

## STUDY OF THE OCCURRENCE OF TAUTOMERIC FORMS OF UREAS AND THIOUREAS BY MASS SPECTROMETRY

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### ABSTRACT

Tautomerism of several ureas and thioureas has been studied by mass spectrometry. Experimental findings are supported by theoretical calculations. Imidolization is noticeable in the case of the thio-derivatives. Semiempirical results indicate that the thioamide-thioimidol equilibrium can be studied by mass spectrometry and ionisation in the ion source has no effect on the position of that equilibrium.

**Keywords:** Tautomerism, Ureas, Thioureas, Mass Spectrometry, Imidolization.

### INTRODUCTION

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<sup>1</sup>. Some authors proposed a zwitterionic structure<sup>2–5</sup> but this concept has also found some criticism<sup>6–8</sup>. However, S-alkyl derivatives of isothiurea (thiomidol form) could be prepared by the action of alkylation agents on thiourea in polar solvents<sup>9</sup>.

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<sup>10–12</sup>. 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<sup>13,14</sup>.

The potentiometric titration of aqueous solutions of thiourea and monophenylthiourea against aqueous alkali<sup>15</sup> shows that only 2% is titrable, *i.e.*, 98% is present in non-acidic form. In addition, the transformation from the non-acidic to the acidic form is found to be very slow.

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The results of the study of this and other related compounds at room temperature in aqueous solutions suggest their existence in two tautomeric forms in equilibrium with each other<sup>16</sup>.

For urea, thiourea and their derivatives in sulphuric acid, IR, UV and NMR data indicate the presence not only of N-protonated but also of O- and S-protonated species<sup>17-20</sup>. When studying the alkylation of ambident nucleophiles having a thiocarbonyl group by the use of alcohol, diethylazodicarboxylate, and triphenylphosphine, selective S-alkylation took place in the cases of N-benzoyl-N-mono- and disubstituted thioureas, while selective N-alkylation occurred in the reaction of N-phenyl-N, N'-diethylthiourea<sup>21</sup>.

Acidity measurements and oxidation potentials of carboxamides, thiocarboxamides and their conjugated bases indicate that the thioamide group donates a hydrogen or an electron more easily than the amide group. This behaviour is associated to the higher ability of sulphur vs oxygen to stabilise the anion, the radical or the radical cation respectively<sup>22</sup>. These evidences are consistent with the higher lability of the CS vs CO double bonds.

Mono-, di- and triprotonation of thiourea were studied by low-temperature <sup>1</sup>H, <sup>13</sup>C and <sup>15</sup>N NMR spectrometry in superacidic systems. In FSO<sub>3</sub>H/SO<sub>2</sub>ClF at -80°C, thiourea is monoprotated exclusively at the sulphur atom giving rise to [(H<sub>2</sub>N)<sub>2</sub>CSH]<sup>+</sup>. The addition of SF<sub>5</sub> to this system increases the acidity of the solution and results in the observation of the diprotated species [H<sub>3</sub>NC(SH)NH<sub>2</sub>]<sup>2+</sup>. No NMR evidence was found for triprotonation under these conditions, although a limited equilibrium concentration should not necessarily be detected<sup>23</sup>.

Mass spectrometry has demonstrated to be useful for the study of tautomerism. Theoretical semiempirical calculations not only support the findings based on the mass spectrometric data but also the fact that ionisation in the mass spectrometer does not affect the tautomeric equilibrium established between the neutral species (at least for monocarbonylic compounds)<sup>24-30</sup>.

In this work, mass spectra of ureas and thioureas (urea, diethyl, ethylene, allyl, dicyclohexyl and diphenyl and their sulphur analogues) were studied to examine the predictive power of mass spectrometry for the occurrence of tautomerism with the additional support of molecular orbital calculations.

## EXPERIMENTAL

### Materials

Methanol, *Carlo Erba* RPE.

Methanol-d<sub>1</sub>, Aldrich 99.5%.

Ureas and thioureas were commercially available and were purified by recrystallization until constant melting point was achieved.

Ethylenethiourea and allylthiourea were synthesized and purified according to published procedures<sup>31</sup>.

### Gas Chromatography–Mass Spectrometry

GC–MS experiments were performed by injection of methanol solutions (1  $\mu$ L) in an HP 5890 Series II Plus chromatograph coupled to an HP 5972 A mass spectrometric detector under the following conditions:

Column: HP5–MS, 30 m x 0.25 mm x 5  $\mu$ m.

Carrier gas: Helium.

Injector temperature: 200°C.

Oven temperature: 80°C, 10°C/min, 200°C.

Interface temperature: 300°C.

Ion source temperature: 185°C.

The pressure in the mass spectrometer,  $1.33 \times 10^{-3}$  Pa, precludes ion–molecule reactions.

Electron energy: 70 eV.

### Computational Procedure

AM1 calculations were performed using the standard HyperChem<sup>®</sup> package<sup>32</sup>. Since we resorted to heat of formation values in order to rationalize experimental findings and the AM1 technique has been especially parameterized to reproduce this sort of experimental data, we deem this choice is a sensible one for the molecular set under study. AM1 was designed to eliminate some problems with MNDO which were considered to arise from a tendency to overestimate repulsion between atoms separated by distances approximately equal to the sum of their *van der Waals* radii.

PM3 calculations were carried out for compounds included in Table 3 since the above mentioned version of the HyperChem<sup>®</sup> package does not include selenium atomic parameters for the AM1 method. PM3 is also based on MNDO and the corresponding Hamiltonian contains essentially the same elements as that for AM1 but the parameters for the PM3 model were derived using an automated parametrization procedure devised by Steward<sup>33</sup>.

## RESULTS AND DISCUSSION

## Gas Chromatography–Mass Spectrometry

Table 1. Relevant Mass Spectral Data for Selected Ureas<sup>a</sup>

Compound	M <sup>+</sup>	[M–XH] <sup>+</sup>	[M–H <sub>2</sub> X] <sup>+</sup>	Others
Urea	359.8	–	–	88.6 <sup>b</sup> , 316.6 <sup>c</sup>
Thiourea	–	5.2	1.2	58.4 <sup>d</sup>
Diethylurea	98.1	1.4	–	125.4 <sup>e</sup>
Diethylthiourea	278.4	18.7	3.9	–
Ethyleneurea	43.7	19.9	–	58.4 <sup>f</sup>
Ethylenehiourea	102.1	69.2	–	58.2 <sup>g</sup>
Allylurea	0.064	0.8	–	213.7 <sup>h</sup>
Allylthiourea	137.3	14.3	–	107.2 <sup>i</sup>
Dicyclohexylurea	849.9	0.3	–	81.5 <sup>f</sup>
Dicyclohexylthiourea	98.3	9.2	–	112.4 <sup>j</sup>
Diphenylurea	57.0	–	1.6	406.6 <sup>k</sup>
Diphenylthiourea	54.2	20.6	79.7	161.2 <sup>k</sup>
Diphenylselenourea <sup>l</sup>	46.7	124.5	65.1	141.3 <sup>k</sup>

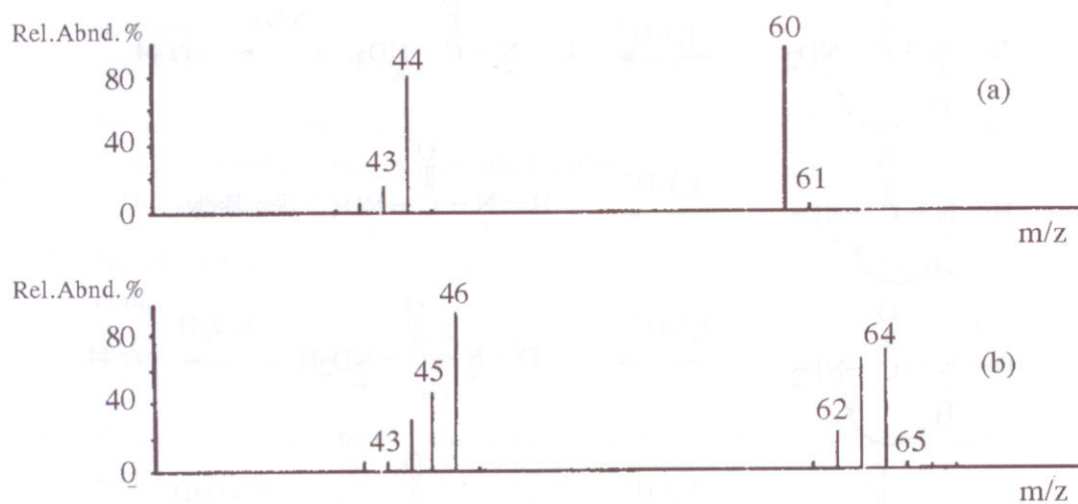
<sup>a</sup> For a better correlation the reported electron impact data are calculated according to the following ratio: Ion abundance x 1000 /  $\Sigma$  abundances; <sup>b</sup> This comes from the loss of 17 amu which can be OH or NH<sub>3</sub> loss; <sup>c</sup> It corresponds to [M–NH<sub>2</sub>]<sup>+</sup>; <sup>d</sup> It corresponds to [M–NH<sub>3</sub>]<sup>+</sup>; <sup>e</sup> Fragment ion from McLafferty rearrangement; <sup>f</sup> It corresponds to [M–CO]<sup>+</sup>, *m/z* 70, assignable to the amido form; <sup>g</sup> It corresponds to [M–CS]<sup>+</sup>, assignable to the amido form; <sup>h</sup> It corresponds to the amido form: [M–NH<sub>2</sub>–CO]<sup>+</sup>, *m/z* 56; <sup>i</sup> It corresponds to the amido form: [M–NH<sub>2</sub>–SC]<sup>+</sup>, *m/z* 56; <sup>j</sup> *m/z* 98, [C<sub>6</sub>H<sub>12</sub>N]<sup>+</sup>; <sup>k</sup> *m/z* 93, [PhNH<sub>2</sub>]<sup>+</sup>; <sup>l</sup> Data obtained from NIST 98 mass spectral database.

Chromatographic separation of amide tautomers has not been attained may be due to the speed of the equilibrium involved. In previous studies on the tautomeric equilibria of  $\beta$ -ketoesters chromatographic separation was reported<sup>28</sup>.

Table 1 shows the most relevant mass spectral data for selected ureas.

In general, the loss of XH or XH<sub>2</sub> (X = O, S, Se) from the molecular ion can be assigned to the imidol form and the loss of NHR or NH<sub>2</sub> to the amido form, but in all these cases it is not possible to assign unambiguously a fragment ion to the keto form since ureas have two amine moieties.

The loss of 17 amu from the molecular ion of urea likely corresponds to  $\text{NH}_3$  loss since an analogue loss of  $\text{NH}_3$  with a similar abundances ratio was found for thiourea. To prove this, the spectra of urea and deuterated urea, after injection in deuterated methanol (Fig. 1), suggest that the loss of 16 amu,  $[\text{M}-\text{NH}_2]^+$  in Fig. 1 a, corresponds, Fig. 1b, i) to the loss of 18 amu  $[\text{M}-\text{ND}_2]^+$ , from  $\text{D}_2\text{N}(\text{CO})\text{NDH}$  to yield  $m/z$  46 and 45 and ii) to the loss of 17 amu  $[\text{M}-\text{NDH}]^+$ , from  $\text{D}_2\text{N}(\text{CO})\text{NDH}$  or  $\text{DHN}(\text{CO})\text{NDH}$  or  $\text{H}_2\text{N}(\text{CO})\text{NDH}$  to yield the same fragment ions. The spectrum for the deuterated urea has been obtained by averaging of the spectra taken during the isotopomers co-elution.

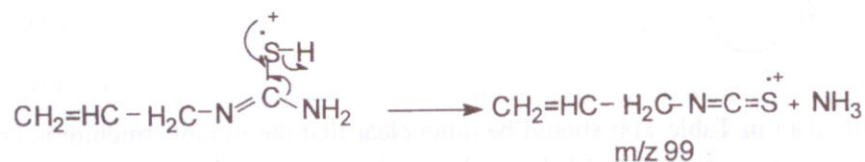


**Figure 1. Mass spectra of urea: (a) hydrogenated and (b) deuterated urea.**

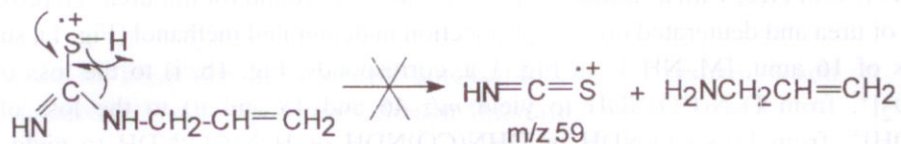
In Fig. 1b ammonia loss (after hydrogen rearrangement) from deuterated and partially deuterated urea (totally hydrogenated urea is not observed) is explained by Scheme 1, so that, the loss of 17 amu in Fig. 1a is supposed to come exclusively from  $\text{NH}_3$  loss ( $m/z$  43).

For allylthiourea (only asymmetrical structure) the favored imidol form is the one that involves the N-substituted side which is proved by the observation of  $[\text{M}-\text{NH}_3]^+$  and the corresponding absence of  $m/z$  59 in the mass spectrum (Eqns. 1 and 2).

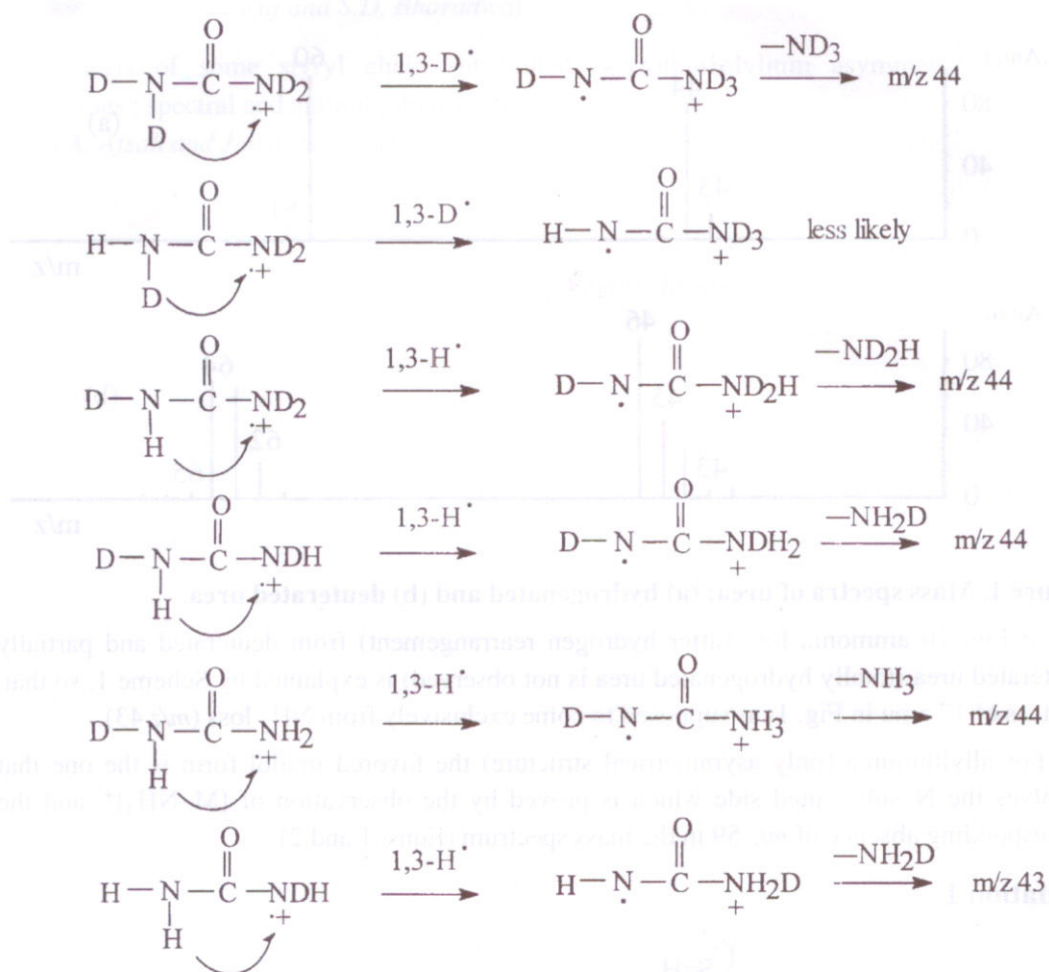
#### Equation 1



## Equation 2



Scheme 1: Ammonia loss from deuterated and partially deuterated urea molecular ions.



From the data in Table 1, it should be quite clear that the imidol structure is favored for thioureas. Taking into account the SH loss, ethylenethiourea might be considered the compound with the highest occurrence of thioimidol form. This would be explained by the ring

stabilization (Eqn. 3) that takes place when going from exocyclic to endocyclic double bond structures<sup>34</sup>.

### Equation 3



The decreasing order for imidol occurrence, thiourea < dicyclohexylthiourea < diethylthiourea, can be justified by hyperconjugation effect due to the sulphur capability for expansion of the valence shell (Eqn. 4).

### Equation 4

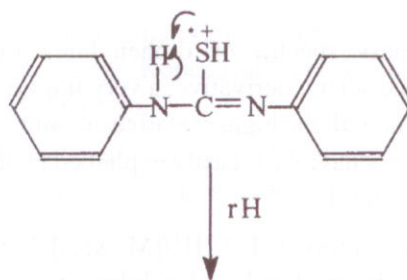


The hyperconjugative canonical forms cannot be postulated for the amido tautomer.

Allylthiourea and diphenylthiourea exhibit an important amount of imidol structure due to conjugation extension. Although the [M-SH]<sup>+</sup> fragment has the highest relative abundance for ethylenethiourea, the diphenylthiourea has a highly abundant [M-SH<sub>2</sub>]<sup>+</sup> fragment which also accounts for the thioimidol structure.

The loss of SH<sub>2</sub> for diphenylthiourea involves a radical carbocation formation which is stabilized by the delocalization of both the charge and the radical sites (Scheme 2). Among the thioureas, the diphenylthiourea has the highest occurrence of thioimidol form according to these data.

### Scheme 2. Stabilization of the radical carbocation fragment after SH<sub>2</sub> loss from diphenylthiourea molecular ion



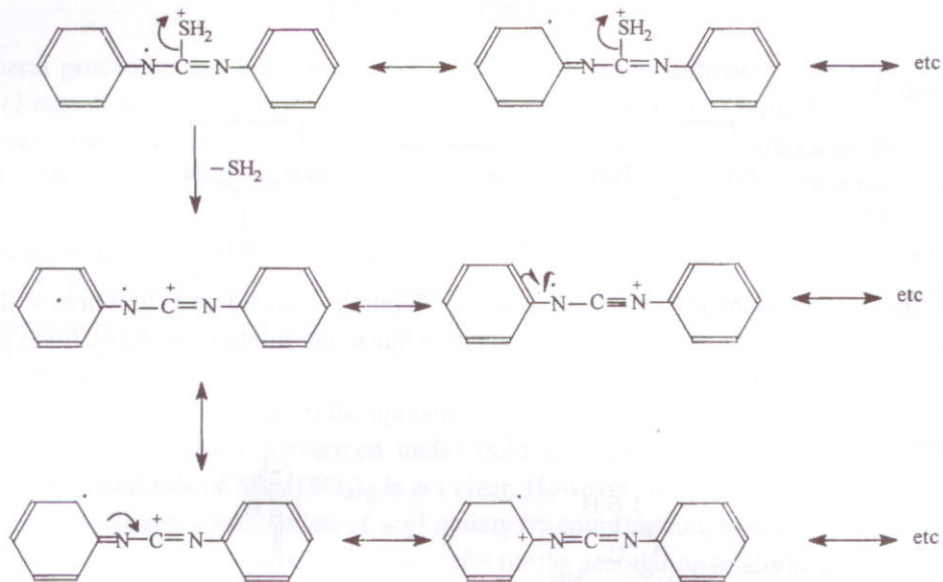


Figure 2: Mass spectra of diphenylurea, diphenylthiourea and diphenylselenourea.

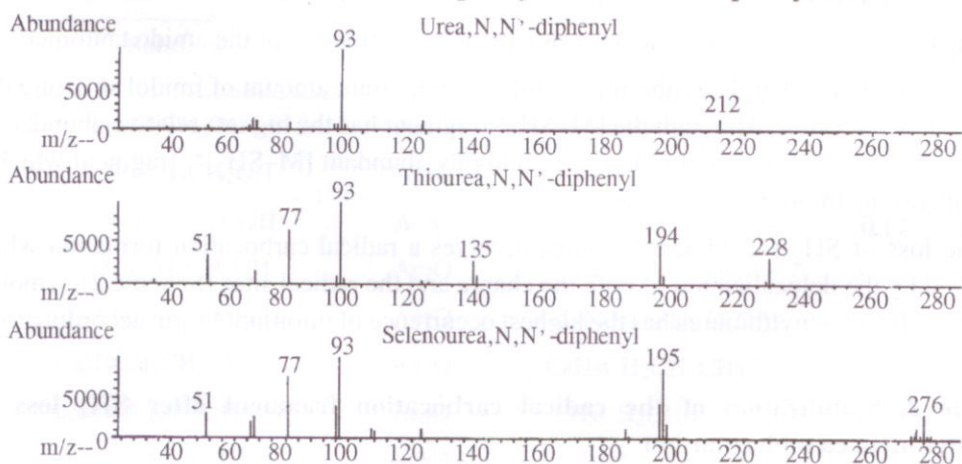


Figure 2 shows the mass spectra of diphenylurea (a), diphenylthiourea (b) and diphenylselenourea (c). For the seleno derivative, a very important loss of 81 amu is observed, [ $m/z$  195]. This fact is consistent with the higher relative amount of imidol form in the tautomeric equilibrium ( $\text{Se} > \text{S} > \text{O}$ ). This behaviour is easily explained by the lower relatively tendency of Se to form double bonds ( $\text{Se} < \text{S} < \text{O}$ ).

The relative abundances ratio  $[\text{M}-\text{XH}]^+ / [\text{M}-\text{XH}_2]^+$  is highly increased for the selenoimidol due to the lower thermodynamical stability of  $\text{H}_2\text{Se}$  compared to  $\text{H}_2\text{S}$  and  $\text{H}_2\text{O}$ .



### Theoretical Calculations

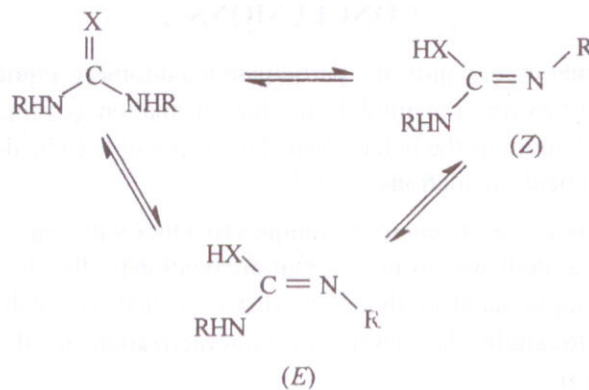
The table 2 shows the difference of heats of formation between the thioamido and thioimidol forms considering the neutral molecules and the corresponding radical cations. Semiempirical calculations have been performed only for the thiocompounds since they are the ones that exhibit an appreciable amount of imidol form.

**Table 2. Difference between the Heats of Formation of Thioamido and Thioimidol Tautomers ( $\text{kJmol}^{-1}$ ) by AM1 Calculations**

Compound	Neutral molecule		Radical cation		
Thiourea	(Z)	49.16	(Z)	82.10	
	(E)	46.15	(E)	65.67	
Diethylthiourea	(Z)	41.76	(Z)	42.34	
	(E)	20.57	(E)	67.38	
Ethylenehiourea		19.06		-68.80	
Allylthiourea	H <sub>2</sub> N-CSH=NR	(Z)	20.98	(Z)	32.31
		(E)	25.16	(E)	39.25
	HN=CSH-NHR	(Z)	49.83	(Z)	90.58
		(E)	34.78	(E)	71.65
Dicyclohexylthiourea	(Z)	30.14	(Z)	80.17	
	(E)	38.37	(E)	48.24	
Diphenylthiourea	(Z)	-6.94	(Z)	26.25	
	(E)	5.68	(E)	43.26	

Taking into account that the equilibrium (*E*)-(*Z*) is fast, due to the planar transition state for the interconversion process at the imide nitrogen atom (Eqn. 5), the heat of formation difference to be considered is that one with the lower energy requirement.

### Equation 5



The relative heats of formation difference can be explained in terms of the stability of the (*E*) and (*Z*) isomers. The values for the (*Z*) forms are preferred for the larger substituents whereas for the smaller ureas the (*E*) forms seem to involve a lower tautomerization energy. For the asymmetrical thiourea, allylthiourea, it is not surprising to find a similar value for both (*E*) and (*Z*) thioimidol tautomers. As observed by mass spectrometry, the most stable form is the imidol that involves the N-substituted side.

A reasonable correlation between the difference of the heat of formation of the tautomers with the mass spectra observations is achieved only in the case of the calculated values for the neutral molecules. Tautomerization might take place after ionization but in such an extent that does not seem to have an impact on the reported correlation. The results for the radical cations do not show a rationalizable trend.

Table 3 accounts for the heteroatom change. The corresponding heats of formation differences were calculated by the PM3 semiempirical method.

**Table 3. Heats of Formation Difference ( $\text{kJmol}^{-1}$ ) for Diphenylurea, Diphenylthiourea and Diphenylselenourea by PM3 Calculations.**

Compound	Neutral molecule		Radical cation	
	Isomer	Heat of formation difference ( $\text{kJmol}^{-1}$ )	Isomer	Heat of formation difference ( $\text{kJmol}^{-1}$ )
Diphenylurea	( <i>Z</i> )	17.60	( <i>Z</i> )	-20.06
	( <i>E</i> )	19.23	( <i>E</i> )	-19.98
Diphenylthiourea	( <i>Z</i> )	0.92	( <i>Z</i> )	-71.60
	( <i>E</i> )	6.35	( <i>E</i> )	-66.88
Diphenylselenourea	( <i>Z</i> )	-28.34	( <i>Z</i> )	82.26
	( <i>E</i> )	-25.50	( <i>E</i> )	73.69

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.

## CONCLUSIONS

For ureas, as for other compounds that participate in tautomeric equilibria, the shift of the tautomeric equilibrium towards the imidol structure formation is remarkable in the series oxygen, sulphur and selenium as the heteroatom. This is possible to be demonstrated by mass spectrometry and theoretical calculations.

The application of mass spectrometry techniques together with semi-empirical theoretical calculations provides a suitable way to analyse tautomerization for the chosen set of ureas. It has been shown that the employment of these two different methods enables one to achieve a predictive capability to study the degree of tautomerization of the compounds under consideration in this work.

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