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AM1 and PM3 studies of some thio analogues of pyrimidine bases in the gas and aqueous phases

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ABSTRACT: Heats of formation, entropies, Gibbs free energies, relative tautomerization energies, dipole moments, and ionization potentials for the tautomers of 2-thiocytosine, 2,4-dithiouracil and 2,4-dithiothymine and their 1-methyl analogues were studied by using semiempirical AM1 and PM3 quantum-chemical calculations at the SCF level both in the gas phase and in aqueous solution, with full geometry optimization. The COSMO solvation model was employed for aqueous solution calculations. The calculations show that the thiol-amino tautomer of 2-thiocytosine is the most stable in the gas phase whereas the thione-amino tautomer is the most stable in the aqueous phase. For 2,4-dithiouracil and 2,4-dithiothymine, dithione tautomers are the most stable in both phases. The results are in good agreement with available experimental and theoretical results. The entropy effect on the Gibbs free energy of the thiopyrimidine bases is very small and has practically no significance for the tautomeric equilibria of thiopyrimidine bases. The enthalpic term is dominant in the determination of the equilibrium constant. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: 2-thiocytosine; 2,4-dithiouracil; 2,4-dithiothymine; tautomerism; dipole moment; ionization potential; AM1; PM3 semiempirical calculations

INTRODUCTION

Pyrimidine bases and their thio derivatives are found in many biological systems and they are very important from the clinical point of view. For example, 2-thiocytosine,[1] 2-thiouracil[2] and 4-thiouracil[3–5] have been identified in tRNA. Numerous sulphur-substituted pyrimidines have found applications as clinically useful drugs.[6–10] It was noted in many of them that the position in which sulphur was present was crucial to the biological activity. For instance, 2-thiouracil, but not 4-thiouracil, inhibits hyperthyroid activity.[11]

The protropic tautomerism of these thiopyrimidine bases results from the migration of liable hydrogens from the ring nitrogens to exocyclic nitrogen or sulphur atoms, hence the thiobases can exist in several tautomeric forms. A knowledge of the relative stabilities of the various tautomeric forms of thiopyrimidine molecules and the tautomeric conversion from one tautomeric form to another is important from the point of view of structural chemistry. They are also important in relation to the biological activity of pyrimidine bases and their thio derivatives. In addition, knowing how these tautomerization energies change in different environments can shed light on the influence of solvent effects on molecular stability.

Although pyrimidine bases (cytosine, uracil and thymine) have been extensively studied,[12–20] thiopyrimidines (thiocytosines, thiouracils and thiothymines) have received much less attention. However, 4-thiouracil has attracted considerably more interest for its photochemical properties.[21,22] Although it is well known that the tautomeric equilibria of a number of pyrimidine bases depend strongly on intermolecular interactions of the bases,[23–26] only a few systematic investigations have been devoted to the tautomerism of thiopyrimidines experimentally[27–34] and theoretically.[35–41] Previously, the structural and tautomeric aspects of 2-thiocytosine and 2,4-dithiouracil were investigated by semiempirical MNDO and MINDO/3 methods[35–38] and by an *ab initio* method[39–41] in the gas phase. However, quantum chemical calculations on 2,4-dithiothymine and their 1-methyl derivatives of thiopyrimidine bases have not been reported in the literature. Also, there are no theoretical data available on the prediction of tautomerism of these molecules in the aqueous phase.

Previous work on thiopyrimidine bases has only considered the relative stabilities of the tautomers of 2-thiocytosine and 2,4-dithiouracil with the neglect of the entropy contributions. However, the analysis of the entropy effect allows for a better understanding of the tautomerization process. If several tautomers exist in comparable concentrations, the entropy contributions are important parts of the relative Gibbs free energies. Because the exact equilibrium concentration depends on

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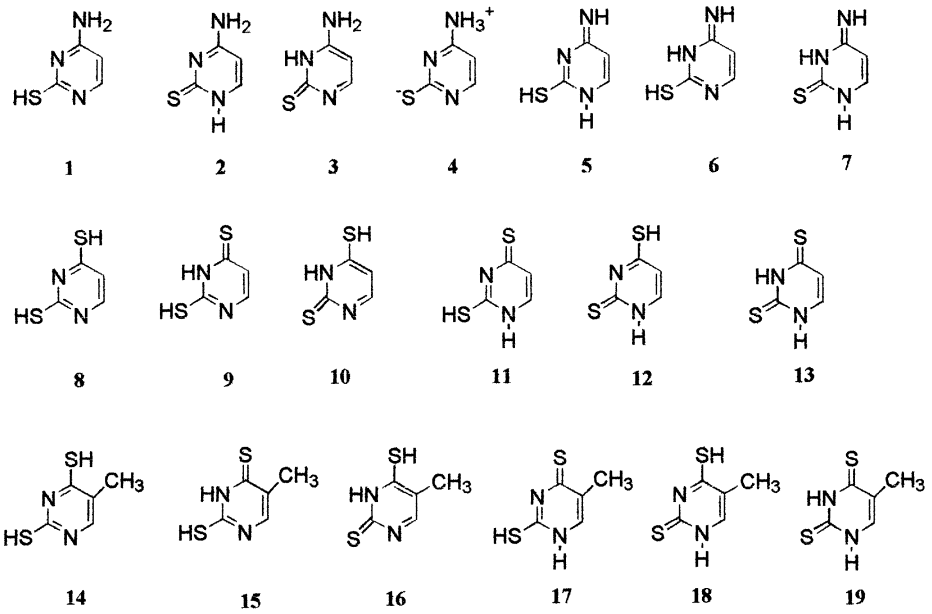

Figure 1. Tautomers of 2-thiocytosine (1–7), 2,4-dithiouracil (8–13) and 2,4-dithiothymine (14–19) considered in the present work

the Gibbs free energies of each tautomer, both entropy and enthalpy should be considered for a proper comparison of the calculated and experimental tautomeric stability of the bases. Experimental information about the relative stability of two tautomeric forms of a molecule (a ↔ b) is obtained from the measurement of the tautomeric equilibrium constant $K_{a,b}(T)$. As a consequence, the Gibbs free energy of the tautomerization $\Delta G_{a,b}(T)$ can be estimated at a given temperature T.

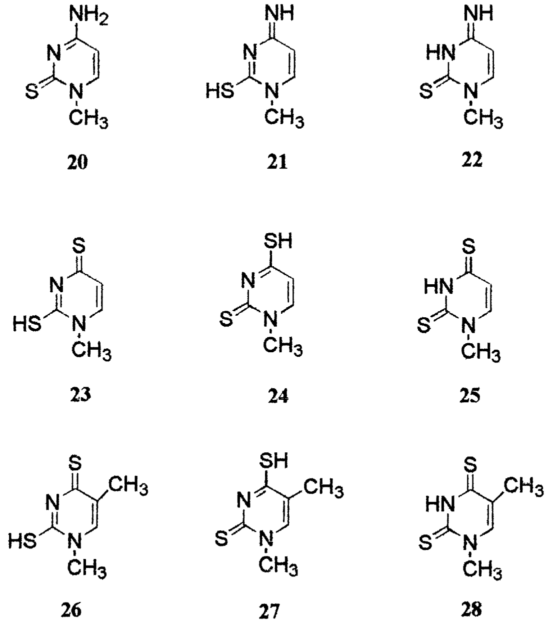

Figure 2. Tautomers of 1-methyl-2-thiocytosine (20–22), 1-methyl-2,4-dithiouracil (23–25) and 1-methyl-2, 4-dithiothymine (26–28) considered in the present work

In Part I of this series, the results of AM1 and PM3 calculations of tautomers of cytosine, uracil and thymine and their 1-methyl analogues were reported.[42] This paper presents the corresponding results for the tautomers of 2-thiocytosine, 2,4-dithiouracil and 2,4-dithiothymine and their 1-methyl analogues, as presented in Figs 1 and 2. We also report a molecular orbital study of thiopyrimidines in an attempt to learn more about the protropic tautomerism of these nucleobases. We also considered the effects of the entropy on the equilibrium and report the properties of thiopyrimidine bases such as heats of formation, entropy, Gibbs free energy, dipole moments and ionisation potentials. The AM1 and PM3 methods were used to predict tautomerism of thiopyrimidine bases. These methods have not been used previously to study tautomerisation of thiopyrimidines.

METHOD OF CALCULATION

Theoretical calculations were carried out at the restricted Hartree–Fock (RHF) level using AM1[43] and PM3[44] semiempirical SCF-MO methods incorporated in the MOPAC 7.0 program,[45] implemented on a Pentium II 300 MHz computer. In aqueous phase calculations, the COSMO (conductor-like screening model) solvation model[46] was used to construct a solvent-accessible surface area based on Van der Waals radii. The relative permittivity of water was taken to be $\epsilon = 78.4$. The model incorporated up to 30 surface segments per atom, and we then set the parameters NPPA to 1082 and NSPA to 30. Initial geometry estimates for all the structures were obtained from a molecular mechanics calculation (CS

Table 1. AM1-calculated thermodynamic properties for the tautomers of 2-thiocytosine, 2,4-dithiouracil and 2,4-dithiothymine and their 1-methyl analogues in the gas phase ($\epsilon = 1$) at 298.15 K

Tautomer	ΔH_f (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	$\Delta G_f{}^a$ (kcal mol^{-1})	$\delta\Delta G_f{}^b$ (kcal mol^{-1})	IP (eV)	μ (D)
1	46.64	83.39	21.78	0.00	8.78	7.64
2	55.36	85.03	30.01	8.23	8.33	3.06
3	56.83	83.24	32.01	10.23	8.47	8.31
4	97.99	86.14	72.31	50.53	7.30	14.70
5	69.57	85.15	44.18	22.40	8.87	4.86
6	62.19	83.73	37.23	15.45	8.82	1.59
7	60.98	82.99	36.24	14.46	9.08	5.33
8	57.69	85.06	32.33	5.46	8.98	3.13
9	58.48	84.30	33.35	6.74	8.81	4.13
10	63.19	85.55	37.68	10.81	8.72	6.80
11	65.56	85.97	39.93	13.06	8.18	6.64
12	62.88	84.96	37.55	10.68	8.65	7.38
13	52.44	85.76	26.87	0.00	8.96	4.70
14	50.46	94.62	22.25	4.74	8.83	3.48
15	51.77	94.12	23.71	6.20	8.74	3.82
16	55.95	94.02	27.92	10.41	8.68	7.10
17	58.61	95.55	30.12	12.61	8.11	6.46
18	56.46	92.26	28.95	11.44	8.60	7.70
19	45.35	93.38	17.51	0.00	8.91	4.85
20	64.94	95.72	36.40	0.00	8.91	7.43
21	75.09	93.73	47.14	10.74	8.69	5.34
22	67.62	92.65	40.00	3.60	9.24	5.57
23	76.93	94.39	48.79	12.65	8.14	8.80
24	69.01	95.01	40.68	4.54	8.47	7.42
25	64.73	95.90	36.14	0.00	8.86	5.31
26	70.01	103.97	39.01	12.34	8.07	8.66
27	62.61	102.57	32.03	5.36	8.43	7.71
28	57.99	105.04	26.67	0.00	8.82	5.36

[a] From $\Delta G_f = \Delta H_f - T\Delta S$.
[b] $\delta\Delta G_f = \Delta G_{f,\,(b)} - \Delta G_{f(a)}$.

Chem Office)[47,] and were followed by full optimization of all geometrical variables. All structures were optimized to a gradient norm of <0.2 in the gas phase and 0.1–2 in the aqueous phase, using the Eigenvector Following (EF) method at the PRECISE level. In order to calculate thermodynamic properties (ΔH_f, ΔS) of the tautomers, the gradient norm was reduced again to a value very close to zero. The entropy term was obtained from FORCE calculations for all the possible tautomers and the Gibbs free energies of the tautomerization (ΔG_f) at 298.15 K were predicted by adding the enthalpic (ΔH_f) and entropic ($-T\Delta S$) terms.

RESULTS AND DISCUSSION

Relative stability

The relative stabilities, enthalpies, entropies and Gibbs free energies for the possible tautomeric forms of the investigated compounds are given in Tables 1–4. The AM1 gas-phase calculation results (cf. Table 1) indicate that the thiol-amino form **1** for 2-thiocytosine is more stable than that of the 1*H*-thione-amino form **2** by about 8 kcal mol^{-1}, and the 3*H*-thione amino form **3** by about 10 kcal mol^{-1} (1 kcal = 4184 kJ). The predominance of the thiol-amino form is in complete agreement with experimental studies[27,30] and with theoretical studies.[38] Mezey and co-workers[48,49] proposed that any species with an energy larger than 10 kcal mol^{-1} above the most stable form would not exist in any appreciable concentration. A correct application of the equation $LnK^0 = -\Delta G/RT$ indicates that only tautomer **1** can exist in appreciable amounts at room temperature. The other tautomers would possess no significant concentration at room temperature. The effects of the polar environment were estimated by the COSMO solvation model. The AM1 aqueous phase calculations (cf. table 2) suggest that the thione-amino forms **2** and **3** are more stable than that of the thiol-amino form **1** by $\approx$13 kcal mol^{-1}. In other words, the thione forms are more stabilized than the thiol forms. This situation is opposite to that found in the gas phase where the thiol form was the predominant tautomer. Here the stabilization of the thiol form over the thione forms in the gas phase is greater, most probably due to electrostatic effects. Table 2 also clearly shows that the 1*H*-thione form **2** is more stable than the 3*H*-thione form **3** by 0.84 kcal mol^{-1}. In fact, Brown and Teitei[50] found that the concentration of the 1*H*-thione form **2** is nearly equal to the concentration of the 3*H*-

Table 2. AM1-calculated thermodynamic properties for the tautomers of 2-thiocytosine, 2,4-dithiouracil and 2,4-dithiothymine and their 1-methyl analogues in the aqueous phase ($\epsilon = 78.4$) at 298.15 K

Tautomer	ΔH_f (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	ΔG_f[a] (kcal mol^{-1})	$\delta\Delta G_f$[b] (kcal mol^{-1})	IP (eV)	μ (D)
1	29.87	83.00	5.08	13.43	9.29	5.24
2	16.39	82.99	−8.35	0.00	9.84	16.82
3	17.70	84.56	−7.51	0.84	9.62	19.14
4	18.98	85.01	−6.37	1.98	9.87	26.95
5	48.20	84.04	23.14	31.49	9.28	12.67
6	45.79	83.88	20.78	29.13	9.16	2.95
7	32.49	82.43	7.91	16.26	9.67	10.66
8	38.10	88.50	11.71	9.72	9.40	5.72
9	32.54	85.76	6.97	4.98	9.52	12.11
10	32.89	85.32	7.45	5.46	9.52	16.79
11	32.30	84.74	7.03	5.04	9.75	21.77
12	29.28	84.32	4.14	2.15	9.71	17.07
13	26.88	83.47	1.99	0.00	9.89	12.06
14	31.56	93.86	3.58	7.52	9.28	5.99
15	27.99	95.37	−0.44	3.50	9.33	11.27
16	27.81	92.39	0.26	4.20	9.38	16.75
17	27.04	92.64	−0.58	3.36	9.52	17.84
18	34.94	92.80	7.27	11.21	9.56	17.32
19	23.49	91.99	−3.94	0.00	9.24	5.45
20	18.50	91.24	−8.70	0.00	9.28	17.79
21	53.53	94.99	25.21	33.91	9.17	9.56
22	43.86	94.47	15.69	24.39	9.60	10.55
23	41.48	92.29	13.96	5.10	9.79	22.11
24	40.28	93.87	12.29	3.43	9.66	16.56
25	36.59	93.00	8.86	0.00	9.84	12.57
26	37.40	102.40	6.87	4.92	9.59	21.37
27	33.50	101.51	3.23	1.28	9.57	16.84
28	33.04	104.27	1.95	0.00	9.63	12.28

[a] From $\Delta G_f = \Delta H_f - T\Delta S$.

[b] $\delta\Delta G_f = \Delta G_{f(b)} - \Delta G_{f(a)}$.

thione form **3**, the former being more stable by about 4 kcal mol^{-1}. This finding, although observed in solution where a number of effects could stabilize a given species, also points to 1*H*-thione-amino **2** as one of the predominant species of 2-thiocytosine. It can also be inferred that the more unstable tautomeric forms are those possessing an imino group in both phases. For these species the relative energies vary between 14.46 and 31.49 kcal mol^{-1}.

Tautomeric equilibrium constants depend on two major external factors: the phase (solid, solution or vapour) and temperature.[51] The position of the tautomeric equilibria can be affected by the nature of the solvent and by the concentration of the solution. The proportion of any one tautomer present in an equilibrium mixture can change if a change in environment alters the relative stabilities of the tautomers by preferentially stabilizing one of them. If one isomer is more polar than another, it will be preferentially stabilized in media with high dielectric constants; e.g. it will be more stable in water than in hydrocarbon solvents. Specific interaction with the solvent, in particular hydrogen bonding with the solvent acting as the hydrogen donor or acceptor, or both, often preferentially stabilizes one tautomer.

There is a difference between predicted values in the gas phase and in aqueous solution. Comparison of the gas-phase equilibrium constants with solution-phase values implies that large changes may occur on going from the gas phase to solution. This result also agrees with what one can expect from the solvent effects. Solvent effects have been ascribed to two major components[52]: electrostatic solvent–solute interaction and hydrogen bonding. The hydrogen bonding effects cannot be estimated quantitatively from the solvation model, without further large-scale calculations. The electrostatic solvent–solute effects, however, are readily estimated by a continuum model such as COSMO.[46] Application of the COSMO model leads to an explanation of the change in order of tautomeric stability on going from the gas phase to solution. However, such a treatment lacks explicit consideration of base–water hydrogen bonding effects[53], thus tautomeric equilibrium constants predicted in water are considerably less reliable than those predicted in the gas phase. Application of the COSMO solvation model to 2-thiocytosine yields the order of stability **2**>**3**>**4**>**1**>**7**>**6**>**5**, while the order of stability in the gas phase is **1**>**2**>**3**>**7**>**6**>**5**>**4**.

For the 2,4-dithiouracil tautomers, the calculations

Table 3. PM3-calculated thermodynamic properties for the tautomers of 2-thiocytosine, 2,4-dithiouracil and 2,4-dithiothymine and their 1-methyl analogues in the gas phase ($\epsilon = 1$) at 298.15 K

Tautomer	ΔH_f (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	ΔG_f[a] (kcal mol^{-1})	$\delta\Delta G_f$[b] (kcal mol^{-1})	IP (eV)	μ (D)
1	37.76	84.22	12.65	0.00	9.09	7.85
2	45.46	83.73	20.49	7.84	8.57	3.18
3	51.38	85.6	25.86	13.21	8.70	8.11
4	57.81	87.09	31.84	19.19	8.84	3.90
5	58.81	84.22	33.70	21.05	8.77	1.23
6	58.38	86.35	32.63	19.98	9.04	5.95
7	65.67	86.66	39.83	27.18	7.5	5.32
8	62.89	86.74	37.03	13.56	9.31	3.87
9	68.15	87.52	42.06	18.59	8.98	4.53
10	70.75	89.68	44.01	20.54	8.91	7.37
11	68.86	87.00	42.92	19.45	8.53	6.98
12	72.76	86.56	46.95	23.48	8.91	8.06
13	49.55	87.46	23.47	0.00	9.28	4.85
14	54.58	96.01	25.95	12.75	9.18	4.23
15	59.56	95.98	30.94	17.74	8.88	4.31
16	62.82	98.83	33.35	20.15	8.81	7.76
17	61.42	94.64	33.20	20.00	8.45	6.90
18	64.94	96.15	36.27	23.07	8.79	8.45
19	41.58	95.20	13.20	0.00	9.24	5.05
20	56.12	94.71	27.88	0.00	8.42	7.69
21	58.20	94.10	30.14	2.26	8.69	4.28
22	59.28	95.05	30.94	3.06	8.91	6.15
23	72.90	96.88	44.02	3.99	8.48	7.53
24	70.69	97.61	41.59	1.56	8.74	8.14
25	69.27	98.07	40.03	0.00	9.20	5.74
26	65.09	105.70	33.58	3.05	8.41	7.47
27	62.93	107.04	31.02	0.49	8.69	8.50
28	61.81	104.92	30.53	0.00	9.15	5.99

[a] From $\Delta G_f = \Delta H_f - T\Delta S$.

[b] $\delta\Delta G_f = \Delta G_{f(b)} - \Delta G_{f(a)}$.

show that the dithione form **13** appears to be the most stable in both the gas and aqueous phases. The predominance of the dithione form is in complete agreement with IR gas matrix experimental studies[28,54] and with theoretical studies.[36,39,40]

For 2,4-dithiothymine, the dithione form **19** is predicted to be at least 3 kcal mol^{-1} more stable than the other tautomers in both phases. Unfortunately, there are no experimental or theoretical data for this compound with which to compare our calculated values. However, looking at the good agreement between our calculated and reported experimental relative stabilities for 2,4-dithiouracil, our results are expected to be fairly reliable. As expected, substitution of 2,4-dithiouracil at the 5-position by CH_3 does not change the order of the stability of the tautomer.

For the 1-methyl derivative of 2-thiocytosine, the thione-amino tautomer **20** predicted to be the most stable form in both phases. In the gas phase, relative stabilities for thione-amino tautomer **20** and 3*H*-thione-imino form **22** are very close, whereas in solution their energy values are substantially different (cf. Tables 1 and 2). These results illustrate the differential effects of a polar medium on the relative stability of tautomers. The AM1 and PM3 calculations for 1-methyl-2,4-dithiouracil and 1-methyl-2, 4-dithiothymine tautomers also show the dominance of the dithione forms **25** and **28** both in the gas phase and in solution (cf. Tables 1–4). Unfortunately, experimentally and theoretically determined tautomerization energies of these compounds are not available, so we cannot compare our calculated values with experimental and theoretical data. The stable methyl tautomers (**20**, **25** and **28**) are the same as the main tautomers (**2**, **13** and **19**). In other words, substitution of thiopyrimidine bases at the N$-$1 position by CH_3 does not change the order of the stability of the tautomers.

The value of any semiempirical method depends not only on the ability of that method to reproduce experimental observations, but also on the particular set of molecules used for optimizing the parameters. Some attempts to test the reliability of semiempirical quantum chemical methods (AM1, PM3, MNDO and MINDO/3) for many organic molecules were performed by Dewar *et al.*[43] and Stewart.[44] They show that there are some problems in the case of molecules containing hetero-atoms (O, N, S), because of the neglect of one-centre overlap in the INDO approximation on which MINDO/3 is based. These problems are avoided in the MNDO

Table 4. PM3-calculated thermodynamic properties for the tautomers of 2-thiocytosine, 2,4-dithiouracil and 2,4-dithiothymine and their 1-methyl analogues in the aqueous phase ($\varepsilon = 78.4$) at 298.15 K

Tautomer	ΔH_f (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	ΔG_f[a] (kcal mol^{-1})	$\delta\Delta G_f$[b] (kcal mol^{-1})	*IP* (eV)	μ (D)
1	21.64	88.01	−4.60	11.53	8.93	7.35
2	8.64	83.10	−16.13	0.00	9.19	18.64
3	9.38	83.62	−15.55	0.58	9.29	22.19
4	13.03	83.80	−11.95	4.18	10.06	27.20
5	38.17	85.51	12.68	28.81	9.01	12.34
6	36.09	85.04	10.74	26.87	9.08	2.73
7	25.99	83.62	1.06	17.19	9.35	11.01
8	33.62	89.05	7.07	6.70	9.71	6.47
9	28.36	84.37	3.21	2.84	9.83	12.42
10	30.75	87.16	4.76	4.39	9.76	17.42
11	29.75	86.18	4.06	3.69	9.89	19.61
12	26.21	85.56	0.70	0.33	9.92	18.25
13	27.06	89.51	0.37	0.00	9.98	13.83
14	25.08	97.42	−3.97	5.95	9.62	6.75
15	21.63	93.8	−6.34	3.58	9.67	11.83
16	23.91	97.25	−5.09	4.83	9.76	17.74
17	21.87	95.23	−6.52	3.40	9.98	21.28
18	26.26	100.03	−3.56	6.36	9.86	18.64
19	18.45	95.17	−9.92	0.00	9.88	14.11
20	18.50	95.04	−9.84	0.00	9.28	17.79
21	38.05	95.77	9.50	19.34	9.00	9.92
22	30.72	93.37	2.88	12.72	9.35	11.71
23	34.55	96.54	5.77	2.89	10.12	22.66
24	33.56	93.75	5.61	2.73	9.83	17.95
25	32.05	97.85	2.88	0.00	10.03	14.21
26	32.55	104.77	1.31	5.90	9.97	22.01
27	29.49	105.19	−1.87	2.72	9.86	18.25
28	25.75	101.77	−4.59	0.00	9.05	13.31

[a] From $\Delta G_f = \Delta H_f - T\Delta S$.

[b] $\delta\Delta G_f = \Delta G_{f(b)} - \Delta G_{f(a)}$.

method, but at the expense of other weaknesses, in particular failure to reproduce hydrogen bonds, energies that are too positive for crowded molecules and too negative for ones containing four-membered rings, and activation energies that tend to be too large. These errors are largely corrected in AM1. However, they also show that the AM1 method performs better than the other methods (PM3, MNDO and MINDO/3) for six-membered nitrogen heterocycles (pyrimidine, pyridazine and pyrazine), most probably due to presence of radial gaussians which modify the core–core repulsion terms and the ability to reproduce hydrogen bonds and the promise of better estimates of activation energies.[55] Part of the improvement in AM1 over MNDO is due to the fact that a better minimum was found, corresponding in particular to different orbital exponents, which have a large effect on activation barriers, and to the ratios of the β parameters for s and p atomic orbitals, which appear to control the bond angles. According to our results, the PM3 method gives the same order of stability for the thiopyrimidine bases and their 1-methyl analogues. With respect to the tautomerism in azines, the PM3 method was found to be comparable in accuracy to AM1.[56] Entropy effects, which are sometimes neglected in quantum mechanical calculations, also affect the calculated results and are important especially in the thione–thiol tautomerism. Comparison of our results with the previous MNDO, MINDO/3 and *ab initio* results is of interest; unfortunately the comparison should be limited to energies, since enthalpies and entropies were not reported. However, as we can see from Tables 1–4, the calculated entropy values are generally small (≈100 cal mol^{-1}). This results indicate that the entropy effect on the Gibbs free energy is very small and the entropy term, that is, the $T\Delta S$ value, can be neglected for the tautomeric equilibria of thiopyrimidine bases. Thus, the entalpic term is dominant in the determination of the equilibrium constant.

Dipole moments and ionization potentials

The calculated dipole moments and the first vertical ionization potentials of the studied thiopyrimidine bases are also listed in Tables 1–4. The comparison between experimental and calculated dipole moments gives additional support for the calculated stabilities of thiopyrimidine bases. The experimental dipole moment

Table 5. Calculated and experimental dipole moments and ionisation potentials of pyrimidine bases with respect to the most stable tautomers

	μ (D)						IP (eV)					
Compound	MNDO[a]	MINDO/3[b]	AM1[c]	PM3[c]	*Ab initio*[d]	Exp.[e]	MNDO[f]	MINDO/3[g]	AM1[c]	PM3[c]	*Ab initio*[h]	Exp.[i]
Cytosine	5.63	—	6.32	6.68	7.11	6.0–6.5	9.54		9.38	9.28	9.00	8.94
2-Thiocytosine	6.73	9.49	7.64	7.85	5.62	—	8.52	8.01	8.78	9.09	9.54	—
Uracil	4.12	4.52	4.29	3.99	4.79	4.16	9.93	9.45	9.97	9.71	11.27	9.50
2,4-Dithiouracil	5.29	7.54	4.70	4.85	5.62	4.67	9.80	8.75	8.96	9.28	—	—
Thymine	4.12	—	4.16	3.97	4.58	4.13	9.78	—	9.60	9.44	9.45	9.18
2,4-Dithiothymine	—	—	4.85	5.05	—	—	—	—	8.91	9.24	—	—
1-Methylcytosine	—	—	5.94	5.58	6.74	6.0–7.0	—	—	9.22	9.03	—	8.65
1-Methylthiocytosine	—	—	7.43	7.69	—	—	—	—	8.91	8.42	—	—
21-Methyluracil	—	—	4.65	4.37	—	—	—	—	9.70	9.52	—	—
1-Methyldithiouracil	—	—	5.31	5.74	—	—	—	—	8.86	9.20	—	—
1-Methylthymine	—	—	4.49	4.20	4.83	4.10	—	—	9.42	9.36	—	—
1-Methyldithiothymine	—	—	5.36	5.99	—	—	—	—	8.82	9.15	—	—

[a] Ref. 17 for cytosine, Ref. 41 for 2-thiocytosine, Ref. 37 for uracil and thymine, Ref. 36 for 2,4-dithiouracil.
[b] Ref. 41 for 2-thiocytosine, Ref. 35 for uracil and 2,4-dithiouracil.
[c] Ref. 42 for cytosine, uracil, thymine and their 1-methyl derivatives; from this work for 2-thiocytosine, 2,4-dithiouracil, 2,4-dithiothymine and their 1-methyl derivatives.
[d] Ref. 18 for cytosine, thymine, 1-methylcytosine and 1-methylthymine, Ref. 15 for 2-thiocytosine, Ref. 41 for uracil, Ref. 40 for 2,4-dithiouracil.
[e] Ref. 18 for cytosine and 1-methylcytosine, Ref. 57 for uracil, 2,4-dithiouracil and thymine.
[f] Ref. 17 for cytosine, uracil and thymine, Ref. 23 for 2-thiocytosine, Ref. 36 for 2,4-dithiouracil.
[g] Ref. 23 for 2-thiocytosine, Ref. 35 for uracil and 2,4-dithiouracil.
[h] Ref. 20 for cytosine and thymine, Ref. 23 for 2-thiocytosine, Ref. 24 for uracil.
[I] Ref. 19 for cytosine, uracil, thymine and 1-methylcytosine.

is available only for 2,4-dithiouracil.[57] The calculated dipole moments for the most stable tautomer of 2-thiocytosine by both the AM1 and PM3 methods are very different from the MNDO (6.73 D) and MINDO/3 predictions (9.49 D).[36] As previously noted, dipole moments are systematically overestimated on average by 0.68 D, probably owing to atomic charges and lone pairs in the MNDO method.[36]

In the case of 2,4-dithiouracil, the AM1- and PM3-calculated dipole moments for the most stable tautomer are 4.70 and 4.85 D, respectively. This result is in good agreement with the experimental value (4.67 D) reported by Schneider and Halvestadt.[57] AM1 and PM3 methods give better values of dipole moments than do other theoretical methods,[35,36,40] such as MNDO (5.29 D), MINDO/3 (7.54 D) and *ab initio* (5.62 D).

The calculated dipole moment of 2,4-dithiothymine is 4.85 D for AM1 and 5.05 D for PM3. No experimental and theoretical data are available for the comparison, but the result obtained for 2,4-dithiothymine is fairly close to the value obtained for 2,4-dithiouracil. Consequently, our result is expected to be reliable, at least semiquantitatively.

There are no available experimental dipole moments for 1-methyl-2-thiocytosine, 1-methyl-2,4-dithiouracil and 1-methyl-2,4-dithiothymine. We cannot compare the calculated dipole moments of the title compounds since no experimental and theoretical dipole moments of the thiopyrimidines have been reported in the literature. However, some indirect support can be derived from experimental and theoretical data on the hydroxypyrimidines. After having well-established data for pyrimidines, we can make some inferences about physico-chemical properties (dipole moments or ionization potentials) of less known thiopyrimidines. As can be seen in Table 5, the dipole moments of the thiopyrimidines are greater than those of the corresponding pyrimidines. The trend of the change in the dipole moments for oxygen-containing molecules is similar to the trend of the change in the dipole moments of the thio analogues. Taking into account the fact that the dipole moment of the C═S bond is higher than that of the C═O bond by 1.1 D, there is therefore a good correlation between the calculated dipole moment values of the pyrimidines and their thio analogues.

As we can see from Tables 1–4, the calculated dipole moments change on moving from the gas phase ($\varepsilon = 1$) to aqueous solution ($\varepsilon = 78.4$) and the dipole moments are sensitive to the polarity of the medium. The calculated dipole moments are substantially higher in a medium of high relative permittivity, mainly owing to the major charge redistribution in the molecule, and also by changes in the distances between the charge separations. The magnitude of the influence of the solvent reaction field on electronic structure is different in different tautomers. This may also explain the great variation of the calculated dipole moments of the tautomers.

According to Koopman's theorem and the calculated results of the AM1 method, the first vertical ionization potentials for the most stable tautomers of 2-thiocytosine, 2,4-dithiouracil and 2,4-dithiothymine are 8.78, 8.96 and 8.91 eV, respectively. The calculated ionization potentials of 1-methyl-2-thiocytosine, 1-methyl-2,4-dithiouracil and 1-methyl-2,4-dithiothymine are 8.91, 8.86, 8.82 eV, respectively, for AM1. No experimental ionization potentials are available for these compounds. Unfortunately, the present results cannot be compared with experimental data. However, there are some theoretical results and our results are different from those of MNDO[36] and MINDO/3 predictions,[35] as shown Table 5. Dewar *et al.*[58] have shown, however, that the MNDO method systematically overestimates the ionization energies of the nitrogen lone-pair orbitals and that a correction of 1 eV should be applied when using MNDO calculations to assign photoelectron spectra. Results obtained by the PM3 method are not very different from those obtained by AM1. The PM3 results for the investigated thiopyrimidine bases are also close to the *ab initio* calculations.[20,23,24]

CONCLUSION

The results clearly indicate that thiopyrimidine bases and their 1-methyl analogues exist predominantly in the thiol-amino or dithione tautomeric forms in the gas phase and the thione-amino or the dithione tautomeric forms in solution. These results are in agreement with available experimental and theoretical data.

Substitution of 2,4-dithiouracil at the 5-position by CH_3 does not change the order of the stability of the tautomers.

Substitution of thiopyrimidine bases at the N-1 position by CH_3 does not change the order of the stability of the tautomers.

The results presented in this paper confirm earlier observations about the applicability of the AM1 method to the prediction of the relative stability of six-membered nitrogen heterocyclic tautomers.

AM1 also gives a good representation of the charge distribution in molecules in terms of calculated dipole moments in both phases.

The inclusion of the solvent reaction field in quantum-chemical theory is obligatory for accurate results in solution.

Use of the COSMO solvation model successfully explains the reordering of the relative tautomeric stability on passing from the gas phase to solution.

The entropy effect on the Gibbs free energy of the thiopyrimidine bases is very small and is of little significance for the tautomeric equilibria of thiopyrimidine bases. The enthalpic term is dominant in the determination of the equilibrium constant.

There is a good correlation between calculated physico-chemical properties (dipole moments and ionization potentials) of pyrimidines and thiopyrimidines.

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