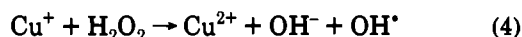# SHORT COMMUNICATIONS
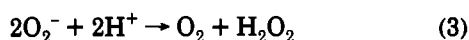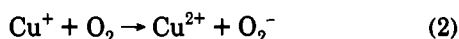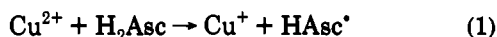
Benzene Production from Decarboxylation of Benzoic Acid in the Presence of Ascorbic Acid and a Transition-Metal Catalyst

INTRODUCTION

Ascorbic acid (vitamin C) is a natural component of many foods and is often added to foods and beverages as a vitamin supplement or fortifier and promoted as an antioxidant. Transition metals, e.g., Cu(II) and Fe(III), can catalyze the one-electron reduction of O_2 by ascorbic acid to produce the superoxide anion radical, which undergoes spontaneous disproportionation to produce hydrogen peroxide. Subsequent metal-catalyzed reduction of H_2O_2 by ascorbic acid can generate the hydroxyl radical (Halliwell and Gutteridge, 1981):

$$Cu^{2+} + H_2Asc \rightarrow Cu^+ + HAsc^\bullet \qquad (1)$$

$$Cu^+ + O_2 \rightarrow Cu^{2+} + O_2^- \qquad (2)$$

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \qquad (3)$$

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^- + OH^\bullet \qquad (4)$$

Sodium benzoate has been widely used as a food preservative for many years and is generally recognized as safe (GRAS). The optimum antimicrobial activity occurs in the pH range 2.5–4.0, indicating benzoic acid (pK_a = 4.2) is the active form of this antimicrobial agent, making it most suitable for foods and beverages which are naturally acidic (Chichester and Tanner, 1968). The sodium salt is preferred because of its much higher solubility in aqueous media. It is generally permitted in beverages at a level of less than 0.1% (ca. 7 mM), although higher levels are permitted for some foods.

Mathew and Sangster (1965) have shown that sodium benzoate is decarboxylated by hydroxyl radical attack. Sagone et al. (1980) used ^{14}C-labeled benzoate decarboxylation to show hydroxyl radical generation by phagocytic leukocytes. Alkoxyl radicals do not decarboxylate benzoate, suggesting benzoate decarboxylation is a relatively

specific indicator of hydroxyl radical (Winston et al., 1983). Although the three isomeric hydroxybenzoates have also been measured as products of hydroxyl radical attack on benzoate (Loeble et al., 1951; Armstrong et al., 1960), we have not found any report of benzene production by this reaction.

The present study shows that hydroxyl radical, generated by the metal-catalyzed reduction of O_2 and H_2O_2 by ascorbic acid, can attack benzoic acid to produce benzene under conditions prevalent in many foods and beverages. Since benzene has been shown to be carcinogenic (Maltoni and Scarnato, 1979), its potential formation in foods during processing and storage should be of some concern.

MATERIALS AND METHODS

Desferal mesylate (desferioxamine) was a gift from Ciba Pharmaceutical Co. Ascorbic acid stock solutions were prepared fresh daily in 0.1 M HCl to stifle autoxidation. Hydrogen peroxide stock solutions were standardized colorimetrically with saturated $TiOSO_4$ solution in 2 M H_2SO_4 (ϵ = 717 M^{-1} cm^{-1} at 410 nm) (Ellis and Sykes, 1973).

High-Performance Liquid Chromatography. The HPLC system consisted of a Knauer 64 pump, a Rheodyne 7125 injection valve with 20-μL sample loop, and a Knauer variable-wavelength UV detector operating at 204 nm with the response recorded on a Kipp and Zonen BD41 strip chart recorder. The analytical column was a 100 × 4.6 mm Spheri-5 reversed-phase C_8 cartridge with a 30 × 4.6 mm guard cartridge of the same packing material (Applied Bioscience, Inc.). The mobile phase was 25% aqueous acetonitrile (spectral quality, Baker Chemical Co.) filtered through 0.2-μm membrane filters prior to use each day and was pumped at a flow rate of 3.0 mL/min.

A 2.0 μM aqueous benzene standard solution was stored refrigerated in a capped container. Dilutions of this standard in water were used as the working standards for HPLC analysis. Benzene was well separated from other components in the reaction mixtures under these conditions and was quantitated by comparison of peak height with the standard solutions.

Reaction Conditions. Reaction mixtures were placed in a constant-temperature bath at 25 °C for 15 min, at which time an aliquot was removed and injected into the HPLC system for analysis. All experimental conditions were prepared and analyzed
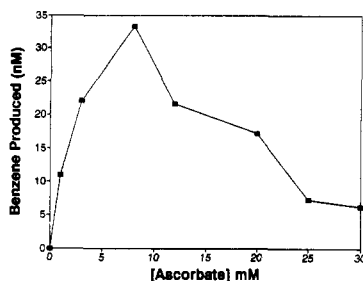
Figure 1. Dependence of benzene production on ascorbic acid concentration. Reaction conditions were as in Table I, with 0.25 mM CuSO₄ and varying ascorbic acid concentrations.
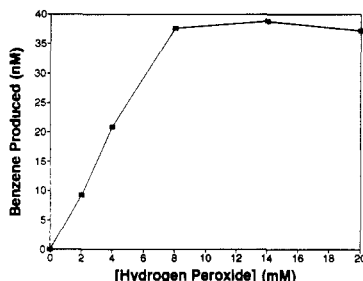

Figure 2. Dependence of benzene production on hydrogen peroxide concentration. Reaction conditions were as in Table I, with 0.25 mM CuSO₄ and varying H_2O_2 concentrations.

in triplicate. Buffers for the pH dependence study contained 50 mM phosphoric acid and 50 mM acetic acid (combined), and pH was adjusted with NaOH. NaClO₄ was added to buffers to give a final ionic strength of 0.10 M, except at pH 7.0 the ionic strength was 0.14 M without added NaClO₄.

GC/MS Analysis of Benzene in Headspace. The reaction mixture was heated to 45 °C, and 500 μL of headspace gas from the reaction vial was analyzed on a Hewlett-Packard (5890/5971, Series II) gas chromatograph/mass spectrometer. A 40 nM benzene standard in water was used as an external standard for comparison of retention time and mass spectrum of benzene in the reaction mixture.

RESULTS AND DISCUSSION

Quantitation of Benzene. The chromatographic system gave a linear response for benzene standards over a concentration range of 0–100 nM ($r^2 > 0.98$), with a retention time of approximately 8 min. The lower limit for detection was approximately 1 nM. A time-dependent study of a typical reaction mixture showed an increase in benzene production up to ca. 10 min, with no further change in benzene concentration up to 40 min. Consequently, all subsequent reaction mixtures were analyzed for benzene at 15 min after mixing (unless indicated otherwise).

Dependence of the Reaction on Ascorbic Acid and Hydrogen Peroxide. Benzene production increased with increasing ascorbic acid concentration at low ascorbic acid concentrations but decreased with increasing amounts of ascorbic acid when its concentration exceeded that of benzoic acid (Figure 1). This dependence on ascorbic acid concentration indicates hydroxyl radical production is dependent on the concentration of ascorbic acid, but the ascorbic acid competes with benzoic acid as a scavenger of the hydroxyl radical at higher concentrations.

Benzene production increased linearly with H_2O_2 concentration until the concentration of H_2O_2 exceeded the ascorbic acid concentration, reflecting a change in the limiting reagent (Figure 2). Under the standard conditions (25 °C and 15-min reaction time), there was no benzene detected when either ascorbic acid or H_2O_2 was omitted from the reaction mixture. However, benzene was pro-

Table I. Effect of Metal Ions and Ligands on Benzene Production from Benzoic Acid

addition	[benzene], nM
none	6.7 ± 1.9
0.1 mM desferal	ND[b]
0.05 mM CuSO₄ + 0.1 mM desferal	23.3 ± 1.7
0.25 mM CuSO₄ + 0.1 mM desferal	27.0 ± 1.1
1.0 mM CuSO₄ + 0.1 mM desferal	37.3 ± 3.1
2.5 mM CuSO₄ + 0.1 mM desferal	29.6 ± 3.4
4.0 mM CuSO₄ + 0.1 mM desferal	29.3 ± 0.6
0.05 mM FeSO₄	21.6 ± 0.5
0.25 mM FeSO₄	13.2 ± 1.9
1.0 mM FeSO₄	11.9 ± 0.6
0.25 mM FeSO₄ + 0.25 mM EDTA	17.6 ± 0.3

[a] Each reaction mixture contained 50 mM sodium phosphate buffer, pH 3.0, 6.25 mM sodium benzoate, 8.0 mM ascorbic acid, 10.5 mM hydrogen peroxide, and the additions indicated in a total volume of 2.0 mL. Reaction mixtures were incubated at 25 °C in sealed vials and aliquots removed at 15 min for analysis of benzene. [b] ND, none detected.
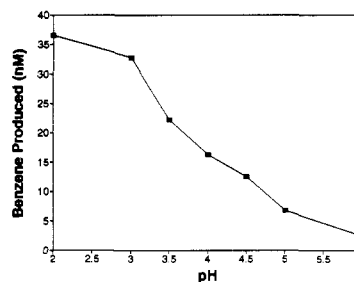

Figure 3. Dependence of benzene production on pH of the solution. Buffer solutions were prepared as described in the text. Reaction conditions were as in Table I, with 0.25 mM CuSO₄ and varying buffer solutions.

duced in the absence of H_2O_2 when the reaction mixture was heated to 50 °C for 3 h (25.0 ± 1.4 nM benzene produced, with 8 mM ascorbic acid, 6.2 mM sodium benzoate, and 0.25 mM CuSO₄ in 50 mM phosphate buffer, pH 3.0). Hydroxyl radical could be formed under these conditions by the sequential reduction of atmospheric O_2 present in solutions, as shown in eqs 1–4.

Dependence of the Reaction on Transition-Metal Catalyst. Benzene production was optimum at 1.0 mM CuSO₄ concentration (Table I); higher concentrations of this metal ion resulted in a small decrease in benzene production. When CuSO₄ was omitted from the reaction mixture, there was a significant amount of benzene produced (6.7 ± 1.9 nM), which could be completely suppressed by addition of 0.1 mM desferioxamine, an iron chelator that suppresses iron-catalyzed production of hydroxyl radical by trace amounts of iron in distilled water and buffer salts (Buettner, 1986).

Addition of 0.05 mM FeSO₄ to the reaction mixture, in the absence of added CuSO₄, gave optimum benzene production; higher concentrations of FeSO₄ resulted in suppression of benzene production. These results suggest these metal ions are necessary to catalyze hydroxyl radical production but at higher concentrations may interfere with benzene production, possibly by catalyzing reactions in the free-radical chain mechanism that do not yield benzene. When an equimolar amount of EDTA was added with 0.25 mM FeSO₄, there was a small increase in benzene production relative to addition of iron without EDTA.

pH Dependence of the Reaction. The benzene yield was measured for this reaction over the pH range 2–7. The maximum amount of benzene was produced at pH 2, with a sharp drop in benzene production from pH 3 to pH 5 (Figure 3), and benzene was not detectable in reaction mixtures at pH 7. These results indicate that hydroxyl

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radical attack on benzoic acid may yield benzene, but attack on the benzoate anion apparently yields other products, such as hydroxybenzoates, phenol, or biphenyl (Loeble et al., 1951; Armstrong et al., 1960; Sugimore et al., 1960; Sakumoto et al., 1961).

Positive Identification of Benzene in Reaction Mixtures by GC/MS Analysis. Complete reaction mixtures were placed in sealed vials and warmed to 45 °C, and the headspace gas was analyzed by GC/MS. There was a major peak corresponding to benzene, in both GC retention time and mass spectrum, in the headspace gas of the reaction vial. This peak was not observed in reaction blanks that were lacking ascorbic acid, nor was it observed in headspace gas of vials containing ascorbic acid but lacking sodium benzoate in the reaction buffer.

To our knowledge, this is the first demonstration of benzene production from benzoic acid in the presence of a hydroxyl radical generating system. The conditions for this reaction suggest that benzene could be produced in food products, especially acidic beverages, containing the combination of ascorbic acid and sodium benzoate. The metal ion catalysts are most likely present in water used in the preparation of these foods. Although the yield of benzene is extremely small (<50 nM or <1 ppb) under conditions selected to approximate relative amounts of these compounds in foods or beverages, the combination of ascorbic acid and sodium benzoate in foods and beverages should be evaluated more carefully, in view of these results.

ACKNOWLEDGMENT

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