forms of a series of 5-R-tetrazoles (Fig. 39) [R = H, CH_3, $C(CH_3)_3$, Ph, Cl, CF_3, NO_2] (anion, 1H-and 2H-neutral tautomers, 1,3-H,$H^{+.}$ and 1,4-H,$H^{+.}$ cation tautomers) in the gas phase were calculated with the DFT/B3LYP method at the 6-31G* level [89]. For tetrazolate anions, a shortening of the 1-2 bond and a simultaneous elongation of the 2-3 bond was observed when the value of the σ_p constant of the substituent increases. A considerable ring aromaticity was exhibited by tetrazolate anions and also by the 2H-form of neutral tetrazoles that correlated well with the σ_p constant of substituents. The relative thermodynamic stability of 2H-forms as compared with 1H-ones did not practically depend on the nature of the substituent R. In contrast, in the case of tetrazolium cations, the most stable form was the 1,4-H, H^+ for the electron-donating substituents and the 1,3-H,H^+ for the electron-withdrawing ones. Good correlations were observed between the energies of protonation of anions and neutral tetrazoles and the experimental pK_a and pK_{BH+} values.

The tautomerism and protonation of 6-aminopyrazolopyrimidine (Fig. 40) (biochemically and pharmacologically important) in the gas phase were studied by means of *ab-initio* methods [90]. The effect of the solvent on the tautomerization and protonation processes was accounted for by using several high-level techniques including molecular dynamics free energy perturbation (MD-FEP), an optimized *ab-initio* Self-Consistent Reaction Field (SCRF) method and two different semiempirical SCRF methods. The results not only provided a complete picture of the tautomerism and protonation of 7-aminopyrazolo pyrimidine but also allowed to test different "state-of-the-art" techniques to represent solvation effects.

Tautomeric equilibria of 2-pyridone (PD) and 2-hydroxypyridine (HP) dimeric forms as well as PD/HP complexes mediated by the conjugated dual hydrogen-bonding (CDHB) formation (Scheme 11) were studied by *ab-initio* molecular orbital calculations up to the 6-31+G** basis set at the Möller-Plesset level [91]. The result in combination with the semiempirical solvation free-energy calculation was reasonably good to predict the relative free energy and consequently the tautomeric equilibria between PD, HP, their corresponding dimers and PD/HP complex in the gas phase as well as in solution. The results also indicated that the strength of dual hydrogen bonding resonantly affects the PD and HP electronic structures upon CDHB formation, resulting in additional stabilization energy. Further calculation showed that the tautomeric equilibria could be fine-tuned by the formation of a hydrogen-bonded complex with guest molecules possessing bifunctional hydrogen bonds. The results lead to a possible mechanism suggesting that the tautomerization of a specific DNA base may be induced by forming a complex with an intruder, i. e., a specific molecule with multiple hydrogen-bonding capability which triggers the mutation process [92].

Theoretical models were used to study pH-dependent equilibria of 2,4-diamino-5-phenylthiazole (Fig. 41) tautomer molecules in water [92]. A complete screening of semiempirical SCF multiple minimum hypersurfaces, corresponding to several solute-water supermolecules, was made. Experimental NMR results were confirmed thus indicating that the native diamine tautomer predominates in aqueous neutral and basic media. This tautomeric structure, protonated either in N3 and N4, also predominates in aqueous acid media with a minor presence of a protonated monoimine tautomer, in agreement with ^1H NMR results in D_2O. High-level *ab-initio* SCF MO of the main structures, where solvent reactions field effects were taken into account with a dielectric constant equivalent to that of



Scheme 11

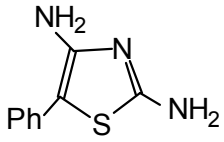


Fig. 41: Structure of 2,4-diamino-5-phenylthiazole

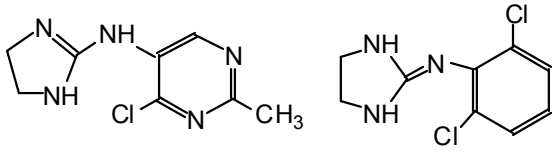


Fig. 42: Structures of moxonidine and clonidine

water, predicted a non-conjugated protonated monoimine tautomer in noprotic solvents, according to 1H NMR data in polar aprotic solvents.

The geometries of various tautomers and rotamers of moxonidine (Fig. 42) in both anionic and protonated forms were optimized using the two layered ONIOM(B3LYP 6-311+G (d,p): AM1) method [93]. The calculations showed that, in agreement with experiments, moxonidine exists in a more stable imino tautomer. The tautomer containing the amino group is less stable by about 19kJ/mol. The computed stable conformation for the moxonidine species is characterized by the pyrimidine and imidazolidine rings being in the mutual gauche conformation to one another. In contrast to the parent neutral molecule of moxonidine, ionization caused considerable geometric changes in the anions compared to the neutral species. In the neutral form and anion of the parent drug, an intramolecular hydrogen bond stabilizes the structure and makes the most stable conformations more planar. The primary protonation site was found to be the imidazolidine part of the drug. The proton affinity of moxonidine was computed to be-1004 kJ/mol. The moxonidine base was found to be less lipophilic than the base of parent clonidine (Fig. 42).

THE AUTHORS´ WORK

Methodology employed

Gas chromatography-mass spectrometry determinations: As it has been demonstrated in the case of keto-enol tautomerism of a series of 1-and 3-substituted acetylacetones [94] and a variety of carbonylic and thiocarbonilic compounds [95-104], there is no significant interconversion of the tautomeric forms in the gas phase following electron impact ionization in the mass spectrometer (molecular ions, $M^{+\cdot}$, 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 (intermolecular, unimolecular) would not seem to take place even with no GC separation (under the selected experimental conditions). These conclusions were 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 [100]. In fact, these equilibria would take place very fast under the working conditions in the GC.

Separation of the tautomers in the analytical column is usually very difficult; consequently, the different pathways of fragmentation of the tautomeric forms have to be used for identification of the individual tautomers [100]. 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 authors´ work. Analogously, it is thought that most of the conclusions could be useful to analyzed spectra registered with mass spectrometers equipped with direct insertion probes.

The results obtained by GC-MS when correlated with those from semiempirical molecular orbitals calculations for amides, ureas, hydantoins, isoquinolinones, ketones, diketones, lactones, demonstrated that mass spectrometry constitutes an adequate tool for predicting tautomeric equilibrium shifts within a family of organic compounds [95-104].

Theoretical methods: The Hartree-Fock (HF) model [105] is a kind of branching point, either 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 towards the exact solution of the electronic Schrödinger equation [106].

The cost of performing a HF calculation 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 [107]. The most obvious way to achieve this reduction is therefore to neglect or/and neglect some of them. Semiempirical methods achieve this in part by explicitly resorting to the valence shell approximation, *i.e.* considering only the valence electrons of the system; the core electrons are subsumed into the nuclear core [108]. The rationale behind this approximation is that the electrons involved in chemical bonding and other chemical phenomena are those located in the valence shell. The 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 [109].

The Austin Model 1 (AM1) method was designed to eliminate the problems with the Modified Neglect of Diatomic Overlap (MNDO) method [110] which were considered to arise from the tendency to overestimate repulsions between atoms separated by 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; the 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 chief advantage of semiempirical molecular orbital programs over *ab-initio* molecular orbital programs is their speed. The simplification to the integrals have the results that the time required for a calculation increases only as N^3, where N is the number of basis functions and so quite large molecules can be studied directly. There is also a helpful additional advantage. Because the methods are parametrized against experimental results and these include the effects of electron correlation, some allowance for this effect is implicit in the calculation [111].

Calculations were run using the HYPERCHEM® package [112]. The authors have chosen the Polak-Ribiére first-order minimization algorithm, which is frequently employed in molecular modeling. This method gradually changes the coordinates of the 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 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 for a quadratic function of M variables, the minimum will be reached in M steps. The root mean square gradient was chosen equal to 0.012 kcal/mol.

Studies on different compounds families: This section covers the authors' contribution to the study of tautomerism by mass spectrometry of several organic compounds families supported in many cases, by semiempirical molecular orbital calculations.

Carbonylic compounds and thio-analogues

Ketones: There has been considerable interest in the enolization of carbonyl compounds for many years [113] and excellent methods exist for the generation of simple enols of aldehydes and ketones in solution [114, 115].

These enols, although thermodynamically unstable with respect to their carbonyl isomers, exist for long enough to be detected by conventional methods. On the other hand, the enol forms of simple carboxylic acids and derivatives such as esters, amides and anhydrides have never been detected in solution. The relative instability of these enols can be attributed to resonance stabilization of the keto isomers.

Tautomerism of organic compounds has been the subject of numerous theoretical studies by the use of several physical and quantum-mechanical approaches. The results of AM1 calculations for tautomerization energies are satisfactory and they exhibit a good correlation with the experimental data [116] so that its use is recommended, in particular for high molecular compounds [117].

Data about stability and the short lifetime of aldehydes and ketones enols can be found in the literature [118]. In addition, for carboxylic acids, the very low relative stability of their enols is a well-known fact [119, 120], although some of these short-life tautomers were observed or even prepared as intermediate compounds [121-125].

A theoretical study [130] has calculated that the energy difference between acetic and ethene-1,1-diol is 10 kcal mol^{-1} greater than that between acetaldehyde and ethenol. This suggests that enols of carboxylic acids (and their derivatives) would have much lower intrinsic stability than enols of aldehydes and ketones.

However, enols can be dramatically stabilized by the introduction of bulky group onto the carbon α to the carbonyl group [126, 127]; in some cases the enol may

then be the thermodynamically stable tautomer [128], not kinetically though [129]. The pentamethylphenyl group is an effective bulk and provides the necessary stability for enediols (enol form of carboxylic acids and esters) [130].

The chemistry of enols, particularly those derived from carbonyl containing compounds other than ketones, including carboxylic acids and esters, has been of some interest [131, 132].

The position of the keto-enol equilibrium is influenced by several factors, particularly electronic and steric effects of the substituents and the nature of the solvent [133]. That the nature of the solvent should affect the position of the keto-enol equilibrium can be understood in the light of the strong tendency of the enol form to H-bond intramolecularly while the keto-form may H-bond to protic solvents, which should thus stabilize it.

In cyclic β-diketones such as 1,3-cyclopentanodione, bulky substituents favored the keto form, but primary alkyl groups favor the enol tautomer [134].

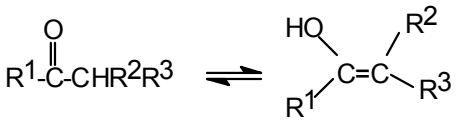
The presence of a bulky α-group might be expected to force the carbonyl oxygen atoms approach each other thus strengthening any hydrogen bond between them and favoring the enol form, but at the same time there will be interaction with β-substituents that can be alleviated by the molecule adopting a diketo conformation E, Z or E,E.

In fact in some cases the enol may then be the thermodynamically more stable tautomer [128, 130].

On the other hand, the tautomerism of organic compounds has been the subject of extensive theoretical studies using various quantum-mechanical statistical-physical approaches [135]. Theoretical calculation has proved to be useful in the assignation of tautomeric structures based on mass spectrometric data. In fact, the route to several fragmentations can only be rationalized by invoking a specific tautomer for the parent ions [62].

Structural studies involving the mass spectra of keto-enol tautomers have been reported for several compounds. Nagraba and coworkers [136] investigated the fragments produced by enol and keto isomers and Holmes and Lossing [137, 138] studied the positive-ion energetics of the enol and keto tautomers and concluded that the enol ions are thermodynamically more stable than the respective keto forms. Notwithstanding, all of these studies were conducted on ions produced in the gas phase.

The enolization degree for ketones is generally favored by the increase in the steric congestion exerted by the substitution α to the carbonyl group. Table 1 shows the most relevant mass spectral data. In general, the loss of OH from the molecular ion can be assigned to the enol form and the loss of R to the keto form (Eq. 10). A relative estimation of the tautomers occurrence could be the ratio $[(M-R)^+]/[(M-OH)^+]$. The analysis of the mass spectra of selected ketones allowed the authors to establish an acceptable correlation between a selected ion abundances ratio and the semiempirical AM1 calculation of the approximate enolization equilibrium constants, just for the neutral species (Table 2) [95].



(10)

Ketones and thioketones: Aliphatic thioketones exist in equilibrium with their enethiols [139]. From theoretical calculations, the keto and thioketo structures of simple carbonyl compounds are 11 and 4 kcal mol^{-1} more stable than the enol and enethiol structures respectively, so that pk thioenol values should be lower than the corresponding pk enol values [140].

As the thiocarbonyl group exhibits characteristic absorption in the UV/VIS region, the thione form is easily detected spectrophotometrically [141].

By nuclear magnetic resonance spectrometry definite and quantitative data for keto-enol equilibria are available [142-144].

The prototropic tautomerism of thiones can also be detected by infrared spectrometry [145] and polarography [146].

The thiol form of 2(1H)-pyridine thione was predicted to be predominant in the gas phase not only by experimental methods (IR) but also by theoretical calculations [147].

In the thione series, the tautomers seem to be relatively slowly interconverted and therefore the proton signals of the tautomeric isomers may be expected to be clearly distinguishable [148, 149].

In polar solvents both tautomeric forms are detectable by both UV and NMR spectrometries [150].

Compared with the oxygen analogues, the thioketones yielded a higher proportion of molecular ions, indicating the greater ability of the sulfur atom to stabilize the radical cation. Additionally, thioketones are of particular interest due to their tendency to shift the tautomeric equilibrium towards the enethiol form. The loss of SH was found to be a characteristic fragmentation. Additionally $(M-SH_2)^{+\cdot}$ can be assigned to the enethiol form while the fragment $(M-CHR^2R^3)^+$ can be assigned to the thioketo tautomer (Table 3) [104].

Table 1: Relevant mass spectral data for selected ketones regarding tautomerism occurrence

Ketone	$[M^+]$	$[(M-OH)^+]$	$[(M-R)^+]$[a]	$\frac{[(M-R)^+]}{[M-OH)^+]}$
1- Acetophenone	3334	10	10000	1000
2- Methylnaphtylketone	4885	43	10000	233
3- *m*-Aminoacetophenone	9542	45	10000	222
4- *o*-Methylacetophenone	3403	80	10000	125
5- Methyl-*iso*-butylketone	1350	83	10000	120
6- Benzylmethylketone	2300	100	10000	100
7- 3-Phenyl-2-butanone	10000	152	9863	65
8- *Iso*-propylphenylketone	741	15	10000	667
9- *Iso*-propylnaphtylketone	1435	303	10000	33
10- Naphtyl 1-*p*-tolylethylketone	190	10000	-	0
11- 4-Phenyl-3-buten-2-one	5346	40	10000	250
12- 2-Methyl-5-phenyl-4-penten-3-one	893	45	10000	222
13- 3-(Phenylmethylene)-2-pentanone	10000	90	6130	68

[a]R is the radical moiety that participates in the enolization process next to the carbonyl group which is CHR^2R^3 in equation 10

Table 2: Keto-enol heats of formation difference (kcal/mol) by AM1 calculation and approximate equilibrium constant values for the enolization reaction (AM1 calculation)

Ketone	Neutral molecule	Radical cation	Keq
1- Acetophenone	10.44	-16.65	$2.33x10^{-8}$
2- Methylnaphtylketone	8.85	-0.99	$3.38x10^{-7}$
3- *m*-Aminoacetophenone	7.71	4.24	$2.32x10^{-6}$
4- *o*-Methylacetophenone	6.87	-12.97	$9.49x10^{-6}$
5- Methyl-*iso*-butylketone	6.87	-4.62	$9.53x10^{-6}$
6- Benzylmethylketone	5.04	-18.89	$2.08x10^{-4}$
7- 3-Phenyl-2-butanone	3.27	-17.99	$4.07x10^{-3}$
8- *Iso*-propylphenylketone	8.41	-16.65	$7.13x10^{-7}$
9- *Iso*-propylnaphtylketone	5.07	-11.39	$1.76x10^{-4}$
10- Naphtyl 1-*p*-tolylethylketone	1.74	-21.78	0.0534
11- 4-Phenyl-3-buten-2-one	10.03	-5.03	$4.64x10^{-8}$
12- 2-Methyl-5-phenyl-4-penten-3-one	4.25	-17.39	$7.80x10^{-4}$
13- 3-(Phenylmethylene)-2-pentanone	3.16	-17.02	$4.85x10^{-3}$

Table 3: Relevant mass spectral data for selected ketones and thioketones[a]

Compound	$M^{+\cdot}$	$(M-XH)^+$	$(M-XH_2)^{+\cdot}$	$(M-CHR^2R^3)^+$	$10^2(M-XH)^+/(M-CHR^2R^3)^+$
Propanone	162	8.5	3.8	500[b]	n.a. (1.7)[c]
Propanothione	218	46.5	13.1	376[b]	n.a. (12.4)
3-Methyl-2-butanone	133	-	-	613	0.0
3-Methyl-2-butanethione	145	71.5	10.7	117	61.1
2,4-Dimethyl-3-pentanone	42.5	-	-	376[b]	0.0
2,4-Dimethyl-3-pentanethione	140.0	87.7	2.8	57.1[b]	n.a. (153.6)
Methylphenylketone	95.1	1.5	-	324	0.5
Methylphenylthioketone	198	66.1	11.6	331	20.0
Methylnaphtylketone	109	2.1	-	227	0.9
Methylnaphtylthioketone	189	84.5	54.9	279	30.3

[a]The reported electron impact ion abundances were calculated according to the following ratio: (10^3 Ion Abundance)/(Total Ion Abundance)

[b]This fragment can be assigned to both the keto and the enol tautomer, so that the calculated ratio in the last column (between parenthesis) could be higher when substracting the enol contribution to this ion abundance

[c]n.a. stands for: not apply

Table 4: Heats of formation difference (kcal mol^{-1}) for the Enol-Ketone and Enethiol-Thioketone transformations by AM1 calculations

Compound	Neutral molecule	Radical cation
Propanone	11.21	-0.74
Propanothione	-1.47	-1.59
3-Methyl-2-butanone	11.86	-1.25
3-Methyl-2-butanothione	-2.76	-1.88
2,4-Dimethyl-3-pentanone	5.13	-30.82
2,4-Dimethyl-3-pentanothione	-7.68	-10.12
Phenylmethylketone	10.67	-1.52
Phenylmethylthioketone	-1.28	3.95
Methylnaphthylketone	5.13	-5.23
Methylnaphtylthioketone	-14.82	-11.53

The results coming from the molecular orbital theory application support the spectrometric data predictive value and the fact they correspond to the tautomeric equilibria between neutral species, so that subsequent ionization in the ion source does not affect the position of those equilibria (Table 4).

The results obtained by mass spectrometry when correlated with those from molecular orbitals for several compounds families demonstrated that mass spectrometry constitute an adequate tool for predicting tautomeric equilibrium shifts [95-104].

The predictive value of this methodology was not only supported by the influence of these compounds nature and size of the substitution on these equilibria but also by the good correlation found between the selected fragments abundances ratio and semiempirical calculation (AM1) of the corresponding heats of tautomerization for the neutral tautomers in equilibrium.

β-Diketones: Proton transfer and hydrogen bonding are two important behavioral aspects regarding structure and reactivity of simple compounds [151-153] and complex substances [154], from water to DNA. β-dicarbonylic compounds exhibit both features and they constitute one of the best examples of keto-enol tautomerism combining in many cases a slow proton transfer process and high concentration of the enol form which is stabilized by intramolecular hydrogen bonding. β-dicarbonyllic compounds have been a source of controversy for decades. Early this century the argument centered around the possibility of tautomerism and the extent to which enolization could occur [151-153].

Keto-enol tautomerism has been studied for many years by means of techniques such as bromine titration and infrared and ultraviolet spectroscopy. Nuclear magnetic resonance spectroscopy, like other spectroscopic methods, provides the opportunity of investigating the tautomeric equilibrium without affecting the position of the equilibrium itself [155].

Again there may be steric reasons for the percentage of the enol form varying according to the nature of the β-substituents, but the relationships are not very clear. Electron withdrawal is also a factor that must be taken into consideration and this favors the enol tautomer. β-substituents, which could enhance the delocalization around the enol ring, were noted also to give sharper signals in the ^{1}H NMR spectrum and this in turn was assumed as favoring a more symmetrical hydrogen bond. In particular, phenyl groups in β-positions should encourage centered hydrogen bonds. The effects of β-substituents are not only felt at the enol proton since the C-α proton's chemical shift is also sensitive to changes and this also can be attributed to anisotropy changes due to variations in the π system [156].

Relevant ^{1}H and ^{13}C NMR data have been reported with regard to tautomerism of β-diketones [157-163].

The hydrogen bonding of the *cis*-enol tautomers of β-diketones is rather strong (50-100 kJmol-1), not very short (2.45-2.55 Å), non-centered and non-linear. The proton of this OHO bond finds itself in a double minimum potential energy well. This hydrogen bond is the key factor in determining many of the chemical properties of β-dicarbonylic compounds including the keto-enol equilibrium [164].

In general Ke is much more sensitive to the nature of the β-substituent in case of β-diketones than the corresponding β-ketoesters or β-ketoamides [165].

The analysis of the mass spectra of selected β-diketones was carried out to predict the occurrence of tautomeric forms and correlate the results to theoretical reactivity studies [99]. The influence of substitution was discussed and found to be consistent with the expected electronic and steric effects (Table 5).

Acceptable correlation between the experimental data and theoretical results was found for the neutrals molecules (Table 6). Both methods provide a useful tool to study the enolization phenomena. Mass spectrometry and semiempirical theoretical calculations (AM1) constituted an appropriate way to analyze the enolization occurrence for the chosen set of β-diketones. It has been shown that the sensible employment of these two different methods provides a tool with predictive capability to study tautomerism for these compounds.

β-Ketoesters: The chemistry of enols has been subject of interest in recent years [166] particularly those derived from carboxylic acids, esters and amides [118].

Table 5: Relevant mass spectral data for selected diketones regarding tautomerism occurrence

β-Diketone	$[M^+]$	$[M\text{-}OH]^+$ (A)	$[M\text{-}H_2O^+]$	$[M\text{-}COR]^{+a}$	$[M\text{-}OH]^+/[M\text{-}COR]^+$	$[M\text{-}O]^+$ (B)	A/B
2,4-Pentanedione	78.5	0.43	0.43	467.0	$9.8x10^{-4}$		-
3-Methyl-2,4-pentanedione	28.5	0.57	-	467.1	0.0010		-
3-Ethyl-2,4-pentanedione	38.2	8.8	32.4	470.7	0.0200		-
3-Phenyl-2,4-pentanedione	149.6	9.2	3.7	184.5	0.0500		-
2,6-Dimethyl-3,5-heptanedione	53.9	0.5	-	227.2	0.0022		-
3,5-Dimethyl-2,4-hexanedione	3.3	10.1	1.1	218.8	0.0460		-
2-(1-oxoethyl) cyclopentanone	106.1	2.7	16.0	149.7		1.9	1.420
2-(1-oxoethyl) cyclohexanone	117.5	1.5	2.8	256.3		27.7	0.054
1,3-Cyclopentanedione	270.7	1.9	0.8		-	51.76	0.037
4,5-Dimethyl-1,3-cyclopentanedione	77.5	3.7	-			29.5	0.125
2,4-Dimethyl-1,3-cyclopentanedione	133.8	2.7	2.7			8.0	0.330
2-Butyl-1,3-cyiclopentanedione	60.5	6.1	-			8.1	0.770
4-*Iso*-propyl-1,3-cyclohexanedione	28.8	1.7	2.8			5.6	0.330
2-Methyl-1,3-cyclohexanedione	94.7	1.1	1.1			132.8	0.078
Bicycle [3.3.0] 2,8-octanedione	254.4	0.5	0.5			26.0	0.019
Bicycle [4.4.0] 2,10-decanedione	122.1	0.7	0.5			175.9	0.039
Bicycle [3.3.1] 2,8-nonanedione	131.5	1.0	4.4			16.9	0.059

[a] COR involves the smallest alkyl moiety

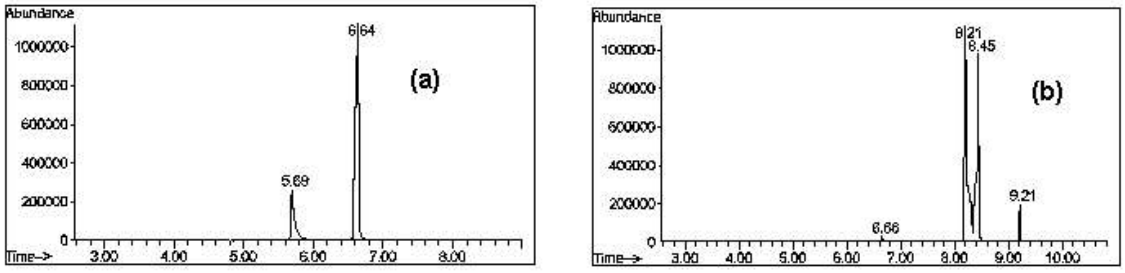


Fig. 43: Gas chromatograms of methylacetoacetate (a) and α-chloromethylacetoacetate (b)

Recently the enol of a carboxylic acid has been isolated (and its structure established) when complexed to an (ethane-1,2-diamine)-platinum(II) fragment [167]. O'Neill and Hegarty have synthesized and characterized enols derived from carboxylic acids and esters, using the Fuson method (developed for ketones) based on the destabilization of the keto form (relative to the enol) by the use of very large substituent groups as for example C_6Me_5 [130].

A series of β-ketoesters: ethylacetoacetate, methylacetoacetate, α-chloroethylacetoacetate and α-chloromethylacetoacetate, was selected for carry out chromatographic and mass spectrometric determinations. Additionally, the feasibility of the gas chromatographic separation of the corresponding tautomer forms was examined and confirmed by the analysis of the corresponding mass spectra (Fig. 43-45 for methylacetoacetate and α-chloromethylacetoacetate). Mass spectrometric detection allowed identification of both keto and enol forms and an estimation of their relative amounts assuming that they exhibit similar response factors (Table 7) [168]. The capability of separating tautomers by GC-MS is feasible in case the usually unstable enols exhibit structural features, like intramolecular hydrogen bonding that would favor the enol occurrence. Electronic and steric effects are consistent with these findings.

Thioesters: Among carbonyl compounds esters are not usually involved in tautomeric equilibrium with the exception of β-ketoesters and thioesters.

Enolization *vs* thioenolization of thioesters and thioxoketones has been studied and the effect of the heteroatom has been demonstrated [169-175]. In contrast to thiones and 1,3-dithiodicarbonyl compounds, simple thionoesters and dithioesters are stable in the thione form.

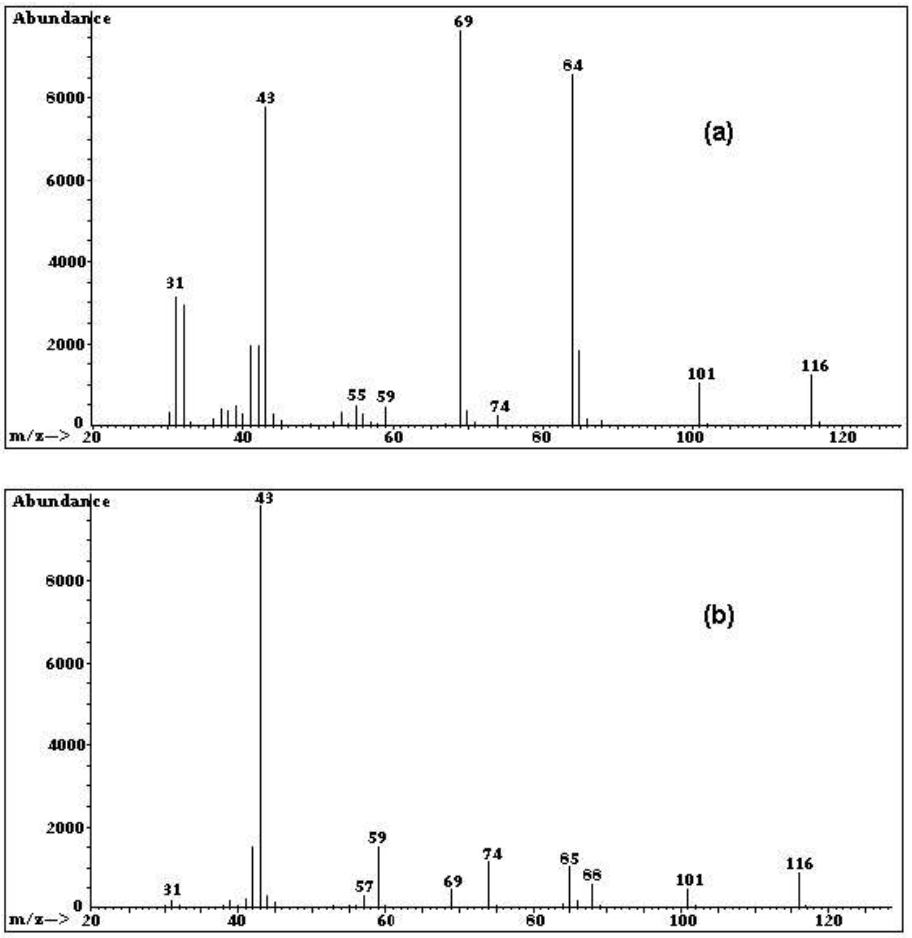


Fig. 44: Mass spectra of methylacetoacetate, enol tautomer (a) and keto tautomer (b)

Table 6: Keto-Enol heats of formation difference (kcal/mol) by AM1

β-Diketone	Neutral molecule[a]	Radical cation[a]
2,4-Pentanedione	-1.5849	36.1294
3-Methyl-2,4-pentanedione	-3.9665	27.2574
3-Ethyl-2,4-pentanedione	-10.8649	7.7339
3-Phenyl-2,4-pentanedione	-17.2963	4.3161
2,6-Dimethyl-3,5-heptanedione	-4.8735	12.7057
3,5-Dimethyl-2,4-hexanedione	-8.3207	26.0732
2-(1-oxoethyl) cyclopentanone	-4.2944	31.9871
2-(1-oxoethyl) cyclohexanone	-4.6139	30.6431
1,3-Cyclopentanedione	-1.3153	18.2197
4,5-Dimethyl-1,3-cyclopentanedione	-1.7795	4.9524
2,4-Dimethyl-1,3-cyclopentanedione	-6.8804	20.599
2-Butyl-1,3-cyclopentanedione	-8.5898	4.2339
4-*Iso*-propyl-1,3-cyclohexanedione	-5.6304	14.5712
2-Methyl-1,3-cyclohexanedione	-6.945	29.9344
Bicycle [3.3.0] 2,8-octanedione	-4.185	28.4801
Bicycle [4.4.0] 2,10-decanedione	-3.8224	22.3279
Bicycle [3.3.1] 2,8-nonanedione	-8.3282	20.9095

[a]For the calculation of the energy differences the most stable enol tautomer was considered in all cases

Table 7: GC/MSD retention times and area percent values for the selected acetoacetate tautomers

	Enol		Keto	
Compound	Rt	Area (%)	Rt	Area (%)
Ethylacetoacetate	6.46	15	7.54	85
α-Chloroethylacetoacetate	8.57	30	8.35	70
Methylacetoacetate	5.69	19	6.64	81
α-Chloromethylacetoacetate	8.45	45	8.21	55

Thionocarbonic esters and dithiocarbonic esters are easily available [176-181] and they are of special interest because the ester group reacts under very mild conditions and often without addition of a catalyst, giving condensation reactions or thioacylations.

In a general study of β-ketodithio acids by NMR and IR spectroscopy [182, 183], a real keto-enol equilibrium was found just as in the oxygen series.

Diphenylthioacetic acid esters have been described as enethiols with no tautomeric equilibrium being detectable, although rearrangement to the thione form occurs in non-polar solvents [184, 185].

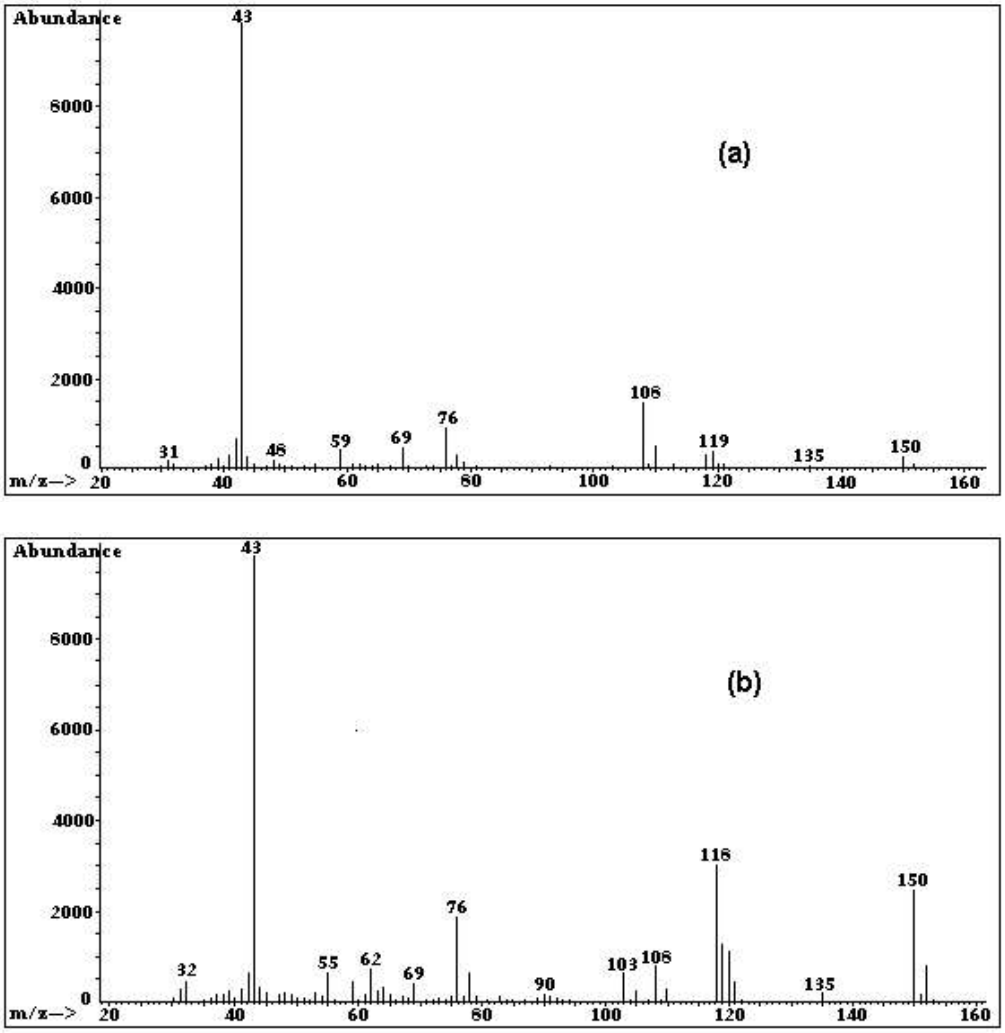
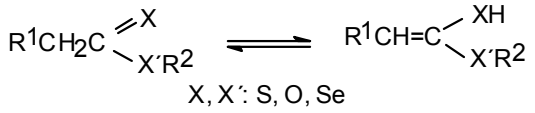


Fig. 45: Mass spectra of a-chloromethylacetate, enol tautomer (a) and keto Tuatomer (b)

Due to the possibility of existence in both tautomeric forms, that is, as thione or thiol, mercaptothiophenes and related compounds are of special interest in this connection, too. A thorough investigation of their NMR and IR spectra has shown that these compounds exist solely in the thiol form, where the thiolene-2-ones exist exclusively in the ketone form [186-189].

Mass spectrometry was used to examine the occurrence of tautomerization for selected esters and their sulfur analogues [102] (Eq. 11).



(11)

It was shown that mass spectrometry data is helpful in demonstrating the occurrence of the enol structure for thioesters, particularly significant for the dithio-analogues.

Table 8 shows the most relevant mass spectral data for selected thioesters.

To weigh the occurrence of both tautomers in the equilibrium (Eq. 11), suitable fragmentations have been assigned to the keto and enol forms. The loss of XH from the molecular ion could be assigned to the enol form and the CH_2R^1 radical loss to the keto form.

Despite the fact that the analyses have been carried out by GC-MS, no chromatographic separation has been observed so that the mass spectra are the result of the mix of both tautomers. Contrarily, previous work reported chromatographic separation of the tautomeric forms for β-ketoesters [168].

Esters are rather unique in regard to their mass spectra and the use of the mass spectrometry data to study their tautomeric equilibria. Ion

Table 8: Relevant mass spectral data for selected esters and thioesters

Compound	$M^{+\cdot}$	$(M-XH)^+$	$(M- CH_2R^1)^+$	$(M-XH)^+/(M-CH_2R^1)^+$
Ethylacetate	16.70	-	17.60	0.00
Ethyldithioacetate	109.00	1.1	0.98	1.10
Allylacetate	0.49	-	0.98	0.00
Allyldithioacetate	56.80	17.0	118.00	0.14
Allylpropanoate	1.10	-	3.20	0
Allyldithiopropanoate	58.40	9.8	-	-
1-Methylallyldithiopropanoate	98.40	14.6	73.0	0.20
3-Methylallyldithiopropanoate	92.70	12.0	60.0	0.20
Ethylphenylacetate	192.00	-	162.0	0
O-Ethylphenylthioacetate	229.00	1.8	1.3	1.40
Propylpropanoate	-	-	45.9	0
O-Propylthiopropanoate	-	1.6	3.0	0.40
Methylbutanoate	6.90	-	43.1	0
Methyldithiobutanoate	113.00	1.1	0.4	2.75
Methylpentanoate	0.80	-	56.9	0.00
O-Methylthiopentanoate	48.60	54.3	42.9	1.30
Ethylbutanoate	5.46	-	33.8	0
O-Ethylselenobutanoate	85.50	13.2	-	>>
Ethyl-4-methylpentanoate	0.30	-	17.9	0
O-Ethyl-4-methylselenopentanoate	82.30	14.5	-	>>
Ethylhexanoate	2.80	-	31.2	0
O-Ethylselenohexanoate	21.40	17.8	-	>>

Table 9: Heats of formation difference (kcal mol^{-1}) between the tautomeric forms of esters by AM1 calculations

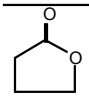
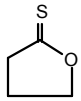
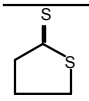
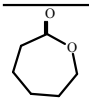
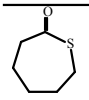
	Neutral molecule		Radical cation	
Compound	Z isomer	Eisomer	Z isomer	E isomer
Ethylacetate	27.10	-22.50		
Ethyldithioacetate	2.50	0.64		
Allyldithioacetate	12.50	-3.90		
Allyldithiopropanoate	8.80	8.20	-26.30	-25.00
O-Ethylphenylthioacetate	0.50	0.30	14.80	15.10
O-Propylthiopropanoate	2.10	2.30	-0.84	-13.60
Methyldithiobutanoate	-1.60	-1.50	1.60	1.40
O-Methylthiopentanoate	-1.05	0.90	-37.20	-38.50
Ethylbutanoate	14.60	12.70	-53.50	-55.60
O-Ethylselenobutanoate	-5.73	-3.84	1.91	3.26
O-Ethyl-4-methylselenopentanoate	-7.50	-5.70	9.50	13.60
O-Ethylselenohexanoate	-14.40	-14.10	-7.40	-0.50

assignments may not be specific to particular tautomers, however, by analyzing Table 8 the enol occurrence is null for oxygenated esters and quite significant for the selected thioesters, particularly with the second exchange in heteroatom (dithio-compounds). When the heteroatom is selenium a strong equilibrium shift towards the enol tautomer is observed. The ease of enol formation increases in the order thiocarbonylesters < dithioesters < selenocarbonylesters and it is consistent with the size of R^1.

AM1 semiempirical molecular orbitals calculations for the neutral molecules are also consistent with the experimental results [102] (Table 9). Additionally, information about Z/E enol isomers stability is provided.

There is a remarkable tendency towards enolic structures formation when sulfur replaces oxygen in the esters reported. This behavior is evidenced not only by mass spectra but also by theoretical calculations and the mutual correlation. Once against, these results constitute an evidence of the feasibility of tautomerism evaluation by mass spectrometry since it seems that 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-ionic species can differ sensibly from the neutral analogues.

Table 10: Relevant Mass Spectral Data for Selected Lactones and Thiolactones[a]

Compound	M^+	$(M-XH)^+$	$(M-CX)^+$	$(M-CX_2)^+$
	186.3	-	-	376.4
	780.0	-	11.6	-
	430.5	13.7	15.5	-
	331.1	48.4	14.5	-
	233.3	0.5	-	249.8
	432.5	0.4	-	-
	370.5	24.6	12.8	-
	218.8	58.6	8.8	5.5

[a]The reported electron impact ion abundances were calculated according to the following ratio: 1000xIon abundance/Σ Ion abundances

Lactones: There are few studies about lactones tautomerism. These family of compounds exhibit great biological interest. In this sense biological activity has been assayed by carrying out studies on substituted heterocyclic rings [190].

In addition, the tautomeric equilibrium of lactones has been studied and evidence of the enol tautomer occurrence has been found by ^{1}H NMR and ^{13}C NMR [191].

Ab-initio calculations provide a reasonable correlation for the tautomerization energy estimation [121-125]. However these calculations are expensive and hard to apply to most of the molecules of real interest, particularly in the biochemical field. Alternatively it is possible to use semiempyrical calculations assuming errors of some kcal/mol for the tautomerization energy values [192].

The enolization degree of lactones and esters is favored by the oxygen-sulfur exchange in the respective functional groups [101]. The analysis of the corresponding mass spectra has allowed unambiguous assignment of some fragments to specific tautomers (Table 10 and 11). Heteroatom and ring size effects are observed.

Once again, AM1 theoretical calculations (Table 12) are consistent with the mass spectral data and support the fact that the equilibria being measured involve the neutral molecules with a minimum impact (if any) of tautomerization between ionized species (radical cations) [101].

Nitrogenated heterocycles

Hydantoins: The hydantoins (Fig. 46) are compounds of considerable practical interest whose tautomeric structures are, as expected, highly dependent on their substitution. In fact, these compounds reactivity is mainly determined by the stability of the corresponding tautomers (8 tautomers for hydantoin).

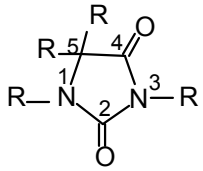
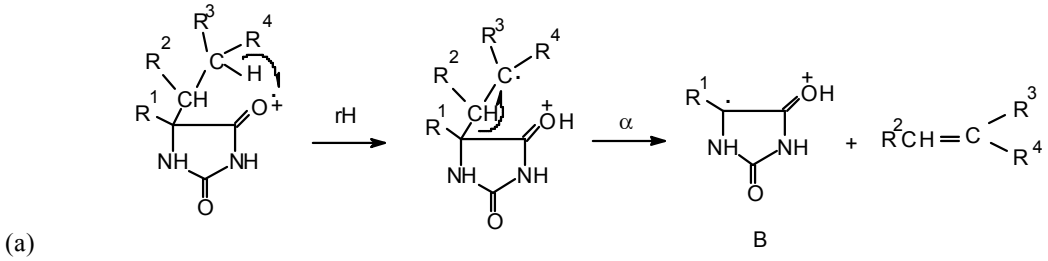


Fig. 46: Structure of hydantoins

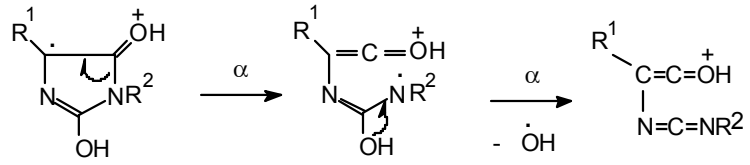
Table 11: Relevant mass spectral data for selected esters and thioesters[a]

Compound	M^+	$(M-XH)^+$	$(M-R)^+$	$(McLafferty)^+$
$CH_3CH_2CH_2COOCH_3$	6.9	-	43.1	184.9
$CH_3CH_2CH_2CSSCH_3$	113.0	1.1	39.2	10.3 [b]
$CH_3CH_2COOCH_2CH=CH_2$	0.5	-	3.2	[c]
$CH_3CH_2CSSCH_2CH=CH_2$	58.4	9.8	-	Not possible

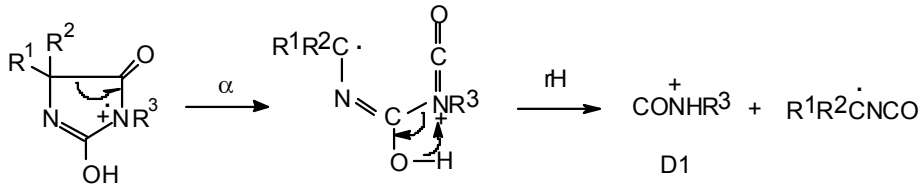
[a]The reported figures (mass spectra from Nist´98 mass spectral database) were calculated according to the following ratio (ion abundance) x 1000/(total ion abundance), [b]Not possible or neglectable due to allylic hydrogen involvement



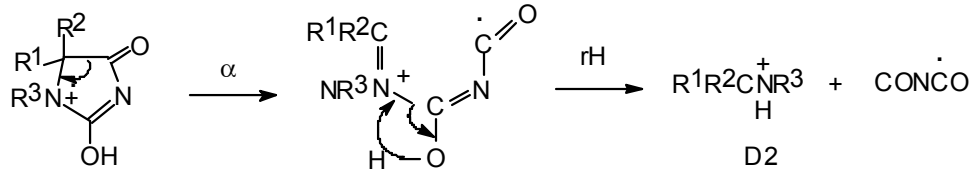
(a)



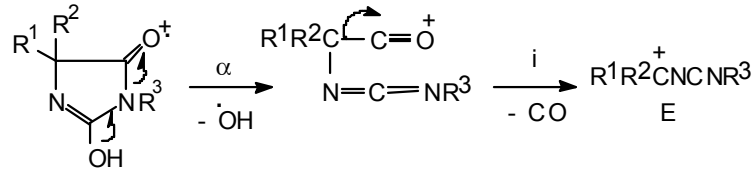
(b)



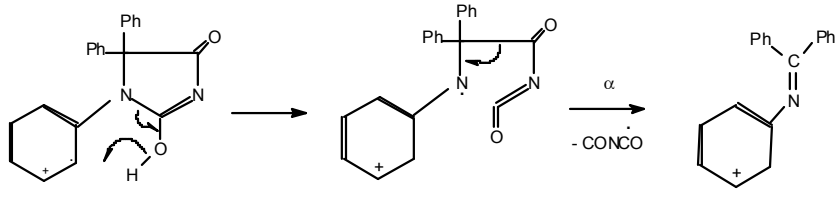
(c)



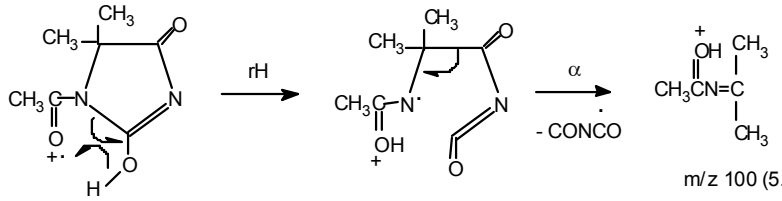
(d)



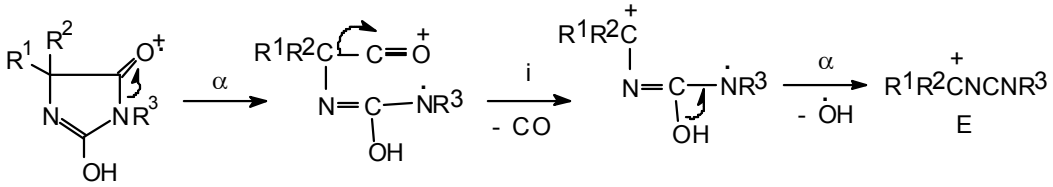
(e)



(f)



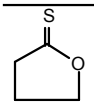
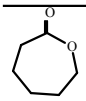
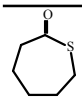
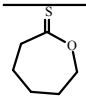
(g)



(h)

Scheme 12

Table 12: Keto-Enol heats of formation difference (kcal mol^{-1}) by AM1 calculations

Compound	Neutral molecule	Radical cation
	22.86	-29.98
	12.87	-13.37
	5.02	-32.98
	1.97	0.41
	23.06	-34.78
	9.09	-12.42
	-1.68	-8.63
	-1.91	-0.71
$CH_3CH_2CH_2COOCH_3$	23.98	$2.5x10^{-3}$
$CH_3CH_2CH_2CSSCH_3$	1.98	-1.83
$CH_3CH_2COOCH_2CH=CH_2$	24.36	-16.04
$CH_3CH_2CSSCH_2CH=CH_2$	2.10	-0.84

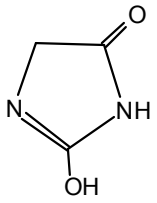


Fig. 47: Structure of the preferred enolic form of hydantoin

Tautomerism in hydantoin derivatives has been widely discussed but there is little experimental evidence concerning the existence of specific tautomeric forms [65, 193] except for the keto form which seems to be dominant for the pure compounds.

The analysis of the mass spectra of some substituted hydantoins was carried out to predict the occurrence of tautomeric forms (Table 13) [96].

Substitution is relevant to tautomerism. It is shown that the C-2 enol is the preferred enolic form (Fig. 47).

For all the N-1 non substituted hydantoins enolization occurs with the corresponding hydrogen. Otherwise enolization involves the hydrogen of N-3.

Furthemore, molecular orbital calculations have been made using the semiempirical AM1 method [110] in looking for additional support regarding hydantoins tautomerism. Theoretical calculations also support the C-2 enol structure and disregard tautomerization between ionic species inside the mass spectrometer.

3-(2H)-isoquinolones: The understanding of the nature of tautomeric equilibria is relevant to the study of processes of both organic chemistry and biochemistry. Extensive studies of the lactam-lactime tautomerism have established that usually the lactam (or carbonyl) tautomer greatly predominates [194]. A number of 3-isoquinolinol derivatives have been shown to be active in the central nervious system [195].

Baumgarten *et al*. [196] based on the pka and IR data of non substituted isoquinolone (solid phase and chloroform solution) concluded that the lactam tautomer is the most stable one.

The conclusions were based largely on spectrophotometric studies, evidences that are supported by IR, NMR, X-ray crystallography and theoretical calculations. In the course of synthetic work related to 1-benzyl-3(2H)-isoquinolinone it appeared that four prototropic isomers were possible [197] demonstrating the complexity of the problem.

The study of the UV spectra of some 3(2H)-isoquinolinones substituted in position 1 with aryl and aralkyl groups in different solvents has been carried out [198].

It has been found that, for example, the 1-β-naphtyl derivative exists exclusively as the lactim tautomer in diethyl ether, carbon tetrachloride and sulfuric acid. On the other hand, this form predominates in benzene, ethanol and chloroform. In the case of the 4-chloro-1-phenyl derivative, the data also supported the lactim structure [199]. The UV spectrum in ethanol showed two maxima at 344 nm (lactim form), whereas in ether only the lactim absorption at ? 345 nm could be observed. This type lactam-lactim tautomerism depends both on the substituents and on the solvent and shows considerable variation [200]. Thus, the compounds can be characterized by their UV spectra or occasionally IR spectra in carbon tetrachloride.

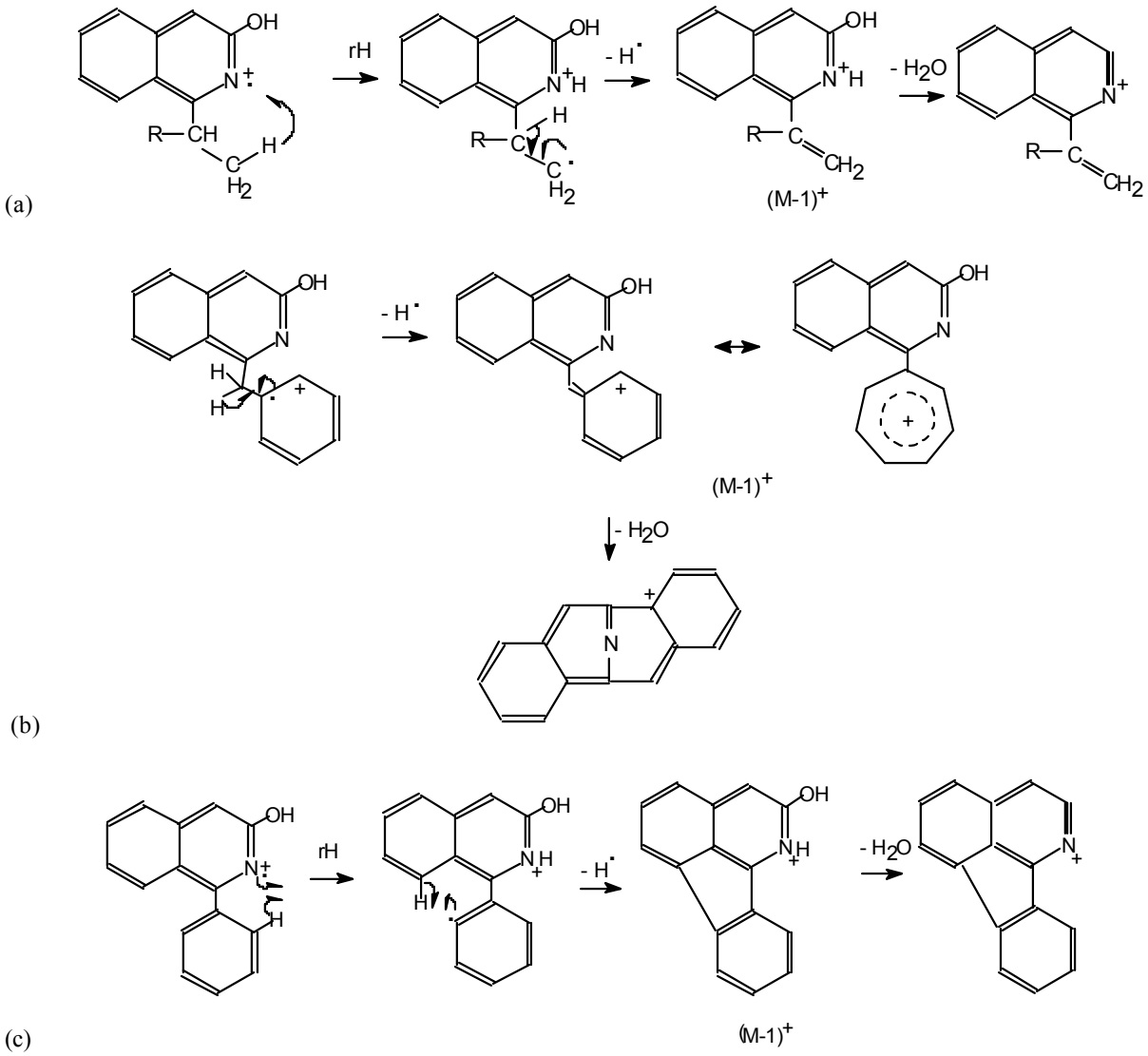
The tautomeric equilibrium isoquinolinone-isoquinolinol was studied experimentally by MS and theoretical semiempirical calculations [97].

The analysis of the mass spectral data of the selected isoquinolinones (Table 14) allows to assign the

Table 13: Percent relative abundances of relevant mass spectral peaks

Hydantoin	$M^{+\cdot}$	A $(M-CO)^{+\cdot}$	B McLaff.	C $(B-OH)^{+}$	E $(A-OH)^{+}$	$(M-OH)^{+}$	(rearr. on N) D1	(rearr. on N) D2
5-phenyl	93	11	n.a.[a]	n.a.			9	3
5-methyl	88	77	n.a.	n.a.			28	28
5-*iso*-propyl	1	1	100	2			28	7.7
3-methyl	100	15	n.a.	n.a.			77	n.a.
3-*n*-butyl	27	1	3			3	23	n.a.
3-*iso*-propyl	48	4	9.5	1[b]	4		4	n.a
3-*n*-hexadecyl	73		10	4[b]		7	11	n.a
1-methyl	100	6	n.a.	n.a.			n.a	35
1-*n*-butyl	34		1				n.a	10
1-carboxamide-N(3,5-dichlorophenyl) -3-*iso*-propyl	7		8	n.a.	n.a.		n.a.	
1-phenylcarbamoyl-3-phenyl	11		n.a.	n.a.	n.a.		n.a.	
5,5-diphenyl	27	37	n.a.	n.a.			1	
5-ethyl-5-methyl	1	71[c]	71[c]	1	21		6	
5-methyl-5-*n*-pentyl			100[c]	4.7			6	100[c]
5-methyl-5-*n*-undecyl	1.5		87	3	1.5	1	1	
5-methyl-5-*iso*-propyl			100	1			9	1
5,5-dimethyl	6.5	3	n.a.	n.a.			19	
5-methyl-5-*ter*-butyl			100	2.2			10	
5,5-diethyl	1	13[c]	13[c]	1	1		13	
3,5-dimethyl	96	44	n.a.	n.a.	2	1	100	n.a
3,5-diethyl	1	25[c]	25[c]				n.a	
3-*n*-pentyl-5,5-diphenyl	8	5.5	5.5				2	n.a
3,5,5-trimethyl	24	4	n.a.	n.a.			n.a	
3-*n*-pentyl-5,5-dimethyl			3				22	n.a
3-methyl-5-ethyl-5-phenyl	3	12	12				49	n.a
3-*p*-nitrobenzyl-5-methyl-5-phenyl	14	3	n.a.	n.a.	1	1	n.a	
1-butyl-5-methyl-5-*n*-pentyl	1		11[d]	1			n.a	1
1-phenethyl-5-methyl-5-*n*-pentyl	2		1				n.a.	100
1,5,5-trimethyl	20		n.a.	n.a.			n.a.	4
1,5-dimethyl-5-ethyl	5	4	4				n.a.	0.5
1-methyl-5,5-diphenyl	27	4	n.a.	n.a.			n.a.	9
1-benzyl-5-methyl-5-phenyl	35	1	n.a.	n.a.	1[e]		n.a.	11
1,5,5-triphenyl[f]	100	3	11	n.a.			n.a.	11
1-acetyl-5,5-dimethyl[g]	12	4	5.5	n.a.			n.a.	5.5
1-benzyl-5-*n*-pentyl-5-methyl	3		1				n.a.	1
1-cyano-3,5-dimethyl-5-phenyl	31		n.a.	n.a.	n.a.	n.a.	n.a.	
3-cyano-1,5-dimethyl-5-phenyl	12	6	n.a.	n.a.	n.a.	n.a.	n.a.	
1-*iso*-propyl-3-methyl-5,5-diphenyl	11		[h]	n.a.	n.a.	n.a.	n.a.	
1-allyl-5-phenyl-5-methyl-3-*p*-nitrobenzyl	3	0.5	[i]	n.a.	n.a.	n.a.	n.a.	

[a] Do not apply, [b] This ion would correspond to the hydroxyl loss from the McLafferty rearrangement fragment, but would not follow the pathway indicated in Scheme 12 (b), [c] There is more than one possibility to render the same m/z, [d]The McLafferty rearrangement involves the *n*-pentyl moiety, [e] This ion would correspond to the hydroxyl loss from the (M-CO) fragment but would not follow the pathways indicated in Scheme 12 (e) or (f), [f] A McLafferty rearrangement depicted in Scheme 12 (g) with a 11% relative abundance could be proposed. The fragment D2 has the same m/z 258 and also supports the same enol structure, [g] For this compound a McLafferty rearrangement can be proposed according to Scheme 12 (h) involving the hydroxyl hydrogen (5.5%) although the fragment D2 from Scheme 12 (d) has the same m/z 100, [h]The *iso*-propyl group does not seem to comply with the proper steric requirement for this fragmentation pathway, [i]The McLafferty rearrangement does not seem to occur due to the extremely tight electrons in the allylic C-H bond



Scheme 13

loss of 28 amu $(M\text{-}CO)^{+\cdot}$ to the lactam structure (except for the mass spectrum of ethylisoquinolinone which can generate the ion $(M\text{-}C_2H_4)^{+\cdot}$ after the McLafferty rearrangement) and the loss of 19 amu to the lactim structure (Scheme 13).

Table 15 shows the difference of heats of formation between the lactam and lactim forms considering the neutral molecules and the corresponding radical cations for the selected isoquinolinones. A reasonable good correlation with the mass spectra observations is achieved only in the case of the neutral molecules.

The application of mass spectrometry techniques together with semiempirical theoretical calculations gave a suitable way to analyze the tautomerism occurrence for the chosen set of isoquinolinones.

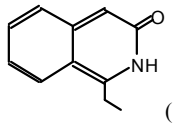
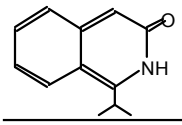
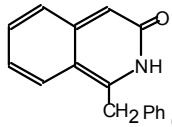
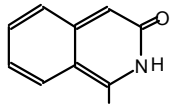
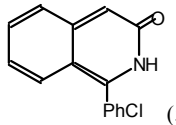
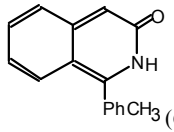
Amides and related compounds

Amides and thioamides: Tautomerism of amides and related compounds has been the focus of several investigations with almost inconclusive results [130, 201].

The solvent effect on the optical rotation of certain amides has been interpreted in terms of the amido-imidol equilibrium [202].

The existence of two crystalline forms of optically active amides was considered to be an experimental evidence although it can be assigned to rotation hindrance and complexation [203]. These equilibria have been studied by a variety of spectroscopic techniques including ^{1}H, ^{13}C, ^{15}N NMR. In general the results were disappointing.

Table 14: Relevant mass spectral data for selected isoquinolinones regarding their tautomerism[a]

Isoquinolinone	[M+·]	[(M-1)+]	[(M-19)+]	[(M-CO)+]	Other peaks	[(M-28)+·]/[(M-19)+]
 (1)	100	33.6	8.6	15.7	[(M-COHN)+]=90	1.82
 (2)	100	36.2	3.6	12.9	[(M-OH)+] = 2.8 [(M-CH3)+]=42 [(M-COHN)+]=32	3.6
 (3)	100	95	8.6	7.1		0.82
 (4)	100	11.4	7.1	39.4		5.5
 (5)	100	6.6	4.7	49.3		10.5
 (6)	100	9.2	3.7	55		14.9

[a]Percent relative abundance data

Table 15: Lactam-Lactim heats of formation difference (kcal/mol) by AM1 calculation

Isoquinolinone	[(M-28)+]/[(M-19)+]	Neutral molecule	Radical cation
(1)	1.82	5.42	-10.43
(2)	3.60	4.90	-11.62
(3)	0.82	5.85	-10.65
(4)	5.50	4.71	6.89

As mentioned before, it is interesting to note that thio-compounds have a relatively higher tendency for the formation of the corresponding imidols. IR spectra of thioamides exhibit typical bond stretching frequencies for C=N and C-S [204].

Regarding the mass spectrometric studies for amides and thioamides, mention t the equilibrium displacement towards the thioimidol form has been made [15].

It is possible to assign certain fragmentations to both tautomers and thus learn their origin, from the molecule or the molecular ion, by examining the temperature effect on the GC-MS system (injection port and ion source temperatures) [5].

Acidity measuraments and oxidation potentials of carboxamides, thiocarboxamides and their conjugated bases indicate that the thio group donates a proton, a hydrogen or an electron more easily than the oxygenated groups, which is associated with the higher ability of sulfur vs oxygen to stabilize the anion,, the radical or the radical cation respectively [205].

These evidences are consistent with the higher lability of the CS vs CO double bonds.

The application of mass spectrometry techniques together with semiempirical theoretical calculations has been proved to be a suitable way to analyze the imidolization mechanism for a chosen set of amides and thioamides (Table 16 and 17) [98].

The temperature effect on equilibria can be used as a methodology for experimental determination of heats of reaction. In order to try to ponderate the use of mass spectrometry as a adequate analytical tool for the study of rapid equilibria, as it is the case of tautomerization, the influence of the sample introduction system temperature was studied in the experimental set up not only for the estimation of the heat of tautomerization but also to prove that enolization takes place before ionization with almost no occurrence between ionic species in the ion source [5].

In order to determine the heat of tautomerization from mass spectral data it is necessary:

Table 16: Relevant mass spectral data for amides and thioamides regarding tautomerism occurrence (Ion Abundancex1000/Total Ion Abundance)

Amide/Thioamide	M^+	$[M-OH/SH]^+$	$[M-H_2O/SH_2]^+$	$[M-NHY]^+$	Others	McLaff.+1	$[M-OH/SH]^+$ /$[M-NHY]^+$
Acetamide	31.4	8.40[a]	3.2[b]	17.8	-	-	-
Thioacetamide	32.9	19.10	6.2	6.7	2.6[c]	-	2.8
Benzanilide	10.1	-	-	39.1[d]	-	-	-
Thiobenzanilide	8.9	4.70	-	24.1[d]	-	-	>0.0
Benzamide	17.9	-	0.7[e]	22.2	-	-	-
Thiobenzamide	21.5	13.50	1.7	6.7	-	-	2.0
N-Cyclohexylbenzamide	7.1	-	-	28.5	-	20.2	-
N-Cyclohexyl-thiobenz	8.3	3.20	-	16.8	-	9.9	0.20
N-benzyl-benzamide	13.9	0.27	0.27	27.2	-	-	0.01
N-benzylthiobenz.	-	7.30	-	[f]	-	-	>>
N-*p*-tolylurea	2.3	-	20.6	0.21	20.6[g]	-	-
N-*p*-tolylthiourea	7.4	2.80	1.3	4.80	14.9[h]	-	0.60
N,N'-dimethylurea	42.7	-	-	-	3.0[i]	-	-
N,N'-dimethyl-thiourea	33.4	5.50	1.0	-	2.0[i]	-	>>
N-methyl-*p*-nitro-benzamide	7.7	3.70	-	17.5[j]	1.6[k]	-	>0
N-acetyl-*p*-nitroaniline	-	-	-	25.8	18.2[k]	-	
Acetanilide	11.0	-	-	12.2	-	-	-
Thioacetanilide	7.9	3.80	0.6	7.7	-	-	0.5

[a]For acetamide the loss of 17 amu is mainly due to the loss of NH_3 formed by rearrangement, [b]For small molecules unspecific rearrangements can occur and should not be taken into consideration as an evidence of the imidol structure, [c]Loss of ammonia is also observed for thioacetamide, [d]The $[M-NHY]^+$ can also be rationalised from the imidol structure by previous hydrogen rearrangement to the aniline ring, [e]For benzamide the loss of 18 amu is due to the formation of a stable nitrile (benzonitrile), [f]m/z 121 is not observed because the rearrangement in the thioimidol structure is not possible (see[d]), [g]The importance of the M-1 peak is due to the formation of a stable cyclic compound, [h]It corresponds to the $[M-CSNH_2]^+$ fragment ion, [i] It corresponds to the loss of 29 amu, [j]The fragment ion can also come from the loss of NO from the molecular ion, [k]McLafferty rearrangement

Table 17: Amido-imidol heats of formation difference (kcal/mol) by AM1 calculation

Compound	Neutral molecule	Radical cation
Acetamide	-3.90	-9.38
Thioacetamide	0.89	-34.80
Benzamide	-3.67	1.93
Thiobenzamide	0.56	3.93
Benzanilide	0.11	6.48
Thiobenzanilide	4.36	0.87
N-Benzylbenzamide	0.92	-5.38
N-Benzylthiobenzamide	5.11	-17.93
N-Cyclohexylbenzamide	-1.33	1.70
N-Cyclohexylthiobenzamide	2.93	-2.78
Acetanilide	-3.67	-1.31
Thioacetanilide	5.14	0.93
N-Methyl-*p*-nitrobenzamide	0.75	1.76
N-Methyl-*p*-nitrothiobenzamide	3.53	$6x10^{-5}$
N-Acetyl-*p*-nitroaniline	-0.72	-0.57
N-Thioacetyl-*p*-nitroaniline	4.53	$-5x10^{-6}$
Urea	-6.73	39.46
Thiourea	-4.08	20.55
N,N'-Dimethylurea	-6.40	8.90
N,N'-Dimethylthiourea	-2.50	-15.56
N-*p*-Tolylurea	-3.51	2.03
N-*p*-Tolylthiourea	-0.32	2.55

to assign fragment ions to specific tautomers to calculate imidol/amido selected fragments abundances ratios for different fragment pairs of the same thioamides (for validation purposes) to repeat these calculations for different sample introduction temperatures.

The use of different ionization electron energies provides additional support for this methodology. The modification of electron energies has impact on the relative abundances of the fragment ions although it should not modify the heat of reactions value. That was the reason for the introduction of this variable (Fig. 48, 49) [110].

Finally, since the enthalpy (or heat) of formation, can be estimated from semiempirical theoretical calculations, the finding of reasonably good correlations with the experimental data constitute an extra piece of support for the evaluation of mass spectrometry as a valuable tool for the study of tautomeric equilibria (Table 18).

Evidence that supports that transformations after ionization do not occur or are insignificant was found not only by the experimental spectrometric strategy but also by the theoretical methodology employed. A reasonable correlation was found between mass spectral

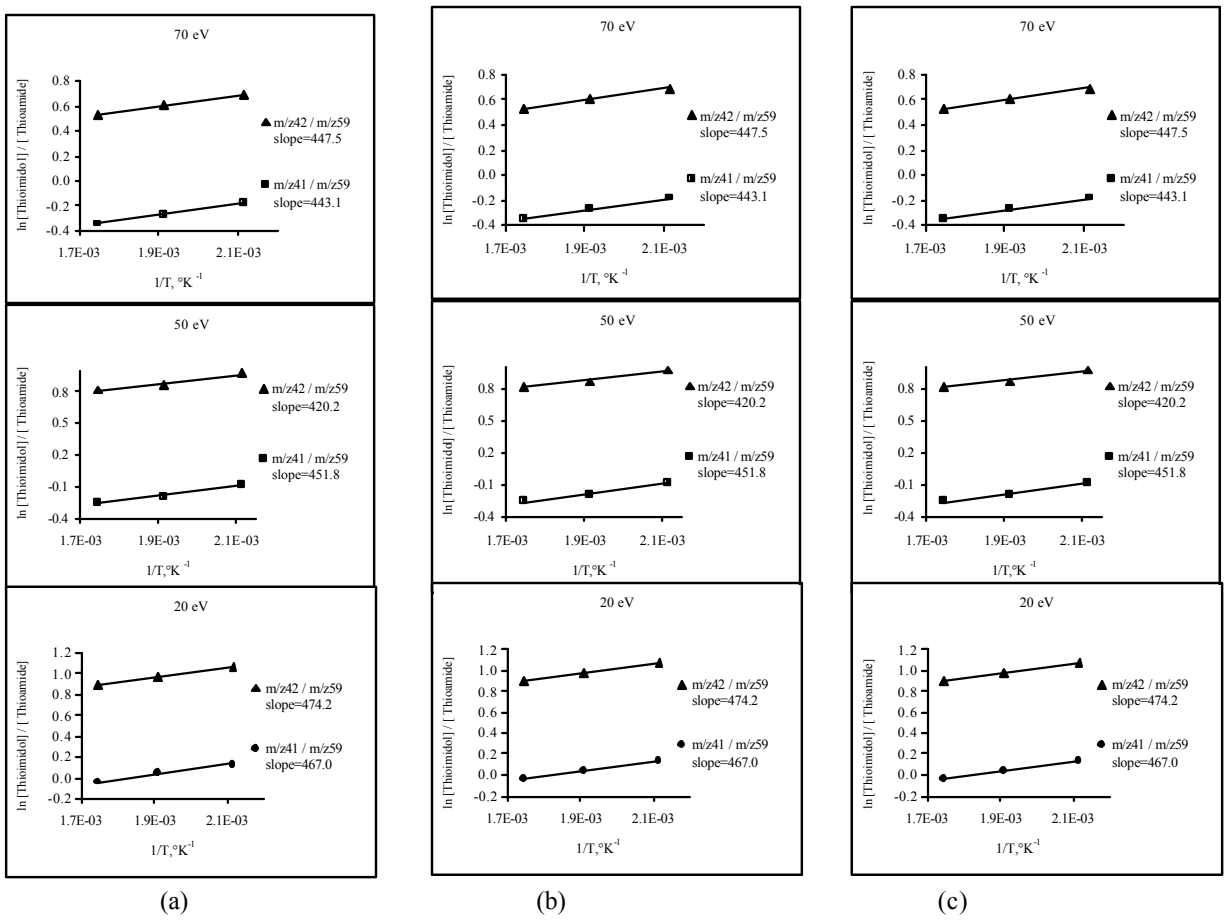


Fig. 48: Effects of the sample introduction system temperature and the electron beam energy on ion abundances ratios for thioacetamide (a), thiobenzanilide (b) and N-cyclohexylthiobenzamide (c)

Table 18: Heat of tautomerization values for thioamides

Compound	Calculated ? H (Kcal mol^{-1})	Experimental ? H (Kcal mol^{-1})
Thioacetamide	0.91	0.89±0.11
Thiobenzanilide	4.36	4.13±0.20
N-Cyclohexylthiobenzamide	2.72	2.61±0.20
Thioacetanilide	5.14	5.28±0.41
p-Nitrothioacetanilide	4.53	4.28±0.21

data and AM1 calculations of the neutral molecules while no correlation could be observed for the corresponding molecular ions.

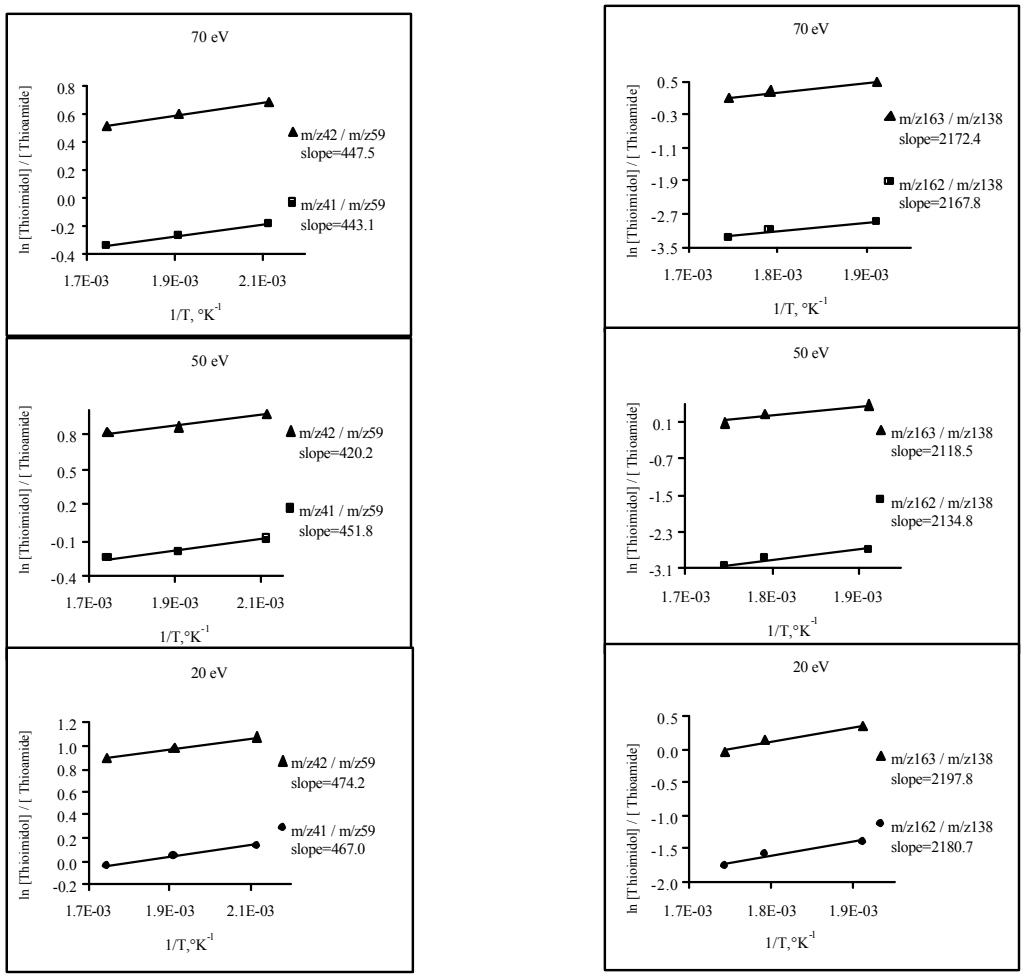
Ureas and thioureas: The occurrence of tautomerism of ureas and related compounds has been controversial. The IR spectrum of urea in polar solvents and the formation of o-alkyl derivatives were considered as experimental evidence of tautomerism [206].

Some authors proposed a zwitterion structures [207-210] but this concept has also found some

Table 19: Relevant mass spectral data for selected ureas[a]

Compound	$M^{+\cdot}$	$(M-XH)^+$	$(M-H_2X)^+$	Others
Urea	359.8	-	-	88.6[b] 316.6[c]
Thiourea	-	5.2	1.2	85.4[d]
Diethylurea	98.1	1.4	-	125.4[e]
Diethylthiourea	278.4	18.7	3.9	
Ethylenurea	43.7	19.9	-	58.4[f]
Ethylenthiourea	102.1	69.2	-	58.2[g]
Allylurea	0.064	0.8	-	213.7[h]
Allylthiourea	137.3	14.3	-	107.2[i]
Dicyclohexylurea	849.9	0.3	-	81.5[j]
Dicyclohexylthiourea	98.3	9.2	-	112.4[j]
Diphenylurea	57.0	-	1.6	406.6[k]
Diphenylthiourea	54.2	20.6	79.7	161.2[k]
Diphenylselenourea[l]	46.7	124.5	65.1	141.3[k]

[a]For a better correlation the reported data are calculated according to the following ratio: Ion abundance x 1000/∑ Abundances, [b]This comes from the loss of 17 amu which can be OH or NH_3 loss, [c]It corresponds to $(M-NH_2)^+$, [d]It corresponds to $(M-NH_3)^+$, [e]Fragment ion from McLafferty rearrangement, [f]It corresponds to $(M-CO)^+$, m/z 70, assignable to the amido form, [g]It corresponds to $(M-CS)^+$, assignable to the amido form, [h]It corresponds to the amido form: $(M-NH_2-CO)^+$, m/z 56, [i]It corresponds to the amido form: $(M-NH_2-SC)^+$, m/z 56, [j]m/z 98, $(C_6H_{12}N)^+$, [k]m/z 93, $(PhNH_2)^+$, [l]Data obtained from NIST`98 mass spectral database



(a) (b)

Fig. 49: Effects of the sample introduction system temperature and the electron beam energy on ion abundances ratios for thioacetanilide (a) and *p*-nitrothioacetanilide (b)

criticism [211, 212]. However, S-alkyl derivatives of isothiourea (thiomidol form) could be prepared by the action of alkylation agents on thiourea in polar solvents [213].

Thiourea is one of the simplest organic molecules containing a thioamide group and its structure and properties have been studied extensively by various experimental and theoretical techniques [214-216].

In biochemistry, considerable interest has been focused on the role of the thioamide group, as it is a fundamental building block in the skeleton of thiopurines and thiopyrimidines [217, 218].

The results of the study for this and other allied compounds at room temperature in aqueous solutions suggest their existence in two tautomeric forms in equilibrium [219].

For urea, thiourea and derivatives in sulfuric acid, IR, UV and NMR data indicate the presence not only of N-protonated but also of O-and S-protonated species [220-223].

Acidity measurements and oxidation potentials of carboxamides, thiocarboxamides and their conjugated bases indicate that the thio group donates a proton, a hydrogen or an electron more easily than the oxygenated group, which is associated to the higher ability of sulfur *vs* oxygen to stabilise the anion, the radical or the radical cation respectively [206]. These evidences are consistent with the higher lability of the CS *vs* CO double bonds.

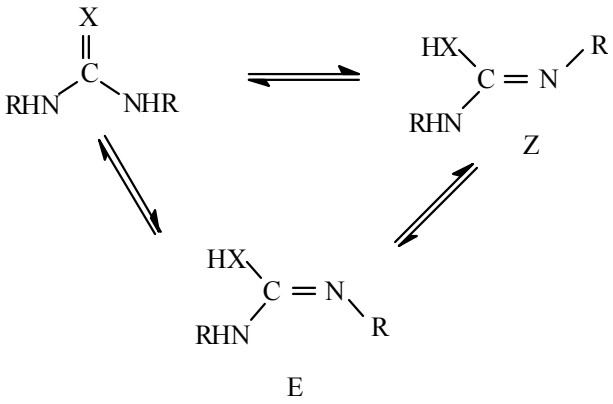
Mass spectra of some ureas and thioureas were studied to analyze the predictive power of this technique for the occurrence of tautomerism with the additional support of molecular orbital calculations (Table 19 and 20) [103]. Since GC-MS instrumentation was employed, chromatographic separation of amide tautomers was not attained. In all these cases it is not

Table 20: Thioamido-thioimidol heats of formation difference (kcal/mol) by AM1 calculations

Compound	Neutral molecule			Radical cation	
Thiourea	Z	11.76		Z	19.64
	E	11.04		E	15.71
Diethylthiourea	Z	9.99		Z	10.13
	E	4.92		E	16.12
Ethylenthiourea	4.56			-16.46	
Allylthiourea	H_2N-CSH=NR	Z	5.02	Z	7.73
		E	6.02	E	9.39
	HN=CSH-NHR	Z	11.92	Z	21.67
		E	8.32	E	17.14
Dicyclohexylthiourea	Z	7.21		Z	19.18
	E	9.18		E	11.54
Diphenylthiourea	Z	-1.66		Z	6.28
	E	1.36		E	10.35

Table 21: Heats of formation difference (kcal/mol) for diphenylurea, diphenylthiourea and diphenylselenourea by PM3 calculations

Compound	Neutral molecule		Radical cation	
Diphenylurea	Z	4.21	Z	-4.80
	E	4.60	E	-4.78
Diphenylthiourea	Z	0.22	Z	-17.13
	E	1.52	E	-16.00
Diphenylselenourea	Z	-6.78	Z	19.68
	E	-6.10	E	17.63



Scheme 14

possible to assign unambiguously a fragment ion to the keto form since ureas have two amine moieties. The use of labeled ureas has been helpful in this sense.

The relative heats of formation difference can be explained in terms of the stability of the E and Z isomers (Scheme 14).

The values for the Z forms are preferred for the bigger substituents while for the smaller ureas the E forms seem to involve a lower tautomerization energy. For the only asymmetrical thiourea, allylthiourea, it is not surprising to find a similar value for both E and Z thioimidol tautomers.

For ureas, as for other tautomerizable compounds, the shift of the tautomeric equilibrium towards the imidol structure formation is remarkable when heteroatom is successively oxygen, sulfur and selenium. This can be easily demonstrated by mass spectrometry and theoretical calculations (Fig. 50 and Table 21). For one seleno derivative a dramatic loss of 81 amu is observed, $(M\text{-}SeH)^+$,. This fact is consistent with the higher relative amount of imidol form in the tautomeric equilibrium (Se>S>O). This behavior is easily explained by the lower relatively tendency of Se to form double bonds (Se<S<O).

The relative abundances ratio $(M\text{-}XH)^+/(M\text{-}XH_2)^{+\cdot}$ is highly increased for the selenoimidol due to the lower thermodynamical stability of H_2Se compared to H_2S and H_2O.

Table 21 accounts for the heteroatom change. The corresponding heats of formation differencies were calculated by the PM3 semiempirical method.

The results for the neutral molecules correlate adequately with the mass spectral data. As observed above, the Z isomers imply the lower energy requirement for the tautomerization.

Once again mass spectrometry has demonstrated to be useful for the study of tautomerism for this compounds families. Theoretical semiempirical calculations AM1 not only support the findings based on the spectrometric data but also the fact that ionization in the mass spectrometer does not affect the tautomeric equilibrium established between the neutral species (at least for monocarbonylic compounds) [95-104]. The results for the radical cations do not show a rationalizable trend.

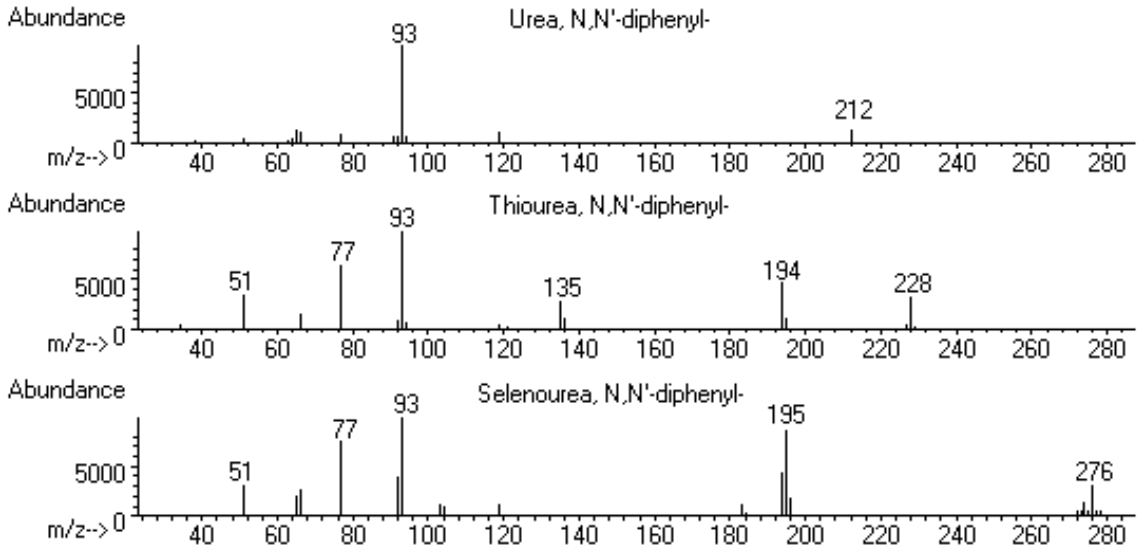


Fig. 50: Mass spectra of diphenylurea, diphenylthiourea and diphenylselenourea

The application of mass spectrometry techniques together with semiempirical theoretical calculations provides a suitable way to analyze tautomerization occurrence. It was shown that the sensible employment of these two different methods enables one to achieve a predictive capability to study the tautomerization degree of several organic compounds families.

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