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Uric and 1-Methyluric Acids: Metabolic Wastes or Antiradical Protectors?

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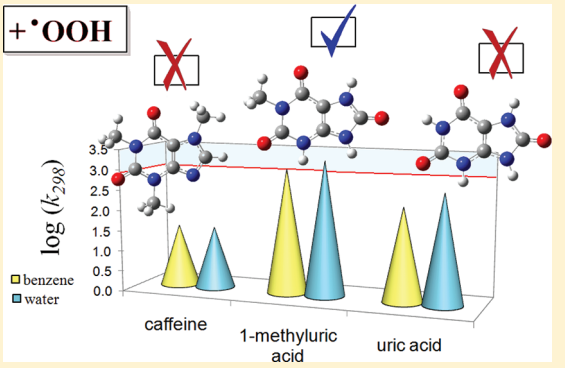
Supporting Information

ABSTRACT: The reactions of uric and 1-methyluric acids in nonpolar environments, as well as those of the corresponding urate anions in aqueous solution, with $^{\bullet}OH$, $^{\bullet}OCH_3$, $^{\bullet}OOH$, and $^{\bullet}OOCH_3$ have been studied using the density functional theory. Different mechanisms of reactions have been taken into account, and their relative importance on the antiradical activity of these compounds is analyzed. Both uric and methyluric acids are better scavengers in aqueous solution than in nonpolar media, which indicates that the urate anions are the most active species. The free radical scavenging activity of the studied compounds was found to be excellent for $^{\bullet}OH$, and very good for $^{\bullet}OCH_3$. In addition, 1-methyluric acid is predicted to moderately protect against peroxyl oxidation, while the protective effects of uric acid against these particular species are not expected to be significant. In addition, 1-methyluric acid was found to be a better radical scavenger than its precursor, caffeine, suggesting that the antiradical activity of the latter might be explained by the action of its metabolites, rather than by its direct activity.



INTRODUCTION

At high concentrations, free radicals are harmful to living organisms. Such high concentrations are caused by the imbalance between the production and the consumption of free radicals, which is commonly referred to as oxidative stress (OS). This chemical stress has attracted great deal of attention in the last decades due to the increasing evidence supporting its role in the development of a large number of health disorders such as cancer,[1] cardiovascular disorders,[2] atherosclerosis,[3] and Alzheimer's disease.[4] Since OS frequently involves reactions between biological molecules and free radicals, the study of compounds with free radical scavenging activity becomes an important area of research aiming to prevent OS and the consequent molecular damage.

Uric acid (HUA) is one of the major degradation products of DNA purines present in blood plasma and has many physiological functions in living organisms.[5] Several isomers of monomethylated HUA are also found in physiologic media, arising from the metabolism of the purines caffeine, theobromine, and theophylline.[6,7] Moreover, based on the relatively high amount of caffeine in coffee, significant amounts of its metabolites are anticipated in blood and might be involved in the physiological effects related to coffee.[8] In particular, 1-methyluric acid (HMUA) is one of the main metabolic products of caffeine in humans, and has been reported to present antioxidant activity.[9] It was found to be particularly effective in inhibiting human low-density lipoprotein oxidative modification.[8] Its protective effect has been reported to be equivalent to that of HUA.[9] HUA is an intriguing compound that is particularly abundant in humans. The mutation of the uricase gene during human evolution resulted in an average plasma urate concentration that is higher than that in most of the other mammals (~0.3 mM).[10] This has led to the hypothesis that this evolutionary strategy represents a counteracting action against the damaging effects of OS, and that urate has an important role as antioxidant in humans.[11−14]

According to their pK_a values (Figure 1) at pH = 7.4, the dominant forms of HUA and HMUA are expected to be the corresponding monoanions, UA^- and MUA^-. Therefore, while in lipid media their neutral forms should prevail, in the aqueous phase under physiological conditions, the urate anions are supposed to be the relevant species to the antiradical activity of these compounds.

Meadows and Smith[17] demonstrated that, among purines and pyrimidines, HUA shows the greatest susceptibility to ozone-induced degradation. It has also been demonstrated that this compound protects unsaturated fatty acids from air oxidation,[18,19] and oxyhemoglobin from sodium nitrite oxidation.[20] It has been reported that HUA is able to scavenge single oxygen,[12,21] peroxinitrite,[22] and hydroxyl[23] radicals. It has also been demonstrated that both urate and methylurates inhibit lipid peroxidation induced by ozone,[24] hydrogen peroxide,[19] and other radical initiators.[25,26] HUA has been recently reported to be more

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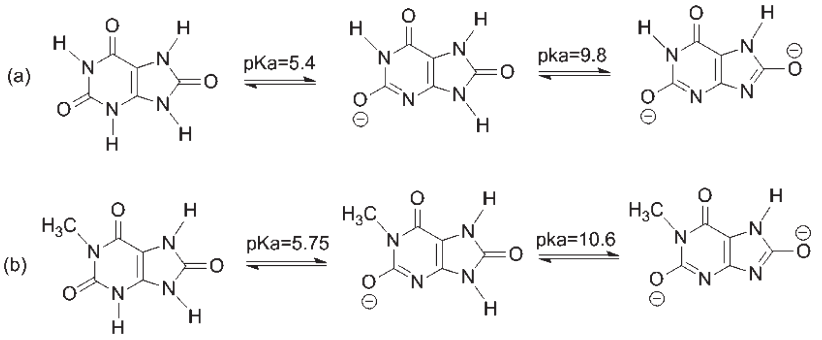

Figure 1. Acid dissociations and pK_a's of (a) HUA and (b) HMUA. (a) Values from ref 15; (b) values from ref 16.

efficient as a NO radical scavenger than caffeic acid, trolox, genistein, glutathione, and *N*-acetylcysteine.[27] In addition, based on the rate of the reaction between HUA and the guanyl radical, Simic and Jovanovic[15] proposed that HUA may also repair oxidative-damaged DNA bases. Differing from these reports, Hershfield et al.[28] concluded that urate is not a major factor controlling OS in vivo.

In addition, concentration is a key factor regarding the beneficial effects of HUA. Peden el al.[29] showed that HUA has antioxidant activity at concentrations larger than 1.5 μM, which is significantly below its concentration in human plasma. On the other hand, it has been demonstrated that excessive amounts of HUA in the blood plasma (hyperuricemia) cause gout[30] and induces OS in adipocytes.[31] Moreover hyperuricemia is considered a strong predictor of mortality in women,[32] elder persons,[33] and those with cardiovascular diseases.[34] In line with this evidence, and with the potential beneficial effects of HUA as free radical scavenger, Ruggiero et al.[35] have recently demonstrated that people with intermediate levels of HUA (0.29−0.32 mM) have a better physical performance than those with higher or lower levels.

Regarding the reaction mechanism involved in the free radical scavenging activity of HUA and HMUA, the available information is rather scarce. Maples and Mason[36] detected the urate free radical using electron spin resonance, and located the unpaired electron on the five-memberd ring, based on ^{15}N isotopic experiments. Simic and Jovanovic[15] proposed that the peroxy free radical scavenging activity of HUA takes place by electron transfer. More recently, Telo[37] proposed that HUA and its methyl derivatives react with primary oxidizing radicals by hydrogen abstraction from an NH group. In addition, Allen et al.[38] used density functional theory calculations and, based on relative energy considerations, proposed that the radical products most likely to be formed by H transfers are those involving sites 9 and 3 of HUA, and site 7 for UA^-. These authors also proposed that the monoanion corresponds to deprotonation of site N3, and the dianion to deprotonations of sites N3 and N9. The numbering of the reaction sites are shown in Figure 2.

According to the information gathered so far, and discussed above, it seems that the antiradical activity of HUA and HMUA deserves further studies since there still are several related open questions. For example, (a) Are the anions in aqueous phase actually more active? (b) Are the neutral forms also active as antiradicals in the lipid phase? (c) How efficient these species actually are for scavenging free radicals of different nature? (d) Which is the preponderant mechanism of reaction involved in their antiradical activity? (e) Does the preponderant mechanism

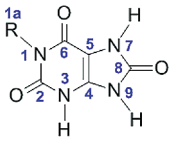

Figure 2. Site numbers. HUA: R═H; and HMUA: R═CH_3.

change depending on the polarity of the media and on the nature of the reacting radical? Therefore this work presents a systematic study on the reactions of HUA and HMUA with $^{\bullet}OH$, $^{\bullet}OCH_3$, $^{\bullet}OOH$, and $^{\bullet}OOCH_3$ radicals, in aqueous solution and in nonpolar environments. The rate constants have been estimated and the contributions of different mechanisms and sites of reaction to the overall radical scavenging activity of these compounds are provided.

COMPUTATIONAL DETAILS

Geometry optimizations and frequency calculations have been carried out using the M05-2X functional and the 6-31+G(d,p) basis set. The electronic energies were improved by single point calculations using the 6-311++G(d,p) basis set. The M05-2X functional has been recommended for kinetic calculations by their developers,[39] and it has been also successfully used to that purpose by independent authors.[40−46]

Unrestricted calculations were used for open shell systems and local minima and transition states were identified by the number of imaginary frequencies (NIMAG = 0 or 1, respectively). Intrinsic reaction coordinate (IRC) calculations have been performed to confirm that the transition states properly connect reactants and products. All the electronic calculations were performed with the Gaussian 03 package of programs.[47] Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. They were calculated using the harmonic oscillator/rigid rotator approximation, as implemented in Gaussian 03. The stationary points were first modeled in gas phase (vacuum), and solvent effects were included *a posteriori* by single-point calculations using polarizable continuum model, specifically the integral-equation-formalism (IEF-PCM)[48] with RADII = UAKS, at the M05-2X/6-311++G(d,p) level of theory. They have been performed using benzene and water as solvents, to mimic nonpolar and polar environments, respectively. This approach has been successfully used before for describing radical−molecule reactions in solution involved in the free radical scavenging activity of different compound.[49−55] Moreover, it was demonstrated for a very similar system (caffeine) that the geometries, energies, and rate constants obtained with this

methodology are very similar to those obtained when geometry optimizations are performed in solution.[56]

Relative Gibbs free energies in solution have been computed using thermodynamic cycles and the Hess law, explicitly including solvation free energies. For example, for $^{\bullet}$OH additions to HUA,

$$\begin{array}{ccccc} HUA_{gas} & + & {}^{\bullet}OH_{gas} & \xrightarrow{\Delta G_{gas}} & HUA{-}OH^{\bullet}_{gas} \\ \uparrow -\Delta G_s(HUA) & & \uparrow -\Delta G_s({}^{\bullet}OH) & & \downarrow \Delta G_s(HUA{-}OH^{\bullet}) \\ HUA_{sol} & + & {}^{\bullet}OH_{sol} & \xrightarrow{\Delta G_{sol}} & HUA{-}OH^{\bullet}_{sol} \end{array}$$

Within this strategy the Gibbs free energies of reaction in solution (ΔG_{sol}) can be obtained as the sum of the Gibbs free energy of reaction in vacuum (ΔG_{gas}) and the difference in the solvation free energies ($\Delta\Delta G_S$):

$$\Delta G_{sol} = \Delta G_{gas} + \Delta\Delta G_S \quad (1)$$

where $\Delta\Delta G_S$ is calculated as

$$\Delta\Delta G_S = \Delta G_S(HUA{-}OH^{\bullet}) - \Delta G_S(HUA) - \Delta G_S({}^{\bullet}OH) \quad (2)$$

with ΔG_S representing the free energies of solvation. In all the cases the reference state is 1 M. The solvent cage effects have been included according to the corrections proposed by Okuno,[57] taking into account the free volume theory.[58] These corrections are in good agreement with those independently obtained by Ardura et al.[59] and have been successfully used before.[60]

The rate constants (k) were calculated using conventional transition state theory (TST)[61] and 1 M standard state as

$$k = \sigma\kappa\frac{k_B T}{h} e^{-(\Delta G^{\ddagger})/RT} \quad (3)$$

where k_B and h are the Boltzmann and Planck constants, $\Delta G^{\ddagger}$ is the Gibbs free energy of activation, σ represents the reaction path degeneracy, accounting for the number of equivalent reaction paths, and κ accounts for tunneling corrections. The latter, which are defined as the Boltzmann average of the ratio of the quantum and the classical probabilities, were calculated using the zero curvature tunneling corrections (ZCT).[62]

Some of the calculated rate constants (k) were found to be close to, or within, the diffusion-limited control. Accordingly, the apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. In the present work we have used the Collins–Kimball theory to that purpose:[63]

$$k_{app} = \frac{k_D k_{act}}{k_D + k_{act}} \quad (4)$$

where k_{act} is the thermal rate constant, obtained from TST calculations (eq 3), and k_D is the steady-state Smoluchowski[64] rate constant for an irreversible bimolecular diffusion-controlled reaction:

$$k_D = 4\pi R D_{AB} N_A \quad (5)$$

where R denotes the reaction distance, N_A is the Avogadro number, and D_{AB} is the mutual diffusion coefficient of the reactants A (radical) and B (scavenger). D_{AB} has been calculated from D_A and D_B according to ref 65, and D_A and D_B have been estimated from the Stokes–Einstein approach:[66]

$$D = \frac{k_B T}{6\pi\eta a} \quad (6)$$

where k_B is the Boltzmann constant; T is the temperature; η denotes the viscosity of the solvent, in our case water ($\eta = 8.91 \times 10^{-4}$ Pa s) and benzene ($\eta = 6.04 \times 10^{-4}$ Pa s); and a is the radius of the solute.

RESULTS AND DISCUSSION

Seventy-four different tautomers have been recently identified for HUA.[67] In this thorough investigation Raczynska et al. found that the tetra-NH species is the lowest in energy, using different levels of theory. At the most accurate one, G2(MP2), this tautomer was found to be 2.7 kcal/mol lower in energy than the following species. This means that the tetra-NH tautomer accounts for more than 98.9% of the total population of HUA. On the basis of these results, this is the only neutral tautomer considered in the present work.

In addition, based on their pK_a values, the monoanionic form is the one relevant for estimating the free radical scavenging activity of the studied acids, in aqueous solution, under physiological conditions. According to the molar fractions, at physiological pH (7.4) the monoanions represent 98.62% and 97.75% of HUA and HMUA, respectively (Figure 1S, Supporting Information). Therefore, in this work, the monoanions are the species considered for the study in aqueous solution. On the other hand, nonpolar environments do not promote enough solvation of the ionic species. Moreover, according to our calculations, the deprotonation of HUA in benzene solution requires ~80 kcal/mol more than the same process in aqueous solution, which means that in such media the deprotonation of this compound is negligible. Consequently, the neutral forms have been used for the study in nonpolar environments. Accordingly, the overall reactions with different free radicals ($^{\bullet}$R=$^{\bullet}$OH, $^{\bullet}OCH_3$, $^{\bullet}OOH$, and $^{\bullet}OOCH_3$) for both HUA and HMUA studied in this work are

$$Acid_{(lipid)} + {}^{\bullet}R_{(lipid)} \rightarrow products_{(lipid)} \quad (I)$$

and

$$Mono-anion_{(aqueous)} + {}^{\bullet}R_{(aqueous)} \rightarrow products_{(aqueous)} \quad (II)$$

We have estimated the relative energies of the monoanions formed by deprotonation from sites N1, N3, N7, and N9 and found that the most stable one is that arising by deprotonation from site N3. Their relative energies, for HUA in aqueous solution, were found to be N1 (8.81 kcal/mol), N3 (0.00 kcal/mol), N7 (7.90 kcal/mol), and N9 (1.06 kcal/mol). As suggested by one of the Reviewers, the energy difference between N3 and N9 has been recomputed at the G2MP2 level to check the energy difference, which is the smallest one. It was found that at this level the energy difference is even larger, 1.7 kcal/mol. These results are in agreement with the proposal by Allen et al.[38] and Kahn et al.[68] that the monoanion corresponds to the species deprotonated at site N3. Accordingly, this is the one used in this work.

The free radical scavenging activity of the studied compounds (HX, with X = UA and MUA) can take place through different mechanisms, as it is also the case for many other compounds.[49−51,53−56] Those considered in this work are

For the reaction involving the neutral species, in lipid medium (**I**):

$$\text{Radical adduct formation (RAF)}: \ HX + {}^{\bullet}R \rightarrow [HX{-}R]^{\bullet}$$

$$\text{Hydrogen transfer (HT)}: \ HX + {}^{\bullet}R \rightarrow HX^{\bullet}_{(-H)} + HR$$

$$\text{Single electron transfer (SET)}: \ HX + {}^{\bullet}R \rightarrow HX^{\bullet+} + R^{-}$$

For the reaction involving the monoanions, in aqueous solution (**II**):

$$\text{Radical adduct formation (RAF)}: \ X^{-} + {}^{\bullet}R \rightarrow [X{-}R]^{-\bullet}$$

$$\text{Hydrogen transfer (HT)}: \ X^{-} + {}^{\bullet}R \rightarrow X^{-\bullet}_{(-H)} + HR$$

$$\text{Single electron transfer (SET)}: \ X^{-} + {}^{\bullet}R \rightarrow X^{\bullet} + R^{-}$$

The Gibbs free energies of reaction (ΔG) corresponding to all the studied paths are reported in Table 1. Even though RAF process on sites 1 to 9 were initially considered, any attempt to obtain the addition products on nitrogen sites (1, 3, 7, and 9) invariably led to structures that correspond to weak-bonded complexes rather than to proper radical adducts. Therefore these channels have been ruled out for yielding viable products of reaction.

As the values in Table 1 show, for the SET mechanism only the reactions with the hydroxyl radical, in aqueous solution, were found to be exergonic. In nonpolar environments, the endergonicity was found to be higher than 75 kcal/mol for the reactions of both scavengers with all the studied radicals. This is a logical result since only polar environments promote enough solvation for the ionic species formed via SET. In addition the electron-donnor capability of the anions (UA^{-} and MUA^{-}) is higher than those of the parent protonated species. The finding that electron transfers to the OH radical are the only ones that were found to be exergonic can be explained based on the higher electrophilicity of this radical, compared to the other three.

Regarding the RAF mechanism in nonpolar environments (**I**), additions to C2 were found to be significantly endergonic for all the scavenger-radical pairs studied in the present work. The additions of $^{\bullet}OH$ to the other RAF sites were found to be exergonic. However, the exergonicity of channel C6 is small, and therefore it is expected to be reversible for both HUA and HMUA. For the RAF reactions involving $^{\bullet}OCH_3$, this particular channel becomes endergonic, while additions to sites C4, C5, and C8 remain thermochemically feasible. For the additions of $^{\bullet}OOH$, only one channel was found to be exergonic, that involving site C4, which is also the channel with the largest exergonicity for the reactions with $^{\bullet}OH$ and $^{\bullet}OCH_3$. The addition of $^{\bullet}OOH$ to C4 was found to be slightly more favored for HUA than for HMUA. In the case of the RAF reactions involving $^{\bullet}OOCH_3$, all the channels were found to be endergonic. Therefore for peroxyl radicals, other than $^{\bullet}OOH$, this mechanism is not expected to contribute to the overall reactivity of HUA and HMUA, in nonpolar environments.

When the RAF processes takes place in aqueous solution (**II**), channel C2 becomes viable for the reaction with $^{\bullet}OH$, but remains unfeasible for the other, less reactive, free radicals. For the RAF reactions with $^{\bullet}OCH_3$, channel C8 was found to be exergonic for both UA^{-} and MUA^{-}, while channel C4 is only viable for the latter. The rest of the addition channels were found to be endergonic. In addition, for both peroxyl radicals all the RAF channels are endergonic. This suggests that the neutral form of the studied acids is more reactive than the anionic one through the RAF mechanism.

The H transfer mechanism was found to be the most favored from a thermochemical point of view. It has the largest exergonicities and presents viable channels of reaction with all the studied free radicals. For the HT reactions involving $^{\bullet}OH$, all the reaction channels were found to be considerably exergonic for both scavengers, regardless of the polarity of the environment, and therefore of the reacting form (neutral or anion). For the reactions with $^{\bullet}OCH_3$, the only HT channel that is predicted to be endergonic is that involving site N1 in HUA. For the reactions of $^{\bullet}OOH$, those channels involving H transfers from sites N3 and N9 in HUA and HMUA were found to be exergonic. The HT processes from sites N7 and N9 in UA^{-} and MUA^{-} were also predicted to be thermochemically viable. For the reactions of $^{\bullet}OOCH_3$, HT is the only mechanism that was found to be feasible. The viable channels are the same than those previously described for the reactions with $^{\bullet}OOH$. Moreover, the exergonicity is slightly increased for the reactions of $^{\bullet}OOCH_3$, compared to $^{\bullet}OOH$. This strongly suggests that the overall reactivity of HUA and HMUA toward peroxyl radicals takes place via HT. On the contrary,

Table 1. Gibbs Free Energies of Reaction (ΔG, kcal/mol) at 298.15 K

	$^{\bullet}OH$		$^{\bullet}OCH_3$		$^{\bullet}OOH$		$^{\bullet}OOCH_3$	
	(I)[a]	(II)[a]	(I)	(II)	(I)	(II)	(I)	(II)
				HUA				
SET	80.29	−14.72	93.17	6.34	101.97	16.58	103.97	19.40
RAF								
C2	10.96	−7.24	26.03	7.62	43.46	22.94	45.89	24.03
C4	−30.35	−12.64	−17.44	0.71	−2.16	16.27	0.41	17.90
C5	−20.13	−12.32	−5.10	1.58	9.53	18.91	12.39	17.87
C6	−1.71	−10.82	14.01	4.11	32.00	18.94	32.52	21.11
C8	−28.96	−16.55	−16.82	−2.05	15.89	12.68	20.80	15.87
HT								
N1	−11.62	−24.61	3.48	−11.26	20.77	5.69	22.32	6.24
N3	−34.63		−19.52		−2.24		−0.68	
N7	−29.37	−37.50	−14.27	−24.15	3.02	−7.20	4.58	−6.65
N9	−35.02	−33.26	−19.92	−19.92	−2.63	−2.96	−1.08	−2.42
				HMUA				
SET	78.04	−16.66	90.92	4.40	99.73	14.64	101.73	17.46
RAF								
C2	10.94	−8.55	26.63	6.72	20.17	18.38	12.00	22.91
C4	−30.72	−13.79	−17.17	−1.34	−1.76	16.26	0.58	16.56
C5	−20.33	−12.95	−5.21	0.89	8.57	15.31	11.33	16.86
C6	−1.08	−11.59	14.90	3.89	31.40	18.89	35.08	21.43
C8	−24.59	−16.66	−10.27	−3.90	17.16	11.37	20.45	11.28
HT								
C1a	−21.61	−24.31	−6.51	−10.97	10.78	5.99	12.33	6.53
N3	−34.86		−19.75		−2.47		−0.91	
N7	−29.68	−38.14	−14.58	−24.80	2.71	−7.84	4.27	−7.30
N9	−35.41	−33.91	−20.31	−20.56	−3.02	−3.61	−1.47	−3.06

[a] (**I**) lipid phase, modeled using benzene; (**II**) aqueous phase.

Table 2. Apparent Rate Constants of the Different Channels, and Overall Rate Coefficient (M^{-1} s^{-1}) at 298 K

	•OH		•OCH_3		•OOH		•$OOCH_3$	
	(I)*	(II)*	(I)	(II)	(I)	(II)	(I)	(II)
				HUA				
SET		7.47×10^9		1.27×10^8				
RAF								
C2		2.02×10^9						
C4	3.00×10^9	2.04×10^9	4.07×10^5		1.26×10^2			
C5	2.92×10^9	2.04×10^9	1.10×10^6					
C6	3.77×10^7	2.04×10^9						
C8	6.26×10^3	2.04×10^9	1.82×10^{-4}	8.19×10^2				
HT								
N1	4.98×10^2	7.20×10^{-1}		3.32×10^6				
N3	2.20×10^8		2.75×10^5		2.29×10^{-1}		3.39×10^{-1}	
N7	1.43×10^8	3.28×10^5	2.41×10^4	1.01×10^3		4.14×10^{-1}		9.88×10^{-4}
N9	3.06×10^7	$\approx 3.28 \times 10^5$	2.73×10^4	1.70×10^{-2}	5.86×10^1	4.43×10^2	4.00×10^1	1.43×10^3
overall	6.35×10^9	1.76×10^10	1.84×10^6	1.30×10^8	1.85×10^2	4.43×10^2	4.03×10^1	1.43×10^3
				HMUA				
SET		7.79×10^9		1.14×10^8				
RAF								
C2		2.04×10^9						
C4	3.00×10^9	2.04×10^9	6.79×10^5	8.63×10^4	1.00×10^3			
C5	2.95×10^9	2.04×10^9	1.24×10^6					
C6	7.46×10^7	2.04×10^9						
C8	1.43×10^4	2.04×10^9	1.00×10^{-2}	5.93×10^3				
HT								
C1a	3.71×10^8	1.97×10^7	2.40×10^3	1.65×10^2				
N3	5.67×10^8		2.50×10^5		4.58×10^{-1}		7.15×10^{-1}	
N7	3.33×10^8	3.45×10^6	2.02×10^4	5.52×10^4		3.16×10^0		5.91×10^{-3}
N9	6.36×10^7	$\approx 3.45 \times 10^6$	4.05×10^4	1.73×10^{-3}	7.82×10^1	1.82×10^3	2.22×10^{-1}	2.11×10^4
overall	7.92×10^9	1.80×10^{10}	2.23×10^6	1.14×10^8	1.08×10^3	1.83×10^3	9.37×10^{-1}	2.11×10^4

* (I) lipid phase, modeled using benzene; (II) aqueous phase.

caffeine is unable to react with •OOH and •$OOCH_3$ by H transfer.[56] Since peroxyl radicals have a relative low reactivity, this suggests that the less methylated analogs of caffeine are more reactive than the parent compound toward free radicals.

The Gibbs free energies of activation ($\Delta G^{\ddagger}$) of the channels of reaction that were found to be exergonic are reported in Table 1S. Those described above as endergonic are not longer considered in the present work, since even if they take place at a significant rate, they would be reversible and therefore the formed products will not be observed. However, it should be noticed that they might still represent significant channels if their products rapidly react with other species with enough exergonicity and at high enough rates. Since this is expected to be the case for the SET products, the kinetic calculations of the SET channels with low endergonicity have also been performed. In addition we were unable to locate the transition states (TS) corresponding to HT from site N9 in UA^- and MUA^- to the OH radical. However, taking into account the similarity in ΔG for channels N7 and N9 in aqueous solution, together with the similarity in $\Delta G^{\ddagger}$ for channels N7 and N9 in benzene solution, the barriers and the rate constants of HT from N9 in UA^- and MUA^- are expected to be very similar of those corresponding to HT from site N7. Accordingly, we have used them as approximated estimations for site N9, in the particular case of the reaction with •OH in aqueous solution. Even though this can be considered as a rough approximation, it is not expected to affect the main conclusions of the present work since the HT mechanism has only minor contributions to the overall reactivity of the studied compounds toward •OH.

In aqueous solution, where the reactions involve the urate anions, the additions to sites C4, C5, C6 and C8 were found to be barrierless, or close, as well as the SET process from MUA^- to •OH (Table 1S, Supporting Information). Accordingly all of these processes are expected to be diffusion-controlled. In general, the RAF channels have lower barriers in aqueous solution than in nonpolar environments. On the contrary, the barriers of the HT channels increase with the polarity of the surroundings for the reactions with •OH and •OCH_3, while they decrease for the reactions with peroxyl radicals. The optimized geometries of the transition states are provided as Supporting Information (Figures 2S to 17S). The TS structures corresponding to the RAF channel at site C5 for the reactions of UA^- and MUA^- with •OH are not reported because according to the minimum energy potential surface they do not exist (Figures 18S and 19S, Supporting Information), i.e. they are strictly barrierless reactions.

The rate constants for the different channels of reaction, in aqueous and benzene solutions, are reported in Table 2, together with the overall rate coefficients, which have been calculated as the sum of the rate constants of each channel. For example, for the UA^- + $^\bullet OH$ reaction, in aqueous solution:

$$k_{overall} = k_{app}^{SET} + k_{app}^{HT} + k_{app}^{RAF}$$

where:

$$k_{app}^{HT} = k_{app}^{N1} + k_{app}^{N7} + k_{app}^{N9}$$

$$k_{app}^{RAF} = k_{app}^{C2} + k_{app}^{C4} + k_{app}^{C5} + k_{app}^{C6} + k_{app}^{C8}$$

According to our results, both HUA and HMUA are better scavengers in aqueous solution than in nonpolar media, suggesting that the urate anions are the most active species. HUA is predicted to react in aqueous solution about 2.8, 70.8, 2.4, and 35.5 times faster than in nonpolar media, with $^\bullet OH$, $^\bullet OCH_3$, $^\bullet OOH$, and $^\bullet OOCH_3$, respectively (Table 2). For HMUA, these ratios were found to be equal to 2.3, 51.0, 1.7, and 2.2 × 10^4. The reactivity of the urate anions toward the OH radical was found to be diffusion-controlled in both media, supporting their excellent $^\bullet OH$ scavenging activity. The overall rate constants of the reactions with $^\bullet OCH_3$ are lower than those with $^\bullet OH$, but they are still very fast. The rate constants of the reactions with the peroxyl radicals are significantly lower, as expected based on their lower reactivity.

The efficiency of the studied compounds as free radical scavengers relative to that of other compounds has also been analyzed. In nonpolar environments, the peroxyl radical scavenging activity of HMUA was found to be lower than that of carotenes (~10^5–10^6 M^{-1} s^{-1}),[69] canolol (6.8 × 10^5 M^{-1} s^{-1}),[70] and sinapinic acid (1.7 × 10^4 M^{-1} s^{-1}).[71] On the other hand it was found to be higher than that of melatonin (3.1 × 10^2 M^{-1} s^{-1}),[72] caffeine (3.2 × 10^1 M^{-1} s^{-1}),[56] and HUA (1.85 × 10^2 M^{-1} s^{-1}, this work). Moreover, taking into account that the rate constants corresponding to the $^\bullet OOH$ damage to unsaturated fatty acids are in the range 1.18–3.05 × 10^3 M^{-1} s^{-1},[73] HMUA is predicted to moderately protect against peroxyl oxidation of lipids, while the protective effects of HUA are not expected to be significant.

In aqueous solution the peroxyl radical scavenging activity of MUA^- was found to be higher than that of caffeine (3.3 × 10^{-1} M^{-1} s^{-1}),[56] melatonin (2.0 × 10^1 M^{-1} s^{-1}),[72] and UA^- (4.4 × 10^2 M^{-1} s^{-1}, this work); and similar to that of allicin (7.4 × 10^3 M^{-1} s^{-1}).[74] On the contrary, it was found to be lower than that of carotenes (~10^4–10^6 M^{-1} s^{-1}),[69] canolol (2.5 × 10^6 M^{-1} s^{-1}),[70] and sinapinic acid (5.4 × 10^5 M^{-1} s^{-1});[71] and significantly lower than that of 2-propenesulfenic acid (2.6 x10^7 M^{-1} s^{-1})[74] and glutathione (2.7 x10^7 M^{-1} s^{-1}),[75] which are excellent $^\bullet OOH$ scavengers. According to these analyses, it seems that the metabolite of caffeine, HMUA, is a better radical scavenger than its precursor. Moreover, they suggest that the antiradical capacity of caffeine might be explained by the action of its metabolites, rather than by its direct activity.

We have used the reactions with $^\bullet OOH$ for these comparisons due to its relative low reactivity. Using very reactive radicals, such as $^\bullet OH$, might lead one to misconclude that a wide variety of compounds have similar reactivity, since the reactions with this radical are often diffusion-controlled.[76] Therefore we prefer, and recommend, to use a less reactive free radical ($^\bullet OOH$) to assess the relative free radical scavenging capacity of chemical compounds.

Table 3. Branching Ratios (Γ) of the Different Channels of Reaction at 298.15 K.

	$^\bullet OH$		$^\bullet OCH_3$		$^\bullet OOH$		$^\bullet OOCH_3$	
	(I)*	(II)*	(I)	(II)	(I)	(II)	(I)	(II)
				HUA				
SET		42.35		97.44				
RAF								
C2		11.43						
C4	47.20	11.55	22.17		68.16			
C5	46.00	11.55	60.07					
C6	0.59	11.55						
C8	~0.00	11.55	~0.00	~0.00				
HT								
N1	~0.00	~0.00		2.56				
N3	3.47	~0.00	14.96		0.12		0.84	
N7	2.26	~0.00	1.31	~0.00		0.09		~0.00
N9	0.48	~0.00	1.49	~0.00	31.72	99.91	99.16	100.00
				HMUA				
SET		43.18		99.87				
RAF								
C2		11.29						
C4	38.02	11.29	30.42	0.08	92.71			
C5	37.49	11.29	55.54	~0.00				
C6	0.95	11.29						
C8	~0.00	11.29	~0.00	0.01				
HT								
C1a	11.31	0.32	0.11	~0.00				
N3	7.19		11.22		0.04		76.27	
N7	4.23	0.02	0.90	0.05		0.17		0.00
N9	0.81	0.02	1.82	~0.00	7.24	99.83	23.73	100.00

*(*I*) lipid phase, modeled using benzene; (*II*) aqueous phase.

The branching ratios of the different channels of reaction in aqueous and benzene solutions are reported in Table 3. They represent the percent contributions of the different channels to the overall reaction, and have been calculated as

$$\Gamma_i = \frac{k_i}{k_{overall}} \times 100 \qquad (7)$$

For the reactions with $^\bullet OH$, in nonpolar environments RAF is predicted to be the main mechanism of reaction accounting for 93.8% and 76.5% of the overall reactivity of HUA and HMUA, respectively. The products that are expected to be formed to a larger extent are those corresponding to $^\bullet OH$ additions to sites C4 and C5. This would explain the formation of the oxidation intermediates hydroxyisourate (HIU), with OH at site C5, previously reported.[68,77] For HUA, the contributions of the other studied mechanisms are small, lower than 10%. For HMUA, on the other hand, the HT mechanism is of minor importance but with significant contributions to the overall reactivity (~23.5%). In this case, the most reactive sites, through HT, were found to be C1a and N3 in that order. In aqueous solution, the branching ratios are quite different. The SET mechanism becomes very important for the reactions of both

UA^- and MUA^- with $^\bullet OH$, accounting for 42.4% and 43.2% of the overall reactivity, respectively. The RAF mechanism remains as a major one, but becomes significantly less important, compared to what was described for nonpolar environments. Its contributions to the overall reactivity lower to 57.6% and 56.5% for UA^- and MUA^-, respectively. In addition, a wide product distribution of the formed radical adducts is expected.

For the reactions involving $^\bullet OCH_3$, the change on the relative importance of the different mechanism with the polarity of the environment, and therefore with the active species (neutral or anionic), is more dramatic. In nonpolar media RAF is the main mechanism of reaction for both HUA and HMUA, accounting for 82.2% and 86.0% of the overall reactivity; while in aqueous solution the SET mechanism becomes the most important one with contributions of 97.4% and 99.9%. In addition it should be noticed that after SET, the formed radical might deprotonated, i.e., the observable products of reaction might be the same yielded from HT.

For the reactions with the studied peroxyl radicals, the number of reactive sites significantly lowers with respect to the $^\bullet OR$ species. When the reacting free radical is $^\bullet OOH$ in nonpolar environments, the RAF mechanism is the major one, accounting for 68.2% and 92.7% of the overall reactivity of HUA and HMUA, respectively. When HUA is the scavenger, the contributions of HT from site N9 are secondary but very significant (~31.7%). In addition, small but not negligible contributions to HT from site N9 were also found for HMUA (~7.2%). In aqueous solution, the formation of the adducts becomes thermochemically unfeasible, and HT becomes the only viable mechanism, with N9 as the most reactive site. For the reactions involving $^\bullet OOCH_3$, only HT processes are viable, regardless of the polarity of the environment, with the H transfer taking place almost exclusively from site N9 in aqueous solution for both UA^- and MUA^-. For HMUA, in nonpolar environments HT from site N3 is the major channel of reaction. This particular site is not active in aqueous solution since it is deprotonated due to the formation of the urate anions.

CONCLUSIONS

The reactions of HUA and HMUA in nonpolar environments, as well as those of the corresponding urate anions (UA^- and MUA^-) in aqueous solution, with $^\bullet OH$, $^\bullet OCH_3$, $^\bullet OOH$, and $^\bullet OOCH_3$ have been studied. Both HUA and HMUA are better scavengers in aqueous solution than in nonpolar media, which indicates that the urate anions are the most active species.

Different mechanisms of reactions have been taken into account. They are RAF, HT, and SET. For the reactions with $^\bullet OH$, RAF is predicted to be the main mechanism in nonpolar environments, while in aqueous solution SET becomes almost equally important. For the reactions with $^\bullet OCH_3$, RAF is also the major mechanism in nonpolar media, while in aqueous solution its contributions to the overall reactivity of the studied compounds become almost negligible, and SET becomes the main mechanism. For the reactions involving peroxyl radicals, on the other hand, the SET mechanism is not feasible. In particular, for the reactions of $^\bullet OOCH_3$, only HT processes are viable, regardless of the polarity of the environment. Therefore it can be stated that the nature of the reacting free radical and the polarity of the environment both influence the relative importance of the different mechanisms of reaction.

The free radical scavenging activity of HUA and HMUA was found to be different depending on the free radical they are reacting with. It is excellent for $^\bullet OH$, and very good for $^\bullet OCH_3$. Regarding peroxyl radicals, HMUA is predicted to moderately protect against peroxyl oxidation of lipids, while the protective effects of HUA are not expected to be significant.

In addition, HMUA was found to be a better radical scavenger than its precursor, caffeine, suggesting that the antiradical activity of caffeine might be explained by the action of its metabolites, rather than by its direct activity.

ASSOCIATED CONTENT

Supporting Information. Gibbs free energies of activation. Distribution diagrams including netrual, monoanionic, and dianionic species. Fully optimized geometries of the transition states. Minimum energy potential surfaces for the $^\bullet OH$ additions to MUA^- and UA^-, at site C5. This material is available free of charge via the Internet at http://pubs.acs.org.

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