Autoxidative Phenolic Ring Opening under Alkaline Conditions as a Model for Natural Polyphenols in Food

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Ring opening occurred when phenanthrenequinone was subjected to alkaline (pH 13) oxidative conditions at room temperature in methanol and 100% oxygen. The reaction was followed by HPLC, and spectra were obtained with a diode array detector. The spectra and retention times of the compounds formed were the same as those of authentic diphenic acid used as a standard and prove ring opening. Similar reactions have been hypothesized for *o*-dihydroxyphenols found in food systems. This research demonstrates its occurrence.

Nonenzymic autoxidative reactions involving polyphenolic compounds in food systems lead to undesirable brown oxidation compounds in the absence of polyphenol oxidase. These reactions occur during processing or storage. Several of these oxidized products have been isolated and identified (Cilliers and Singleton, 1990). Certain oxidative conditions could mimic enzymic ring opening (Pandell, 1983), a reaction common in catabolism of specific phenols by microorganisms, plants, and animals. The possible opening of the aromatic ring in polyphenols during autoxidation under alkaline conditions has been suggested by Cilliers and Singleton (1989) and Tulyathan et al. (1989) using caffeic and gallic acids as models for naturally occurring phenols. This would account for the very high amount of oxygen taken up by these phenols. Particularly in the gallic acid case it is not possible to account for the oxygen uptake without a reaction of this nature.

In this research we used phenanthrenequinone as a model for *o*-dihydroxyphenols to test for ring opening. This compound was chosen as the only readily available tetrasubstituted *o*-quinone to prevent competitive polymerization and substitution reactions.

MATERIALS AND METHODS

Phenanthrenequinone (0.1 g) from Aldrich Chemical Co. (Gold label, Milwaukee, WI) was oxidized in 50 mL of methanol (HPLC grade from Fisher Scientific, Pittsburgh, PA) with a magnetic stirrer at room temperature in a stoppered Erlenmeyer flask. The pH was adjusted to 13 with 10% KOH and 100% oxygen bubbled through the solution to be oxidized. Samples were taken at different times up to 100 h with 100% oxygen bubbled through after each sampling and the flask resealed. The reaction was stopped in the subsamples by acidifying with 2 μL of concentrated H_2SO_4 per 1.5 mL of sample in a vial.

HPLC. The HPLC conditions are as described by Cilliers and Singleton (1989). A Hewlett-Packard (Santa Clara, CA) 1090M HPLC with a diode array detector was used. It was connected to a Hewlett-Packard 9000 series 300 ChemStation for data handling. Three wavelengths (200, 280, and 320 nm) were monitored simultaneously, and scanning was done from 200 to 400 nm with the acquisition wavelength 200 nm. A Microsorb (Rainin Instrument Co. Inc., Woburn, MA) column (10 × 0.46 cm) packed with 3 μm C_{18} reversed-phase packing material was used for analysis. All separations were carried out at 40 °C after injection of 20 μL of sample.

Mobile phase A was 0.05 M ammonium phosphate containing 0.001 M triethylamine. The pH was adjusted to 2.6 with 85% phosphoric acid. Mobile phase B was 80% acetonitrile and 20% mobile phase A. The linear gradient used was started with 30% B to 70% B in 20 min. It was then run isocratic at 70% B for 5 min and then back to the initial conditions.

As authentic standard for the ring-opened product, biphenyl-2,2′-dicarboxylic acid or diphenic acid (Aldrich) was used to establish retention times and UV–visible spectra.

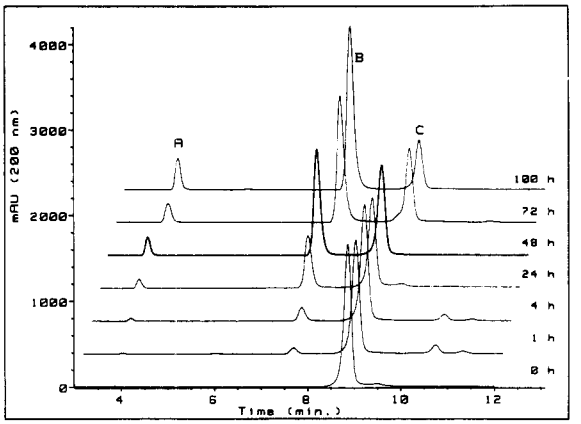

Figure 1. Stacked chromatograms of the oxidation of phenanthrenequinone (C) in methanol for 0–100 h at pH 13. Reversed-phase HPLC separation and detection at 200 nm show the oxidation products diphenic acid (A) and its dimethyl ester (B).

RESULTS AND DISCUSSION

Increased oxygen uptake by certain phenols, e.g., gallic acid, which is much greater than explainable by simple quinone formation, can be explained if opening of the aromatic ring occurs (Tulyathan et al., 1989). When this happens, muconic acid derivatives containing two new carboxylic groups will form. This will account for two additional atoms of oxygen taken up per molecule oxidized. Oxidizing caffeic acid under alkaline conditions leads to an increase in the concentration of one compound much more polar than caffeic acid or its dimers and believed to be an open-ring compound (Cilliers and Singleton, 1989). To prove ring opening does occur under alkaline conditions, we used phenanthrenequinone. It did form the ring-opened, oxidized product *o*-diphenic acid. Formed diphenic acid was identified by its retention time and UV–visible spectrum with the authentic standard. Diphenic acid formed did produce an additional compound lower in polarity (on the reversed-phase system it increased retention time) which gave the identical spectrum and is identified as the dimethyl ester form of diphenic acid since this reaction was carried out in 90+% methanol owing to

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the insolubility of phenanthrenequinone in water. This is shown in Figure 1.

We conclude that ring opening of *o*-quinones does occur during alkaline oxidation by air with no special additions. Hydrogen peroxide should not be formed as in the case of *o*-dihydroxyphenols (O_2 should add directly) and, in any case, would not be involved in this reaction since it would be in the unreactive sodium salt form at this high pH (pK_a H_2O_2 = 12). Tulyathan et al. (1989) provided evidence that H_2O_2 formed in quinone production but not in ring splitting during gallic acid oxidation.

The use of phenanthrenequinone simplified the system since this quinone is "locked in" and can only give rise to a very limited number of reactions involving the quinone. Ring opening was found to be the case and lends support to the general importance of reactions of this type in wine and other foods (Singleton, 1987).

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