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Stability, Tautomerism and Acidity of Xanthine by the Density Functional Theory (DFT)

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Abstract

This work is a contribution of theoretical chemistry to the knowledge of xanthine's properties. Its aim first consists in checking the chemistry's results related to the exploitation of semi-empirical methods, it provides theoretical data on the acidity of xanthine tautomers. To do this, the DFT method with the B3LYP functional, associated with the 6-311+G (d, p) basis set was used. The aqueous phase was modeled with the polarizable continuum model (PCM). The results show that in the gas one, xanthine can exist as a mixture of two tautomers, in decreasing order of stability, it is X (1,3,7) and X (1,3,9). In the aqueous one, four are identified, in the same order, those are X (1,3,7), X (1,3,9), X (1,7,10) and X (1,9,10). This work establishes that the tautomer X (1,9,10) comes from the X (1,3,7) via the X(1,3,9) one. It demonstrates that the acidity of the most stable tautomer's nitrogen X (1,3,7) decreases in the order 7>3>1 regardless of its physical state. It provides data to elucidate the mechanisms to understand biological activities of xanthine.

Keywords: Xanthine; Stability; Tautomerism; Acidity; B3LYP

Introduction

The xanthine is part of the oxopurine family. It exists in nature, in large quantities, especially in meat [1]. The studies have shown that compound and its derivatives have very important biological properties. In terms of stability, this compound is an intermediate in the degradation of adenine and guanine purines' bases [2] xanthine oxidase converts it to uric acid [3,4]. The alkylated one (caffeine, theophylline and theobromine) is used as mild stimulants, it also serves for their bronchodilators effects, especially in the treatment of asthma [5,6]. The thio derivatives of xanthine (2-thioxanthine, 6-thioxanthine, 2,6-dithioxanthine) has anti-cancer activities [7,8]. Several experimental studies are reported on the tautomerism of xanthine in literature. Indeed xanthine has 14 shapes through a keto-enol transformation and a proton exchange between nitrogen atoms. UV spectroscopy in aqueous solution shows that xanthine exists in the N7-H dioxo tautomeric form [9]. This result is confirmed by NMR measurements [10]. On the other hand, X-ray diffraction experiments establish that the sodium salt of

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xanthine is predominantly in the solid state as the N9-H dioxo tautomer [11]. Although this molecule has been the subject of several researches, the theoretical ones of Pervin Unal Civcir have given a renewed interest. It has been studied by the semi-empirical calculation methods AM1 and PM3 xanthine [12], 2-thioxanthine [13], 6-thioxanthine [14] and 2,6-dithioxanthine [15]. The research establishes that xanthine exists in the gaseous and aqueous phases, in the form of two predominant tautomers. X (1,3,7) and X (1,3,9) [12].

The verification of semi-empirical methods' chemistry results remains at the centre of our team's concern. To do this, we use DFT method with the B3LYP functional. This process yields convincing results for 6-thioxanthine [16]. In this impetus, the present research aims to question those deducted from semi-empirical methods relating to xanthine. Specifically, it focused on the stability, tautomerism and acidity of its tautomers.

Methods of Calculation

All calculations were carried out with DFT (B3LYP functional) using 6–311+G (d, p) [17] basis set, as incorporated in the GAUSSIAN-03 program [18]. In aqueous phase, the solvation model Polarizable Continuum Model (PCM) was used [19,20]. The geometries of tautomers, transition states and intermediates have been fully optimized. The Gibbs free energies are obtained from the calculation of the frequencies. Frequency analyses were proceeded to confirm the structure being a minimum or a transition state (i.e without or with solely an imaginary frequency). To name the 14 tautomeric forms we have used the following notations: X (i, j, k) where i, j and k stand for the amount of the nitrogen or oxygen atoms to which the hydrogen is attached (FIG. 1).

Moreover, to establish the presence of a tautomerism, this work determines, first of all, the possible equilibriums. Then, it highlights those that are likely to exist. It calculates their constants and their activation energies. The following section presents the results obtained.

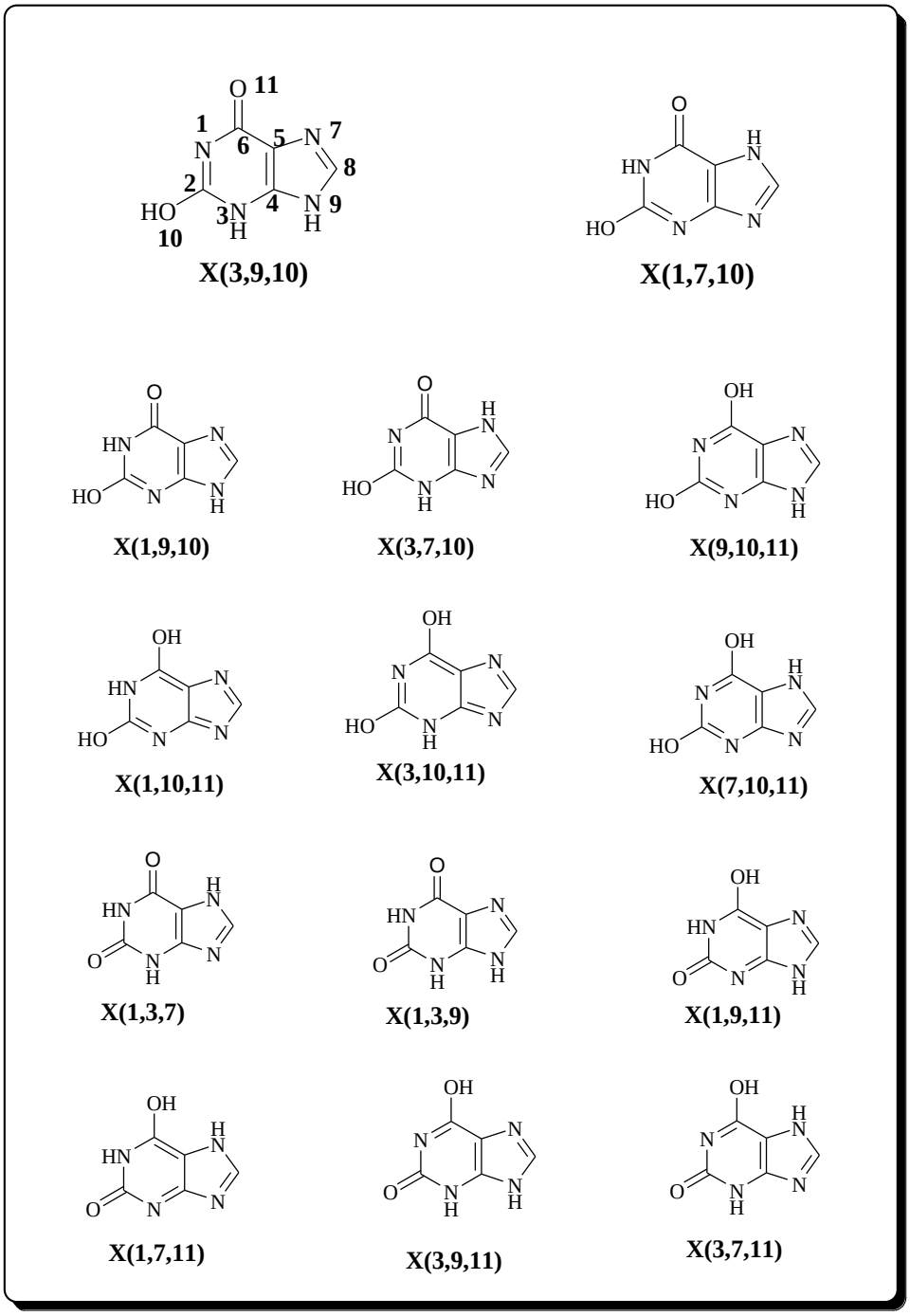

FIG. 1. **Tautomers of xanthine.**

Results of Calculations

The results concern the tautomerism of xanthine and the acidity of its heteroatoms. Previously, they focus on its stability.

Stability of xanthine's tautomers

The Gibbs free energy, at computational level B3LYP/6-311+G (d, p), helps to discuss the relative stability of the 14 tautomers shown in FIG. 1. TABLE 1 summarizes its values; ΔG is the difference between the Gibbs free energy of the most stable tautomer X (1,3,7) and those of the others) [21,22].

TABLE 1. **Gibbs free energy G (a.u) and ΔG (kcal/mol) at T=298, 15 K.**

Tautomeres	Gas phase		Aqueous phase	
	Gg	**ΔGg**	**Gaq**	**ΔGaq**
X (3,9,10)	-562.489414	29.20	-562.55287	16.81
X (1,7,10)	-562.517318	11.69	-562.563624	10.06
X (1,9,10)	-562.515286	12.96	-562.564186	9.70
X (3,7,10)	-562.508221	17.40	-562.558144	13.50
X (9,10,11)	-562.508457	17.25	-562.552681	16.93
X (1,10,11)	-562.483525	32.90	-562.535321	27.82
X (3,10,11)	-562.48974	29.00	-562.54095	24.29
X (7,10,11)	-562.505558	19.07	-562.551431	17.71
X (1,3,7)	-562.535947	0.00	-562.579661	0.00
X (1,3,9)	-562.522414	8.49	-562.576392	2.05
X (1,9,11)	-562.494511	26.00	-562.553553	16.38
X (1,7,11)	-562.483495	32.91	-562.549607	18.86
X (3,9,11)	-562.501725	21.47	-562.557418	13.96
X (3,7,11)	-562.509883	16.36	-562.559704	12.52

Note: ΔGg and ΔG in the gas phase, ΔGaq and ΔG in the aqueous phase.

Tautomerism of xanthine

To evaluate the tautomerism of xanthine, we first consider the possible equilibriums between the potential tautomers and then we calculate the equilibrium constants and activation energies.

Probable tautomeric equilibriums in xanthine

Most therapeutic activities of purines depend on potential equilibriums between their tautomeric forms [23,24]. Here, it is accepted that the exchange of hydrogen atoms remains possible only between neighboring heteroatoms or separated by at most two atoms. Moreover, this research prohibits simultaneous transfers of more than two hydrogen atoms. Under these conditions, there are four potential equilibriums between the most stable tautomers of xanthine. FIG. 2 below summarizes them. In addition, this work calculates their equilibrium constants.

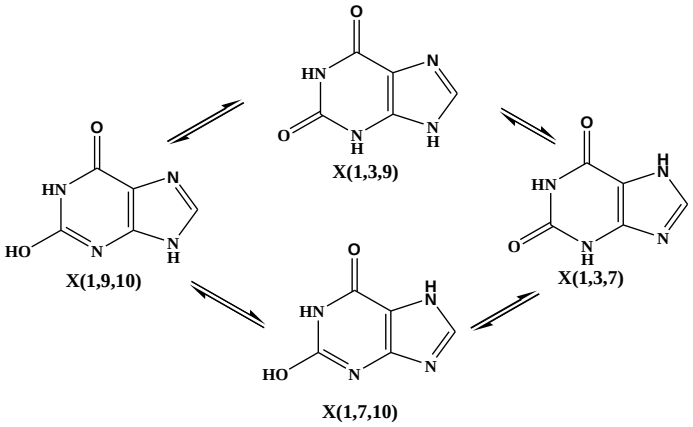

FIG. 2. **Possible equilibriums between the most stable tautomers of xanthine.**

Tautomeric equilibrium constants

The calculation of K_T makes it possible to highlight the real xanthine's tautomeric equilibrium. It characterizes an AB equilibrium (A $\rightleftharpoons$ B). K_T's values are obtained with the relation:

$$K_T = \exp\left(-\frac{\Delta G}{RT}\right)$$

ΔG is the difference of Gibbs free energy between tautomers in equilibrium. When $K_T<10^{-4}$, the form A exists alone. If $K_T>10^4$, compound B predominates. The equilibrium becomes effective if K_T ranging from 10^{-4} to 10^4. TABLE 2 collects the K_T values in gas or aqueous phase at B3LYP/6–311+G (d, p).

TABLE 2. **Equilibrium Constants K_T at T=298, 15 K.**

Equilibrium	**K_{Tg}**	**K_{Taq}**
X (1,9,10) $\rightleftharpoons$ X (1,3,9)	$1.90.\ 10^3$	$4.12.\ 10^5$
X (1,3,9) $\rightleftharpoons$ X (1,3,7)	$1.68.\ 10^6$	$3.19.\ 10^1$
X (1,9,10) $\rightleftharpoons$ X (1,7,10)	8.61	$5.51.\ 10^{-1}$
X (1,7,10) $\rightleftharpoons$ X (1,3,7)	$3.71.\ 10^8$	$2.38.\ 10^7$

Activation energies of possible tautomeric equilibriums

The FIG. 3 illustrates two access pathway to the most stable tautomer X (1,3,7) starting from X (1,9,10). The TABLES 3 and 4 respectively show the Gibbs free energies of all the species involved in tautomerism as well as the imaginary frequencies of the different transition states; these last quantities characterize them. The preceding results make it possible to discuss the xanthine's heteroatom acidity.

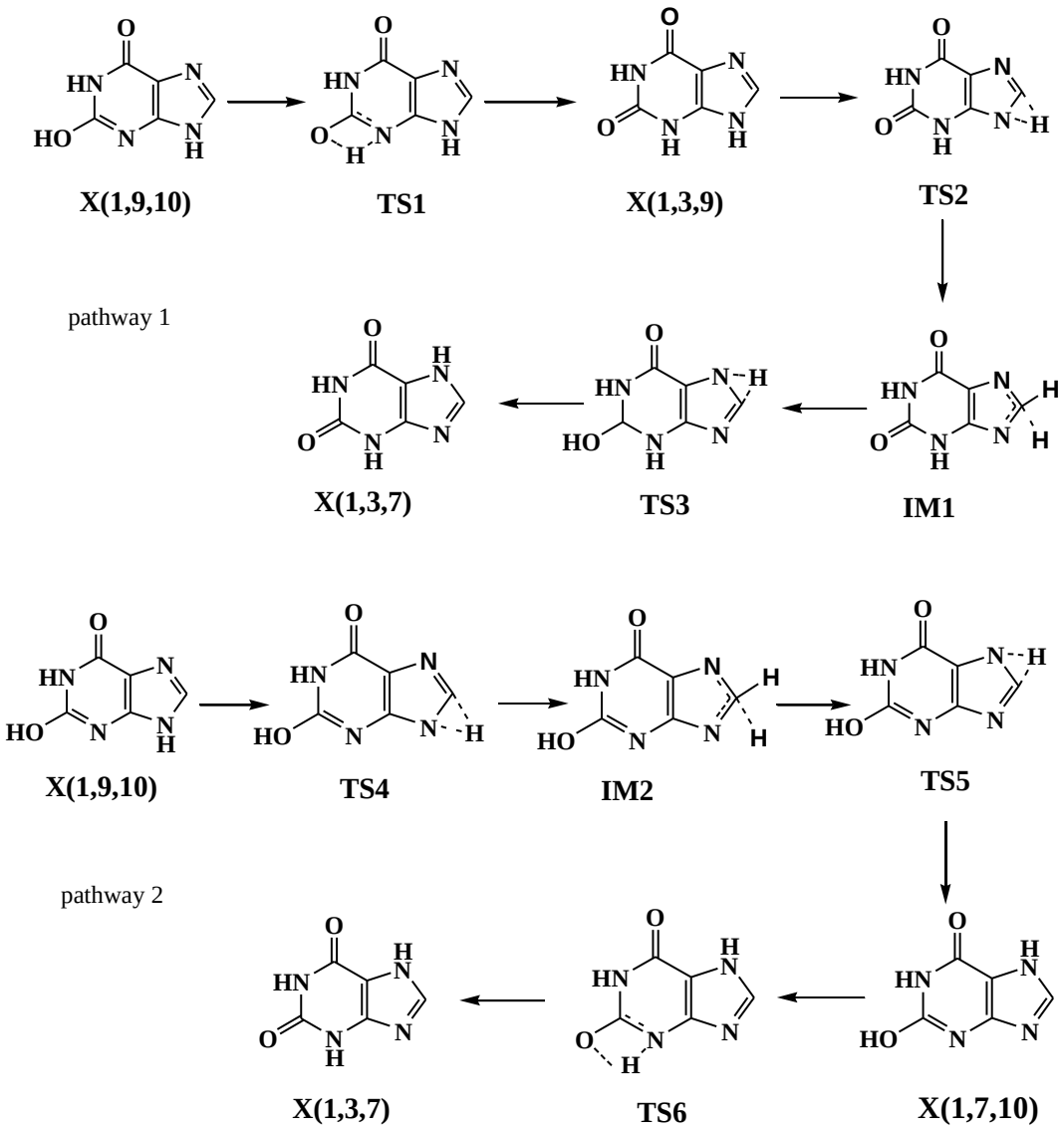

FIG. 3. **Potential tautomerism of xanthine.**

TABLE 3. **Gibbs free energies and imaginary frequencies.**

Especes	G (au)	ΔG (kcal/mol)	Imaginary frequencies
X (1,3,7)	-562.535947	0.00	-
X (1,3,9)	-562.522412	8.49	-
X (1,7,10)	-562.51732	11.69	
X (1,9,10)	-562.515281	12.97	-
IM1	-562.501667	21.51	-
IM2	-562.476794	37.12	-
TS1	-562.459337	48.07	-190122
TS2	-562.447305	55.62	-156055
TS3	-562.456111	50.1	-149578
TS4	-562.429935	66.52	-152081
TS5	-562.435251	63.19	-147512
TS6	-562.465168	44.41	-191692

TABLE 4. **Activation energies Ea (kcal/mol) in gas phase at T=298, 15 K.**

TS	Reactions	Ea (kcal/mol)
TS1	X(1,9,10) → X(1,3,9)	35.10
TS2	X(1,3,9) → IM1	47.13
TS3	IM1→ X(1,3,7)	28.59
TS4	X(1,9,10) → IM2	53.55
TS5	IM2 → X(1,7,10)	26.07
TS6	X(1,7,10) → X(1,3,7)	32.72

Note: This article also explains the energy profile of xanthine's tautomerism (FIG. 4).

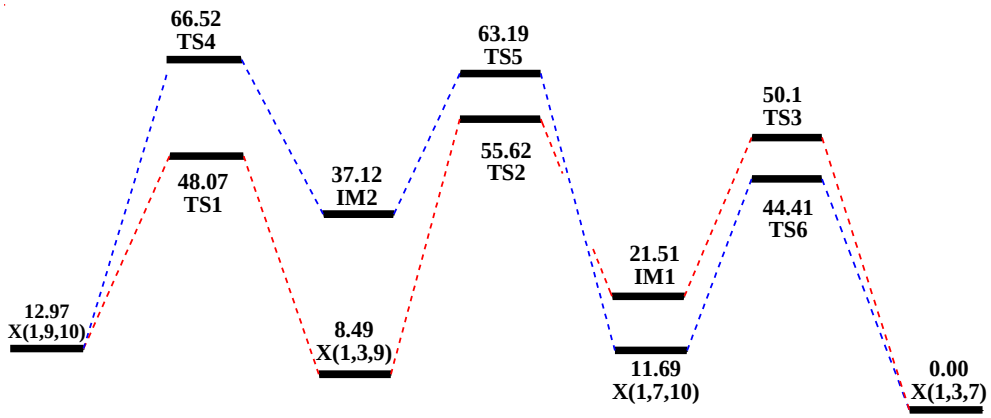

FIG. 4. **The relative energy profiles of the two pathways tautomerization of xanthine.**

Acidity of the potential tautomers

The tautomers of xanthine have hydrogenated sites that may be deprotonated. The choice of these depends on the acid's strength [25]. The relative Gibbs free energy (ΔG) of the associated general reaction ($AH \rightarrow A^- + H^+$) helps evaluate the xanthine's heteroatom acidity.

$\Delta G = \Delta H - T\Delta S$

$\Delta G = G(A^-) + G(H^+) - G(AH)$

In gas phase, $G_g(H^+) = 2.5\ RT — TS^\circ(H^+) = 1.48 - 7.76 = -6{,}28$ kcal/mol [25]

In aqueous phase, $G_{aq}(H^+)$ is calculated from the relation:

$G_{aq}(H^+) = G_g(H^+) + G_{solv}(H^+)$

G_{solv} is the Gibbs free energy of solvation, $G_{solv}(H^+) = -263.47$ kcal/mol [26].

TABLE 5 presents the results of the calculations.

TABLE 5. **ΔG (Kcal/mol) of potential tautomers.**

Tautomers	Δ**Gg**	Δ**Gaq**
X (1,3,7)		
N_1	355.29	23.85
N_3	328.85	20.38
N_7	319.56	19.00
C_8	355.26	49.37
X (1,3,9)		
N_1	338.01	24.46
N_3	316.92	16.92
N_9	311.06	16.94
C_8	359.64	51.52
X (1,7,10)		
N_1	328.15	20.33
N_7	326.30	20.80
O_{10}	316.53	10.55
C_8	363.29	51.41
X (1,9,10)		
N_1	330.04	21.07
N9	325.03	21.12
O_{10}	312.45	9.27
C_8	367.34	53.28

Note : All the results obtained suggest the discussion of this work.

Discussion

Stability

With regard to the stability of xanthine, the discussion focuses on the gas phase at first. The results in TABLE 1 show that the diketo tautomer X (1,3,7) is the most stable of the 14 tautomers. X (1,3,9) follows it. Their energy differs from 8.49 kcal/mol. The third most stable tautomer corresponds to X (1,7,10). Its relative energy is 11.69 kcal/mol. According to Mesey et al. (1979), 10 kcal/mol is a reasonable limit for predicting the presence of stable species. This work suggests that xanthine can exist in the gas phase under a mixture of only two tautomers that are X (1,3,7) and X (1,3,9). This result is consistent with that of Civcir [12]. Comparing the previous finding with those of the aqueous phase, it is observed that the Gibbs free energy difference is positive for all the tautomers. This result suggests that the presence of water helps to stabilize them. In this state, according to Mezey et al. [21,22], tautomers X (1,9,10) and X (1,7,10) can also exist. The Gibbs free energy difference of tautomers X (1,3,9), X (1,9,10) and X (1,7,10) with the most stable tautomer X (1,3,7) is 2.05; 9.70 and 10.06 kcal/mol. Our results corroborate those of Civir. Berdys-Kochanska [27] and Michael et al. [28] with respect to the relative stability of X (1,3,7), X (1,3,9) and X (1,7,10). Moreover, they establish the existence of X (1,9,10) for the first time.

Tautomerism of xanthine

The presence of these xanthine compounds represents the basis of the discourse relating to its tautomerism. From the outset, this work lists their possible equilibrium. It analyzes their constant and activation energy.

Possible tautomeric equilibrium of xanthine

The FIG. 2 shows that the most stable tautomer X (1,3,7) can be in equilibrium with X (1,7,10) or X (1,3,9). The tautomer X (1,7,10) can also be in equilibrium with X (1,9,10), which is itself with X (1,3,9). Their actual existence suggests calculating their constants and activation energies.

Equilibrium constants

A chemical equilibrium between two tautomers A and B ($A \rightleftharpoons B$) is characterized by its constant K_T. The values of this one are calculated using the relation:

$$K_T = \exp\left(-\frac{\Delta G}{RT}\right)$$

ΔG is the Gibbs free energy difference between tautomers in equilibrium. The results are shown respectively in TABLES 2 and 3. The probable equilibrium was determined from the K_T values:

-if $K_T<10^{-4}$, only A exists.

- if $10^{-4}<K_T<10^{4}$, there is an equilibrium

- if $K_T>104$, only B exists

The K_T value of the X (1,3,9) $\rightleftharpoons$ X (1,3,7) and X (1,7,10) $\rightleftharpoons$ X (1,3,7) processes are respectively 1.68 10^6 and 3.71 10^8 in the gas phase; they suggest a clear stability of X (1,3,7) in equilibrium; it can't be transformed into the forms X (1,3,9) and X (1,7,10). The X (1,9,10) $\rightleftharpoons$ X (1,3,9) and X (1,9,10) $\rightleftharpoons$ X (1,7,10) processes have respective K_T values of 1.90 10^3 and 8.61. These values indicate that the tautomers are present in comparable quantities. The transformations X (1,3,9) $\rightleftharpoons$ X (1,3,7) and X (1,9,10) $\rightleftharpoons$ X (1,3,9) depend on the physical state of the compounds in contrast to the other two. Indeed, the K_T

value of these transformations passes respectively 1.68 10^6 and 1.90 10^3 in the gas phase at 3.19 10^1 and 4.12 10^5 in the aqueous one. This is due to the clear stabilization of tautomer X (1,3,9) in this latter one. Nevertheless X (1,3,7) remains the most stable.

Activation energies of potential tautomerism

In the first pathway (FIG. 4), the hydrogen atom bonded to the oxygen atom O_{10} of the tautomer X (1,9,10) transfers onto the nitrogen atom N3. This result carried out through the transition state TS1 with an energy barrier of 35.10 Kcal/mol to give X (1,3,9). Then, in X (1,3,9), the hydrogen atom bonded to the nitrogen atom N9 firstly transfers onto the carbon atom C8 via the transition state TS2, with an energy barrier of 47.13 Kcal/mol to give intermediate IM1. In the second step, the same hydrogen atom passes from this latter one onto tautomer X (1,3,7) via the transition state TS3, this process corresponds an energy barrier of 28.59 Kcal/mol.

In the other pathway, in X (1,9,10), the hydrogen atom bonded to the nitrogen atom N9 firstly transfers onto the carbon atom C8. This displacement is realized through the transition state TS4, with an energy barrier of 53.55 Kcal/mol to give intermediate IM2, then it passes from IM2 onto tautomer X (1,7,10) via the transition state TS5, with a barrier energy of 26.07 Kcal/mol. Finally, the hydrogen atom bonded to the oxygen atom O_{10} in X (1,7,10) transfers onto the nitrogen atom N3 via the transition state TS6, with an energy barrier of 32.72 Kcal/mol to give X (1,3,7).

The highest activation energy in both pathways is obtained at the breaking of the bond N9-H (47.13 and 53.55 Kcal/mol respectively). That of the second is 6.42 Kcal/mol higher than that of the first. This latter remains thus the most favourable for the reaction between xanthine's tautomers which may exist. In other words, the tautomerism is preferentially realized by the first pathway.

Acidity of potential tautomers

The acidity of tautomeric sites is related to the value of ΔG [25]. The weaker this is, the more the heteroatom of the position concerned is acidic. Under these conditions, in the gas phase, the nitrogen N7 of the imidazole ring is the most acid site of the tautomer X (1,3,7). The nitrogen N9 is the most acid one of X (1,3,9) and Oxygen O_{10} that of X (1,7,10) and X (1,9,10). In the aqueous one, nitrogen N7 remains the most acid site of X (1,3,7) and oxygen O_{10} that of X (1,7,10) and X (1,9,10). At the same time, in X (1,3,9), the nitrogen N3 of the pyrimidine ring becomes the most acid. In the end, the acidity of the most stable tautomer X (1,3,7) decreases in the order 7>3>1>8, in both phases. This result helps to elucidate the contradiction observed with the experimental data. It refutes the conclusion of Lichtenberg et al. [10]; these authors establish a similar degree of acidity for N3 and N7, this work shows that N7 is more acid than N3. Moreover, it confirms the values of Cavalieri et al. [9]. Its results indicate that the acidity of the sites of the other three tautomers evolves according to their physical states. The acidity of the X (1,3,9) decreases in the order of the positions 9>3>1>8 and 3>9>1>8 respectively in gas and aqueous phases. These are distinguished by the relative position of the sites 9 and 3. In the gas phase, the acidity is more marked on the 9-position. A similar situation occurs for the tautomers X (1,7,10) and X (1,9,10). The acidic degree of the heteroatoms decreases in the order of 10>7>1>8 and 10>1>7>8 in the gas and aqueous phase respectively in the tautomer X (1,7,10). In X (1,9,10), it drops the order 10>9>1>8 and 10>1>9>8 in these two states. These results lead to the conclusion of this work.

Conclusion

The study concerns the stability, tautomerism and acidity of xanthine from literature results. In particular, it relies on elaborate methods of theoretical chemistry to take stock of its tautomeric forms and the acidity of its heteroatoms.

Its results establish that xanthine can exist in gas phase as a mixture of two tautomers X(1,3,7) and X(1,3,9) and in aqueous one as an association of four X (1,3,7) X (1,3,9), X (1,7,10) and X (1,9,10). This work highlights this last form for the first time.

The equilibrium constant reveals that in the gas phase, only the tautomeric equilibrium X (1,9,10) $\rightleftharpoons$ X (1,3,9) and X (1,9,10) $\rightleftharpoons$ X (1,7,10) are possible. In the presence of water, the equilibrium X (1,3,9) $\rightleftharpoons$ X (1,3,7) is superimposed on that of X (1,9,10) $\rightleftharpoons$ X (1,7,10). This work suggests that the biological activity of xanthine relies on these two mainly tautomeric equilibrium. Activation energies in the gas phase show that the xanthine's tautomerization is realized by the transformation of tautomer X (1,9,10) to X (1,3,7) through X (1,3,9).

In terms of acidity, research establishes that in the gas phase, N7 represents the most acidic atom of tautomer X (1,3,7). The nitrogen N9 corresponds to that of X (1,3,9) whereas the oxygen O_{10} that of X (1,7,10) and X (1,9,10). In aqueous phase, this work shows that the results are preserved except those of X (1,3,9); for the latter form, N3 becomes the most acidic. In addition, this study indicates that the acidity of the most stable tautomer X (1,3,7) decreases in the order 7>3>1>8 regardless of its state. Moreover, this work opens up new perspectives; in particular, its results could help to identify the mechanisms underlying specific biological properties of xanthine.

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