Is Usnic Acid a Promising Radical Scavenger?

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Article

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ABSTRACT: Usnic acid (UA) is a natural product found in the lichen genera. Because of the phenolic groups in its structure, UA is suspected to be an antioxidant. Therefore, in this study, the radical scavenging of UA was investigated in physiological environments in silico by using kinetic calculations. It was found that the overall rate constant for the hydroxyl radical scavenging activity was approximately 10^9 M^−1 s^−1 in all environments, whereas the HOO^• and CH_2OO^• radical scavenging activities were only significant in the polar environments with k in the range of 10^3–10^4 M^−1 s^−1. The results also revealed that the HO^• scavenging activity followed the single electron transfer (SET) and radical adduct formation mechanisms; however, the SET pathway (for the diazonium HU2) played a dominant role in the scavenging of other studied radicals, including CH_3O^•, CCl_3O^•, CCl_3OO^•, NO_2, SO_4^2−, and N_3^−. The activity of UA against these radicals was as high as that of typical phenolic acids such as ferulic acid, p-coumaric acid, caffeic acid, dihydrocaffeic acid, and sinapinic acid (k_1 ~ 10^8 M^−1 s^−1) in polar solvents. Thus, UA is a promising natural antioxidant in aqueous environments.

1. INTRODUCTION

Usnic acid (UA), a natural product that is isolated from lichens, has potential antibacterial effects as well as other biological activities such as anti-inflammatory, anticancer, and antioxidant properties.1–4 The antioxidant activity of UA was assessed by in vivo and in vitro studies.5–8 It was reported that UA could reduce oxidative damage by increasing glutathione peroxidase activity, constitutive nitric oxide synthase, superoxide dismutase activity, and total glutathione activities.6,8,10 UA showed strong antioxidant capacity in oxygen radical absorbance capacity assay, indicating significantly reduced radical oxygen species production;11,12 whereas it showed no antioxidant activity in 2,2-diphenylpicrylhydrazyl assay.13,14 This ambiguous behavior warrants a theoretical investigation of the free-radical scavenging activity of UA including a detailed evaluation of the mechanism of its action to understand its biological role. UA exists in two enantiomers: (+)-UA and (−)-UA, depending on the configuration of the methyl group at the 1’ position (Figure 1). It was shown that the (+) enantiomer was more stable and exhibited higher biological activity in, for example, its antiviral and antibacterial functions than the (−) enantiomer.2,13,16 Thus, in this study, the (+)-UA (Figure 1) structure was used to evaluate the radical scavenging of UA.

This study aims to investigate the radical scavenging activity of UA in aqueous and lipid media by (i) thermodynamic calculations to identify the most likely mechanism of action and (ii) kinetic calculations to evaluate the activity of UA in HO^•, HOO^•, CH_3O^•, CCl_3O^•, HOO^•, CH_2OO^•, CCl_3OO^•, NO, NO_2 (free radical), O_2^•−, SO_4^2−, Br_2^−, and N_3^− radical scavenging reactions.

2. RESULTS AND DISCUSSION

2.1. Thermodynamic Study. 2.1.1. Acid—Base Equilibria. The pK_a values for UA are pK_a1 = 4.4 (O3−H), pK_a2 = 8.8 (O7−H), and pK_a3 = 10.7 (O9−H).17,18 Thus, under physiological conditions (pH 7.40), UA exists in both monovalent anionic state (O3−H bond, HU2, 96.1%), and dianionic state (O3−H and O7−H bonds, HU^−, 3.9%), and therefore, the H_2U^− and HU^− forms were used to evaluate the radical scavenging of UA in aqueous solution. In the lipid environment, the neutral UA (H_3U) was modeled.

In this study, the radical scavenging of UA was evaluated following three main antioxidant pathways: single electron transfer (SET), formal hydrogen transfer (FHT), and radical adduct formation (RAF) in the studied solvents according to the following reactions:19

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Figure 1. Structure of UA.
The nuclear reorganization energy \( \lambda \) can be calculated using the relationship \( \lambda = f \times k_{app} \). For example, for \( \lambda(H_2UA^2- \rightarrow H_2UA^-) = 0.961 \), \( \lambda(H_2UA^2-) = 0.039 \).

In the aqueous solution
\[ \begin{align*}
    &H_2U^- + R^* \rightarrow H_2U^* + R^- \quad \text{(SET-2)} \\
    &H_2U^- + R^* \rightarrow H_2U^* + RH \quad \text{(FHT)} \\
    &H_2U^- + R^* \rightarrow [H_2U - R^*] \quad \text{(RAF)}
\end{align*} \]

Also, for the divalent anionic form:
\[ \begin{align*}
    &H_2U^2- + R^* \rightarrow H_2U^+ + R^- \quad \text{(SET-2)} \\
    &H_2U^2+ + R^* \rightarrow H_2U^* + RH \quad \text{(FHT)} \\
    &H_2U^2+ + R^* \rightarrow [H_2U - R^*] \quad \text{(RAF)}
\end{align*} \]

where \( R^* = HO^*, HOO^* \). To model FHT, the direct hydrogen atom transfer pathway was calculated.

### 2.1.2. Mechanism Valuation

The \( \Delta G^0 \) values of the UA + HO\(^*\)/HOO\(^*\) reactions were used for evaluating the preferred antioxidant mechanism. \( \Delta G^0 \) values were calculated following each mechanism, and the results are presented in Table 1. It was found that the hydroxyl radical scavenging of UA is almost always spontaneous (\( \Delta G^0 < 0 \)) in the studied media, with the exception of the SET and RAF mechanisms at the C9' position. However, the reaction of UA with HOO\(^*\) radical was only spontaneous for the SET-2 mechanism. This suggests that the HOO\(^*\) radical scavenging in the aqueous solution only occurs following the SET-2 mechanism. Thus, the overall rate constants can be calculated according to eqs 8–11.

In the aqueous solution
\[ k_{overall}(\text{OH}) = 0.039 \times k_{\text{SET-2}} + 0.961 \left( k_{\text{SET-1}} + \sum k_{\text{FHT}} + \sum k_{\text{RAF}} \right) \quad \text{(8)} \]

In the nonpolar solvent
\[ k_{overall}(\text{OOH}) = 0.039 \times k_{\text{SET-2}} \quad \text{(9)} \]

### Table 2. Gibbs Free Energies of Activation (\( \Delta G^0 \), kcal/mol), Tunneling Corrections (\( \kappa \)), Rate Constants (\( k_{app}, \text{M}^{-1} \text{s}^{-1} \)), and Branching Ratios (\( \Gamma \), %) at 298.15 K for the UA Oxidation by HO\(^*\) and HOO\(^*\) Radicals in the Studied Environments

| Mechanism | Water        | Pentyl Ethanoate |
|-----------|--------------|------------------|
| HAT       | \( \Delta G^0 \) | \( k \) | \( k_{app} \) | \( \Gamma \) | \( \Delta G^0 \) | \( k \) | \( k_{app} \) | \( \Gamma \) |
| OH\(^*\)   | 7.7          | 6.9             | 1.50 \times 10^7 | 1.44 \times 10^7 | 0.2           | 17.7      | 18.7        | 1.21 \times 10^4 | 0.0 |
| Set-2     | 3.9          | 5.7             | 4.10 \times 10^8 | 1.60 \times 10^8 | 1.7           | 3.9       | 16.5        | 1.49 \times 10^4 | 0.0 |
| C10       | 15.3         | 25.8            | 9.63 \times 10^5 | 9.26 \times 10^5 | 0.0           | 17.6      | 19.6        | 1.49 \times 10^4 | 0.0 |
| C9'       | 5.7          | 5.2             | 1.93 \times 10^4 | 1.86 \times 10^4 | 20.2          | 7.1       | 3.5         | 3.50 \times 10^8 | 15.8 |
| C12       | 7.2          | 5.9             | 1.00 \times 10^4 | 9.65 \times 10^4 | 10.5          | 9.4       | 5.4         | 1.40 \times 10^7 | 0.6 |
| C14       | 8.3          | 8.5             | 3.15 \times 10^4 | 3.03 \times 10^4 | 3.3           | 10.1      | 4.5         | 3.20 \times 10^6 | 0.1 |
| C15       | 8.6          | 12              | 1.46 \times 10^7 | 1.40 \times 10^7 | 0.2           | 10.4      | 1.3         | 3.80 \times 10^3 | 0.0 |
| C3        | 9.6          | 1.0             | 2.20 \times 10^8 | 2.11 \times 10^8 | 23            | 6.6       | 1.1         | 1.80 \times 10^4 | 8.1 |
| C4'       | 6.6          | 1.1             | 3.22 \times 10^8 | 3.10 \times 10^8 | 3.4           | 7.1       | 0.6         | 4.50 \times 10^7 | 2.0 |
| C6        | 7.3          | 1.2             | 1.25 \times 10^8 | 1.20 \times 10^8 | 1.3           | 8.5       | 0.8         | 5.78 \times 10^6 | 0.3 |
| C7        | 9.7          | 1.0             | 9.80 \times 10^7 | 9.41 \times 10^7 | 0.0           | 8.2       | 1.1         | 1.40 \times 10^7 | 0.6 |
| C8        | 5.9          | 1.2             | 8.89 \times 10^7 | 8.54 \times 10^7 | 9.3           | 9.3       | 1.2         | 2.10 \times 10^6 | 0.1 |
| C9        | 8.3          | 1.1             | 3.47 \times 10^7 | 3.33 \times 10^7 | 0.4           | 10.1      | 1.3         | 6.32 \times 10^3 | 0.0 |
| OOH\(^*\) | 9.5          | 17.2            | 7.20 \times 10^3 | 2.81 \times 10^4 | 100           | 9.18 \times 10^9 | 2.22 \times 10^9 | 0.0 |
Melatonin, dopamine, indolinalonic hydroxylamine, ramosom, and Trolox in all of the studied nonpolar solvent. The hydroxyl radical scavenging of UA is somewhat lower than that of typical antioxidants such as melatonin, dopamine, indolinalonic hydroxylamine, ramal, indole-3-carbinol, and Trolox in all of the studied media. Thus, UA is a moderate hydroxyl radical scavenger.

It is important to note that the SET-2 mechanism played a deciding role in the HOO• radical scavenging of UA with \( k_{\text{app}} = 7.20 \times 10^5 \text{ M}^{-1} \text{ s}^{-1} \) (\( k_f = 2.81 \times 10^4 \text{ M}^{-1} \text{ s}^{-1} \)) in the polar solvent; however, the mechanism did not occur with UA in lipid media. Based on these results, UA has a similar activity to Trolox (\( k = 8.96 \times 10^4 \text{ M}^{-1} \text{ s}^{-1} \)) in the HOO• scavenging reaction in polar environments.

### Table 3: Calculated Kinetic Parameters of the Reaction between HU\(^{2-}\) and Selected Radicals Following the SET-2 Mechanism in Aqueous Solution at pH 7.4

| radical | \( \Delta G^\circ \) | \( \lambda \) | \( k_0 \) | \( k_{\text{app}} \) | \( k_i \) |
|---------|------------------|---------|-------|---------|-------|
| HO•     | 3.9              | 5.7     | \( 8.40 \times 10^9 \) | \( 4.10 \times 10^9 \) | \( 1.60 \times 10^8 \) |
| CH\(_3\)O• | 2.8             | 7.7     | \( 8.10 \times 10^9 \) | \( 7.10 \times 10^9 \) | \( 2.77 \times 10^8 \) |
| CCl\(_3\)O• | 0.2             | 54.8    | \( 7.60 \times 10^9 \) | \( 7.60 \times 10^9 \) | \( 2.96 \times 10^8 \) |
| HOO•    | 9.5              | 17.2    | \( 8.20 \times 10^9 \) | \( 7.20 \times 10^9 \) | \( 2.81 \times 10^8 \) |
| CH\(_2\)OO• | 11.0          | 16.6    | \( 8.00 \times 10^9 \) | \( 5.80 \times 10^4 \) | \( 2.26 \times 10^3 \) |
| CCl\(_3\)OO• | 0.5            | 18.3    | \( 6.80 \times 10^9 \) | \( 6.80 \times 10^9 \) | \( 2.65 \times 10^8 \) |
| NO      | 96.4             | 16.2    | \( 8.40 \times 10^9 \) | \( 1.30 \times 10^{-36} \) | \( 5.07 \times 10^{-40} \) |
| NO\(_2\) | 2.0              | 30.0    | \( 8.20 \times 10^9 \) | \( 7.80 \times 10^9 \) | \( 3.04 \times 10^8 \) |
| O\(_2\)• | 55.3             | 19.1    | \( 8.60 \times 10^9 \) | \( 1.80 \times 10^{-28} \) | \( 7.02 \times 10^{-30} \) |
| SO\(_4\)• | 3.0              | 21.0    | \( 7.80 \times 10^9 \) | \( 6.50 \times 10^9 \) | \( 2.54 \times 10^8 \) |
| N\(_2\)• | 2.7              | 4.9     | \( 8.10 \times 10^9 \) | \( 7.10 \times 10^9 \) | \( 2.77 \times 10^8 \) |

\( k_i = f \times k_{\text{app}}(\text{H}_2\text{UA}^{2-}) = 0.039 \).
caffeic acid, dihydrocaffeic acid,20 and sinapinic acid,27 the predicted activities for UA against CH3O•, CCl3OO•, CCl3OO•, NO2•, SO4•−, and N4• radicals were as high as those of these compounds (k ≈ 10^8 M^−1 s^−1). Thus, UA is a promising natural antioxidant in aqueous solution.

3. CONCLUSIONS

The radical scavenging of UA was investigated in aqueous and lipid (pentyl ethanoate) media. It was found that UA had moderate HO2• and CH3OO• radical scavenging activities in aqueous solution (k ≈ 10^8−10^9 M^−1 s^−1), whereas activity in lipid media was generally low. The overall rate constants for the hydroxyl radical scavenging were k_{overall} = 9.18 × 10^9 and 2.22 × 10^10 M^−1 s^−1 in polar and nonpolar solvents, respectively. The HO• antiradical activity mainly followed the SET and RAF mechanisms; however, the SET-2 pathway played a deciding role in the radical scavenging activity of the other studied radicals. UA exhibited activities against CH3O•, CCl3OO•, CCl3OO•, NO2•, SO4•−, and N4• radicals, which were as high as those of typical phenolic acids such as ferulic acid, p-coumaryl acid, caffeic acid, dihydrocaffeic acid, and sinapinic acid (k ≈ 10^8 M^−1 s^−1) in polar solvents. Thus, UA is a promising radical scavenger in aqueous solution.

4. COMPUTATIONAL METHODS

In this study, the quantum mechanics-based test for the overall free-radical scavenging activity protocol was applied to perform the kinetic calculations in water and pentyl ethanoate environments by using the M05-2X/6-311++G(d,p) method with the solvation model based on density that has been widely used for evaluating the radical scavenging activity of antioxidants because of low errors compared with experimental data (k_{calc}/k_{exp} ratio = 1−2.9).23,26,28−51 Rate constants were computed according to the TS theory (at 298.15 K, 1 M standard state) following the equation

\[
k = \frac{k_{B}T}{\hbar} e^{-(\Delta G^\ddagger)/RT}
\]

where σ is the reaction symmetry number,38,39 κ contains the tunneling corrections calculated using the Eckart barrier,40 k_B is the Boltzmann constant, h is the Planck constant, and ΔG^\ddagger is the Gibbs free energy of activation.

The Marcus reactivity barriers were corrected following the Marcus theory,6,42 whereas the apparent rate constants (k_{app}) in solvents were computed following the literature.6,44 Okuno’s corrections61 of the free volume theory following the Benson correction were also applied to minimize overpenalizing entropy in solution.62 All of the studied species (molecules, radicals, anions, and TSs) were treated by the hindered internal rotation method to obtain the lowest energy conformers that were used in the further studies.47 Intrinsic coordinate calculations were carried out to confirm that the TSs connect the reactants and products. All of the calculations were performed by using the Gaussian 09 program package,68 and the Eyringpy program49,50 depending on each individual task.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02306.

Cartesian coordinates of TSs in all of the studied environments (PDF)

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Notes

The authors declare no competing financial interest.

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