Are the Thiouracils Sulfur Bases in the Gas-phase?

M. Lamsabhi,[†] M. Alcamí,[‡] O. Mó,[‡] W. Bouab,*[,†] M. Esseffar,[†] J. L.-M. Abboud,[§] and M. Yáñez*[,‡]

Departement de Chimie, Faculté des Sciences Semlalia Université Cadi Ayyad., Marrakech, Morocco, Departamento de Química, C-9, Universidad Autónoma de Madrid, Cantoblanco, and Instituto "Rocasolano", C.S.I.C., Serrano, 119, 28006-Madrid, Spain

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The gas-phase proton affinities of 2- and 4-thiouracil and 2,4-dithiouracil have been measured by means of Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. High-level ab initio calculations, in the framework of the G2(MP2) theory, have been carried out to establish the nature of the protonation site. Thiouracils behave as bases of rather similar moderate strength in the gas phase, the 2,4-dithiouracil being the most basic of the three. In all cases, the protonation takes place at the heteroatom attached to position 4, hence although, in general, thiocarbonyls are stronger bases than carbonyls in the gas phase, 2-thiouracil behaves as an oxygen base. For 2-thiouracyl and 2,4-dithiouracil, the most stable protonated conformer is the enol−enethiol form that cannot be formed by either direct protonation of the corresponding neutral or a unimolecular tautomerization of the oxygen or sulfur protonated species. We have shown that alternative mechanisms involving the formation of hydrogen bonded dimers between the protonated form and the neutral form, followed by appropriate proton transfers within the dimer, can be invoked to explain the formation of the most stable conformer.

Introduction

The reactivity of uracil thio derivatives presents a great interest in chemical investigations because of their biological and pharmacological activities. 2-Thiouracil and 4-thiouracil have been identified as minor components of t-RNA, and they can be used as anticancer and antithyroid drugs.[1] Also, their existence in many tautomeric forms, like other nucleoside bases, seems to be crucial in order to explain the mutation occurring during DNA duplication.[2−5]

During recent years, a large amount of experimental and theoretical work has been carried out in order to elucidate different aspects of thiouracil tautomerism. Indeed, each of them can exist in the six tautomeric forms shown in Figure 1. Earlier studies demonstrate that 2-thiouracil, 4-thiouracil, and 2,4-dithiouracil exist as planar dioxo tautomers not only in the gas-phase but also in solution and in the solid state.[6−11] The enhanced stability of the dioxo forms has also been established by means of ab initio calculations.[12−15]

Although the tautomerism in this set of compounds has been intensively investigated, there is an almost complete lack of information regarding their intrinsic reactivity, and to the best of our knowledge, only the gas-phase basicity of 2-thiouracil has been experimentally measured.[9] Hence, the aim of this paper is to investigate the gas-phase protonation of the aforementioned compounds in order to establish both their intrinsic basicities and the nature of the protonation site and to elucidate the possible role that the tautomerization processes may play in the gas-phase protonation.

To achieve these goals we combined the experimental information obtained from ion cyclotron resonance spectrometry (FT ICR)[16] studies with the results of high-level ab initio calculations devoted to explore the potential energy surfaces (PES) associated with both the neutral and the protonated species.

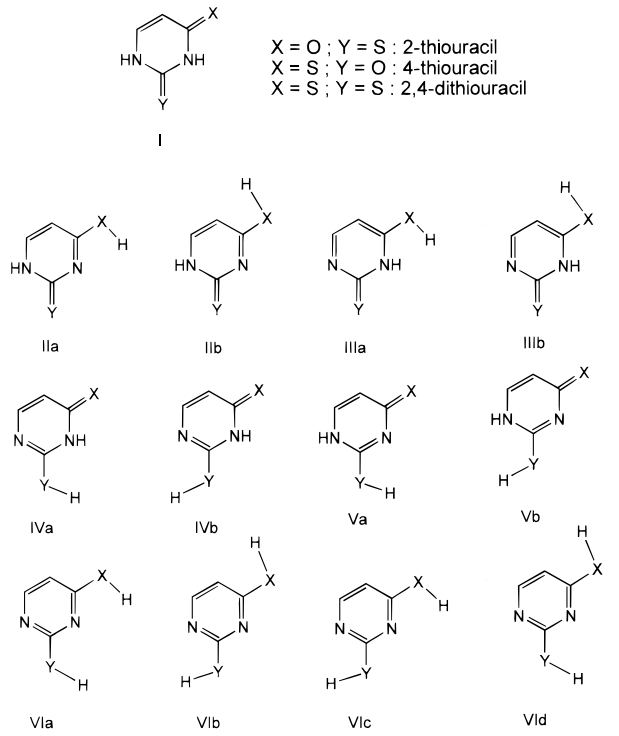

Figure 1. Different tautomeric forms of neutral thiouracils.

Experimental Section

A. The FT ICR Spectrometer. In this work, use was made of a modified Bruker CMS 47 FT ICR mass spectrometer. A

* Corresponding authors.
[†] Faculté des Sciences Semlalia Université Cadi Ayyad.
[‡] Universidad Autónoma de Madrid.
[§] Instituto "Rocasolano", C.S.I.C.

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TABLE 1: Experimental Determination of the Gas-Phase Basicities of 2,4-Dithiouracil and 2-Thiouracil[a,b]

compd	B_{ref}(GB)	$\delta\Delta G^\circ_{H+}$	GB(B), average
2,4-DTU	isophorone (205.9)	1.50	204.40
			204.2 ± 0.2
	2-fluoropyridine (203.8)	−0.28	204.08
2-TU	di-*n*-butylsulfide (201.3)	−0.99	202.29
			202.1 ± 0.2
	4-cyanopyridine (202.9)	0.97	201.93

[a] Magnitudes defined in the text. [b] All values in kcal mol^{-1} (1 kcal = 4.184 kJ).

detailed description of the most relevant features of the original instrument is given in ref 17. The instrument has already been used in a number of studies.[18,19] The field strength of its supraconducting magnet, 4.7 T, allows the monitoring of ion−molecule reactions for relatively long periods of time. The instrument is also fitted with a direct insertion probe enabling the study of solid materials endowed with very low vapor pressures.[20]

B. Materials. 2-Thiouracil and 2,4-dithiouracil were commercial (Aldrich) products. They were twice recrystallized from ethanol (mp 339−341 and 279−281 °C, respectively).

4-Thiouracil was obtained according to Mizumo et al.[21] The evolution of the reaction was monitored by IR. The crude product was column-chromatographed (silicagel, dichloromethane/methanol 60:40) and further recrystallized from ethanol (mp 326−328 °C).

The purity of the three compounds was assessed by GLC (C. P. Sil5 column). No impurities were detected by FT ICR.

C. Experimental Determination of Gas-Phase Basicities. The gas-phase proton basicity, GB, of a base B is defined as the standard Gibbs energy change, ΔG°_{H+} for reaction 1 in the gas phase

$$BH^+(g) \rightarrow B(g) + H^+(g)\ \Delta G^\circ_{H+} \qquad (1)$$

The FT ICR experiments provide the standard Gibbs energy change, $\delta\Delta G^\circ_{H+}$, for reaction 2 in which B is the relevant thiouracil and B_{ref} is a reference base of known GB

$$BH^+(g) + B_{ref}(g) \rightleftharpoons B(g) + B_{ref}H^+(g) \quad K_p,\ \delta\Delta G^\circ_{H+} \qquad (2)$$

K_p (dimensionless) is given by $K_p = [P(B)P(B_{ref}H^+)]/[P(BH^+)P(B_{ref})]$, wherein P stands for the partial pressures of the various species and $\delta\Delta G^\circ_{H+} = -RT \ln K_p$.

The GBs of 2-thiouracil and 2,4-dithiouracil were determined by direct equilibration with the corresponding reference bases [see, e.g., ref 18a]. Protonation of the various species was carried out by chemical ionization. The experimental results are reported in Table 1. The GB values for the reference bases are taken from the most recent critical compilation.[22] In all cases, double-resonance-like experiments confirmed the existence of an equilibrium.

Notice that, because of the extremely low vapor pressure of 4-thiouracil, the samples of this compound had to be introduced by means of the direct insertion probe. This technique has the drawback of not allowing the control of the pressure of the sample. Also, the partial pressure of 4-thiouracil being quite low ($<10^{-7}$ mbar), the relative uncertainties on this magnitude are large. This prevented us from directly determining K_p values. Instead, a bracketing method was used. The strongest base able to transfer a proton to 4-thiouracil and the weakest base protonated by 4-thiouracil (as established by ion-selection experiments) were found to be, respectively, diisopropylsulfide

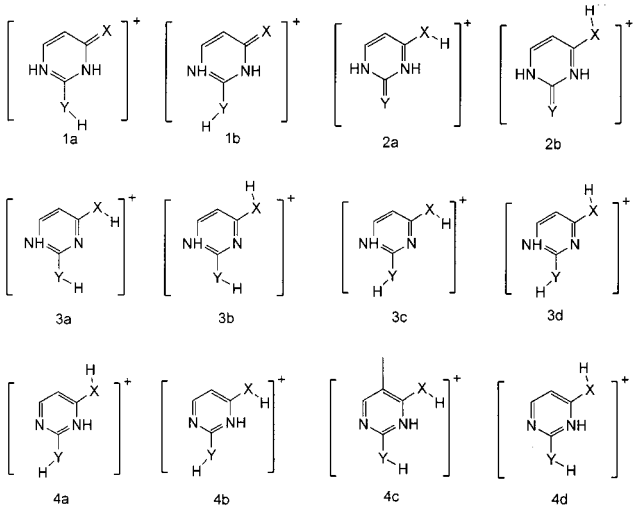


Figure 2. Different tautomeric forms of protonated thiouracils.

(GB = 202.3 kcal mol^{-1}) and 2-fluoropyridine (GB = 203.8 kcal mol^{-1}). The GB of 4-thiouracil is thus estimated at 203.1 ± 2.0 kcal mol^{-1}.

Some comments on the uncertainties affecting these GB values seem in order, because the comparison with the calculated values is of great importance in this study. In the case of GB values obtained by direct equilibration, the uncertainties on the *absolute values* of these magnitudes are estimated at ca. 2 kcal mol^{-1}. The uncertainties on the relative values, as in the difference between 2-thiouracil and 2,4-dithiouracil, are much smaller, generally lower than 0.5 kcal mol^{-1}. This is so because the comparison method, involving a gas-phase basicity scale constructed over some thirty years through multiple overlaps involving very small steps (essentially because of the small dynamic range of ICR and FT ICR) and cross-checks of the data, has become fairly reliable. This applies, in particular, to the range of GBs relevant to this work. In the case of 4-thiouracil, the uncertainty is larger, about 2 kcal mol^{-1}, because of the bracketing method involved. Here, however, the reduced dynamic range of the method and the ion-selection technique keep the uncertainty within these reasonable limits. In short, for the comparison with the computed GB values, the *overall* uncertainties for the GBs of 2-thiouracil and 2,4-dithiouracil can be estimated at 2.1 kcal mol^{-1}. In the case of 4-thiouracil, this value is 2.8 kcal mol^{-1}.

To obtain the proton affinities (PAs) from the measured GBs, we have used the entropy values obtained in our ab initio calculations, at the HF/6-31G* level, for the corresponding neutral and protonated species. For H^+ a value of $S = 26.039$ cal. mol^{-1} K^{-1} was employed.

Computational Details

Standard ab initio calculations have been carried out by means of the *Gaussian 94* series of programs.[23]

The geometries of the six different tautomers of 2-thiouracil, 4-thiouracil, and 2,4-dithiouracil were initially optimized at the HF/6-31G* level. In all cases the different conformers of each tautomer were considered, so in the whole the 13 different structures schematized in Figure 1 were optimized for each of them. Similar geometry optimizations were carried out for the corresponding protonated species. In this case we considered also all possible tautomers and all possible conformations, so that for each species the 12 different structures shown in Figure 2 were fully optimized. The corresponding harmonic vibrational frequencies were evaluated at the same level of theory to asses

TABLE 2: Total Energies (a.u.) Calculated at MP2/6-31G* Level, ZPE Corrections, without Scale Factor (a.u.) Calculated at HF/6-31G* Level, and Relative Energies with Respect the Most Stable Tautomer at MP2/6-31G* Level, Taking into Account ZPE Scaled by 0.893 (ΔE in kcal/mol) for the Neutral and Protonated Thiouracils Considered in This Study

	2-thiouracil			4-thiouracil			2,4-dithiouracil		
	energy	ZPE	ΔE	energy	ZPE	ΔE	energy	ZPE	ΔE
				Neutral Forms					
I	−736.214047	0.092248	0.0	−736.216746	0.092185	0.0	−1058.798737	0.089730	0.0
IIa	−736.191914	0.091821	13.6	−736.192769	0.088157	12.8	−1058.774520	0.085595	12.9
IIb	−736.178668	0.091292	21.7	−736.188684	0.088094	15.3	−1058.770219	0.085532	15.5
IIIa	−736.170580	0.090499	26.3	−736.182108	0.087318	19.0	−1058.763563	0.084731	19.3
IIIb	−736.175729	0.090540	23.1	−736.183674	0.087693	18.2	−1058.764835	0.085076	18.7
IVa	−736.190735	0.087991	12.2	−736.184525	0.091148	19.6	−1058.776990	0.085627	11.3
IVb	−736.194845	0.088161	9.8	−736.198732	0.091971	11.2	−1058.780921	0.085748	8.9
Va	−736.179227	0.087628	19.3	−736.183079	0.091419	20.7	−1058.763867	0.085082	19.3
Vb	−736.173331	0.087361	22.8	−736.163872	0.089856	31.9	−1058.757844	0.084846	22.9
VIa	−736.194650	0.087957	9.8	−736.193644	0.087906	12.1	−1058.780217	0.081720	7.1
VIb	−736.186001	0.087663	15.0	−736.191795	0.088024	13.3	−1058.777930	0.081801	8.6
VIc	−736.195459	0.088010	9.3	−736.194618	0.087984	11.5	−1058.780660	0.081758	6.9
VId	−736.186103	0.087657	15.0	−736.191792	0.087997	13.3	−1058.777979	0.081792	8.6
				Protonated Forms					
1a	−736.540503	0.101821	5.4	−736.537192	0.104922	11.9	−1059.124228	0.099188	7.2
1b	−736.539476	0.101823	6.1	−736.535311	0.104814	13.0	−1059.123345	0.099160	7.8
2a	−736.542531	0.105181	6.0	−736.552693	0.102338	0.7	−1059.133048	0.099624	1.9
2b	−736.547528	0.105416	3.0	−736.553841	0.102372	0.0	−1059.134163	0.099637	1.2
3a	−736.549461	0.102155	0.0	−736.551467	0.102198	1.4	−1059.132961	0.096078	0.0
3b	−736.541086	0.101944	5.1	−736.549165	0.102261	2.9	−1059.130546	0.096144	1.6
3c	−736.545187	0.102190	2.7	−736.536407	0.101598	10.5	−1059.128499	0.096115	2.8
3d	−736.535759	0.101952	8.5	−736.533060	0.101621	12.6	−1059.125509	0.096165	4.7
4a	−736.536762	0.101450	7.6	−736.542634	0.101776	6.7	−1059.123915	0.095630	5.4
4b	−736.528874	0.100997	12.3	−736.540260	0.101628	8.1	−1059.121431	0.095480	6.9
4c	−736.522536	0.100863	16.2	−736.522200	0.100686	18.9	−1059.115524	0.095396	10.6
4d	−736.531468	0.101422	10.9	−736.525637	0.101029	16.9	−1059.118503	0.095602	8.8

that all of the structures found corresponded to local minima of the PES and to estimate the corresponding zero point energy (ZPE) corrections which were scaled by the empirical factor 0.893.

The geometries so obtained were refined at the MP2/6-31G-(d) level to include electron correlation effects. A similar procedure was adopted to locate the transient species associated with the tautomerization processes for both neutral and protonated species.

The final energies for the most stable neutral tautomer and for the two most stable protonated tautomers were obtained in the framework of the G2(MP2) theory.[24] This is a composite method that corresponds effectively to calculations at the QCISD(T)/6-311+G(3df,2p) level assuming that basis set effects on the correlation energies are additive. A small empirical correction (HLC) to accommodate remaining deficiencies is finally added as well as the corresponding zero point energy (ZPE) correction, estimated at the HF/6-31G* level. The reader is addressed to ref 24 for a complete description of this method. The reliability of the G2(MP2) procedure to estimate absolute proton affinities is well documented.[25−27].

The charge distribution of the different neutrals has been analyzed by means of the atoms in molecules (AIM) theory of Bader.[28]

Results and Discussion

The MP2/6-31G(d) total energies, as well as the ZPE corrections obtained at the HF/6-31G(d) level, for each of the species investigated are given in Table 2. The optimized geometries of the 75 structures investigated are available from the authors upon request. The total energies for the most stable neutral tautomer and for the two most stable protonated forms, namely **2b** and **3a**, obtained at the G2(MP2) level are summarized in Table 3. In this table we have also compared the G2(MP2) calculated proton affinities and the corresponding

TABLE 3: G2(MP2) Energies, *E* (a.u.) and Calculated and Experimental Proton Affinities, PA (kcal/mol)

compound	tautomer	*E*	$PA_{calc.}$ (G2(MP2))[a]	$PA_{exp.}$
2-thiouracil	**I**	−736.770512	205.4; 208.6	210.3
	2b	−737.095397		
	3a	−737.100610		
4-thiouracil	**I**	−736.773387	209.8; 208.3	211.1
	2b	−737.105365		
	3a	−737.103004		
2,4-dithiouracil	**I**	−1059.357498	209.8; 210.8	212.0
	2b	−1059.689480		
	3a	−1059.691050		

[a] The first value corresponds to the direct protonation of the neutral to yield species **2b** while the second value corresponds to the formation of the tautomer **3a**, which cannot be obtained by direct protonation.

experimental values, which indicate that the three thiouracil investigated are bases of moderate strength in the gas phase. The strongest base among the three is 2,4-dithiouracil, but the basicity gap is extremely small and 2-thiouracil, which is the least basic compound, has a PA only 2 kcal/mol smaller than that of 2,4-thiouracil. It is worth mentioning that the value of the PA determined by us for 2-thiouracil is slightly higher than that reported previously in the literature[9] obtained by means of the bracketing method.

To establish the nature of the basic center it is necessary first to establish which is the most stable tautomeric form of the neutral. The results obtained in our theoretical survey can be summarized as follows:

For 2-thiouracil the stability order found was **I** > **VIc** ≈ **IVb** > **IIa** > **Va** > **IIIb**. These results are in agreement with previous semiempirical[13] and ab initio[10,14] calculations that also concluded that the oxo-thione tautomer is the most stable one. However, in contrast with previous studies,[10] our calculations show that tautomers **IVb** and **VIc** are almost degenerate. Although experimentally only the oxo-thione tautomer has been

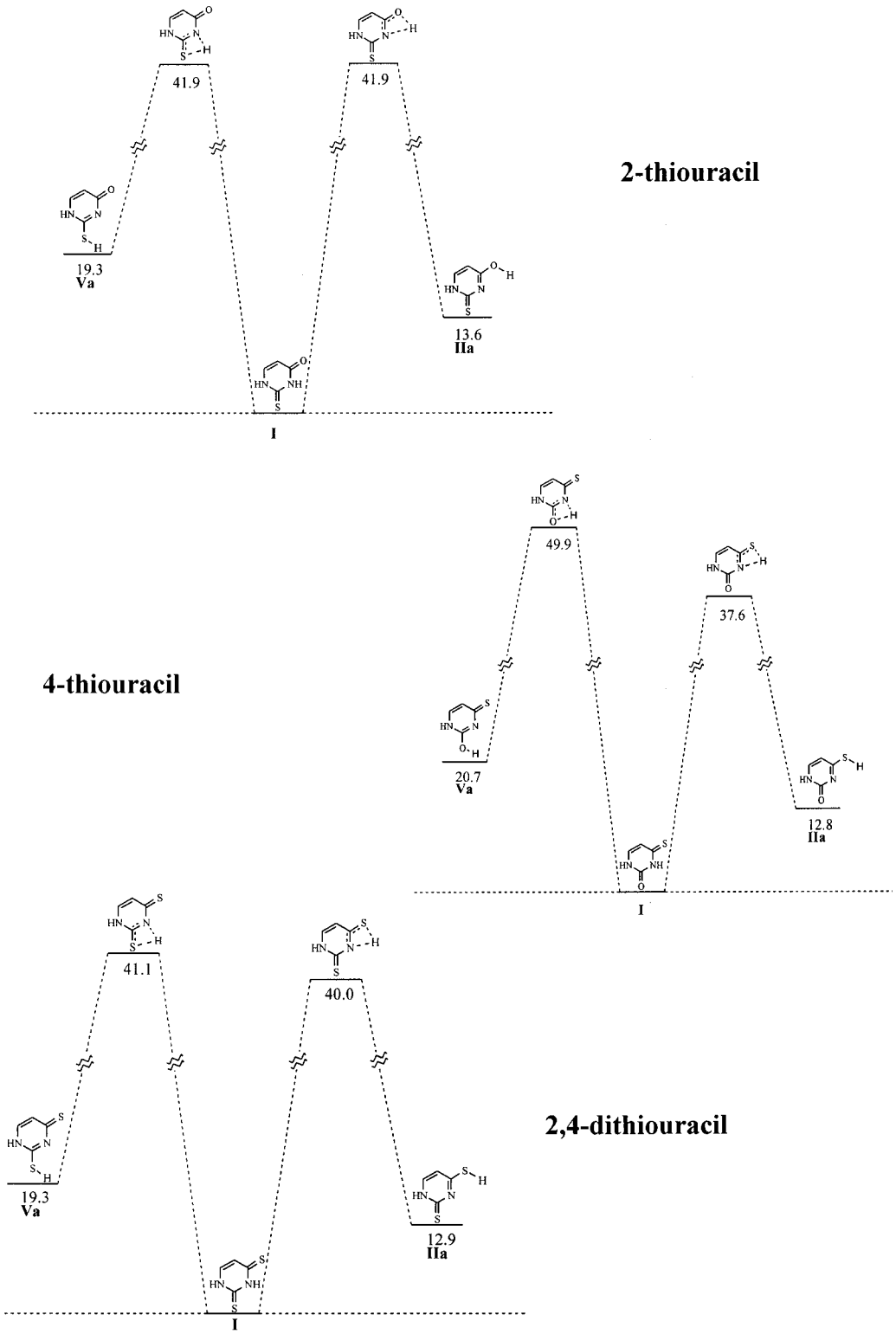


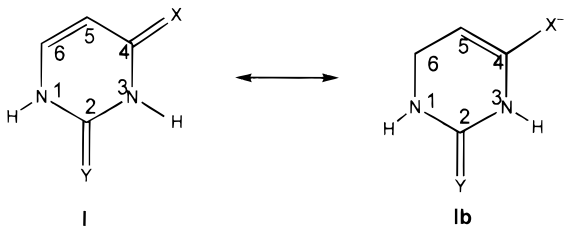
Figure 3. Energy profiles corresponding to the unimolecular tautomerization processes of neutral thiouracils. All values in kcal/mol.

observed in the gas phase,[29,30] the stability order of the different tautomers deduced from the IR study of the different alkyl derivatives[30] nicely agrees with our predictions.

For 4-thiouracil the stability order **I** > **IVb** ≈ **VIc** > **IIa** > **IIIb** > **Va** is rather similar to that found for 2-thiouracil, with the only difference being that the relative stabilities of tautomers **III** and **V** appear reversed. These predictions are also in agreement with the recent study of Rubin et al.[31] who have classified the 4-thiouracil tautomeric forms at both MP4(SDQ)/6-311G(2d,2p) and MP2/6-311++G(2d,2p) levels of theory using the MP2/6-31G(d,p) reference geometries.

For 2,4-dithiouracil, the stability order found was **I** > **VIc** > **IVb** > **IIa** > **IIIb** >**Va**. Again, these stability trends are in agreement with those reported before by Leszczynski and Lammertsma.[15] It must be mentioned, however, that according to our results the conformer **Va** is 3.6 kcal/mol more stable than the conformer **Vb**, which is the only one reported by these authors.

SCHEME 1



I Ib

In summary, in what concerns the gas-phase basicity of these systems, the most important conclusion that can be attained from the previous results is that for 2- and 4-thiouracil the most stable tautomer is the oxo-thione form. Similarly, for 2,4-dithiouracil the dithione form is the most stable tautomer. On the other hand, as shown in Figure 3, the energy barriers connecting the different tautomers are very high, and therefore we can safely conclude that only the aforementioned tautomers will exist in the gas phase. This reduces the possibilities of gas-phase protonation to two sites: the oxygen or the sulfur atom in the case of 2- and 4-thiouracil and to the two sulfur atoms in the case of the 2,4-dithiouracil.

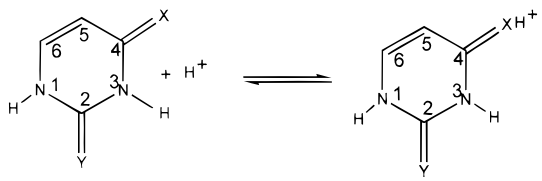
Our calculations show that in all cases the protonation is more favorable at the heteroatom, X, attached to position 4 (see Table 2) to yield tautomer **2b**. This implies that although, 4-thiouracil is a sulfur base in the gas phase, 2-thiouracil is an oxygen base. This is somehow an unexpected result, since it seems well established, both on experimental and theoretical grounds,[18] that thiocarbonyl derivatives are stronger bases in the gas phase than are the corresponding carbonyl analogues. Hence, the first question that needs to be addressed is why this position exhibits an enhanced intrinsic basicity. An AIM analysis of the charge distribution of the corresponding neutral and protonated species shows that the charge density at the C4−C5 bond critical point significantly increases upon protonation, while that of the C4−C5 bond sizably decreases. Consistently, the C4−C5 length significantly shortens, while the C5−C6 considerably lengthens. This charge redistribution points to a significant contribution of the zwiterionic resonance structure **Ib** (see Scheme 1), which would be the main factor explaining the enhanced basicity of the X heteroatom attached to position 4.

It must be observed, however, that although for 4-thiouracil and 2,4-thiouracil, the calculated proton affinity is reasonably close to the experimental value, for 2-thiouracil the calculated value to give **2b** is off by 5.0 kcal/mol. If one takes into account that the error of the proton affinities estimated at the G2(MP2) level is typically smaller than 2.0 kcal/mol, the significant gap between the experimental and the calculated value seems to point to the existence of a different structure for the protonated species.

In this respect, it must be considered that, as illustrated in Figure 2, the proton attack on thiouracils can lead to four tautomers distributed into twelve conformers. A systematic study of the stability of these twelve conformers reveals that for the 2-thiouracil and 2,4-dithiouracil, the most stable one corresponds to an enol−enethiol or dienethiol tautomer (**3a**), respectively, and only for 4-thiouracil the enethiol-oxo form (**2b**) is the most stable one. In all cases the tautomers **1a**,**b** and **4a**−**d** are significantly less stable. More specifically, at the G2(MP2) level of theory, form **3a** is estimated to be 3.2 and 1.0 kcal/mol more stable than form **2b**, for 2-thiouracil and 2,4-dithiouracil, respectively. The other tautomeric forms, **1a** and **4a** are less stable than **3a** by 5.2 and 7.1 kcal/mol for 2-thiouracil and by 7.2 and 5.4 kcal/mol for 2,4-thiouracil, at the MP2/6-31G* level. The form **2b** of protonated 4-thiouracil is estimated to be 1.5 kcal/mol more stable than structure **3a** at the G2(MP2) level. The energy gap with respect to forms **1a** and **4a**, at the MP2/6-31G* level of theory, are 11.9 and 6.7 kcal/mol, respectively. It is also worth noting that the energy differences between the different protonated tautomeric forms are systematically lower than for the neutrals. Actually, while for 2-thiouracil, 4-thiouracil, and 2,4-dithiouracil the energy gap between the most stable tautomer and the next one is 9.3, 11.2, and 6.9 kcal/mol, respectively, for the protonated species, the gap between the two most stable tautomers is only 5.1, 1.4 and 1.2 kcal/mol, respectively.

According to these results it is evident that if structure **3a** is the one formed upon protonation in the gas phase, the calculated proton affinities for 2-thiouracil and 2,4-thiouracil would be in a fairly good agreement with the experimental values. The question that remains to be answered is whether that tautomer can be formed in the gas phase. As we have discussed above, theory and experiment show unambiguously that for the neutrals only the oxo-thione forms should be found, and therefore the direct protonation of these species can only yield tautomers **1** and **2**, while structures **3** and **4** can be formed only by appropriate tautomerization of the first two ones. To investigate if these tautomerization processes are feasible under the experimental conditions, we have systematically studied the activation barriers which connect the most stable conformer of each tautomer. The results obtained have been schematized in Figure 4. Let us discuss in more detail the particular case of 2-thiouracil. As shown in Figure 4a the sulfur-protonated species (**1a**) is connected with the global minimum (**3a**) by a 1,3H shift that implies a barrier of 34.7 kcal/mol. To go from the oxygen protonated species, **2b**, to the global minimum, **3a**, two different mechanisms can be envisaged, depending on the relative order of the two steps necessary to connect structures **2b** and **3a**. The one that implies lower activation barriers is that in which the first step is the internal rotation of the OH group of form **2b** to yield **2a**, through an activation barrier of 5.9 kcal/mol, followed by a 1,3H shift from the NH group toward the thiocarbonyl group, which implies an activation energy of 27.1 kcal/mol. The alternative mechanism, in which the first step is the 1,3H shift followed by the internal rotation of the OH group, involves energy barriers slightly higher (32.3 and 7.4 kcal/mol, respectively) and therefore it is less favorable.

In any case, the tautomerization processes for protonated species involve activation barriers only slightly smaller than those estimated for the corresponding neutrals (See Figures 3 and 4). Hence, the main conclusion we can obtain from these results is that the protonation of thiouracils can be then represented by the equilibrium (3). This means that the most basic center of the thiouracils is the heteroatom at position 4 so species **2b** should be the only one formed, while the other tautomeric forms can be reached only if a significantly large amount of energy is communicated to the system.



(3)

Hydrogen Transfer Assisted Mechanisms. Assuming, as mentioned above, that the unimolecular tautomerization **2b**↔**3a** requires a very large activation barrier and therefore it should not take place under normal experimental conditions, one should reach the conclusion that the gas-phase basicity measured for

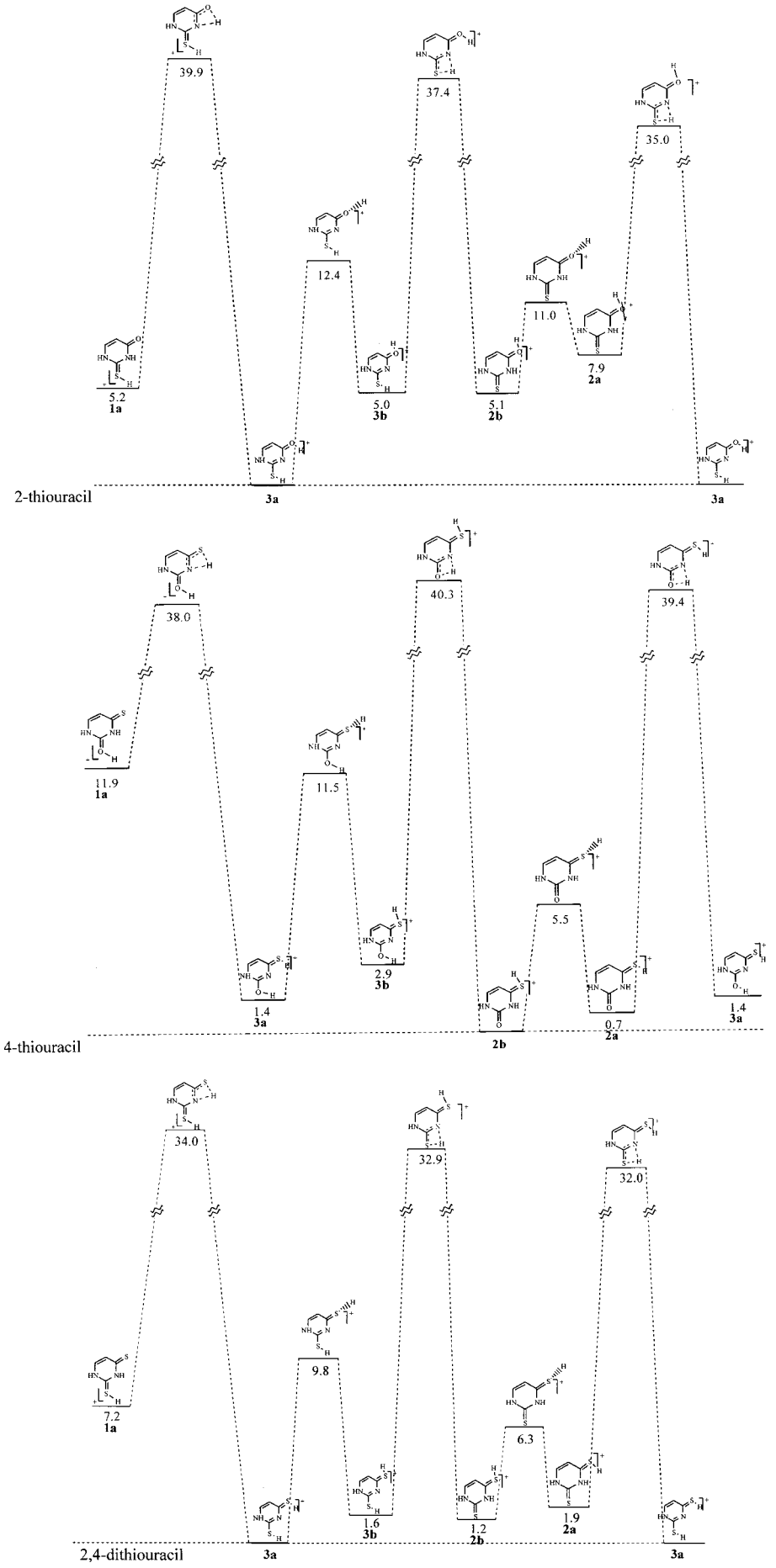


Figure 4. Energy profiles corresponding to the unimolecular tautomerization processes of protonated thiouracils. All values in kcal/mol.

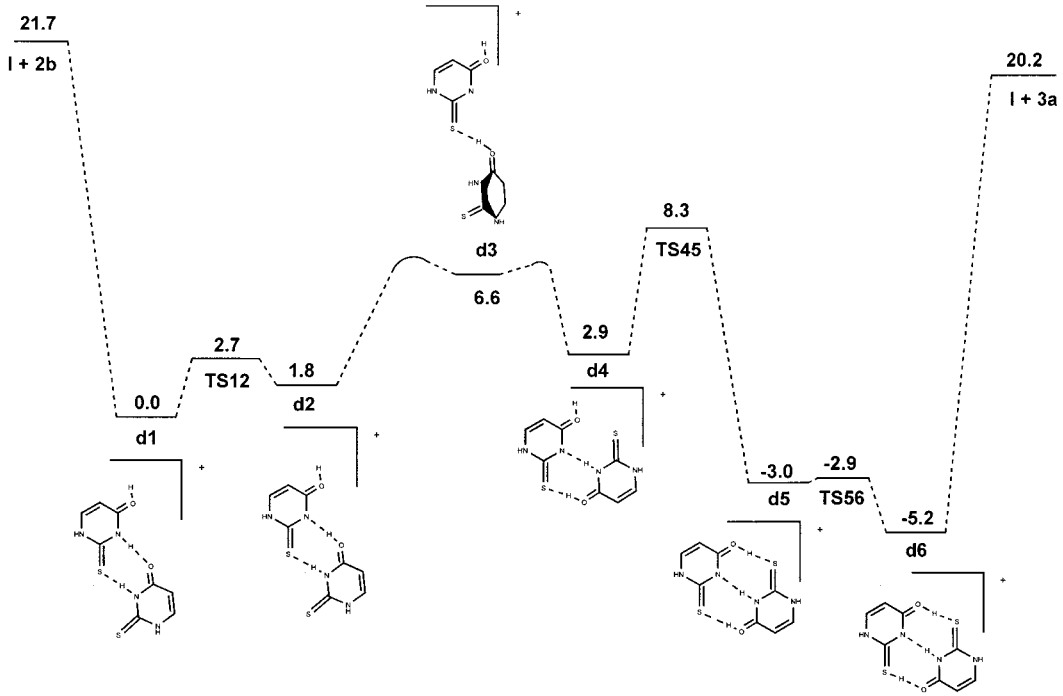


Figure 5. Potential energy surface associated with the tautomerization mechanism from the form **2b** of the 2-thiouracil to the most stable tautomer **3a**, through the formation of heterodimers involving neutral and protonated monomers. All values in kcal/mol.

2-thiouracil and 2,4-dithiouracil should be slightly smaller, unless alternative pathways to connect both tautomers are possible. In general, proton transfer processes require much lower activation barriers when they take place between two partners, i.e., between two species connected by one or more hydrogen bonds. Therefore, one possibility of having low-barrier proton transfer processes, under normal experimental conditions, would require the formation of dimers. Under this assumption, two possibilities would be open, either the neutrals form dimers easily and then these dimers undergo protonation, or once the monomer becomes protonated it forms a hydrogen bonded complex with the unprotonated species. Since these latter dimers should be, in principle, more stable than those involving only neutral species because of the ionic nature of the hydrogen bonds, we shall start our survey by considering the possible proton transfers within neutral-protonated dimers that can connect tautomers **2b** and **3a**. We shall have the opportunity later on to show that the conclusions reached from this survey can be easily applied also to the first case, i.e., to the protonation of the neutral dimers. The gas-phase tautomerization mechanism based on the formation of dimers in the gas-phase has been extensively used in the past decade, in particular to offer a rationale of these processes in neutral nucleic acid bases,[32] as well as in other systems such as pyrazoles,[33] formamide[34] and related compounds,[35] formic acid,[36] etc. To the best of our knowledge, however, the problem of the dimerization of thiouracil derivatives was only envisaged in the paper of Sponer et al.,[37] and it was restricted to the neutral species.

Since the main discrepancy between theory and experiment appears for the case of 2-thiouracil, we will take this system as a suitable example. The size of these compounds would make very time-consuming the geometry optimizations of their dimers at the MP2 level, hence, for this particular study, we shall use the B3LYP density functional method,[38] which has been shown[39] to be a reliable method for the treatment of these kinds of complexes. The geometries of the different dimers involved in our survey were optimized using a 6-31G(d) basis set expansion. The same basis set was used to evaluated the harmonic vibrational frequencies, the corresponding zero point energy corrections as well as the final energies. The energy profile associated with the tautomerization process under consideration has been schematized in Figure 5. To asses the reliability of the relative stability of the different dimers included in this mechanism, we have used the largest basis set expansion of the G2 theory in B3LYP/6-311+G(3df,2p) test calculations carried out for four of them, namely **d1**, **d2**, **d4**, and **d6**. The changes observed in their relative stabilities were not greater than 0.5 kcal/mol, and therefore we may safely conclude that the values given in Figure 5 are reasonably correct.

As shown in this figure, the first dimer that can be formed, **d1**, involves the neutral 2-thiouracil and its oxygen protonated form **2b**. It can be observed that in its equilibrium conformation the carbonyl oxygen of the neutral moiety behaves as hydrogen bond acceptor, while in the protonated moiety the thiocarbonyl sulfur is the one that behaves as HB acceptor. In both moieties the NH group at position 3 is the one which behaves as a hydrogen bond donor. All the other arrangements explored lead to less stable complexes or to structures that finally collapse toward the aforementioned complex.

Once this dimer is formed, one can expect that a synchronous proton transfer from the NH group of the protonated form toward the O atom of the neutral moiety and from the NH group of the neutral toward the sulfur atom of the protonated monomer would yield a new dimer between the protonated tautomer **3a** and the neutral tautomer **IIa** . However this process cannot compete with a single hydrogen transfer from the NH of the protonated moiety toward the oxygen atom of the neutral, which is practically barrierless, leading to a new dimer **d2** which is only 1.8 kcal/mol above dimer **d1** (see Figure 5). One could expect the dimer **d2** to be much less stable than dimer **d1** since it corresponds to the interaction of the **IIIb** neutral tautomer and the **2a** protonated one, which are much less stable than the monomers involved in dimer **d1** (see Table 2). The enhanced stability of dimer **d2** is essentially due to a significant increase

in the dipole moment of the neutral moiety and to a favorable orientation of this dipole moment with respect to the protonated moiety.

An in-plane internal rotation of the protonated subunit with respect to the neutral one would lead form dimer **d2** to a new conformation, **d3**, in which the hydrogen bond appears between the OH group and the sulfur atom. We have found, by scanning the rotation angle that this processes requires a very low activation energy. Complex **d3** evolves through a practically null activation barrier to yield a new conformation **d4** which lies almost 4 kcal/mol below in energy. The enhanced stability of this new conformation is due to the formation of a new N−H···N intermolecular HB. An internal rotation of the OH group of complex **d4** favors the formation of a third intermolecular HB between the OH group of one of the subunits and the thiocarbonyl group of the other one. The result is a very stable complex **d5** which lies almost 6 kcal/mol below complex **d4** and which is already 3.0 kcal/mol more stable than the initial dimer **d1**. The proton transfer which connects structures **d5** and **d6** is practically barrier-free. It can be observed that **d6** is a dimer which involves the most stable protonated tautomer of 2-thiouracil (**3a**) and the most stable neutral form (**I**).

The most important feature of the aforementioned mechanism is that it permits to evolve from the oxygen protonated species of 2-thiouracil, **2b**, to the most stable tautomer, **3a**, which cannot be formed by direct protonation of the neutral, through successive steps that involve quite low activation barriers and that lie well below the entrance channel. We have envisaged other alternative pathways to connect dimers **d1** and **d6**, but all attempts led either to higher activation barriers or to structures that finally collapsed to one of those included in Figure 5.

In summary we may conclude that although the unimolecular tautomerization of the protonated species of 2-thiouracil is not possible under normal experimental conditions it can take place through the formation of mixed dimers between the corresponding neutral and protonated forms obtained by its direct protonation, because the proton-transfer processes within these dimers require much lower activation energies. More importantly, all of them lie much lower in energy than the entrance channel, i.e., the formation of the **d1** dimer is exothermic enough as to permit its evolution to yield complex **d6** and its eventual dissociation into **3a** + **I**.

Similar mechanisms can also reasonably explain the formation of the **3a** tautomer of 2,4-dithiouracil.

It is worth of noting that the mechanism suggested above would also explain the formation of species **3a** if we assume that dimerization is previous to protonation. In other words, if the neutrals can easily form dimers in the gas phase, the subsequent protonation of the dimer would lead to the formation of complex **d1** and the reaction path leading to the formation of tautomer **3a** as product of the reaction will be the same discussed above.

The important consequence of the feasibility of the **2b** → **3a** tautomerization process is that the PA of 2-thiouracil and 2,4-dithiouracil would be 3.2 and 1.0 kcal/mol larger, respectively, than those corresponding to the direct protonation of the neutral, and therefore in much better agreement with the corresponding experimental values. In other words, we may conclude that for 2-thiouracil and 2,4-dithiouracil, the protonation of the neutrals is followed by a tautomerization which leads to the formation of the enol−enethiol form which is the most stable protonated tautomer.

Conclusions

Thiouracils behave as bases of moderate strength in the gas phase. The proton affinities of the three compounds investigated are rather similar, the 2,4-dithiouracil being the most basic of the three. An ab initio study of the relative stability of the protonated forms shows that in all cases the protonation takes place at the heteroatom attached to position 4. The enhanced basicity of this site seems to be associated with a certain zwiterionic character which accumulates a large electron density on it. The most important consequence is that while 4-thiouracil and 2,4-dithiouracil are sulfur bases in the gas phase, 2-thiouracil behaves as an oxygen base only slightly less basic than the other two thiouracil derivatives. This result was difficult to anticipate since, in general, thiocarbonyl derivatives are stronger bases in the gas phase than are their carbonyl analogues.[18] On the other hand, it is worth emphasizing that the easy and rapid[32b] evolution from form **2b** to form **3a** of 2-thiouracil, through the formation of heterodimers between neutral and protonated species, renders this compound as basic as the other two thiouracil derivatives, although the latter are sulfur bases.

The lower intrinsic basicity of carbonyl vs thiocarbonyl groups would explain our finding that the oxygen protonation of 2-thiouracil is 4.4 kcal/mol less exothermic than the sulfur protonation of 4-thiouracil and 2,4-dithiouracil. Despite this, as mentioned above, the three compounds exhibit rather similar proton affinities, which indicates that after protonation the systems may evolve to yield the enol−enethiol form which is the most stable tautomer but which cannot be formed either by direct protonation of the neutral or by a unimolecular tautomerization of the protonated species. We have shown that alternative mechanisms which favor the evolution toward the most stable tautomer imply the formation of hydrogen bonded dimers between the protonated form and the neutral form, followed by appropriate hydrogen transfers within the dimer, which involve rather low activation barriers.

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