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Antioxidant Activity and Xanthine Oxidase Inhibition Activity of Reductic Acid: Ascorbic Acid Analogue

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Abstract—Reductic acid (2,3-dihydroxy-2-cyclopentenone, **1**) decreased the ESR signal of 5,5-dimethyl-1-pyrroline 1-oxide (DMPO)-OH produced by hydroxyl radical and DMPO. **1** also inhibited lipid peroxidation initiated by cytochrome P450 and *tert*-butyl hydroperoxide. **1** inhibited xanthine oxidase activity, while ascorbic acid and 2-hydroxytetronic acid, an ascorbic acid analogue without side chain, did not. © 2000 Elsevier Science Ltd. All rights reserved.

Recently, a number of studies have reported the important role of active oxygen species in developing and/or progression of more than 50 human diseases.[1] Active oxygen species are formed continuously in the human body.[2] They are removed by enzymatic and non-enzymatic antioxidative defense systems under normal conditions.[2] One of the most important sources of active oxygen species is xanthine oxidase. This enzyme is suspected to play an important role in oxidative stress in living organisms.[3] Xanthine oxidase is a highly versatile enzyme that is widely distributed among species (from bacteria to humans).[3] Oxidative stress, occurring when antioxidant systems are inadequate and/or active oxygen species are overproduced, can damage lipids, proteins, and nucleic acids.[2]

Xanthine oxidase produces uric acid, which plays a crucial role in gout. Therefore, xanthine oxidase inhibitor works as a medicine for gout.

Ascorbic acid (**2**) is a natural, very effective, low-molecular-weight free-radical quencher.[4] It has often served as a starting structure for the design and synthesis of pharmacologically effective antioxidants.[5] It has a unique 2,3-enediol moiety, which is conjugated with the carbonyl group in a five-membered lactone ring (Fig. 1). Its anion form has a strong electron-donating ability and is one of the most effective active oxygen quenchers. Many *O*-acyl, *O*-alkyl, and imine analogues of **2** have been synthesized and studied for their antioxidative activity.[5] However, few papers have reported the effect of lactone ring modification on the activity of ascorbic acid.

Reductic acid (2,3-dihydroxy-2-cyclopentenone, **1**) also has a 2,3-enediol moiety conjugated with a carbonyl group in a five-membered carbon ring (Fig. 1).[6] Like ascorbic acid, **1** exhibits reducing activity, for example, in the reduction of aldehyde and iodine, but its antioxidant activity has not been reported.

In this report, we studied hydroxyl radical quenching activity, effects on lipid peroxidation, and xanthine oxidase inhibition activity of **1**.

Synthesis of 1 and 3

1 was synthesized from cyclopentanone by the method of Hesse.[6] 2-Hydroxytetronic acid (3-hydroxyfurane-2,4 (3*H*,5*H*)-dione, **3**), analogue of **2** without side chain (Fig. 1), was prepared by the method of Ramage.[7] DMPO was obtained from Dojin Chemical Co. Xanthine oxidase (from cow milk) from Boehringer Mannheim.

Hydroxyl Radical Quenching Activity

Produced by the Fenton reaction,[8] the hydroxyl radical was detected by the ESR spin-trapping method. The hydroxyl radical reacts with 5,5-dimethyl 1-pyrroline-1-

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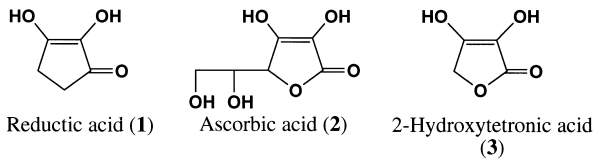

Figure 1. Structures of ascorbic acid and its analogues.

oxide (DMPO) to give DMPO-OH, which is ESR detectable.[9] Reaction mixtures contained ferrous sulfate (0.5 mM), H_2O_2 (0.5 mM), DMPO (10 μL, 0.4 M), and the indicated amount of sample in 0.21 mL of 0.1 M sodium phosphate buffer pH 7.4. The reaction was initiated by the addition of H_2O_2. ESR spectrum was taken 2 min after the addition of H_2O_2.

The addition of **1**, **2** or **3** decreased the signal intensity of DMPO-OH in a dose-dependent manner (Fig. 2A). The effects of **2** and **3** were the same but **1** was less effective.

Inhibition of Lipid Peroxidation

1–**3** were evaluated for the inhibition of lipid peroxidation in rat liver microsomes, initiated by cytochrome P450 and *tert*-butyl hydroperoxide.[10] Rat liver microsomes were prepared from a phenobarbital-treated Wistar rat as previously described.[11] Incubation mixtures contained microsomes (0.8 mg protein), *tert*-butyl hydroperoxide (1.0 mM), and the indicated amount of sample in 1.0 mL of 0.1 M sodium phosphate buffer pH 7.4. The reaction was initiated by the addition of *tert*-butyl hydroperoxide and was continued for 5 min at 37 °C. The incubation was terminated by the addition of 10 mM 2,6-di-*tert*-butyl-4-methylphenol. The solution was then mixed with 2.0 mL of 15% trichloroacetic acid and 0.375% thiobarbituric acid in 0.25 M HCl. After incubation at 90 °C for 15 min, the precipitate was removed by centrifugation, and the absorbance at 535 nm was recorded.[12]

Ascorbic acid and its analogues effectively inhibited the lipid peroxidation in a dose-dependent manner. Different from hydroxyl radical quenching activity, almost no differences were observed between the three samples (Fig. 2B). **1** may be easier to penetrate into the lipid layer than **2** and **3** because **1** is more lipophilic than the others. This is the reason for the same inhibition activity in lipid peroxidation but **1** was less effective in the homogeneous system; hydroxyl radical quenching.

Xanthine Oxidase Inhibition Activity

Xanthine oxidase catalyzes xanthine oxidation to produce uric acid. At the same time, dioxygen is converted to superoxide and hydrogen peroxide.[3] Usually, xanthine oxidase activity is measured in terms of uric acid formation, monitored by absorbance change at 292 nm. Since absorption of **1** interferes with the measurement of absorbance at 292 nm, the enzyme activity was measured by dioxygen consumption, which was monitored polarographically with a Clark-type electrode at 37 °C. The reaction solution contained 0.1 mM EDTA and 50 μM xanthine in 50 mM potassium phosphate pH 7.8.

The addition of xanthine oxidase caused dioxygen consumption in the presence of xanthine (initial consumption rate was 36.1 μM/min under our conditions). This consumption rate slowed and finally the consumption stopped at about 5 min after the addition of the enzyme. At this point, all xanthine was converted to uric acid. The consumption was re-started by the addition of another xanthine solution (Fig. 3, solid line). In the presence of 50 μM **1**, the initial dioxygen consumption rate (34.7 μM/min) was almost the same as the rate in the absence of **1**,

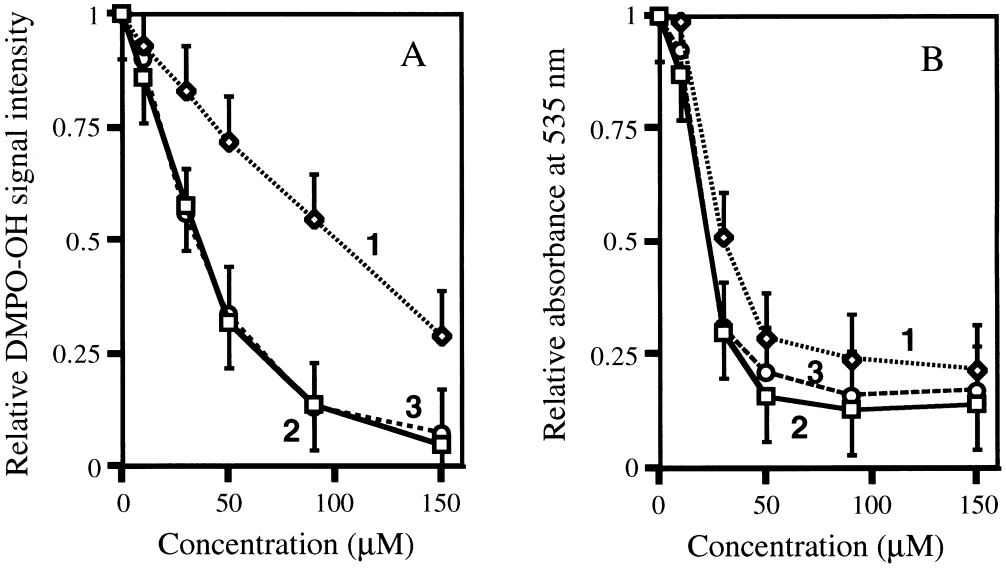

Figure 2. Hydroxyl radical quenching activity (A) and inhibition of lipid peroxidation (B).

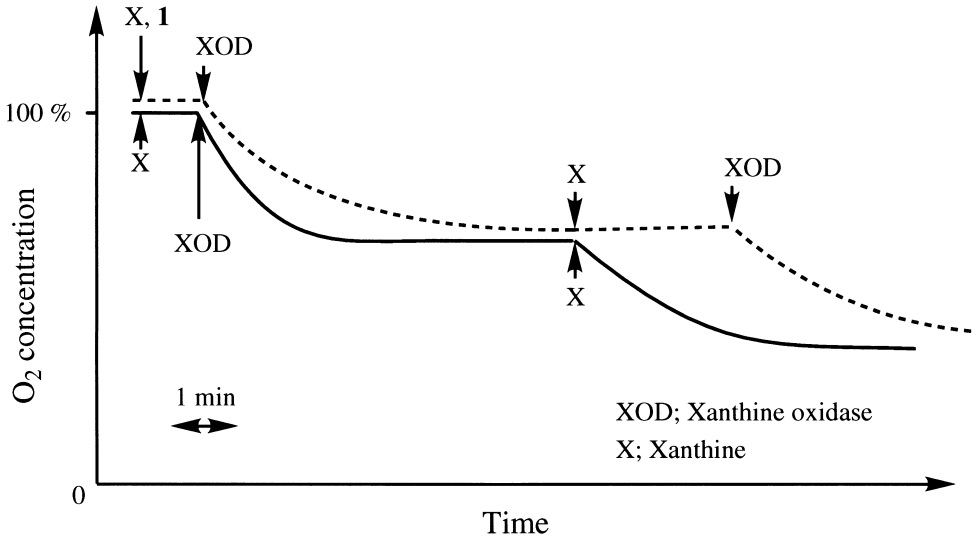

Figure 3. Effect of **1** on xanthine oxidase activity.

but the reaction was gradually inhibited with time. After stopping the reaction, the addition of another xanthine solution did not cause dioxygen consumption. This result indicated that xanthine oxidase was inhibited completely by **1** at that time. Subsequently, another xanthine oxidase addition caused the consumption (Fig. 3, dashed line). These data show that **1** is a time-dependent inhibitor of xanthine oxidase. **2** and **3** did not inhibit xanthine oxidase activity under the same conditions (data not shown). These results showed that the lactone ring interrupted xanthine oxidase inactivation.

There are two possible explanations for the time dependency of inhibition. First, xanthine oxidase or superoxide oxidizes **1**, and the oxidation product inhibits xanthine oxidase. Second, **1** is a suicide inhibitor: **1** binds to xanthine oxidase during their reaction. Further studies for the xanthine oxidase inhibition mechanism have been undertaken.

In conclusion, reductic acid (**1**), an ascorbic acid analogue, exhibited antioxidant activities in hydroxyl radical quenching and in lipid peroxidation inhibition. **1** was an inhibitor of xanthine oxidase, whereas ascorbic acid (**2**) and 2-hydroxytetronic acid (**3**) were not. **1** decreases active oxygen production and quenches free radicals. **1** is thought to be a more potent inhibitor of active oxygen toxicity than **2**. It has been reported that flavonoids inhibit xanthine oxidase and have active oxygen quenching activities.[13] Flavonoids are thought to be a promising inhibitor for oxidative stress and remedy for gout. Therefore, **1** serves as a new starting structure for the design of pharmacologically effective compounds.

Acknowledgements

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References

1. Halliwell, B. *Drugs* **1991**, *42*, 569.
2. Yu, B. P. *Biol. Rev*. **1994**, *74*, 139.
3. Moser U.; Bendich A. In *Handbook of Vitamins*, 2nd ed.; Machlin, L. J., Ed.; Marcel Deckker Inc.: New York, 1991; pp 195–232.
4. Kato, K.; Terao, S.; Shimamoto, N.; Hirata, M. *J. Med. Chem*. **1988**, *31*, 793.
5. Andrews G. C.; Crauford T. In *Ascorbic Acid; Chemistry, Metabolism, and Uses*; Seib, P. A., Tolbert, B. M., Eds.; American Chemical Society: Washington, DC, 1982; pp 59–80.
6. Hesse, G.; Triedrich, H. *Liebigs Ann. Chem*. **1970**, *736*, 134.
7. Ramage, R.; Griffiths, G. J.; Shutt, F. E. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1539.
8. Walling, C. *Acc. Chem. Res*. **1975**, *8*, 125.
9. Finkelstein, E.; Rosen, G. M.; Rauckman, E. J. *Arch. Biochem. Biophys*. **1980**, *200*, 1.
10. Minnotti, G. *Arch. Biochem. Biophys*. **1982**, *273*, 144.
11. Ohe, T.; Mashino, T.; Hirobe, M. *Arch. Biochem. Biophys*. **1994**, *310*, 402.
12. Bernheim, F.; Berheim, M. L.; Wilkur, K. M. *J. Biol. Chem*. **1948**, *174*, 257.
13. Cos, P.; Ying, L.; Calomme, M.; Hu, J. P.; Cimanga, K.; Poel, B. V.; Pieters, L.; Vlietinck, A. J.; Berghe, D. V. *J. Nat. Prod*. **1998**, *61*, 71.