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ANTIOXIDANT ACTIVITY OF ASCORBIC ACID ANALOGS CONTAINING A NITROGEN ATOM IN THE RING

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Abstract – Ascorbic acid ($AscH_2$) is a powerful antioxidant and protects the human body from oxidative stresses by scavenging reactive oxygen species. $AscH_2$ analogs containing a carbonyl conjugated ene-diol structure and endocyclic nitrogen atom were evaluated for their oxidation potentials by cyclic and differential pulse voltammetry. The oxidation potentials of $AscH_2$ analogs in which the endocyclic oxygen atom of $AscH_2$ was replaced by a single nitrogen atom were lower than those of $AscH_2$ under both non-aqueous and aqueous conditions. The results demonstrate that these analogs can have powerful antioxidant activities under physiological conditions.

INTRODUCTION

Reactive oxygen species (ROS) serve as signaling molecules to regulate biological and physiological processes;[1] however, ROS can react with DNA, proteins, and other cellular components to become problematic.[2] ROS are strongly implicated in the pathophysiology of diseases, such as cancer, neurodegenerative, and cardiovascular disease, as well as in the degenerative process associated with aging.[3-6] The cells withstand and counteract ROS occurrence by the use of several different defense mechanisms ranging from radical scavengers, such as ascorbic acid ($AscH_2$), α-tocopherol, and glutathione, to antioxidant enzymes, such as catalase, superoxide dismutase, and various peroxidases.[7] Thus, antioxidant compounds and antioxidant enzymes have been extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress.[8, 9]

Antioxidant compounds can act as reducing agents and, in solutions, they tend to be easily oxidized on inert electrodes. The relationship between the electrochemical behavior of compounds and their antioxidant activity is very interesting because low redox potentials correspond to high antioxidant activity.[10] $AscH_2$ is known for its powerful reducing properties, being easily oxidized to dehydroascorbic acid.[11] Thus, many derivatives of $AscH_2$ have been developed to improve stability and lipophilicity.[12-14]

In our previous paper, we reported the synthesis and radical scavenging activity against the galvinoxyl radical of $AscH_2$ analogs **1–6** (Figure 1) containing a carbonyl conjugated ene-diol structure.[15] In this study, the redox potential of $AscH_2$ analogs in methanol and phosphate buffer (pH 7.4) was evaluated to understand the chemical properties of the $AscH_2$ analogs with a nitrogen atom in the ring, for antioxidant activity.

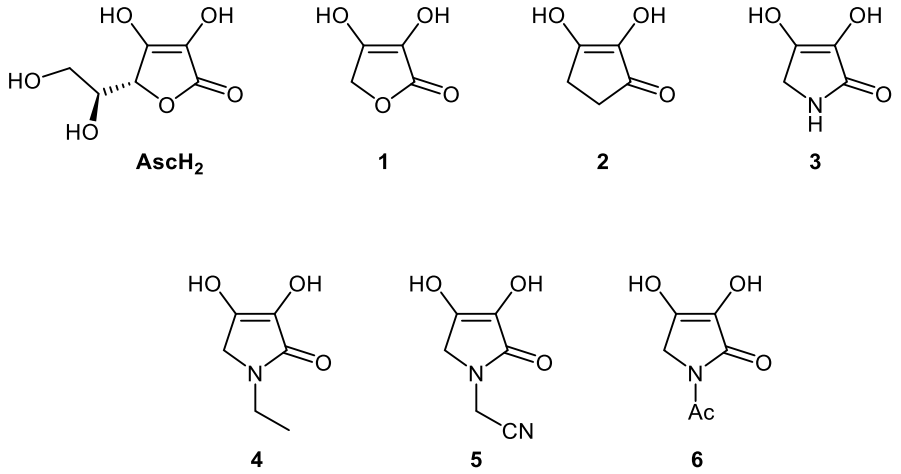


Figure 1. Structures of $AscH_2$ analogs

RESULTS AND DISCUSSION

Synthesis of $AscH_2$ analogs

$AscH_2$ analogs were prepared by the method shown in our previous paper.[15]

Redox potential of $AscH_2$ analogs

To determine the antioxidant activity of $AscH_2$ analogs with a nitrogen atom in the ring, the redox potentials of the compounds were determined by cyclic voltammetry in deaerated methanol and phosphate buffer (pH 7.4). Because polar antioxidants are known to be more effective in less polar media, the chemistry of $AscH_2$ and $AscH_2$ analogs in both non-aqueous and aqueous media was vital for understanding their antioxidant activities.[16] The cyclic voltammograms of $AscH_2$ and its analogs show only an anodic oxidation peak (Figure 2).[17] The oxidation of $AscH_2$ analogs at the electrode was irreversible; thus, we evaluated the oxidation potentials by differential pulse voltammetry (Figure 3). The differential pulse width method can decrease the effect of charging current interference;[18] therefore, it is far more sensitive than cyclic voltammetry for examining the electrochemical behavior of reactant molecules that bind to the electrode surface or irreversibly react with the electrode.[19, 20] The measured oxidation potentials of all compounds are summarized in Table 1.

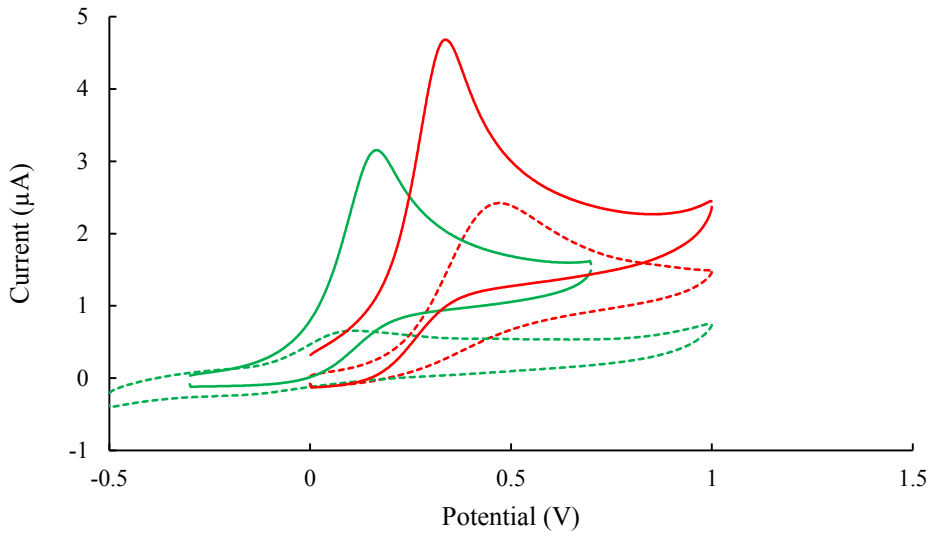
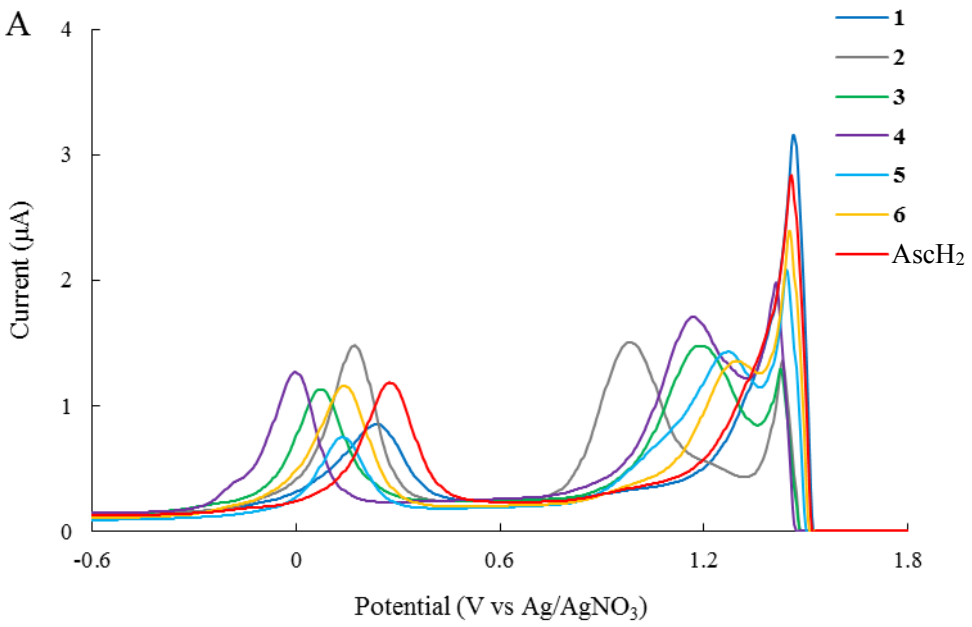


Figure 2. Representative set of cyclic voltammograms obtained for 1 mM $AscH_2$ (red line) and compound **3** (green line) in deaerated methanol at 25 °C vs $Ag/AgNO_3$ as well as 1 mM $AscH_2$ (red hash) and compound **3** (green hash) in deaerated phosphate buffer (pH 7.4) at 25 °C vs Ag/AgCl



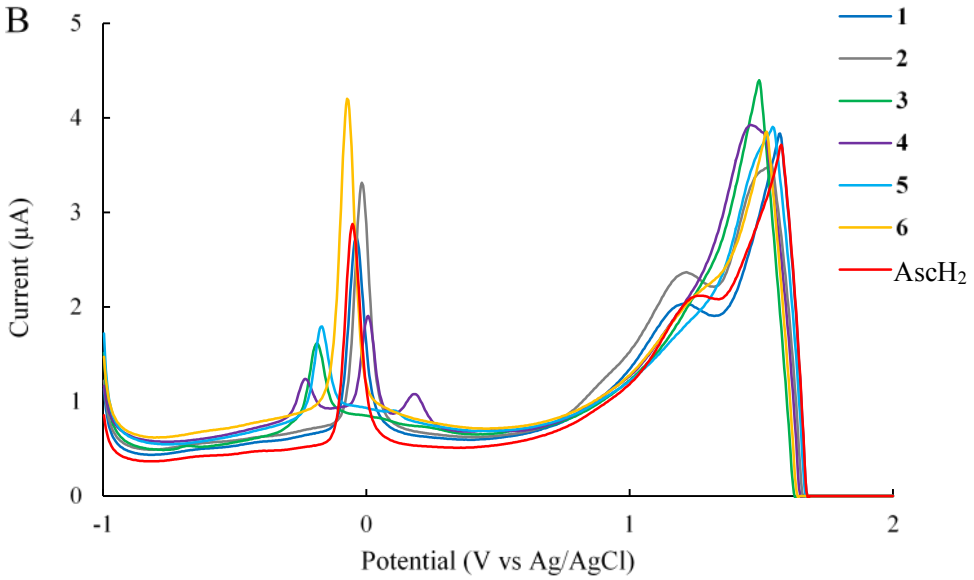


Figure 3. Differential pulse voltammograms obtained for 1 mM $AscH_2$ and its analogs in (A) deaerated methanol at 25 °C vs $Ag/AgNO_3$ and (B) deaerated phosphate buffer (pH 7.4) at 25 °C vs Ag/AgCl

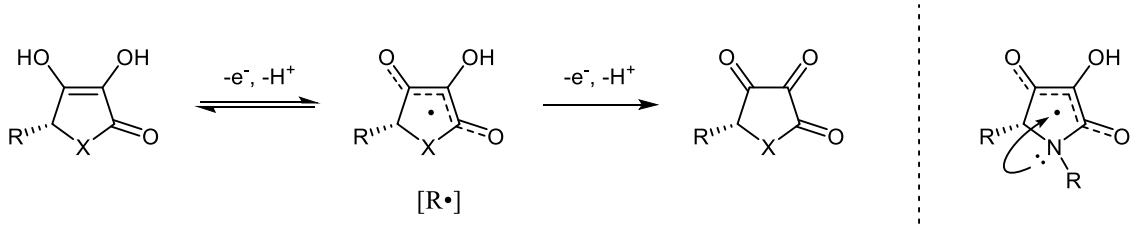
Table 1. Oxidation potential and galvinoxyl radical scavenging activity of $AscH_2$ analogs

Compound	Oxidation potential				Galvinoxyl radical scavenging rate k_i[d, e 15]
	In methanol			In phosphate buffer (pH 7.4)	
	E_{pa1}[a]	E_{pa2}[a]	ΔE[a, b]	E_{pa1}[c]	
1	0.24	–	–	−0.04	20
2	0.17	0.62	0.57	−0.02	23
3	0.07	1.30	1.23	−0.19	618
4	0.00	1.27	1.13	−0.23	n.d.
5	0.14	1.20	1.06	−0.17	815
6	0.14	1.17	1.09	−0.07	240
$AscH_2$	0.28	–	–	−0.05	18

[a] $E_{pa1,2}$, ΔE; V vs $Ag/AgNO_3$, [b] ΔE; the distance between two oxidation potentials, [c] E_{pa1}; V vs Ag/AgCl, [d] k_i; $M^{-3/2}s^{-3/2}$, [e] galvinoxyl radical scavenging rate were evaluated in methanol, n.d.; not detected.

The oxidation potentials in methanol decrease in the following order: $AscH_2 \geq$ **1** > **2** > **6** = **5** > **3** > **4** (Figure 3A, Table 1). The first oxidation potentials (E_{pa1}) represent the one electron transfers with the formation of intermediate radicals. $AscH_2$ and **1** showed only one oxidation potential wave; however, the other $AscH_2$ analogs **2**–**6** showed two oxidation waves. The observation of two oxidation waves for **2**–**6** indicate that their intermediate radicals (Scheme 1) are more stabilized than that of $AscH_2$ and **1**. The

separation between the two oxidation potentials (ΔE) of nitrogen-containing $AscH_2$ analogs **3**–**6** are much larger than that for **2** (Table 1). These results indicate the extent of stabilization of the nitrogen-containing $AscH_2$ analog radicals results from overlap between the lone pair of the endocyclic nitrogen atom and the π-electron system of the carbonyl and conjugated ene-diol groups (Scheme 1).[21,22] Among the nitrogen-containing $AscH_2$ analogs, *N*-ethylated **4** had the lowest oxidation potential, resulting from the electron-donating inductive effect. In contrast, **5** and **6** substituted with electron-withdrawing groups show higher oxidation potentials than **3**. These results indicate that a nitrogen atom with a more electron-donating resonance effect than an oxygen atom better stabilizes the electron-deficient intermediate radical [R•] of $AscH_2$ analogs, weakening the O–H bond and accelerating the oxidation reaction (Scheme 1).[23]



Scheme 1. Mechanism of the oxidation reaction in methanol

We previously reported that the galvinoxyl radical scavenging activity of $AscH_2$ analogs increase in the following order: $AscH_2$ ≤ **1** ≤ **2** << **6** < **3** < **5** (Table 1).[15] The lower oxidation potential of $AscH_2$ analogs correlates with the galvinoxyl radical scavenging activity (Figure 4). This correlation is in accordance with the relationship of low redox potential corresponding to high antioxidant activity.[10]

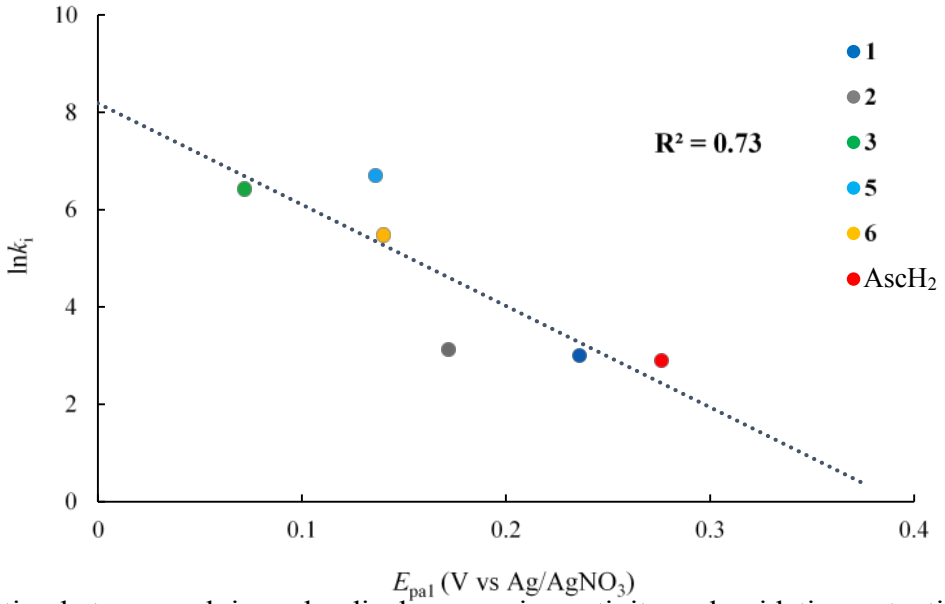
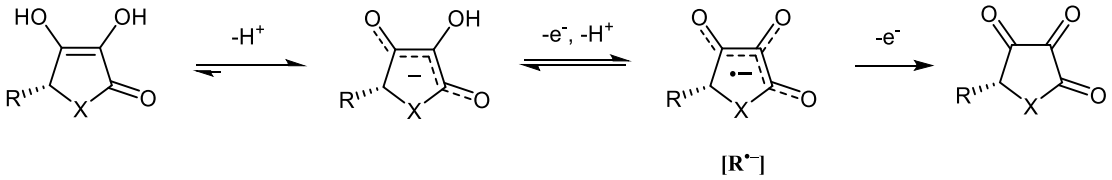


Figure 4. Correlation between galvinoxyl radical scavenging activity and oxidation potential in methanol

The oxidation potential in phosphate buffer (pH 7.4) decreased in the following order: **2** > **1** ≥ $AscH_2$ > **6** > **5** ≥ **3** > **4** (Figure 3B, Table 1). The oxidation potentials of $AscH_2$ and its analogs under neutral conditions were lower than those in methanol. The ionized form of $AscH_2$ and its analogs have more powerful antioxidant activity than those in the protonated form.[23] Under neutral conditions, oxidation proceeds via intermediate $AscH_2$ analog radical anions [$R^{\bullet-}$] (Scheme 2).[24] The first oxidation potentials (E_{pa1}) show the one electron transfers with the formation of intermediate radical anions.



Scheme 2. Mechanism of the oxidation reaction in an aqueous phase, pH 7.4[24]

The oxidation potential of **1** was slightly higher than that of $AscH_2$. Therefore, the side chain of $AscH_2$ is not necessary for the oxidation reaction. **2** has a higher oxidation potential than **1**. An oxygen atom, with a more electron-withdrawing inductive effect than a carbon atom, better stabilizes the radical anion of **1** than that of **2**; however, **3**–**6**, with more electron-donating resonance effects from the nitrogen atom, have lower oxidation potentials than **1**. Through the electron-donating resonance effect, the lone pair of the nitrogen atom in the ring destabilizes the anions of **3**–**6** more than the anion of **1**. Thus, the nitrogen $AscH_2$ analogs **3**–**6** have a lower oxidation potential than **1** in neutral aqueous solutions. The oxidation potential of **6** was the highest among these compounds. The electron-withdrawing resonance effect of the acetyl group stabilized the anion of **6**. These results indicate that the lone pair of an endocyclic nitrogen atom is important for promotion of the oxidation reaction of $AscH_2$ analogs under neutral conditions. Compared with **3**, **4** has a lower oxidation potential and **5** has a higher oxidation potential. The electron-donating ethyl group in **4** increased the electron density of the endocyclic nitrogen atom and destabilized the anion of **4**, relative to **3**. Conversely, the electron-withdrawing cyanomethyl group in **5** stabilized its anion and increased its oxidation potential.

CONCLUSION

We have previously reported the rate constant of the reaction between $AscH_2$ analogs and galvinoxyl radicals,[15] and the radical scavenging activity and oxidation potential in methanol was found to have a correlation. This result indicated that the oxidation reaction of $AscH_2$ analogs proceeds by hydrogen atom transfer via their $AscH_2$ analog radicals. The stabilization of the $AscH_2$ analog radical was the key factor

for decreasing their oxidation potential in this mechanism. In addition, the oxidation potential of the nitrogen $AscH_2$ analogs, under neutral conditions, are lower than any other $AscH_2$ analogs. The nitrogen $AscH_2$ analogs have a low oxidation potential in both non-aqueous and aqueous solutions. The synthesized nitrogen $AscH_2$ analogs should have powerful antioxidant activities under physiological conditions.

EXPERIMENTAL

Reagents

MeOH was obtained from Dojindo (Kumamoto, Japan). Tetrabutylammonium perchlorate was obtained Tokyo Chemical Industry (Tokyo, Japan). Another reagents and solvents were purchased from Wako pure Chemical Industries (Tokyo, Japan).

Synthesis

$AscH_2$ analogs **1**–**6** were prepared as previously reported.[15]

Electrochemistry

The cyclic and differential pulse voltammetry was measured using a potentiostat with a glassy carbon working electrode, a platinum counter electrode, and a $Ag/AgNO_3$ reference electrode in MeOH, or a Ag/AgCl reference electrode in 0.1 M phosphate buffer. The glassy carbon electrode (1 mm diameter) was carefully polished with alumina powder (0.3 μm) on a polishing cloth before each measurement. Voltammograms were obtained in deaerated MeOH using tetrabutylammonium perchlorate (100 mM) as an electrolyte and phosphate buffer (pH 7.4), at 25 °C. For compounds displaying reversible and irreversible redox chemistry, cyclic voltammograms were obtained using a scan rate of 100 mV/s and differential pulse voltammograms were obtained using a pulse amplitude of 50 mV, a pulse width of 60 mV, a pulse period of 0.02 s, and a scan rate of 20 mV/s.[25]

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