Found: C, 52.25; H, 4.20; N, 20.44. IR spectra: $-CON_3$ 2140 cm^{-1} and -O-CO- 1727 cm^{-1}. Identity of this product with the sample prepared through the hydrazide was established by comparison of their IR spectra.

Note 1. Progress of the reaction was followed by thin-layer chromatography on silica gel (Kieselgel G Merck) in the solvent system of benzene as recorded by Honda, *et al.*[11] Ceric sulfate was used to locate the compounds. The red brown color was observed when a solution of ceric sulfate (1 g) in 10% sulfuric acid (100 ml) was sprayed on a chromatographic plate, which was heated with the gentle flame on a asbestos sheet, as for the detection of alkaloids and terpens: *p*-methoxybenzyl alcohol *Rf* 0.03; *p*-methoxybenzyl chloroformate *Rf* 0.61; *p*-methoxybenzyl azidoformate *Rf* 0.40.

Note 2. When a large excess of phosgen was employed, formation of some unidentified product (*Rf* 0.55, IR 1700 cm^{-1}) was noted. This can be separated easily since it remained in the mother liquid of the desired crystalline azidoformate.

Note 3. Pyridine of the above procedure can be substituted with triethylamine; yield 82%.

Note 4. The use of hydrazoic acid and pyridine, instead of sodium azide, gave the azidoformate largely contaminated with the unidentified product mentioned in note 2.

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Inhibition of the Copper-catalyzed Oxidation of Ascorbic Acid by 1,10-Phenanthroline

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In the course of the copper-catalyzed oxidation of ascorbic acid, copper ion is turned over, depending probably on the redox potential, between cupric and cuprous ions. The turn-over of copper ion, which is expected to controll the rate of the oxidation, may be expressed briefly as follows;

$$Cu^{2+} + AH^- \longrightarrow Cu^+ + \cdot AH \quad (1)$$

$$Cu^+ + O_2 + H^+ \longrightarrow Cu^{2+} + \cdot O_2H \quad (2a)$$

$$2Cu^+ + O_2 + 2H^+ \longrightarrow 2Cu^{2+} + H_2O_2 \quad (2b)$$

where AH^- and $\cdot AH$ represent the monoionic ascorbate anion and the ascorbate free radical, respectively. The complexation of copper ion, which interferes either step (1) or (2), retards generally the oxidation.[2] In the previous paper, it was elucidated that the inhibition by inorganic anions was partly due to the complex formation between cupric ion and the inorganic anion, by which the interaction of copper ion to the ascorbate anion to form an active inter-

1) Location: *Anagawa-4, Chiba.*

2) R. Flitman and E. Frieden, *J. Am. Chem. Soc.*, **79**, 5198 (1957); S. Isaka, *Nature*, **179**, 578 (1957); E. Tanner, W. Schuler and R. Meier, *Helv. Chim. Acta*, **42**, 445 (1959); V.S. Butt and M. Hallaway, *Arch. Biochem. Biophys.*, **92**, 94 (1961).

mediate was interrupted, and partly due to the formation of a stable cuprous complex, which shifts the redox potential of copper and hence the reoxidation of the cuprous complex may be retarded.[3] In the present paper, some evidence was presented that 1,10-phenanthroline, which retarded the oxidation as a whole, hindered only step (2) but not inhibit, probably rather accelerate, step (1).

Experimental

Manometric Measurement of the Oxidation——The oxidation of ascorbic acid was followed at 25° by the consumption of oxygen, which was measured with the conventional Warburg manometric technique using air as the gaseous phase. The main compartment of the reaction vessel contained 2.0 ml of 2.00×10^{-4}M copper nitrate and 2.0 ml of 0.2 M acetate buffer, pH 5.5, containing various concentrations of glycylglycine or 1,10-phenanthroline. The side arm of the vessel contained 1.0 ml of 3.00×10^{-2} M sodium ascorbate prepared just before the measurement. All the solutions were prepared by dissolving in twice distilled water from all glass apparatus. The ionic strenght of the reaction mixtures was adjusted at 0.1 with $NaNO_3$. The ascorbate solution was tipped from the side arm after the temperature equilibration for 10 min. The manometer was closed just before tipping the substrate, and the oxygen consumption was read every 1 min thereafter.

Spectrophotometric Measurement of the Reaction——The reduction of cupric ion was followed spectrophotometrically in the reaction system containing both chelating agents. The mixtures of copper nitrate, glycylglycine and 1,10-phenanthroline in acetate buffer, pH 5.25, was thermostatted at 25° in a glass stoppered cylindrical cell with 50 mm cell length. After the temperature equilibration for 15 min, 1 ml of 7.5×10^{-2} M sodium ascorbate was added rapidly into the cell. After the cell was closed, the solution was applied to the spectrophotometric measurement. At the start, the concentrations of each component were as follows; 8.00×10^{-4} M $Cu(NO_3)_2$, 5.00×10^{-3}M glycylglycine, 4.80×10^{-4} or 5.60×10^{-4}M 1,10-phenanthroline, 5.00×10^{-3}M sodium ascorbate and 8.00×10^{-2}M acetate buffer. The optical absorbance was recorded rapidly as possible from 740 mμ to 360 mμ with a Cary 14 recording spectrophotometer.

Results and Discussion

The oxygen consumption increased linearly with time from the start. Both glycylglycine and 1,10-phenanthroline appeared to reduce the rate of the ascorbate oxidation, which was expressed as the consumption of molecular oxygen. The retardation effect of varying concentrations of those chelating agents on the oxygen consumption was shown in Fig. 1.

The retardation by 1,10-phenanthroline is resulted undoubtedly from the formation of the cuprous complex, because cuprous 1,10-phenanthrolinate displaying an absorption maximum at 410 mμ is formed in parallel with the reaction. On the other hand, it seems uncertain that the retardation by glycylglycine is ascribed to the copper complex formation, because acetate ion, which is present excessively in the reaction mixtures as compared with glycylglycine, competes with glycylglycine for the complex formation. In order to clarify this question, the pH dependence of the reaction rate was examined. At high pH, where the glycylglycine complex is

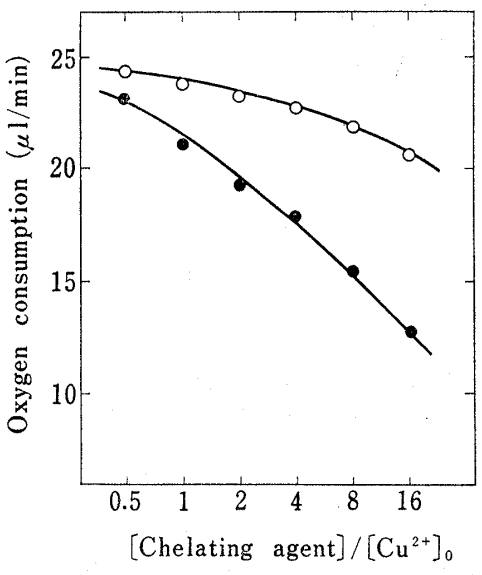

Fig. 1. Retardation of the Ascorbate Oxidation by Varying Concentrations of Chelating Agents

—○—○— : glycylglycine
—●—●— : 1,10-phenanthroline
The details are described in experimental section.

3) A. Hanaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1840 (1969).

apt to be formed, the rate was reduced significantly as shown in Table I. This fact indicates that glycylglycine also reduce the oxidation by forming the cupric complex.

Table I. pH Dependence of the Oxygen Consumption

Chelating agent	pH	Oxygen consumption (μl/min) 4.00	4.60	5.50
None		16.4	22.8	25.9
Glycylglycine		16.1	21.3	21.9
1,10–Phenanthroline		9.5	13.0	15.6

The oxygen consumption was measured manometrically at 25° in the reaction mixtures shown as follows; 8.00×10^{-5} M copper nitrate, 6.40×10^{-4} M chelating agents and 6.00×10^{-3} M sodium ascorbate in 0.08 M acetate buffer.

Copper ion is turned over during the oxidation. When the oxidation was followed spectrophotometrically in the reaction system containing both chelating agents, the electronic absorption band due to cupric glycylglycinate, λ_{max} 650 mμ, was diminished a little with the reaction time, and the band due to cuprous 1,10-phenanthrolinate, λ_{max} 410 mμ, was increased instead. If 1,10-phenanthroline was not added into the reaction mixtures, the solution became turbid and the precipitates of cuprous oxide was produced. A typical example for the spectral changes during the reaction was shown in Fig. 2. The rate of the cuprous complex formation

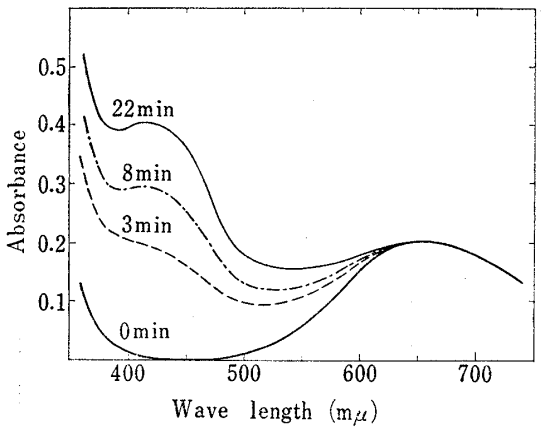

Fig. 2. Spectral Changes in the Reaction Mixtures during the Ascorbate Oxidation

The figures on the curves indicate the time after the start of reaction.

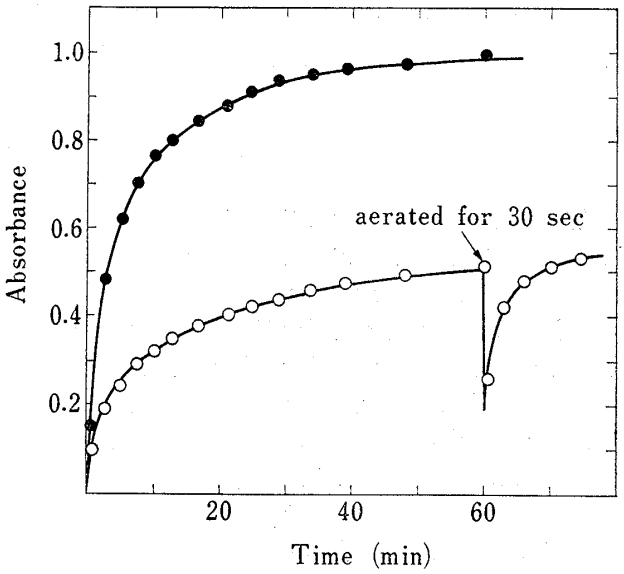

Fig. 3. Formation of Cuprous 1,10-Phenanthrolinate by Ascorbic Acid

1,10-phenanthroline; —●—●— : 5.6×10^{-4} M
—○—○— : 4.8×10^{-4} M
Total concentrations of copper ion: 8.0×10^{-4} M

expressed by $\Delta E_{410m\mu}/\Delta t$ depended on the concentration of 1,10-phenanthroline as shown in Fig. 3, which indicates that this chelating agent accelerates the reduction of the cupric complex. The amounts of the cuprous complex reached to a constant level after 1 hr, and in this stage, where the mixtures are probably in an anaerobic state, the cupric complex was still present excessively as compared with the cuprous complex. When the reaction mixtures in this equilibrium state was aerated by shaking the cell vigorously, $E_{410m\mu}$ was diminished instantaneously. The absorption at 410 mμ, when the solution was kept standing in a stoppered cell, increased gradually thereafter. This typical spectral changes could be repeated as far as ascorbic acid was present in the reaction mixtures.