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ORIGINAL PAPER

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Theoretical investigation of the structure and acid–base properties of potential 2-thiolumazine tautomeric forms using the AM1 semiempirical method

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Abstract The relative stabilities of potential tautomers, in both gas and aqueous phases, have been calculated taking into account the entropy effects over the tautomeric equilibria, in order to determine the structure and acid–base properties of the most stable tautomers of 2-thiolumazine in different pH conditions. In each medium, the tautomer with the lowest energy must be the most representative form at the corresponding pH. Knowledge of the effect of the medium in the tautomerization energies allows us to evaluate the possible effect of the medium on the molecular stability. Clearly, the results show that in the gas phase the basicity of the potential donor atoms is N5<N8<O4<S2<N1<N3, and in the aqueous phase S2<(O4~N5)<N8<N1<N3, with the higher basicity of N3 and N1 being common to the two phases. In the aqueous phase, the sulfur atom is usually found in the thiol form, whereas the oxygen atom is in the keto form only in the most stable species. Moreover the acid–base character of 2-thiolumazine in aqueous solution has been evaluated from the corresponding AM1 thermodynamic parameters. The results agree well with the experimental data. Electronic supplementary material to this paper can be obtained by using the Springer Link server located at http://dx.doi.org/10.1007/s00894-002-0094-9.

Keywords AM1 · Lumazine · Semiempirical methods · Tautomerism

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Introduction

The study of tautomeric phenomena from both experimental and theoretical points of view has been developed in many chemistry and biochemistry areas, mainly because the presence of unusual tautomers may be of biological importance. For example, some DNA mutagenesis processes could be related to either keto–enol or amino–imino tautomeric equilibria in the different bases, these reactions being influenced by the interaction between these molecules and the environment.

In this way, xanthine oxidase is an important and broadly studied enzyme and some pteridine derivatives (e.g. molibdopterine and lumazine derivatives) may act as essential cofactors, [1] substrates, [2, 3] and inhibitors. [4] Moreover, xanthine oxidase is a source of superoxide radicals in vivo and in vitro, which increases the interest in studying the interactions between pteridine derivatives and this enzyme to clarify whether these compounds directly interact with the enzyme or whether they control the generation and the function of the free radicals. [5] Thus, lumazine derivatives are of interest because they can make charge transfer species in the reduction of xanthine oxidase. [6, 7, 8, 9] In these molecules, the replacement of an oxygen by a sulfur atom induces changes in the structure and biological properties. Changes in the tautomeric equilibria must also be expected.

For these reasons, the relative stabilities of potential tautomers of 2-thiolumazine (Fig. 1) have been calculated in both gas and aqueous phases, taking into account the entropy effects on the tautomeric equilibria. In each medium, the tautomer with the lowest energy must be the most representative form at the corresponding pH and knowledge of the effect of the medium on the tautomerization energies allows us to evaluate the possible effect of the medium on the molecular stability. Moreover, the AM1 semiempirical method has been used to evaluate the acid–base character of 2-thiolumazine from the corresponding thermodynamic parameters.

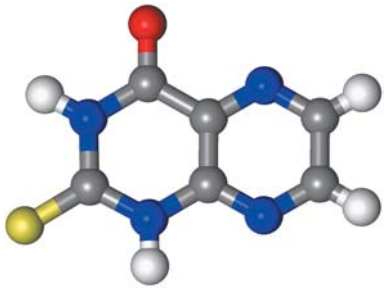

Fig. 1 Ball and stick view of the structure of 2-thiolumazine (color key is as usual)

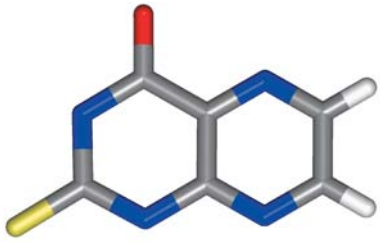

Fig. 2 Potential protonation sites on the 2-thiolumazine skeleton. The tautomers are named with the prefix TLM followed by numbers labeling the protonation positions

Computational methods

Calculations were carried out at the restricted Hartree–Fock level (RHF) using the AM1 [10] semiempirical SCF-MO method as implemented in AMPAC 6.7. [11] All structures were generated with the Ampac Graphic User Interface (AGUI) and fully optimized (bond lengths, bond angles and dihedral angles), without any constraint, to a gradient norm of <0.01 kcal mol^{-1} Å^{-1} in the gas phase and 0.1–0.5 kcal mol^{-1} Å^{-1} in the aqueous phase, using the eigenvector following method; configuration interaction was not taken into account. To calculate the thermodynamic properties (ΔH_f and ΔS) of the tautomers, the gradient was reduced to a close-to-zero value and the absolute entropy was obtained from a complete vibrational analysis at room temperature (298 K), assuming no internal rotations.

In the aqueous phase, calculations were carried out using the COSMO model [12] to construct a solvent accessible surface area based on the van der Waals radii. The relative permittivity corresponding to water was 78.355 and the COSMO total surface was around 90 Å^2.

Finally, it must be pointed out that the AM1 calculations on highly charged systems are only trustworthy to a limited extent; ab initio methods show that diffuse and polarization functions are essential for the reproduction of anions and dianions and such functions are not used in AM1.

Results and discussion

In view of the molecular structure of 2-thiolumazine, three consecutive protonation equilibria must be expected:

$$[\mathrm{TLMH_3}]^+ \rightleftarrows [\mathrm{TLMH_2}] \rightleftarrows [\mathrm{TLMH}]^- \rightleftarrows [\mathrm{TLM}]^{2-}$$

from which the first must take place at such an acid pH that it has not been detected in the study of the pH dependence (0 to 14 pH range) of the electronic spectrum. The two subsequent deprotonation processes have been clearly observed at pH values in accordance with the reported pK_2 and pK_3, 6.49 and 11.90. [13] Thus, in aqueous solution over the full pH range, four different forms could be expected: $[\mathrm{TLMH_3}]^+$ (cation), $[\mathrm{TLMH_2}]$ (neutral), $[\mathrm{TLMH}]^-$ (monoanion), and $[\mathrm{TLM}]^{2-}$ (dianion). In view of the pK values, the absorption maxima at selected pH values are given in Table 1.

Table 1 Absorption maxima of 2-thiolumazine aqueous solution (5×10^{-5} M) at selected pH values

pH	λ_{max}(nm)	log ε
0.15	286	4.42
	346	3.99
4.05	286	4.45
	346	4.01
9.05	242	3.92
	306	4.39
	363	3.84
13.47	244	4.03
	287	4.38
	379	3.92

Spectra of the neutral, monoanionic and dianionic species can be clearly detected but not that corresponding to the monoprotonated one because the comparison between the spectra at pH 0.15 and pH 4.05 shows only small differences in the absorptivity values, not in the wavelength maxima. The structures of the potential tautomers are shown in Fig. 2.

Following the above methodology, the heat of formation (ΔH_f), the absolute entropy (ΔS), the Gibbs free energy (ΔG_f) and the relative stability ($\delta\Delta G_f$) for each tautomer have been calculated in both gas and aqueous phases. The relative stabilities obtained from ΔG_f are very similar to those calculated from ΔH_f since the absolute entropy values are, in gas and aqueous phases, ca. 100 cal K^{-1} mol^{-1}. Thus, the entropy factor only needs to be taken into account if the heats of formation of the tautomeric forms are very close.

Likewise, the tautomerization equilibrium constants (in terms of pK_T) between each form and the most stable tautomer of the group and the mole fraction are given. The pK_T values were calculated for the reaction

$$A_1 \rightleftarrows A_i \quad K_T = \exp[-\delta\Delta G_f/RT]$$

where $\delta\Delta G_f$ is the difference between the Gibbs free energy of the tautomers considered. The mole fraction of n coexisting species as follows

$$A_1 \rightleftarrows A_2 \rightleftarrows A_3 \rightleftarrows \ldots \rightleftarrows A_n$$

can be easily calculated using the following relations:

$$N_{A1} = 1/(1+K_{12}+K_{13}+\ldots+K_{1n})$$
$$N_{A2} = K_{12}/(1+K_{12}+K_{13}+\ldots+K_{1n})$$
$$N_{A3} = K_{13}/(1+K_{12}+K_{13}+\ldots+K_{1n})$$
$$\vdots$$
$$N_{An} = K_{1n}/(1+K_{12}+K_{13}+\ldots+K_{1n})$$

Finally, in order to evaluate structural features of the tautomers studied, the C–S and C–O bond orders have been listed. These values are a measurement of the thione–thiol or keto–enol character of these groups; the bond length (d) may be calculated from the bond order (bo) using the following empirical relation, inferred from data reported in the present paper:

$$d(\mathrm{C-S}) = 0.1072[\mathrm{bo}]^2 - 0.5440[\mathrm{bo}] + 2.1847\ \text{Å}\ (R^2 = 0.9916; \mathrm{rms} = 0.006\ \text{Å})$$
$$d(\mathrm{C-O}) = 0.1297[\mathrm{bo}]^2 - 0.5644[\mathrm{bo}] + 1.8345\ \text{Å}\ (R^2 = 0.9975; \mathrm{rms} = 0.003\ \text{Å})$$

In this way, the analysis of the MDL MOL files of the optimized structures with the WebLab ViewerLite 4.0 program [14] was also a powerful tool, since it easily allows us to obtain a real representation of each structure without needing to draw them in terms of canonical forms.

Tautomerism in the gas phase

Thermodynamic and structural features for the possible tautomers in the gas phase are given in Table 2. From the

Table 2 Thermodynamic properties and C–O and C–S bond orders for the possible tautomers of 2-thiolumazine in the gas phase

Tautomer[a]	ΔH_f (kcal mol^{-1})	ΔS (kcal mol^{-1} K^{-1})	ΔG_f (kcal mol^{-1})	$\delta\Delta G_f$ (kcal mol^{-1})	pK_T	Bond order C2–S2	Bond order C4–O4
TLM238 (0.999)	203.64	94.64	175.44			1.18	1.91
TLM123 (0.001)	208.18	95.73	179.65	4.22	3.09	1.15	1.95
TLM235 (0.001)	207.89	94.19	179.82	4.38	3.21	1.15	1.82
TLM234	210.04	94.52	181.87	6.44	4.71	1.11	1.21
TLM124	210.24	94.48	182.08	6.65	4.87	1.19	1.23
TLM134	210.85	93.80	182.90	7.46	5.46	1.77	1.23
TLM135	212.02	94.01	184.01	8.57	6.27	1.73	1.81
TLM248	214.12	94.09	186.08	10.64	7.79	1.25	1.19
TLM138	216.00	95.10	187.66	12.22	8.95	1.73	1.90
TLM125	219.65	95.73	191.12	15.69	11.49	1.10	1.87
TLM348	221.34	93.67	193.43	17.99	13.17	1.81	1.21
TLM128	227.75	98.30	198.46	23.02	16.86	1.07	1.95
TLM358	228.92	94.43	200.78	25.34	18.56	1.78	1.80
TLM148	229.21	95.11	200.87	25.43	18.62	1.83	1.15
TLM245	229.54	93.41	201.70	26.27	19.24	1.22	1.12
TLM258	231.82	94.91	203.54	28.10	20.58	1.18	1.87
TLM145	238.09	93.59	210.20	34.76	25.46	1.80	1.10
TLM345	242.09	92.74	214.45	39.02	28.57	1.80	1.13
TLM458	257.05	94.25	228.96	53.53	39.20	1.80	1.07
TLM158	257.97	95.37	229.55	54.11	39.63	1.73	1.80
TLM13 (0.991)	45.82	94.01	17.81			1.55	1.84
TLM23 (0.009)	48.85	94.71	20.63	2.82	2.07	1.04	1.82
TLM24	56.5	94.11	28.46	10.65	7.80	1.08	1.12
TLM12	59.23	97.22	30.26	12.45	9.12	1.03	1.87
TLM14	59.72	93.74	31.79	13.98	10.24	1.63	1.12
TLM38	61.46	94.13	33.41	15.60	11.43	1.62	1.82
TLM34	67.56	93.21	39.78	21.98	16.10	1.58	1.13
TLM28	68.56	95.17	40.20	22.39	16.40	1.07	1.87
TLM35	72.27	93.12	44.52	26.72	19.56	1.61	1.72
TLM25	75.11	93.75	47.17	29.37	21.51	1.08	1.75
TLM48	80.46	94.85	52.19	34.39	25.19	1.70	1.10
TLM15	83.57	93.31	55.76	37.96	27.80	1.52	1.72
TLM18	86.78	94.93	58.49	40.69	29.80	1.48	1.84
TLM45	102.91	93.29	75.11	57.30	41.97	1.67	1.05
TLM58	106.59	94.77	78.35	60.54	44.34	1.60	1.70
TLM3 (1.000)	–4.03	92.66	–31.64			1.35	1.72
TLM1	2.54	93.49	–25.32	6.32	4.63	1.31	1.76
TLM2	7.72	93.88	–20.26	11.39	8.34	1.01	1.73
TLM4	15.51	93.18	–12.26	19.39	14.20	1.42	1.06
TLM8	29.05	94.65	0.84	32.49	23.79	1.38	1.74
TLM5	38.99	92.68	11.37	43.01	31.50	1.42	1.62
TLM (1.000)	52.90	92.14	25.44			1.21	1.64

[a] In parentheses, mole fraction. When it is zero, this value has been omitted

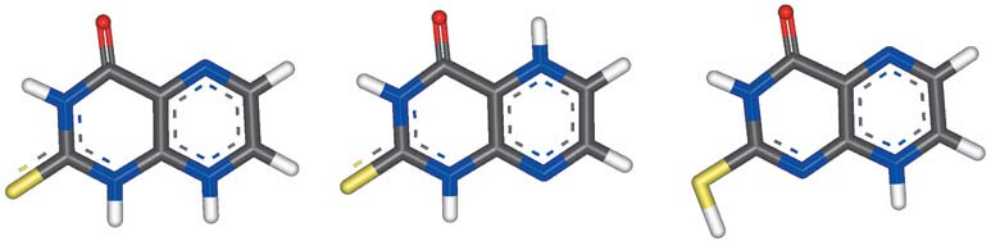

Fig. 3 Structures of the cations TLM138 (*left*), TLM135 (*middle*) and TLM238 (*right*) in aqueous solution

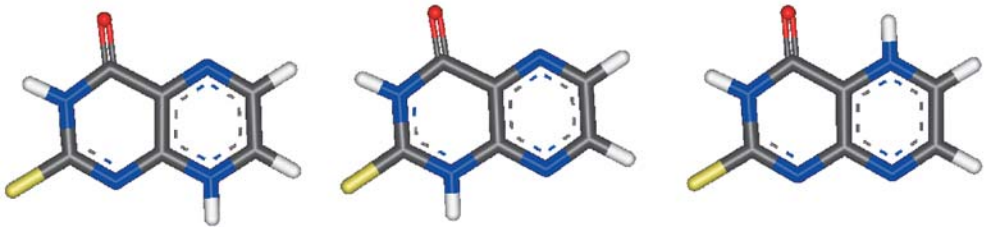

Fig. 4 Structures of the neutral TLM38 (*left*), TLM13 (*middle*) and TLM35 (*right*) in aqueous solution

20 possible tautomers for monoprotonated 2-thiolumazine (Fig. 2), those four resulting from the protonation of the TLM23 are the most stable but the keto–thiol TLM238 form appears to be the most representative (mole fraction=0.999).

For the neutral 2-thiolumazine, the keto–(thiolato–thiona) TLM13 form has the lowest energy, although TLM23 is only 2.82 kcal mol^{-1} and TLM24 is too high (10.65 kcal mol^{-1}). The respective mole fraction values show that TLM13 is present to 99.1%, TLM23 0.9% and TLM24 is not present at all. This is in accordance with previously reported assumptions, since following Mezey et al., [15, 16] species with energy higher than 10 kcal mol^{-1} with respect the most stable one probably do not exist in appreciable amounts, although we think the above-cited energy gap is too broad, since if two forms in equilibrium are ca. 4 kcal mol^{-1} different in energy, the higher energy form will be present in amounts lower than 0.1%.

With respect to the monoanionic tautomers, the most favored is that protonated at the N3 atom (TLM3) which, in view of the C–O and C–S bond orders (see Table 2), must be a keto form with the sulfur atom intermediate between the thiol and thione. The TLM1 form (6.32 kcal mol^{-1} higher) displays similar structural features but, due the energy gap between the TLM3, its mole fraction in the TLM3 ⇄ TLM1 tautomeric equilibrium is 0.000.

Finally, the fully deprotonated form (TLM) shows similar structural features to the TLM3 monoanionic one but both thiol and hydroxy characters of the sulfur and oxygen atoms, respectively, are increased, as can be concluded from the small decrease of the corresponding bond orders.

Tautomerism in the aqueous phase

Thermodynamic and structural features for the possible tautomers in the aqueous phase are given in Table 3. It must be pointed out that upon hydration the main structural effect takes place in the C–S bond, which in most cases displays a roughly pure thiol character (the C–S bond order ranges between 1.04 and 1.29), in clear contrast to the results obtained in the gas phase, in which the C–S bond orders lie between 1.81 and 1.01. This weakening could be due to the high polarizability of the sulfur atom, since the C–O bond displays the same effect but to a smaller extent; in the aqueous phase, the C–O bond order is 1.14–1.74, whereas in gas phase it is 1.05–1.91.

The monoprotonated tautomers with non-zero mole fractions are TLM138 (57.0%), TLM135 (42.2%) and TLM238 (0.7%); their structures are depicted in Fig. 3.

Despite all of them being 4-one forms with a fully aromatic pyrizine ring, some differences can be pointed out. Both TLM138 and TLM135 are nitrogen protonated forms, with the sulfur atom displaying certain thione character. The TLM238 form is clearly a thiol–one tautomer with one hydrogen atom bound to the sulfur atom. The higher stability of the TLM138 tautomer indicates the highest basicity of the N1, N3 and N8 atoms.

The most stable neutral tautomers are TLM13 and TLM38 which are only 0.42 kcal mol^{-1} apart in energy. They are followed by TLM35, but the energy gap (see Table 3) is large enough that this form is present to only 0.3%. The optimized geometries suggest the structures shown in Fig. 4.

In view of the mole fraction for each tautomer, it can be concluded that the neutral form of 2-thiolumazine, detected in aqueous solution at pH<5, must be a mixture of TLM38 and TLM13 in a 3:1 molar ratio.

The data listed in Table 3 suggest that, on deprotonation of the most stable neutral tautomers (TLM38 and TLM13), only the mononegative TLM3 form is obtained, indicating the higher proton stabilization and the higher basicity of this position. The relative stabilities of the possible monoanionic tautomers indicate the following increasing basicities: S2<(O4~N5)<N8<N1<N3. Thus, the higher proton affinity of the pyridine nitrogens,

Table 3 Thermodynamic properties and C–O and C–S bond orders for the possible tautomers of 2-thiolumazine in aqueous phase (COSMO solvation model)

Tautomer[a]	ΔH_f (kcal mol^{-1})	ΔS (kcal mol^{-1} K^{-1})	ΔG_f (kcal mol^{-1})	$\delta\Delta G_f$ (kcal mol^{-1})	pK_T	Bond order C2–S2	Bond order C4–O4
TLM138 (0.570)	125.93	93.44	98.08			1.29	1.74
TLM135 (0.422)	126.18	93.68	98.26	0.18	0.13	1.27	1.72
TLM238 (0.007)	128.67	93.99	100.66	2.58	1.89	1.12	1.68
TLM235	130.79	94.75	102.55	4.47	3.27	1.10	1.66
TLM358	129.80	88.88	103.31	5.23	3.83	1.16	1.68
TLM123	131.74	94.89	103.46	5.38	3.94	1.22	1.72
TLM125	136.08	95.51	107.62	9.53	6.98	1.11	1.65
TLM158	135.55	93.10	107.81	9.72	7.12	1.15	1.68
TLM128	135.90	94.11	107.86	9.77	7.16	1.11	1.67
TLM348	136.42	93.25	108.63	10.55	7.72	1.19	1.24
TLM148	137.81	92.84	110.14	12.06	8.83	1.25	1.21
TLM134	138.85	93.45	111.00	12.92	9.46	1.35	1.28
TLM248	140.37	93.51	112.50	14.42	10.56	1.11	1.16
TLM234	141.34	95.58	112.86	14.77	10.82	1.11	1.23
TLM145	141.10	93.16	113.34	15.25	11.17	1.23	1.20
TLM258	141.46	93.85	113.49	15.41	11.28	1.09	1.60
TLM124	141.59	94.11	113.55	15.46	11.32	1.19	1.20
TLM345	141.70	93.58	113.81	15.73	11.52	1.18	1.22
TLM245	145.15	94.48	116.99	18.91	13.85	1.09	1.14
TLM458	146.39	92.88	118.71	20.63	15.11	1.13	1.16
TLM38 (0.666)	15.63	96.90	–13.25			1.13	1.67
TLM13 (0.331)	15.25	94.23	–12.83	0.42	0.30	1.23	1.71
TLM35 (0.003)	17.54	92.72	–10.09	3.16	2.31	1.12	1.65
TLM18	21.00	92.86	–6.67	6.57	4.81	1.13	1.67
TLM15	21.17	91.86	–6.20	7.04	5.16	1.12	1.65
TLM23	22.14	94.68	–6.07	7.17	5.25	1.07	1.65
TLM12	27.28	97.87	–1.89	11.36	8.32	1.09	1.64
TLM14	26.50	92.68	–1.12	12.13	8.88	1.19	1.20
TLM34	27.42	92.33	–0.09	13.15	9.63	1.14	1.22
TLM48	28.42	92.13	0.97	14.21	10.41	1.10	1.16
TLM28	29.97	92.87	2.29	15.54	11.38	1.07	1.58
TLM58	29.87	91.81	2.51	15.76	11.54	1.08	1.60
TLM25	31.91	92.73	4.28	17.52	12.83	1.06	1.55
TLM24	33.26	92.99	5.55	18.80	13.76	1.07	1.14
TLM45	32.94	91.58	5.65	18.90	13.84	1.10	1.14
TLM3 (0.998)	–90.60	91.83	–117.97			1.10	1.64
TLM1 (0.002)	–87.10	90.64	–114.11	3.85	2.82	1.10	1.63
TLM8	–81.19	91.31	–108.40	9.56	7.00	1.06	1.58
TLM5	–79.43	88.74	–105.87	12.09	8.85	1.05	1.55
TLM4	–78.36	91.29	–105.56	12.40	9.08	1.07	1.14
TLM2	–73.42	93.17	–101.18	16.78	12.29	1.04	1.53
TLM (1.000)	–184.33	91.05	–211.46			1.04	1.53

[a] In parentheses, mole fraction. When it is zero, this value has been omitted

N3 and N1, in aqueous solution is in accord with the data obtained in the gas phase and with those previously published for uracil, thymine, cytosine and xanthine, [17, 18] for which the N1–H hydrogen is always found to have a higher acidity than N3–H.

For the structure of the tautomers (see Fig. 5), the C–O bond orders (Tables 2 and 3) indicate that, in both gas and aqueous solution, the O4 atom is mainly carbonylic, whereas the C–S bond, which is thiolato–thione intermediate in the gas phase, becomes pure thiolato in aqueous solution.

Finally, the electronic spectra at different pH values show that up to pH=11, the spectrum corresponding to the dianionic TLM appears. The calculated data for this form suggest that the geometry is very similar to that of TLM3, but the phenolato character of the C–O bond is increased, as shown in Fig. 6.

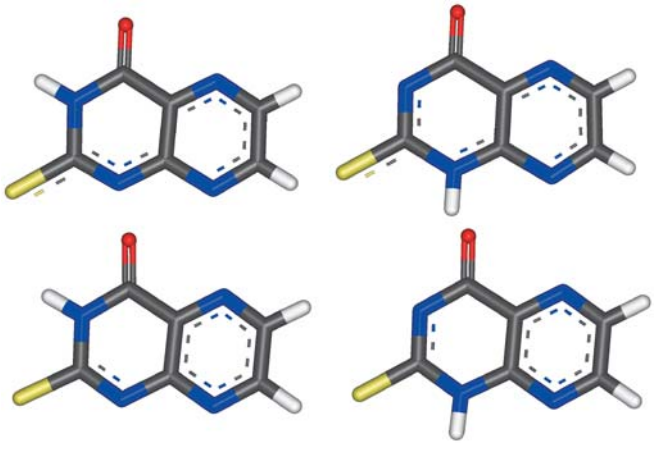

Fig. 5 Structures of the monoanions TLM3 (*left*) and TLM1 (*right*) in gas (*top*) and aqueous medium (*bottom*)

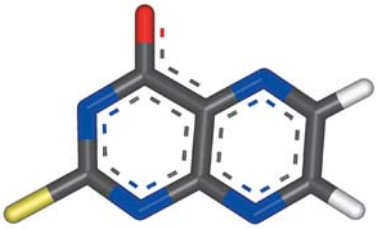

Fig. 6 Structure of the dianion TLM in aqueous medium

Acid–base properties

Finally, a semiempirical study (AM1-COSMO) of the acid–base character of 2-thiolumazine was carried out. From the experimental point of view, the property usually used to describe the acid–base character of a substance is the acidic dissociation constant K_a. As is well known, it is impossible to measure this parameter for an individual tautomer since they are usually mixed with other tautomers, especially if they have similar energies. It is also sometimes difficult to prepare all the fixed model compounds of the individual tautomers. Therefore, it is a matter of interest to predict the K_a values in aqueous solution by means of quantum-chemical methods, such as AM1. Moreover, a good agreement between the experimental and calculated K_a values may allow us to identify which tautomers are predominant in the studied system.

In the aqueous phase, the K_a value of a compound BH could be calculated for the equilibrium:

$$BH\ (aq.) + H_2O \rightleftarrows B^-(aq.) + H_3O^+\ (aq.)$$

using the equation:

$$K_a = \exp[-\Delta G_a/RT]$$

where ΔG_a is the increment of the Gibbs free energy calculated as follows:

$$\Delta G_a = \Delta G_f[B^-(aq.)] + \Delta G_f[H_3O^+(aq.)] - \Delta G_f[BH(aq.)] - \Delta G_f[H_2O]$$

In aqueous solution, heats of formation of H_3O^+ and H_2O were calculated using the AM1 semiempirical method with the COSMO solvation model, these values being 44.3 and –68.5 kcal mol^{-1}, respectively. The heats of formation were converted into Gibbs free energies using the absolute entropy values, calculated in the same way, which were 46.3 cal K^{-1} mol^{-1} (H_3O^+) and 45.1 cal K^{-1} mol^{-1} (H_2O). [19]

Once these parameters are known, the three consecutive deprotonation equilibria for the 2-thiolumazine can be written as

$$[TLMH_3]^+\ (aq.) + H_2O \rightleftarrows [TLMH_2]\ (aq.) + H_3O^+\ (aq.)\ (1)\ K_1$$

$$[TLMH_2]\ (aq.) + H_2O \rightleftarrows [TLMH]^-\ (aq.) + H_3O^+\ (aq.)\ (2)\ K_2$$

$$[TLMH]^-\ (aq.) + H_2O \rightleftarrows [TLM]^{2-}\ (aq.) + H_3O^+\ (aq.)\ (3)\ K_3$$

and the corresponding pK values for those in which both the acid and the conjugate base contribute to the corresponding form (cation, neutral, monoanion or dianion) in a non-zero mole fraction computed.

Thus, for the equilibrium (1), there are five possible acid/conjugate base pairs whose pK_1 values are as follow:

TLM138/TLM38 p$K_1 = 0.82$
TLM138/TLM13 p$K_1 = 1.13$
TLM135/TLM13 p$K_1 = 1.00$
TLM135/TLM35 $pK_1 = 3.00$
TLM238/TLM38 $pK_1 = -1.06$

The two last are given in italics to point out that they must be insignificant because TLM35 only represents 0.3% to the neutral 2-thiolumazine and TLM238 is only 0.7% of monoprotonated 2-thiolumazine. For these reasons, the pK_1 for the monoprotonated 2-thiolumazine, which will be the averaged value of the three first equilibria, could be estimated to be around 1; this value is in accordance with the one reported for the related 2-thiolumazine derivatives. [20] This result may account for why the study of the pH dependence of the electronic spectrum did not show any changes at very acid pH; furthermore, the unsuccessful attempts to isolate any compound containing the protonated 2-thiolumazine are also accounted for.

For the equilibrium (2), the following acid/conjugate base pairs can be written:

TLM38/TLM3 p$K_2 = 5.66$
TLM13/TLM3 p$K_2 = 5.36$
TLM13/TLM1 $pK_2 = 8.18$
TLM35/TLM3 $pK_2 = 3.35$

As above, those given in italics need not be taken into account because both involve species with mole fractions lower than 1%; the individual pK values for the two first equilibria allow us to estimate an averaged pK_2 value at around 5.5, which is in good agreement with the reported value of 6.48. [13] Also, this agreement supports our finding that the strongest proton affinity corresponds to that of the N3 atom.

Finally, for the total deprotonation of 2-thiolumazine, equilibrium (3), there are the two following possibilities:

TLM3/TLM p$K_3 = 13.88$
TLM1/TLM $pK_3 = 11.06$

Of them, only the first may be of significance. The calculated pK_3 of 13.88 is about 2 units higher than the experimental value of 11.90. [13] However, it must be pointed out that this deviation, at 298 K, may be due to an error of only 3 kcal mol^{-1} in the ΔG_f calculation; for this reason, the calculated pK could be a good tentative value taking into account that the above-cited error is the sum of the errors in the ΔG_f values of two species, assuming the ΔG_f values for the water and proton are constants that do not contribute to the results.

Supplementary material

Structural data of the optimized tautomers in both gas and aqueous phases are supplied (84 MOL format files).

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