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Note

Preparation of dehydro-L-ascorbic acid dimer by air oxidation of L-ascorbic acid in the presence of catalytic amounts of copper(II) acetate and pyridine

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Abstract—The catalytic system $Cu(AcO)_2$–pyridine 1:4 mol % in methanol, slowly catalyses the air oxidation of ascorbic acid to the 2-methyl hemi-ketal of dehydroascorbic acid **5**, and hydrogen peroxide. However, with $Cu(AcO)_2$–pyridine 3:4 mol % the air oxidation is quite fast and no hydrogen peroxide is present at the end of the reaction. Removal of the catalyst and refluxing the foamy **5** in MeCN gives the oxidized, dimeric, dehydroascorbic acid in very good yields (~70%) contaminated by ~1–2% MeCN.

Keywords: Ascorbic acid; Dehydroascorbic acid dimer; Copper(II) acetate/pyridine; Dioxygen activation

Ascorbic acid, **1**, is a weak acid and a powerful two one-electron reducing agent. In water, various species resulting from acid/base and redox equilibria are expected on the way to its oxidation product, dehydroascorbic acid.[1]

The structure of dehydroascorbic acid is not properly represented as **2** or **3** but it is **4**, **5**, or **6** depending on the solvent in which ascorbic acid is oxidized. Thus, in water, the bicyclic hydrated form[2] **4** can be isolated in ~70% purity.[3] Oxidation of ascorbic acid in methanol by iodine,[4] chlorine,[5] air/Pd–C,[6] O_2/active charcoal,[7] or arsenic acid/iodine[8] affords the two isomers[9] of the 2-methyl hemi-ketal **5**, which can be isolated as a solid.[5] Finally, oxidation of ascorbic acid by *p*-benzoquinone, chloranil, or mercuric(II) acetate in *N*,*N*-dimethylformamide, dimethyl sulfoxide, or *N*,*N*-dimethylacetamide[10] produces the two anomers[11] of the dimerized dehydroascorbic acid **6**, and by adding a cocktail of formic acid, oxalic acid, and phthalic acid the 'insoluble'[11] symmetrical[12] anomer precipitates out in ~60% yield. The 'insoluble' form of **6** is the crystalline dehydroascorbic acid of commerce and it can also be produced from **5** by thermal elimination of methanol either by 'baking' at 100 °C under diminished pressure (yields 23%,[4] ~40%[5]) or refluxing in methyl ethyl ketone (64%)[7] or acetonitrile (56%).[8] By evaporating a nitromethane solution of **5**, crystalline **6** was obtained in 90% yield,[6] but its purity has been questioned.[7]

In vivo, dehydroascorbic acid is reduced back to ascorbic acid either nonenzymatically (e.g., by glutathione) or enzymatically (by proteins which have dehydroascorbic acid reductase activity and also use glutathione as reducing agent).[13] A few aspects of the dehydroascorbic acid biochemistry have recently been uncovered. Thus, dehydroascorbic acid inhibits a few enzymes, for example, hexokinase, glucose-6-phosphate dehydrogenase,[14] it accumulates in some diseased states,[13] prevents apoptosis, which is induced by oxidized low-density lipoproteins by oxidizing their free –SH groups,[15] and inhibits (at high concentrations) the growth of tumor cells (HeLa and T98 G) by delaying their entry into mitosis.[16]

In this communication, we describe the fast and quantitative oxidation of ascorbic acid to the 'methanolate' **5** using air and catalytic amounts of copper(II) acetate

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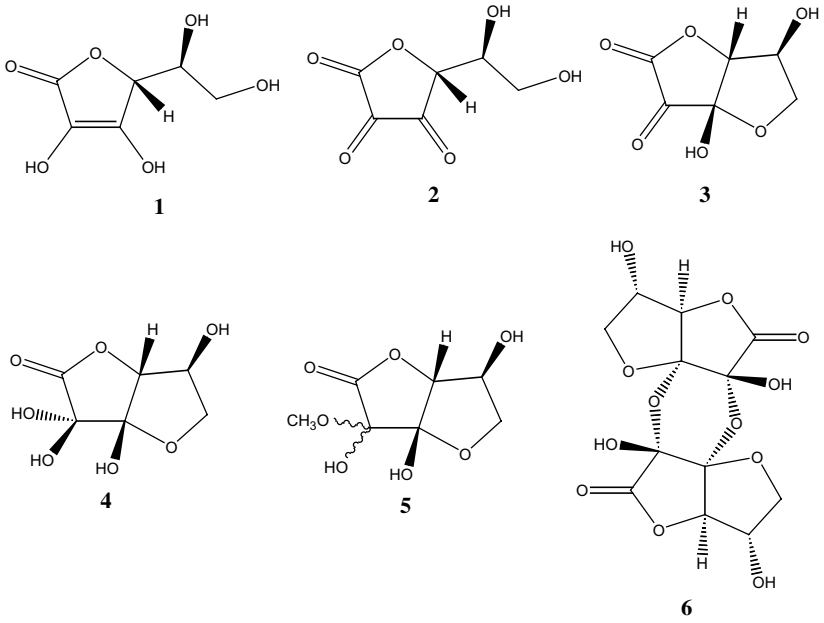

and pyridine and the dimerization of **5** to the quite expensive **6** in boiling acetonitrile.

For the preparation of dehydroascorbic acid on a small scale, the oxidizing agents mentioned above are not objectionable, although the isolation of pure crystalline **5**[5] or **6**[4,8,10] may be cumbersome. The cheapest oxidant is dioxygen in air, whose reaction with ascorbic acid is catalyzed by transition metal ions, particularly monomeric copper(II).[17] Activation of dioxygen in air by palladium-on-charcoal has been used for the preparation of dehydroascorbic acid.[6]

The air oxidation of ascorbic acid catalyzed by monomeric Cu(II) in water, Eq 1 or in methanol, Eq 2:

$$\mathbf{1} + O_2 \xrightarrow{Cu(II)} \mathbf{4} + H_2O_2 \quad (1)$$

$$\mathbf{1} + O_2 + MeOH \xrightarrow{Cu(II)} \mathbf{5} + H_2O_2 \quad (2)$$

can be subdivided into two parts:[18]

$$\mathbf{1} + 2Cu^{2+} \rightarrow \mathbf{4} + 2Cu^{+} + 2H^{+} \quad (3)$$

$$2Cu^{+} + O_2 + 2H^{+} \rightarrow 2Cu^{2+} + H_2O_2 \quad (4)$$

As a catalyst we used dimeric copper(II) acetate monohydrate and in order to keep the produced Cu(I) in solution, we used complexing agents such as pyridine, 2,2′-bipyridine (bipy), and phenanthroline (phen). We found that in the systems containing $Cu(AcO)_2$–bipy 1:2 or $Cu(AcO)_2$–phen 1:2 or $Cu(AcO)_2$–pyridine 1:4 mol %, the oxidation of ascorbic acid was incomplete after 4 h stirring in air and, more significantly, hydrogen peroxide was always present. This seems to be in line with the finding[19] that the 1:1 complexes Cu(II)/bipy and Cu(II)/phen were catalytically inactive for the oxidation of ascorbic acid by pure dioxygen. For the catalytic system 1:4 mol/mol $Cu(AcO)_2{\cdot}H_2O$–pyridine in methanol the nature of the complexes formed during the catalytic cycle (Eqs. 3 and 4) is not known. It is only known[20] that a solution of copper(II) acetate in alcoholic pyridine contained less than 3 molecules of pyridine per Cu(II) and later it was found that the dimer $[Cu(AcO)_2{\cdot}py]_2$ in pyridine[21] or in chloroform in the presence of pyridine[22] is in equilibrium with the monomer $Cu(AcO)_2{\cdot}py_2$.

The reactions 1 and 2 produce hydrogen peroxide, which has oxidizing ability. However, its reaction with ascorbic acid is a slow process.[5,18] Hydrogen peroxide can be activated by Fe^{2+} (Fenton reagent),[23] $O_2^{\cdot-}$ (uncatalyzed Haber–Weiss reaction),[23] Cu(I),[24] and I^- (although the reaction is fairly slow for analytical purposes, it can be catalyzed by molybdate.[25]) In fact, Pecherer[5] prepared impure **4** by oxidation of ascorbic acid with hydrogen peroxide in water in the presence of a catalytic amount of iodine, but failed to obtain a product with Fe^{3+} catalysis. Since the catalytic systems: $Cu(AcO)_2$–pyridine–I_2 1:2:1 mol %, CuI–bipy or CuI–phen tested at 1:1 or 1:2 mol % levels oxidized very slowly and incompletely the ascorbic acid (10–20%/20 h and 40–50%/3 h, respectively), we turned our attention to a copper activator of hydrogen peroxide because the reaction of Fe(II)/H_2O_2 is slower than the reaction of Cu(I)/H_2O_2.[23,24]

By using some excess of $Cu(AcO)_2$ pyridine, we found that ascorbic acid was quickly oxidized without leaving H_2O_2 at the end of the reaction. The catalytic systems $Cu(AcO)_2$–pyridine x:4 mol %, with $3 < x < 16$ showed

no significant difference in the rate of oxidation, this being completed in about 1–1.5 h at 1–5 mmol scale. With $Cu(AcO)_2$–bipy or $Cu(AcO)_2$–phen 3:2 mol %, as catalysts, the reaction was complete in ~1 h. For preparation purposes, therefore, we selected as catalyst the cheaper system $Cu(AcO)_2$/pyridine 3:4 mol %. It seems that the free $Cu(AcO)_2$ works by oxidizing the ascorbic acid according to Eq. 3 and also is involved in the consumption of H_2O_2 probably via Eq. 5:[24]

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + HO^{\bullet} + HO^{-} \quad (5)$$

The $HO^{\bullet}$ radical is now being scavenged by the ascorbic acid to give **5** according to Eq. 6:

$$\mathbf{1} + 2HO^{\bullet} \xrightarrow{MeOH} \mathbf{5} + 2H_2O \quad (6)$$

The reaction 6 is not inhibited by 50 mol % *tert*-butanol, implying that ascorbic acid is more efficient in scavenging the hydroxyl radicals.

When the reaction was over, evaporation and drying under diminished pressure gave the crude compound **5** as a dark green semi-solid. In order to get **6**, complete removal of copper was necessary and this was achieved by a short column chromatography. The white foam obtained consisted (^{1}H NMR analysis) of the two isomers[9] of **5** (~80%), **4** (~15%), ethyl acetate (~5%), and free methanol. Compound **4** most likely arose from partial hydrolysis of **5** on the column because more silica gel causes diminution of **5** in the eluate which, in turn, is reflected to lower yields of **6**.[8] The free methanol, likewise, was formed by partial hydrolysis of **5** by the water in Me_2SO-d_6 because after 2 days the intensity of its peak increased with concomitant decrease of the peaks of CH_3O– in **5**. Refluxing the white foam with acetonitrile, the dimeric **6**[11] was obtained in 60–75% yields. From the melting point, a purity of >97% can be inferred[5] and from the IR and ^{1}H NMR spectra a ~98–99% purity is estimated. The supernatant, after evaporation of the acetonitrile, drying and treating it again[7,8] with fresh acetonitrile did not afford more **6** when the scale was 1–10 mmol or gave more **6** (~3%) at 0.1 mol scale. The final acetonitrile solution either did not contain any **4** or **5** or it contained (^{1}H NMR analysis) only ~40% **5**, the form which easily gives the dimer **6**.[8] This method also works with deteriorated (yellowish) samples of ascorbic acid with 55–65% yields.

1. Experimental

1.1. General

L-Ascorbic acid and 2,2′-dipyridyl (Aldrich), silica gel for column chromatography and 1,10-phenanthroline (Serva), copper(II) acetate monohydrate (Ferak), and Silica Gel 60 H for TLC (E. Merck) were used. Titanyl sulfate, $TiOSO_4$, was prepared by fusing titanium dioxide with sodium hydrogen sulfate[26] and used for H_2O_2 detection. Acetonitrile was dried over 4 Å molecular sieves, pyridine was distilled from NaOH pellets, and nondried MeOH was used. TLC was run on microslides using EtOAc as developing solvent and spots were made visible by spraying with 35% H_2SO_4 acid and charring (R_f values: ascorbic acid 0.18, streaks, compound **5** 0.58). Melting points were obtained using an Electrothermal Model IA9100 apparatus in capillaries, the heating starting at 25 °C.[5] IR spectra were taken on a Perkin–Elmer Model 16PC FT-IR spectrometer and NMR spectra were run on a Bruker DPX Avance spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) as described.[8]

1.2. Crystalline dimeric dehydroascorbic acid (6)

To a soln of ascorbic acid (17.60 g, 0.1 mol) in MeOH (300 mL) in a 0.5 L beaker, a dark blue methanolic soln (25 mL) containing copper(II) acetate monohydrate (200 mg, 1 mmol) and pyridine (0.33 mL, 4 mmol), and solid copper(II) acetate monohydrate (400 mg, 2 mmol) were added to give a clear brown-green soln, which was vigorously stirred at room temperature for 2.5 h. The brown-green color changed to brown in 1 min, then to yellow in 2 min and finally an olive-green, slightly opalescent soln was obtained. The latter color implied complete oxidation of ascorbic acid, as checked by TLC, and hydrogen peroxide was not detected (three drops of saturated aqueous $TiOSO_4$ added to one drop of the soln diluted with 0.5 mL water). Evaporation of MeOH (rotary, 40 °C) and brief drying under diminished pressure gave a dark green semi-solid (23.40 g), which was dissolved in EtOAc (100 mL) and chromatographed [silica gel (30 g, 2 × 17 cm column) in EtOAc; elution with EtOAc (500 mL); at the end of the elution the copper(II) acetate covered the 2/3 of the column]. Evaporation of the clear, colorless soln, and drying under diminished pressure gave a white foam (19.30 g; expected 20.60 g **5**). ^{1}H NMR (Me_2SO-d_6): δ 4.37 (d, 1H, J 4.4 Hz, H-4), 4.23 (m, 1H, H-5), 4.17 and 3.84 (m, 2H, H-6), 3.41 (s, 1.2H) and 3.27 (s, 0.8H) for CH_3O– in **5**, 3.16 (s, 0.2H) for free MeOH and signals from EtOAc. The white foam was dissolved in MeCN (150 mL), placed in an oil bath at 100 °C and refluxed for 30 min. After cooling to room temperature, filtration (sintered glass, porosity 3), washing with cold water (2 × 10 mL) and cold acetone (2 × 10 mL), and drying over P_2O_5 under diminished pressure gave **6** (11.50 g, 66%) as a white solid containing ~2% MeCN (^{1}H NMR): mp 205 °C starts darkening and at 228 °C turns black (lit.[4] 225 °C dec; lit.[5,6] 220–225 °C dec; lit.[7] 230–232 °C dec; lit.[8] ~190 °C darkens, 224–227 °C turns black; lit.[10] 222–223 °C dec, 223–225 °C dec, 231–232 °C dec). Its IR (KBr), is the same as that reported[7] (see also spectral

data base: SDBS; available from www.Aist.go.jp/RIODB/SDBS) but does not have a weak broad band (probably due to water impurity) at 1637 or 1660 cm^{-1}. Its ^{13}C NMR (Me_2SO-d_6) showed the 18 lines expected from the symmetric and asymmetric forms of **6**:[11] δ C-1 167.74, 168.35, and 168.82; C-2 91.32, 99.35, and 103.05; C-3 103.78, 105.44, and 113.44; C-4 72.82, 73.03, and 73.27; C-5 87.90, 88.72, and 89.88; C-6 74.87, 75.84, and 76.45.

The clear slightly yellow MeCN supernatant was evaporated and dried to give a yellowish hygroscopic foam (4.13 g). Dissolution in MeCN (30 mL) and treatment as above gave more **6** (565 mg, 3%) contaminated by ~1.5% MeCN and having the same mp, IR and NMR spectra as **6** of the first crop. The dark yellow MeCN soln gave a dark yellow foam (3.0 g) the ^1H NMR (Me_2SO-d_6) of which did not contain **4** or **5**.

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