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Radicals derived from uric acid and its methyl derivatives in aqueous solution: an EPR spectroscopy and theoretical study

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The oxidation of uric acid and of four *N*-methyluric acids in aqueous solution was studied by EPR spectroscopy. The primary oxidising radicals react with uric acid and its methyl derivatives by formal hydrogen abstraction from an NH group to yield radical-anions in neutral or moderately basic solutions and the respective radical-dianions in basic media. In the case of uric acid, the radical-trianion was detected at very high pH. The pK_a values of the radical-anions were determined to be in the range 9.5–11.2. The pK_a of uric acid radical-dianion was estimated to be 13.0. DFT calculations were performed to assign the hyperfine coupling constants and to determine the predominant tautomeric structure of the radicals. The uric acid radical-anion exists as the N1H, N9H tautomer, while in the radical-dianion the N1H structure is the most stable one. The intrinsic acidity of the NH protons both in uric acid and in its radicals seems to follow the order N1H < N9H < N3H

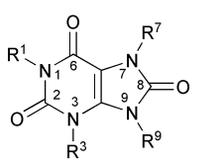
Introduction

Uric acid is one of the major degradation products of DNA purines present in blood plasma. Several isomers of methyluric acid are also found in physiologic media, resulting from the metabolism of the methylated purines caffeine, theobromine and theophyline present in the human diet.[1,2]

It is now well established that uric acid is an important antioxidant.[3] The urate anion, which is the species present at physiologic pH, is able to scavenge not only oxygen free radicals, but also singlet oxygen[3,4] and peroxynitrite.[5] Both urate and methyl urates inhibit lipid peroxidation in human erythrocyte membranes induced by ozone,[6] by hydrogen peroxide[7] or by other radical initiators.[8] Urate was also shown to prevent the oxidation of oxyhemoglobin by sodium nitrite,[9] to protect thymine, guanine and uracil from degradation by ozone,[10] and to inhibit L-DOPA-Cu(II) mediated DNA cleavage.[11] Urate was even shown to be more effective than ascorbate in preventing lipid peroxidation in bovine milk.[12]

Although the antioxidant properties of urate have received extensive attention in recent decades, few studies have been published on the characterization of urate radicals, which are the primary products of the one-electron oxidation of uric acid and its anions. Maples and Mason oxidised uric acid with horseradish peroxidase/hydrogen peroxide and hematin/hydrogen peroxide using a rapid-mixing/continuous flow technique and detected a broad (linewidth 0.27 G) EPR spectrum attributed to the urate radical-anion.[13] Shortly after, Simic and Jovanovic studied the optical, electrochemical and acid–base properties of uric acid radicals by pulse radiolysis.[14] These authors measured a value of pK_{a1} = 3.1 for the neutral radical and pK_{a2} = 9.5 for the radical-anion and unequivocally showed that the uric acid radical-anion is the species present at neutral pH.

In this work, radicals derived from uric acid (**1**) and from four of its methyl derivatives (**2**–**5**) are studied by EPR spectroscopy in aqueous solution in a wide pH range. Theoretical calculations are performed to assign the hyperfine coupling constants and compare the stability of the different tautomers in equilibrium.



1 R^1=R^3=R^7=R^9=H

2 R^1=CH_3 R^3=R^7=R^9=H

3 R^3=CH_3 R^1=R^7=R^9=H

4 R^7=CH_3 R^1=R^3=R^9=H

5 R^9=CH_3 R^1=R^3=R^7=H

Experimental

Uric acid (Aldrich, 99+%) was recrystallised from a large volume of distilled water. 1-Methyl-, 3-methyl- and 9-methyluric acid (Sigma) were used without further purification. 7-Methyluric acid[15] and 4-mercaptopyridine-*N*-oxide[16] were prepared by literature procedures.

Solutions for EPR spectroscopy were typically 2 mM in uric acid, 2 mM in 4-mercaptopyridine-*N*-oxide (or 25 mM potassium persulfate, see text) and 50 mM in phosphate buffer. The KOH solutions of 0.1 M, 0.5 M and 1 M were prepared under argon using distilled water previously boiled and Fixanal® ampoules (Riedel-de Haën).

The solutions were deaerated by bubbling with argon and allowed to flow at a constant rate into a quartz flat cell placed in the EPR cavity. The *in situ* photolysis was performed with an optically focused high-pressure Hg–Xe UV lamp. X-band EPR spectra were recorded in a Bruker ESP300E spectrometer.

Density functional theory (DFT) calculations were performed with the GAUSSIAN 98 package,[17] using the UB3LYP functional. The structures were optimised using the standard 6-31G(d) basis set with no symmetry constraints. The spin densities were calculated with the Mulliken population analysis. The absence of imaginary frequencies was used as a criteria to assure that the optimised structures represent minima in the potential energy surface. Subsequent single-point calculations of the hyperfine coupling constants were performed with the polarised and diffuse 6-31+G(2d,p) basis set. Electronic energies were corrected for zero point energy.

Results and discussion

EPR spectroscopy

Uric acid (1). The photolysis of aqueous solutions of uric acid (pK_{a1} = 5.4 and pK_{a2} = 9.8)[14] in the presence of 4-mercaptopyridine-*N*-oxide, potassium persulfate or hydrogen

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Table 1 Experimental and calculated *a* coupling constants (G) of uric acid and experimental coupling constants of methyluric acid radicals

Radical	N1	N3	N7	N9	H1	H3	H7	H9
$\mathbf{1}^{\bullet -}$	0.732	1.149	3.132	1.322	0.0[c]	—	—	0.161
$\mathbf{1}^{\bullet -}$ (ref. 13)	0.74	1.18	3.20	1.34	0.0[c]	—	—	0.0[c]
$\mathbf{1(1H9H)}^{\bullet -}$ [a b]	−0.75	1.18	3.11	−1.28	−0.11	—	—	−0.17
$\mathbf{1}^{\bullet 2-}$	0.788	0.365	3.025	0.645	0.231	—	—	—
$\mathbf{1(1H)}^{\bullet 2-}$ [a b]	−0.66	−0.08	3.66	−0.60	−0.44	—	—	—
$\mathbf{1}^{\bullet 3-}$ [d]	0.414	0.878	2.701	0.521	—	—	—	—
$\mathbf{1}^{\bullet 3-}$ [a]	−0.44	0.79	2.60	−0.99	—	—	—	—
$\mathbf{2}^{\bullet -}$	0.70	1.28	3.05	1.18	0.0[c] (3H)	—	—	0.0[c]
$\mathbf{2}^{\bullet 2-}$	0.410	0.820	3.036	0.634	0.120 (3H)	—	—	—
$\mathbf{3}^{\bullet -}$	0.0[c]	0.47	2.95	0.41	0.0[c]	1.16 (3H)	—	—
$\mathbf{3}^{\bullet 2-}$	0.228	0.570	2.520	0.228	—	0.908 (3H)	—	—
$\mathbf{4}^{\bullet -}$	0.50	1.60	3.62	0.56	0.0[c]	—	5.13 (3H)	—
$\mathbf{4}^{\bullet 2-}$	0.19	3.14	1.23	0.67	—	—	5.01 (3H)	—
$\mathbf{5}^{\bullet -}$	0.20	1.06	2.94	1.50	0.0[c]	—	—	0.60 (3H)
$\mathbf{5}^{\bullet 2-}$	0.43	2.55	1.43	1.29	—	—	—	0.43 (3H)

[a] DFT calculation, see text. [b] Most stable tautomer. [c] Constant within linewidth. [d] At pH 14. Constants are an weighted average with 10% $\mathbf{1}^{\bullet 2-}$.

peroxide at pH values between 5 and 9 resulted in the observation of an EPR spectrum with hyperfine coupling constants, shown in Table 1, from four non-equivalent nitrogen atoms and one hydrogen. This spectrum was attributed to the uric acid radical-anion $\mathbf{1}^{\bullet -}$ (Fig. 1a). The hydrogen coupling constant

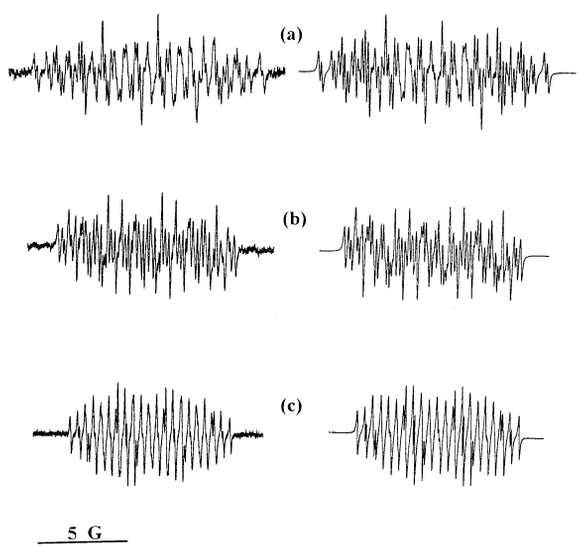


Fig. 1 Experimental EPR spectra (left-hand side) and computer simulations (right-hand side) of radicals derived from uric acid: (a) spectrum at pH 6.7 attributed to the radical-anion $\mathbf{1}^{\bullet -}$; (b) spectrum at pH 11.0 attributed to the radical-dianion $\mathbf{1}^{\bullet 2-}$; (c) spectrum at $[OH^-]$ = 1 M attributed to the radical-trianion $\mathbf{1}^{\bullet 3-}$ (with a 10% contribution from $\mathbf{1}^{\bullet 2-}$, see text).

disappeared when the reaction was performed in D_2O. This indicates that the hydrogen is in a labile NH (or OH) position and exchanges rapidly with the solvent. The nitrogen coupling constants are similar to the ones measured by Maples and Mason. These authors could not detect the hydrogen splitting due to the high intrinsic linewith of the spectrum.[13]

The same spectrum attributed to $\mathbf{1}^{\bullet -}$ was previously observed by us when a solution of xanthine and excess of 4-mercaptopyridine-*N*-oxide was photolysed at low flow rates.[18] Under these high conversion conditions, the xanthine 8-OH radical adduct primary formed disproportionates to give uric acid and regenerate xanthine. Further oxidation of uric acid yields $\mathbf{1}^{\bullet -}$.

At pH values lower than 5 the low solubility of the neutral form of uric acid prevented the observation of any EPR spectrum.

Solutions of uric acid and potassium persulfate turned yellow at pH values higher than 9, even in the absence of light. Attempts to photolyse this solution resulted either in the absence of EPR signal, or in the observation of weak lines resulting clearly from more than one species. Similar spectra were obtained when allantoin, a known decomposition product of uric acid, was photolysed under the same conditions.[21] Positive results at pH > 9 were obtained only when oxidations of uric acid were carried out by photolysis in the presence of 4-mercaptopyridine-*N*-oxide. At pH 11.0 a spectrum attributed to the uric acid radical-dianion $\mathbf{1}^{\bullet 2-}$ was detected (Fig. 1b). The spectrum is characterised by four nitrogen and one hydrogen coupling constant (Table 1).

At pH values in between 9 and 10.2 radicals $\mathbf{1}^{\bullet -}$ and $\mathbf{1}^{\bullet 2-}$ exist together and the EPR spectrum consists in the overlap of the two individual spectra. This confirms the value of pK_a = 9.5 obtained by pulse-radiolysis for $\mathbf{1}^{\bullet -}$.[14]

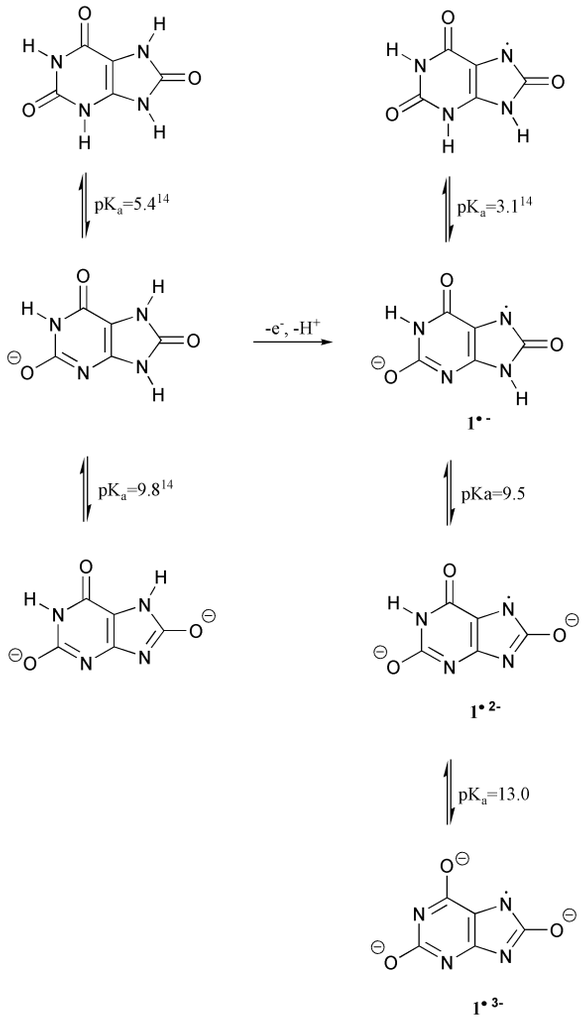
The hyperfine coupling constants of the uric acid EPR spectra recorded above pH 11.2 vary with the pH of the solution. At pH 12 the four nitrogen coupling constants measured have values similar to the ones found for $\mathbf{1}^{\bullet -}$, but no hydrogen coupling constant is detected. As the concentration of hydroxide increases, the rate of exchange of the NH proton of $\mathbf{1}^{\bullet 2-}$ with the solvent becomes faster. When the rate of exchange exceeds the hyperfine coupling constant, taken in frequency units, the hydrogen splitting is no longer detected.

In very basic solutions the nitrogen coupling constants change continuously as the pH increases. At $[OH^-]$ = 1 M these constants are a_N = 2.70, 0.878, 0.521 and 0.414 G (Fig. 1). At this concentration the activity coefficient of OH^- is γ = 1.072 and pH ~ H_- = 14.[22] No significant decomposition of uric acid was apparently detected under these extreme conditions. The EPR spectrum was intense and no extra lines were visible. Lowering the pH of the $[OH^-]$ = 1 M solution regenerated the spectra of $\mathbf{1}^{\bullet 2-}$ with no decrease in intensity. The results were thus tentatively interpreted in terms of a further deprotonation of the radical-dianion, yielding $\mathbf{1}^{\bullet 3-}$.

The equilibrium constant for deprotonation of $\mathbf{1}^{\bullet 2-}$ can be determined from the changes in the magnitude of the coupling constants as the pH is varied. The change in the observed coupling constants a_{obs} can be related to the known coupling of $\mathbf{1}^{\bullet 2-}$, a_{2-}, the unknown coupling constant of $\mathbf{1}^{\bullet 3-}$, a_{3-}, the equilibrium constant K_a and the proton concentration (eqn. (1)).

$$1/(a_{2-} - a_{obs}) = 1/(a_{2-} - a_{3-}) + [H^+]/K_a(a_{2-} - a_{3-}) \quad (1)$$

When plotting the data for the change in the N7 coupling constant of $\mathbf{1}^{\bullet 2-}$ between pH 12.2 and 14, a linear fit was obtained (r = 0.999, 7 points) where the a_{3-} value recovered was 2.67 and pK_a = 13.0. This result shows that the spectrum at pH 14 still has a contribution of around 10% of $\mathbf{1}^{\bullet 2-}$. Scheme 1 summarises the results obtained for uric acid.



Scheme 1

Methyluric acids (2–5). The oxidation of methyluric acids **2**–**5** was also studied by EPR spectroscopy. Photolysis of buffered aqueous solutions containing 4-mercaptopyridine-*N*-oxide resulted in the observation of weak spectra at neutral or moderately basic solutions, attributed to the radical-anions, and more intense spectra at high pH, attributed to the radical-dianions. The hyperfine coupling constants of these radicals are shown in Table 1. Spectra derived from 1-methyluric acid (**2**) and from 7-methyluric acid (**4**) are shown as examples in Fig. 2.

The magnitude of the hydrogen coupling constants of the methyl groups decrease in the order **4** > **3** > **5** > **2**, both for the radical-anions and for the radical-dianions. This may suggest the assignment of the biggest nitrogen coupling constant to N7. Maples and Mason have already shown by isotope labelling that the main nitrogen coupling constant of **1**$^{\bullet -}$ was either N7 or N9.[13] No hydrogen coupling constant from an NH group was detected in any of the methylated radical-anions studied.

The pK_a values of the radical-anions were estimated from the dependence of the relative concentrations of the acid and basic forms on the pH. The complexity of the spectra suggest an uncertainty of ±0.2 in the pK_a values. Furthermore, the relative concentrations of the radical-mono- and dianions may be distorted due to their different stabilities. Since polyanions are more stable toward bimolecular decay processes than monoanions, the pK_a values estimated here may be lower than the real ones. **2**$^{\bullet -}$ and **3**$^{\bullet -}$ have pK_a values of 10.6 and 9.7, respectively. The radicals with methyl groups at the nitrogens of the imidazole ring show slightly higher values: pK_a = 11.2 for **4**$^{\bullet -}$ and pK_a = 11.0 for **5**$^{\bullet -}$. All the pK_a values are larger than the corresponding value of 9.5 for **1**$^{\bullet -}$. This is due to the electron-releasing effect of the methyl groups. This effect unstabilises the radical-dianions preferentially as compared to the monoanions, shifting the pK_a to more basic values.

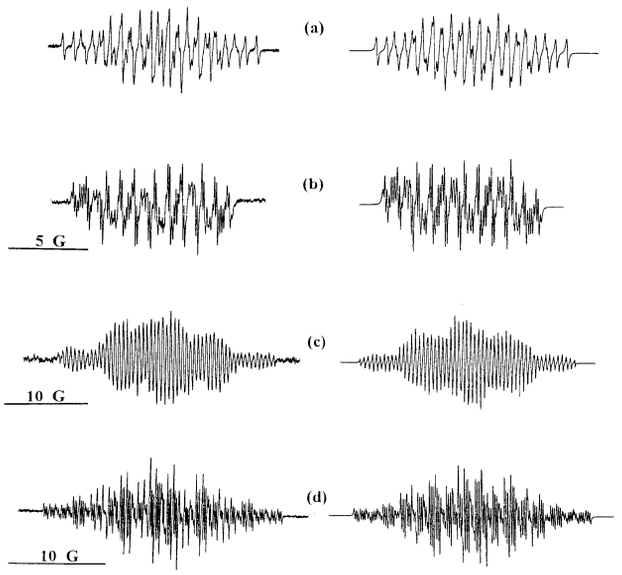


Fig. 2 Experimental EPR spectra (left-hand side) and computer simulations (right-hand side) of radicals derived from (a) 1-methyluric acid (**2**), at pH 8.2, attributed to the radical-anion **2**$^{\bullet -}$; (b) 1-methyluric acid, pH 11.6, attributed to the radical-dianion **2**$^{\bullet 2-}$; (c) 7-methyluric acid (**4**), at pH 10.8, attributed to the radical-anion **4**$^{\bullet -}$, and (d) 7-methyluric acid, at pH 13, attributed to the radical-dianion **4**$^{\bullet 2-}$.

Theoretical calculations

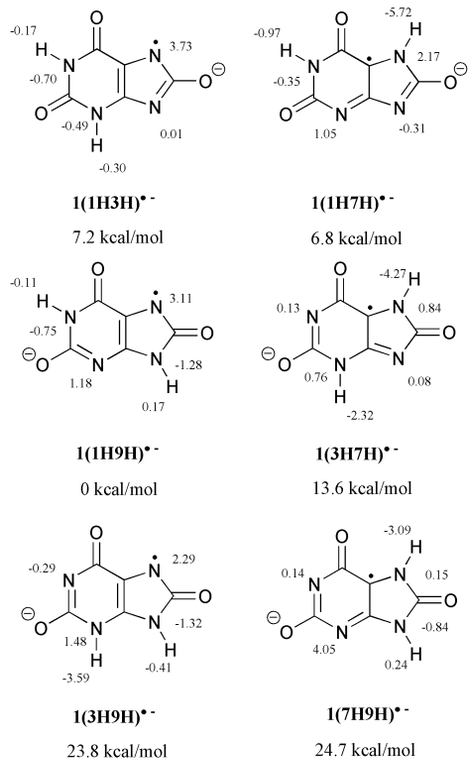
Uric acid (1). DFT calculations have been shown to reproduce successfully the hyperfine coupling constants of pyrimidine[19] and purine[20] radicals and the relative stability of their tautomers. Therefore, a DFT approach was used to compare the stability of the different tautomers of uric acid radicals in equilibrium. The optimised structures of all radicals are planar.

The theoretical calculations of the several possible tautomeric structures for **1**$^{\bullet -}$ showed the N1–H, N9–H tautomer, **1(1H9H)**$^{\bullet -}$, to be the most stable one, 6.8 and 7.2 kcal mol^{-1} lower in energy than **1(1H7H)**$^{\bullet -}$ and **1(1H3H)**$^{\bullet -}$, respectively. The structures with one O–H bond (lactim structures) were found to be less stable than any of the N–H lactam structures. The calculated hyperfine coupling constants of the six lactam tautomers and their relative energies are shown in Scheme 2.

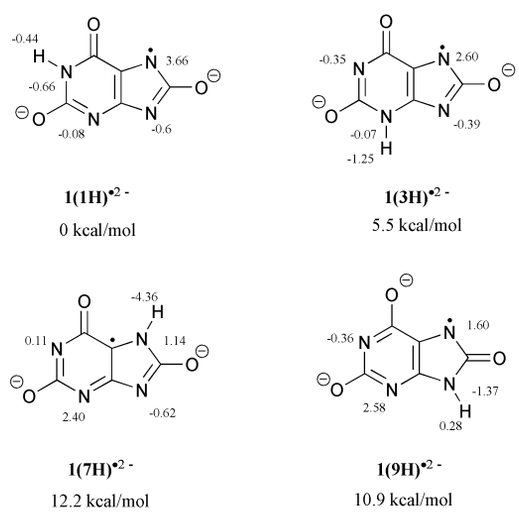
Concerning the radical-dianion, the **1(1H)**$^{\bullet 2-}$ is the dominant tautomer, being 5.5 kcal mol^{-1} more stable than **1(3H)**$^{\bullet 2-}$, the second most stable tautomer. Structures with the hydrogen atom on the N7 or N9 of the imidazol ring proved to be less stable. As in **1**$^{\bullet -}$, all lactim structures were found to be higher in energy than the lactam structures. The theoretical results for the lactam tautomers are summarised in Scheme 3.

Calculated hyperfine coupling constants (Schemes 2 and 3) of the most stable structures are in good agreement with the experimental ones (Table 1). As suggested before, the main nitrogen coupling constant is assigned to N7. This agrees with the findings that for maximum antioxidant activity, uric acid derivatives must have a hydrogen at N7. Rates of reactions of uric acids with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were shown to increase in 15–20% with methylation either at N1, N3 or N9, but the 7-methyl derivative **4** reacted with DPPH at a rate that was only 9% that of **1**. Similar conclusions could be withdrawn from the rates of reaction of dimethyluric acids.[23]

Both experimental determinations and theoretical predictions suggest that the urate anion is the N3-deprotonated species and that the dianion is deprotonated at N3 and N9 (Scheme 1).[24] Oxidation of uric acid and of its anions thus corresponds formally to a hydrogen abstraction at N7–H, as shown in Scheme 1.



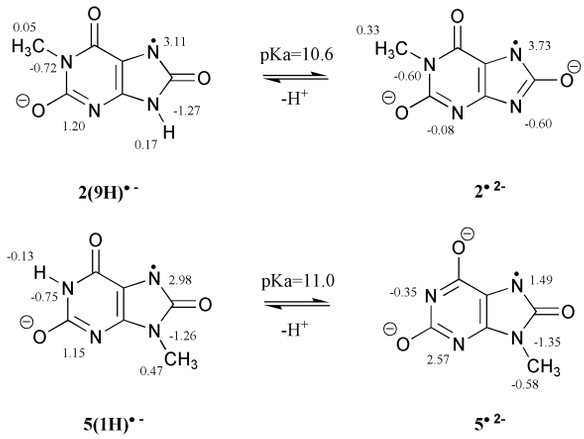
Scheme 2 Calculated hyperfine coupling constants and relative energies of $\mathbf{1}^{\bullet -}$ tautomers.



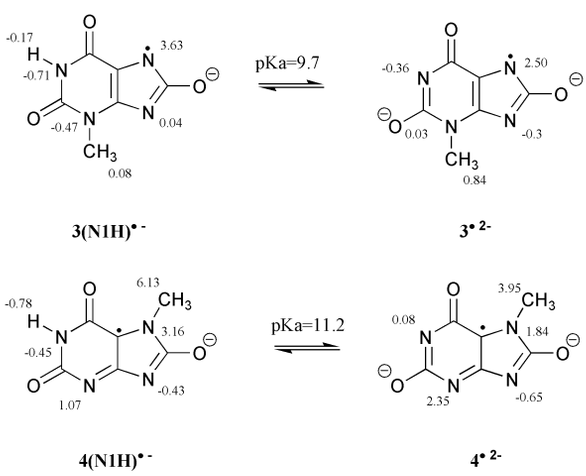
Scheme 3 Calculated hyperfine coupling constants and relative energies of $\mathbf{1}^{\bullet 2-}$ tautomers.

Methyluric acids (2–5). DFT calculations were also performed in the case of the methylated radical-anions to unravel their predominant tautomeric structure and correlate it with their pK_a values. The results show that the 1-methyl- and 9-methyluric acid radical-anions retain the N1,N9 substitution pattern of $\mathbf{1}^{\bullet -}$: the predominant structures for these radicals are the $\mathbf{2(9H)}^{\bullet -}$ and the $\mathbf{5(1H)}^{\bullet -}$ tautomers (Scheme 4). Since the intrinsic acidity of the protons both in uric acid and in its radicals increases in the order N1H < N9H < N3H (see Scheme 1), the pK_a of radical $\mathbf{5(1H)}^{\bullet -}$ is higher than the one from $\mathbf{2(9H)}^{\bullet -}$.

The 3-methyl and 7-methyl isomers have structures that do not correlate directly with $\mathbf{1}^{\bullet -}$. Their most stable structures are the $\mathbf{3(1H)}^{\bullet -}$ and $\mathbf{4(1H)}^{\bullet -}$ tautomers (Scheme 5). Although both the isomers deprotonate from N1H, the pK_a values are quite different, $\mathbf{3(1H)}^{\bullet -}$ being more acidic than $\mathbf{4(1H)}^{\bullet -}$. This may reflect a higher stability of $\mathbf{3}^{\bullet 2-}$ as compared to $\mathbf{4}^{\bullet 2-}$ due to a higher extent of delocalisation of the negative charges through the three carbonyl groups.



Scheme 4 Calculated hyperfine coupling constants for the most stable tautomers of radicals derived from **2** and **5**.



Scheme 5 Calculated hyperfine coupling constants for the most stable tautomers of radicals derived from **3** and **4**.

Conclusions

The reaction of uric acid with mild oxidants results in the observation of EPR spectra attributed to the uric acid radical-anion, radical-dianion or radical-trianion, depending on the pH of the solution. The reaction corresponds formally to an H-atom abstraction from N7H. The pK_a values of the radical-anion and radical-dianion are 9.5 and 13.0, respectively.

Gas-phase DFT calculations showed the N1H, N9H tautomer to be the preferred form for the radical-anion, and the radical-dianion to exist as the N1H tautomer. Although the unpared electron is highy delocalised, the main spin density is located at C5 and N7.

N-Methyluric acids also yield the corresponding radical-anions and radical-dianions upon oxidation. The pK_a values of the radical-anions were determined to be in the range 9.7–11.2. These species were shown to exist predominantely as the N1H tautomer, except for the 1-methyl compoud that exists in the N9H form. Therefore, the intrinsic acidity of the NH protons both in uric acid derivatives and in its radicals seems to follow the order N1H < N9H < N3H.

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References

1 J. P. Telo and A. J. S. C. Vieira, *J. Chem. Soc., Perkin Trans. 2*, 1997, 1755.
2 J. F. Lu, X.M. Cao, T. Yi, H. T. Zhuo and S. S. Ling, *Anal. Lett.*, 1998, **31**, 613.
3 B. N. Ames, R. Cathcart, E. Scwiers and P. Hochstein, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 6858.
4 E. W. Kellogg and I. Fridovich, *J. Biol. Chem.*, 1977, **252**, 6721.
5 R. B. Kean, S. V. Spitsin, T. Mikheeva, G. S. Scott and D. C. Hooper, *J. Immunol.*, 2000, **165**, 6511.
6 Y. Nishida, *J. Pharm. Pharmacol*, 1991, **43**, 885.
7 R. C. Smith and L. Lawing, *Arch. Biochem. Biophys.*, 1983, **223**, 166.
8 R. C. Smith and V. Nunn, *Comp. Biochem. Biophys.*, 1986, **84C**, 79; E. Niki, Y. Yamamoto and Y. Kamiya, *Chem. Lett.*, 1985, 1267.
9 R. C. Smith and V. Nunn, *Arch. Biochem. Biophys.*, 1984, **232**, 348.
10 J. Meadow and R. C. Smith, *Arch. Biochem. Biophys.*, 1986, **246**, 838.
11 S. Singh, F. Asad and S. M. Hadi, *Neurosci. Lett.*, 1998, **258**, 69.
12 H. Ostdal, H. J. Andersen and J. H. Nielsen, *J. Agric. Food Chem.*, 2000, **48**, 5588.
13 K. R. Maples and R. P. Mason, *J. Biol. Chem.*, 1988, **263**, 1709.
14 M. G. Simic and S. V. Jovanovic, *J. Am. Chem. Soc.*, 1989, **111**, 5778.
15 H. Biltz, K. Marwitzky and M. Heyn, *Liebigs Ann. Chem.*, 1921, **423**, 122.
16 E. Shaw, J. Bernstein, K. Losee and W. A. Lott, *J. Am. Chem. Soc.*, 1950, **72**, 4362; D. H. R. Barton, D. Crich and G. Kretzschmar, *J. Chem. Soc., Perkin Trans. 1*, 1986, 39.
17 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Rob, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, and J. A. Pople, *Gaussian 98*, Revision A.7, Gaussian, Inc., Pittsburgh PA, 1998.
18 A. J. S. C. Vieira, J. P. Telo and R. M. B. Dias, *J. Chim. Phys.*, 1997, **94**, 318.
19 S. Naumov, K. Hildenbrand and C. von Sontag, *J. Chem. Soc, Perkin Trans. 2*, 2001, 1648.
20 S. D. Wetmore, R. J. Boyd and L. A. Eriksson, *J. Phys. Chem. B*, 1998, **102**, 10602; J. Gu, A. Tian, W. K. Li and N.B. Wong, *J. Phys. Chem. B*, 2000, **104**, 10692; J. Reynisson and S. Steenken, *Phys. Chem. Chem. Phys.*, 2002, **4**, 527.
21 A. J. S. C. Vieira and J. P. Telo, unpublished results.
22 H. Taniguchi and R. H. Schuler, *J. Phys. Chem.*, 1985, **89**, 335.
23 R. C. Smith, J. Reeves, M. L. McKee and H. Daron, *Free Radical Biol. Med.*, 1987, **3**, 251.
24 K. Kahn, P. Serfozo and P. A. Tipton, *J. Am. Chem. Soc.*, 1997, **119**, 5435.