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Note

Oxidation of L-ascorbic acid by hydrogen peroxide: preparation of L-threonic acid*

HORACE S. ISBELL AND HARRIET L. FRUSH
Department of Chemistry, American University, Washington, D.C. 20016 (U.S.A.)
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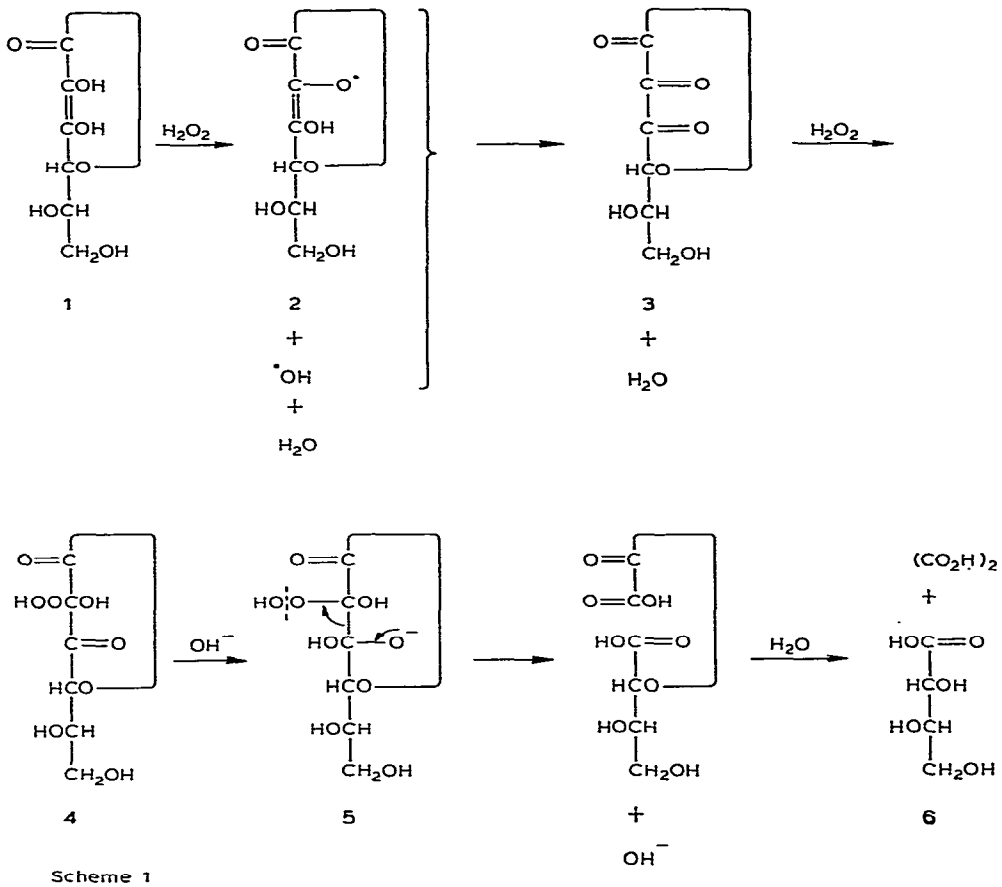
L-Ascorbic acid (**1**) has been extensively studied by prior workers with reference to its biological behavior, its role as an antioxidant, and its enhancement of the oxidative power of hydrogen peroxide[2,3]. Much attention has been devoted to its oxidation–reduction properties, and its capacity, in conjunction with hydrogen peroxide, to cause depolymerization of algins, pectins, mucoitin, and hyaluronic acid[4,5].

It is reported that, at pH 4, aqueous hydrogen peroxide containing a trace of iodine converts **1** into dehydro-L-ascorbic acid (**3**) in high yield, with formation of little or no oxalic acid. However, at pH $\geqslant$ 7, compound **3** is oxidized further, with formation[5] of oxalic acid and L-threonic acid (**6**). The reactions involved are similar to those considered in prior papers of this series[1,6–10]. Hence, it was of interest to examine the reactions in light of our work on the degradation of carbohydrates with alkaline hydrogen peroxide.

Our results confirm the observation[11] that treatment of **1** with an excess of alkaline hydrogen peroxide affords oxalic acid and L-threonic acid (**6**) in high yield. Apparently, the process takes place in two stages. The first stage is the oxidation of **1** to dehydro-L-ascorbic acid (**3**). This process is represented in Scheme 1 as involving reduction of a molecule of hydrogen peroxide by **1**, with formation of an L-ascorbic acid radical (**2**), a hydroxyl radical, and a molecule of water; the hydroxyl radical oxidizes **2** to **3**. In the second stage of the the process, addition of hydrogen peroxide takes place at C-2 (or -3) to yield adduct **4** or its equivalent. Decomposition of **4** involves addition of hydroxyl ion to the adjoining carbonyl group to give compound **5**, and elimination of hydroxyl ion from the hydroperoxide entity of **5**, with cleavage of the carbon–carbon bond. Hydrolysis of the intermediate produces oxalic acid and L-threonic acid (**6**).

Addition of hydrogen peroxide to C-2 and -3 would yield a dicarbonyl adduct that would decompose to peroxyoxalic acid and L-threonic acid (**6**), or to oxalic acid

*Reactions of Carbohydrates with Hydroperoxides. XI. For Part X, see ref. 1.



Scheme 1

and peroxy-L-threonic acid. Decomposition of the peroxy acids would yield carbon dioxide and, ultimately, formic acid. Thus, it was found, that, under some conditions, carbon dioxide and formic acid are produced in small proportions. Production of L-threonic acid (**6**) from L-ascorbic acid (**1**) by the process developed in this investigation is so satisfactory that we recommend it as a laboratory preparation for the instruction of students.

The principles developed in our investigation of the reactions of carbohydrates with hydroperoxides are of interest in relation to the role of **1** as an antioxidant, and its seemingly contradictory action in accelerating the oxidation of many compounds by hydrogen peroxide. As shown in eqs. *1* and *2*, the antioxidative properties of **1** may be ascribed to the elimination of hydrogen peroxide (or other peroxides) in the medium, with formation of the relatively inert compound **3**. The process is highly efficient, because radical **2** is a strong reducing agent, whereas the hydroxyl radical is a powerful oxidizing agent. Reaction of **2** with a hydroxyl or other radical yields inert compound **3**.

$$\mathbf{1} + H_2O_2 \rightarrow \mathbf{2} + \cdot OH + H_2O \quad (1)$$
$$\mathbf{2} + \cdot OH \rightarrow \mathbf{3} + H_2O \quad (2)$$
$$H_2O_2 + Fe^{2+} \rightarrow \cdot OH + OH^- + Fe^{3+} \quad (3)$$
$$Fe^{3+} + \mathbf{2} \rightarrow \mathbf{3} + Fe^{2+} \quad (4)$$

Enhancement of the oxidative power of hydrogen peroxide by **1** in the presence of an iron salt may be rationalized by a chain reaction based on eqs. *1*, *3*, and *4*. The chain is initiated by the Haber–Weiss reaction of ferrous ion with hydrogen peroxide[12], giving a hydroxyl radical and a ferric ion (eq. *3*). In contrast to radical **2** of eq. *1*, ferric ion is not a reducing agent, but rather, a strong oxidizing agent. It is reduced by radical **2** to ferrous ion, with production of dehydro-L-ascorbic acid (3). The sum of eqs. *1*, *3*, and *4* may be expressed as follows:

$$\mathbf{1} + 2\,H_2O_2 \rightarrow 2\,\cdot OH + 2\,H_2O + \mathbf{3} + OH^-.$$

Thus, the reactions in eqs. *1*, *3*, and *4* lead to a supply of hydroxyl radicals, whereas the reaction in eq. *2* results in their elimination.

EXPERIMENTAL

General methods. — Samples of L-ascorbic acid (**1**) were treated with aqueous hydrogen peroxide–alkali under various conditions. After suitable reaction-times, formic acid, oxalic acid, carbon dioxide, and "residual acid" were determined by the methods described in ref. 8. The residual acid was shown to be largely L-threonic acid by preparation of the previously known L-threonic phenylhydrazide and calcium L-threonate[13]. A new lithium salt was also obtained.

Oxidation of L-*ascorbic acid by aqueous sodium peroxide.* — A 0.2M solution of **1** (10 mL) was added to ice-cold, 1.0M sodium peroxide (90 mL). The mixture was kept for 20 h at 4°, and then diluted to 100 mL with carbon dioxide-free water. Determinations of carbon dioxide, formic acid, oxalic acid, and "residual acid" were made on aliquots of the solution. The following results, expressed in moles of the constituent per mole of **1**, were obtained: formic acid, 0.18; oxalic acid, 0.96; carbon dioxide, 0.13: and residual acid, 0.98. The "residual acid" was converted into lithium L-threonate in 94% yield.

Preparation of calcium L-*threonate.* — Calcium carbonate (2 g) was added to a solution of **1** (1.36 g, 10 mmol) in water (25 mL). The mixture was cooled in an ice bath, and shaken gently during addition of 30% hydrogen peroxide (4 mL) in small portions. The mixture was kept for 30 min at 30–40°, and then treated with 0.4 g of activated carbon (Norit), and heated on a steam-bath to decompose the excess of hydrogen peroxide. When evolution of oxygen had ceased, the hot mixture was filtered, and the filtrate was concentrated at 40° under diminished pressure to ~10 mL. Methanol was added to incipient turbidity, and the microscopic, prismatic crystals that formed in the course of several hours were separated by filtration, washed with 80% aqueous methanol, and air-dried; wt, 1.46 g. The product, re-

crystallized from water, had $[\alpha]_D^{25}$ +13.8° (*c* 1.0, H_2O). This optical rotation differs widely from the value (+7.63°) reported in ref. 13 for calcium L-threonate · H_2O. The value reported, for a crude product, is presumably in error.

Anal. Calc. for $(C_4H_7O_5)_2Ca \cdot H_2O$: C, 29.27; H, 4.91; Ca, 12.20. Found: C, 29.60; H, 4.87; Ca, 11.94.

A sample of the calcium salt, after removal of the calcium, and treatment with phenylhydrazine, yielded a crystalline phenylhydrazide; m.p. 157–158°, $[\alpha]_D^{25}$ +31° (*c* 2, H_2O). These properties, as well as the i.r. spectrum (KBr), of the product agreed with the properties of the phenylhydrazide derived from authentic L-threonic acid[13].

Preparation of lithium L-threonate. — A solution of calcium L-threonate, prepared from L-ascorbic acid (10 mmol) by the procedure described in the preceding section, was passed through Amberlite IR-120 (H^+) cation-exchange resin (20 mL). The resulting solution was neutralized with 1.0M lithium hydroxide. Concentration of the solution under diminished pressure produced prismatic crystals (1.3 g, 92%). The product, recrystallized from water, and dried over calcium chloride, had $[\alpha]_D^{25}$ +17° (*c* 4, H_2O).

Anal. Calc. for $C_4H_7LiO_5$: C, 33.82; H, 4.97; Li, 4.89. Found: C, 33.68; H, 5.16; Li, 4.70.

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REFERENCES

1 H. S. Isbell and H. L. Frush, *Carbohydr. Res.*, 59 (1977) c25–c31.
2 A. Szent-Györgi, *Annu. Rev. Biochem.*, 32 (1963) 1–14.
3 H. von Euler and B. Eistert, *Chemie und Biochemie der Reductone und Reductonate*, Ferdinand Enke, Stuttgart, 1957, pp. 319–324.
4 W. W. Pigman, G. Matsumura, and A. Herp, *Proc. Int. Congr. Rheol. Symp. Biorheol., 4th*, (1965) 505–519.
5 H. von Euler and H. Hasselquist, *Ark. Kemi*, 9 (1955) 147–155.
6 H. S. Isbell, H. L. Frush, and E. T. Martin, *Carbohydr. Res.*, 26 (1973) 287–295.
7 H. S. Isbell and H. L. Frush, *Carbohydr. Res.*, 28 (1973) 295–301.
8 H. S. Isbell, H. L. Frush, and Z. Orhanovic, *Carbohydr. Res.*, 36 (1974) 283–291.
9 H. S. Isbell, E. W. Parks, and R. G. Naves, *Carbohydr. Res.*, 45 (1975) 197–204.
10 H. S. Isbell, H. L. Frush, and E. W. Parks, *Carbohydr. Res.*, 51 (1976) c5–c9.
11 S. Kamiya and T. Nakabayashi, *Bitamin*, 13 (1957) 246–249; *Chem. Abstr.*, 54 (1960) 7797.
12 F. Haber and J. Weiss, *Proc. R. Soc. London, Ser. A*, 147 (1934) 333–351.
13 J. U. Nef, O. F. Hedenburg, and J. W. E. Glattfeld, *J. Am. Chem. Soc.*, 39 (1917) 1638–1652.