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Importance of CH tautomers in the tautomeric mixture of uric acid

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ABSTRACT

To understand the formation and degradation processes of uric acid (**UA**) in living organisms we considered all possible tautomers–rotamers of **UA** by semiempirical (AM1) method, and next we used the *ab initio* (HF, MP2, and G2) and DFT methods to the most stable five isomers of **UA** selected at the AM1 level. Our calculations show evidently that at least two CH–NH forms should be considered in the tautomeric mixture of **UA** to understand the formation of the intermediate 5-hydroxyisourate (HIU) upon the **UA** oxidation by uricase. Intramolecular interactions and π-electron delocalization for UA are very similar to those occurring for its building blocks {purine (**P**), uracil (**U**), and 2-imidazolone (**I**)}. Tautomeric equilibrium constants for NH → OH, NH → CH, and OH → CH conversions in **UA** are close to those for **U** and **I**. Tautomeric equilibrium constants for NH → NH conversions in UA are close to those for **P**. Favored tautomers for **UA**, U and **I** are strongly stabilized by intramolecular interactions between the amide groups, whereas that for **P** is stabilized by π-electron delocalization and $N^9H\cdots N^3$ intramolecular interaction. Aromaticity seems to be very important factor that influences the tautomeric preference for the systems which do not possess the *exo* functional groups.

1. Introduction

Uric acid (**UA**) – isolated from human urine more than 200 years ago [1] – presents very complex physicochemical, chemical, biochemical and biological properties, which have been extensively studied during the last five decades. As one of the end products of the metabolism for purine nucleotides in humans [2], **UA** causes various opposite effects on human health, positive effects for substantial concentrations (antioxidation, anticarcerogen properties, and longevity) and negative effects for elevated quantities in blood, tissues and urine (hyperuricemia) [3].

Similar to building blocks (Fig. 1): purine (**P**), uracil (**U**), and 2-imidazolone (**I**), **UA** exhibits prototropic tautomerism [4,5]. It possesses four hydrogens that can move as protons between seven heteroatoms (three *exo*-oxygens and four *endo*-nitrogens) and also between heteroatoms and five *endo*-carbon atoms. Consequently, combinations of various types of tautomeric conversions in **UA** such as amide-iminol, keto-enol, amine-imine, and/or imine–enamine lead to 24 NH–OH tautomers and 50 CH–NH–OH tautomers (Table 1). Solely in the solid state, the structures of dihydrate and anhydrous **UA** and its salts (urates) have been experimentally determined [6,7]. Urates in the solid state are mainly monoanions [7]. Uric acid in the solid state takes the tetra-NH (tri-oxo) form (**UA1**, Table 1) with protons at the N^1, N^3, N^7, and N^9 atoms [6]. The **UA1** form seems to be also favored in the gas phase and in solution [8,9].

Experimental investigations of prototropy for heterocycles are exceptionally difficult to carried out, because tautomeric conversions are very fast processes, and also, because most of experimental techniques are incapable to detect less than 0.1% of minor tautomers [4]. Fortunately, quantum-chemical methods can be applied even for very complicated systems. These methods give the possibilities to optimize the structure of all possible tautomeric forms and to study all tautomeric equilibria. They make also possible to estimate thermodynamic parameters such as the enthalpy (H_T), entropy (S_T), Gibbs free energy (G_T), tautomeric equilibrium constant (pK_T) for each tautomeric conversion, and the percentage content of each tautomer in the tautomeric mixture.

To our knowledge [10], all 74 tautomers possible for **UA** have been never considered in the literature [8,9]. Shukla and Mishra [8] chose only three NH–OH forms for their semiempirical calculations (MNDO, AM1, and CNDO/s-Cl). Demir et al. [9a] reported the semiempirical (MNDO, AM1, and PM3) results for 15 NH–OH tautomers. The same isomers were considered by Monard and co-workers [9b,c] who used the semiempirical (AM1 and PM3), *ab initio* (HF and MP2), and DFT methods. Using the HF and DFT methods, Jiménez and Alderete [9d] analysed 16 NH–OH forms. Leszczynski and co-workers [9e] reported the DFT results for 18 NH–OH isomers. Twenty-four NH–OH tautomers and additionally

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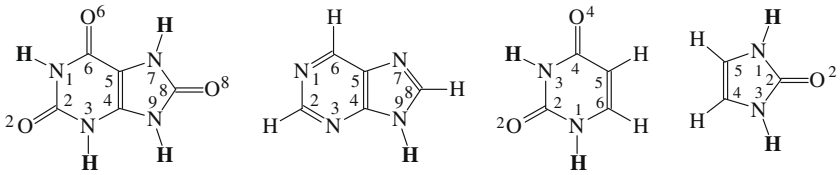

Fig. 1. Uric acid (**UA**) and its building blocks: purine (**P**), uracil (**U**), and 2-imidazolone (**I**). Moving protons are marked in bold.

Table 1
Positions of moving protons for prototropic tautomers of uric acid.

Isomer[a]	Type of tautomer	Position of H	Isomer[a]	Type of tautomer	Position of H
UA1	Tetra-NH	N1N3N7N9	**UA38a,b**	Mono-CH-di-NH-mono-OH	C6N1N3O6
UA2a,b	Tri-NH-mono-OH	N1N7N9O2	**UA39a,b**	Mono-CH-di-NH-mono-OH	C6N1N9O6
UA3a,b	Tri-NH-mono-OH	N3N7N9O2	**UA40a,b**	Mono-CH-di-NH-mono-OH	C5N1N3O8
UA4a,b	Tri-NH-mono-OH	N1N3N7O6	**UA41a,b**	Mono-CH-di-NH-mono-OH	C4N1N3O8
UA5a,b	Tri-NH-mono-OH	N1N7N9O6	**UA42a,b**	Mono-CH-di-NH-mono-OH	C5N1N7O8
UA6a,b	Tri-NH-mono-OH	N3N7N9O6	**UA43a,b**	Mono-CH-di-NH-mono-OH	C5N1N9O8
UA7a,b	Tri-NH-mono-OH	N1N3N7O8	**UA44a,b**	Mono-CH-di-NH-mono-OH	C8N1N3O8
UA8a,b	Tri-NH-mono-OH	N1N3N9O8	**UA45a,b**	Mono-CH-di-NH-mono-OH	C8N1N9O8
UA9a–d	Di-NH-di-OH	N1N7O2O6	**UA46a–d**	Mono-CH-mono-NH-di-OH	C5N7O2O6
UA10a–d	Di-NH-di-OH	N3N7O2O6	**UA47a–d**	Mono-CH-mono-NH-di-OH	C4N9O2O6
UA11a–d	Di-NH-di-OH	N7N9O2O6	**UA48a–d**	Mono-CH-mono-NH-di-OH	C2N3O2O6
UA12a–d	Di-NH-di-OH	N1N7O2O8	**UA49a–d**	Mono-CH-mono-NH-di-OH	C2N9O2O6
UA13a–d	Di-NH-di-OH	N1N9O2O8	**UA50a–d**	Mono-CH-mono-NH-di-OH	C6N1O2O6
UA14a–d	Di-NH-di-OH	N3N7O2O8	**UA51a–d**	Mono-CH-mono-NH-di-OH	C6N3O2O6
UA15a–d	Di-NH-di-OH	N3N9O2O8	**UA52a–d**	Mono-CH-mono-NH-di-OH	C6N9O2O6
UA16a–d	Di-NH-di-OH	N1N3O6O8	**UA53a–d**	Mono-CH-mono-NH-di-OH	C5N1O2O8
UA17a–d	Di-NH-di-OH	N1N7O6O8	**UA54a–d**	Mono-CH-mono-NH-di-OH	C4N1O2O8
UA18a–d	Di-NH-di-OH	N1N9O6O8	**UA55a–d**	Mono-CH-mono-NH-di-OH	C5N3O2O8
UA19a–d	Di-NH-di-OH	N3N7O6O8	**UA56a–d**	Mono-CH-mono-NH-di-OH	C4N3O2O8
UA20a–d	Di-NH-di-OH	N3N9O6O8	**UA57a–d**	Mono-CH-mono-NH-di-OH	C5N7O2O8
UA21a–h	Mono-NH-tri-OH	N1O2O6O8	**UA58a–d**	Mono-CH-mono-NH-di-OH	C5N9O2O8
UA22a–h	Mono-NH-tri-OH	N3O2O6O8	**UA59a–d**	Mono-CH-mono-NH-di-OH	C2N1O2O8
UA23a–h	Mono-NH-tri-OH	N7O2O6O8	**UA60a–d**	Mono-CH-mono-NH-di-OH	C8N1O2O8
UA24a–h	Mono-NH-tri-OH	N9O2O6O8	**UA61a–d**	Mono-CH-mono-NH-di-OH	C8N3O2O8
UA25	Mono-CH-tri-NH	C5N1N3N7	**UA62a–d**	Mono-CH-mono-NH-di-OH	C8N9O2O8
UA26	Mono-CH-tri-NH	C5N1N7N9	**UA63a–d**	Mono-CH-mono-NH-di-OH	C5N3O6O8
UA27	Mono-CH-tri-NH	C4N1N3N9	**UA64a–d**	Mono-CH-mono-NH-di-OH	C4N3O6O8
UA28a,b	Mono-CH-di-NH-mono-OH	C5N1N7O2	**UA65a–d**	Mono-CH-mono-NH-di-OH	C5N7O6O8
UA29a,b	Mono-CH-di-NH-mono-OH	C4N1N9O2	**UA66a–d**	Mono-CH-mono-NH-di-OH	C5N9O6O8
UA30a,b	Mono-CH-di-NH-mono-OH	C5N3N7O2	**UA67a–d**	Mono-CH-mono-NH-di-OH	C6N1O6O8
UA31a,b	Mono-CH-di-NH-mono-OH	C4N3N9O2	**UA68a–d**	Mono-CH-mono-NH-di-OH	C8N3O6O8
UA32a,b	Mono-CH-di-NH-mono-OH	C5N7N9O2	**UA69a–d**	Mono-CH-mono-NH-di-OH	C8N9O6O8
UA33a,b	Mono-CH-di-NH-mono-OH	C2N1N3O2	**UA70a–h**	Mono-CH-tri-OH	C5O2O6O8
UA34a,b	Mono-CH-di-NH-mono-OH	C2N1N9O2	**UA71a–h**	Mono-CH-tri-OH	C4O2O6O8
UA35a,b	Mono-CH-di-NH-mono-OH	C5N3N7O6	**UA72a–h**	Mono-CH-tri-OH	C2O2O6O8
UA36a,b	Mono-CH-di-NH-mono-OH	C4N3N9O6	**UA73a–h**	Mono-CH-tri-OH	C6O2O6O8
UA37a,b	Mono-CH-di-NH-mono-OH	C5N7N9O6	**UA74a–h**	Mono-CH-tri-OH	C8O2O6O8

[a] Rotamers of the OH group(s) are named as **a**, **b**, etc.

11 zwitterionic structures were considered by Chen et al. [9f] who applied the AM1, HF, and DFT methods. The zwitterionic structures, however, cannot be treated as prototropic tautomers because of charge separation.

To well understand the physicochemical, chemical, biochemical, and biological properties of uric acid, particularly its formation and degradation processes in living organisms, we considered here all possible 74 tautomers of **UA** (Table 1) and their different conformations (Fig. S1, Supplementary material). First, we used the semiempirical Austin Model 1 (AM1), introduced by Dewar et al. [11] to study the proton-transfer reactions [12]. Next, to well establish the stability order of the most stable five forms of **UA** selected at the AM1 level, we used the *ab initio* (HF, MP2, and G2) and DFT methods [13]. The AM1 method reproduces well the experimental data for model compound of nucleobases, 2-hydroxypyridine (OH form) → 2-pyridone (NH form) [14,15]. It also predicts the amounts of the OH and NH forms similar to those calculated at the Gaussian-2 (G2) level, recommended for the proton-transfer reactions in the gas phase [16]. We analysed tautomeric conversions, intramolecular interactions and π-electron delocalization for **UA**, and we compared them with those occurring for its building blocks (**P**, **U**, and **I**).

2. Methods

2.1. Quantum-chemical calculations

All possible tautomers–conformers for uric acid (Fig. S1, Supplementary material) and all possible tautomers–conformers for building blocks (Schemes S1–S3, Supplementary material), purine, uracil, and 2-imidazolone, were considered and the heats of formations (H_f at 298.15 K) calculated using the semiempirical (AM1) method [11] and the HyperChem program [17]. High level quantum-chemical calculations were also performed for five isomers of **UA**, selected at the AM1 level, using the HF, MP2, G2, and