Oxidation Kinetics of an Antiasthmatic, 2-[(4-Hydroxyphenyl)amino]-5-methoxybenzenemethanol, and Stabilization with Ascorbic Acid

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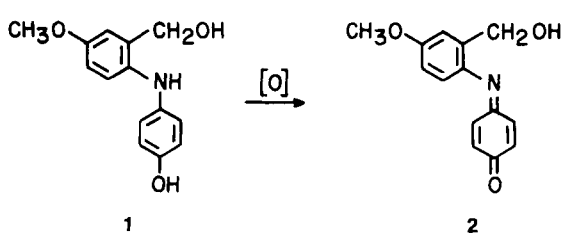
Abstract □ A novel antiasthmatic, 2-[(4-hydroxyphenyl)amino]-5-methoxybenzenemethanol (**1**), oxidizes to the corresponding iminoquinone in aqueous solutions. The reaction was monitored by a paired-ion reversed-phase HPLC method. The oxidation rate was highly dependent on the solution pH, with a large rate increase occurring above pH 6.1. In nonaqueous solution, the oxidation reaction was significantly slower. Ascorbic acid protects **1** from oxidation. The aqueous solution of the decomposed product is reduced to **1** in the presence of ascorbic acid.

2-[(4-Hydroxyphenyl)amino]-5-methoxybenzenemethanol (**1**) is a novel antiasthmatic that acts through inhibition of lipoxygenase product formation in the arachidonic acid pathway.[1] These lipoxygenase metabolic products, leukotrienes, are potent contractile agents of vascular and pulmonary smooth muscle.

Compound **1** turns red in solution due to its oxidation to 4-[[2-(hydroxymethyl)-4-methoxyphenyl]imino]-2,5-cyclohexadien-1-one (**2**) (Scheme I). The reaction is predicted based on the reactivity of the *p*-*N*-arylaminophenol moiety of the molecule. For example, the oxidation of *p*-aminophenol to a transient intermediate, *p*-quinonimine, which in aqueous solution hydrolyzes to *p*-quinone, is a well-known reaction.[2] Rapid autoxidation of *p*-hydroxydiphenylamine (HDPA) to give *N*-phenyl-*p*-benzoquinoneimine in mixtures of water and acetonitrile was reported and a mechanism was postulated involving electron transfer to oxygen from the HDPA anion.[3] Ascorbic acid and sodium metabisulfite were previously reported to be used as general antioxidants.[4,5] The two agents were investigated to stabilize **1** in this study. The objective of our study was to determine the oxidation rates of **1** under different pH conditions, characterize the oxidation product, and stabilize the molecule in aqueous solution.

Experimental Section

Materials—All reagents were analytical grade. 2-[(4-Hydroxyphenyl)amino]-5-methoxybenzenemethanol (**1**) and 4-[[2-(hydroxymethyl)-4-methoxyphenyl]imino]-2,5-cyclohexadien-1-one (**2**) were supplied by the Medicinal Chemistry Group of Sterling Research Group.



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Scheme I—Oxidation of **1** to **2** in aqueous solution.

High-Performance Liquid Chromatographic Assay Method—The chromatographic system consisted of a Varian 5000 liquid chromatograph, Vari-Chrom variable wavelength detector, and a Varian model 9176 recorder. The mobile phase was delivered at a flow rate of 2 mL/min from two reservoirs and consisted of 80% A (1% acetic acid and 0.005 M heptane sulfonate sodium) and 20% B (methanol). All reagents were analytical grade. A Zorbax-TMS column (25 cm × 4.6 mm) was used with UV detection at 284 nm. The lower limit of sensitivity for the assay of **1** is 0.5 μg/mL at 0.01 AUFS. Solutions were diluted to within 50–100 μg/mL with nitrogen-purged distilled water and then injected into a Rheodyne 7125 injector with a 20-μL loop.

Kinetic Study—Solutions of **1** (50 μg/mL) were prepared in 0.2 M lactate buffers of pH 2.5 and 4.6 and 0.2 M phosphate buffers of pH 6.1, 7.4, 7.9, and 8.3. These solutions were immediately assayed and then stored at room temperature (~25 °C). At various time intervals up to 260 min, the solutions were assayed. Also, unbuffered aqueous solutions of 50 μg/mL of **1** in 0.1% ascorbic acid or sodium metabisulfite were prepared and analyzed within 24 h. Additionally, two 0.5-mg/mL samples of **1** in ethanol and a 0.5-mg/mL sample of **1** in distilled water (pH 7.1) were also prepared and stored at room temperature for 1 month. Both the ethanol and aqueous samples were scheduled for assay after 4 and 30 days, respectively (the 30-day aqueous sample was not assayed as all the compound was decomposed after 4 days). Also, a 50-μg/mL aqueous solution of **1** in distilled water (pH 7.1) was prepared and assayed about every half hour for 4 h to determine short-term stability. Two 50-μg/mL samples of **1**, stabilized with 0.1% ascorbic acid in pH 7.9, 0.2 M phosphate buffer and distilled water (pH 7.1), respectively, were also assayed periodically for up to 4 h at room temperature. Two 1-mg/mL sample solutions of **1** were prepared in dimethylsulfoxide and assayed after 5 days of storage at room temperature.

Solubility Study—Saturation solubility of **1** was determined in 0.2 M aqueous phosphate (pH >6) or lactate buffers (pH <6), stabilized with 0.1% ascorbic acid. All buffer solutions were purged with nitrogen prior to shaking. Equilibration was achieved with 4 h of shaking with excess drug. The samples were then filtered and assayed by the HPLC method. The pH of the filtrates was measured with an Orion pH meter.

Spectrophotometric Measurements—Absorption spectra were obtained using a Spectronic 2000 spectrophotometer (Bausch and Lomb). Spectra were recorded from 400 to 200 nm for 5-μg/mL solutions of **1** and **2** (Figure 1) in absolute ethanol.

Results and Discussion

Chromatographic Method—The separation of **1** and **2**, the oxidized product, is shown in Figure 2. Due to the rapid oxidation of **1**, all standard solutions were prepared in 0.05% ascorbic acid. No interference in the assay was caused by the ascorbic acid which elutes very quickly. The retention time for **1** is 2.7 min and that for **2** is 5.7 min. The mean and standard deviation for five replicate assays of an independently prepared 0.3-mg/mL aqueous solution of **1** (diluted to 50 μg/mL during analysis) is 99.8 ± 0.91% of the nominal value. The detection wavelength was at 284 nm, the maximum for **1** (Figure 1, absorptivity of 52). Compound **2**, which has a

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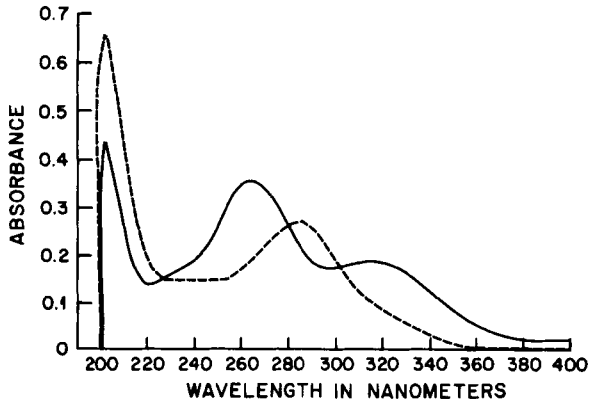

Figure 1—The UV spectrum of **1** (- - - -) and oxidized product, **2** (———); 5 mg/mL in absolute ethanol.

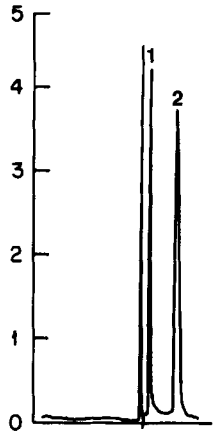

Figure 2—Chromatogram showing separation of **1** and **2**.

maximum at 264 nm (absorptivity of 72), also absorbs strongly at 284 nm.

Kinetic Study—The decrease in the concentration of **1** with time is first order, as shown in Figure 3. The reaction is pH sensitive, with the slowest rate of oxidation occurring at pH 6.1 and a rapid increase in rate when the pH is increased. For example, the reaction rate at pH 8.3 is ~635 times faster than at pH 6.1. A smaller rate increase occurs when the pH is lowered. At pH 2.5 the reaction rate is ~27 times faster than at pH 6.1. A listing of these first-order rate constants is given in Table I.

A minimum in the solubility of **1** also is seen at ~pH 6.5 (Table II). It is apparent that ionization of the phenol group is accompanied by a large increase in solubility and oxidative rate, whereas smaller increases occur in ionization of the amine function. A great increase in the oxidation rate of *p*-hydroxydiphenylamine in the presence of oxygen was also shown by Ram et al.[3] when the pH was increased from 7.5 to 10.0 in an acetonitrile:water (1:1) mixture. Their mechanism involves formation of the phenolate anion which readily oxidizes, forming the corresponding benzoquinonimine.

In nonaqueous solvents, the oxidation rate is slower; for example, only 16% of **1** reacts after 30 days at room temperature when dissolved in absolute ethanol (0.5 mg/mL), whereas 85% reaction occurs for a solution in water after 4 h (pH 7.1). This great acceleration of reaction rate with the addition of water has been shown for *N*-phenyl-*p*-aminophenol, where the addition of 80% water to acetonitrile increased the rate 767 times.[3] This rate acceleration could be due to enhanced ionization or an effect on a polar transition state. There is no evidence of general base catalysis involvement in the phosphate-buffered solution. Also, after 5 days at room

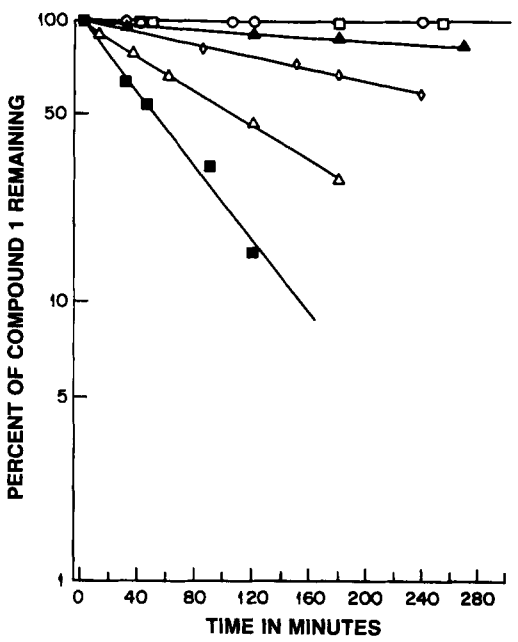

Figure 3—Compound **1** decomposition at 25 °C in various buffered solutions. Key: (▲) pH 2.5; (○) pH 4.6; (□) pH 6.1; (◇) pH 7.4; (△) pH 7.9; (■) pH 8.3.

temperature, no reaction occurred for a 1-mg/mL solution of **1** in dimethylsulfoxide.

The decrease in **1** and the increase in the oxidized product (**2**) with time is shown in Figure 4 for a distilled water solution (pH 7.1) and a phosphate buffer solution (pH 7.9). The stoichiometry of the reaction is good, indicating the formation of only one product throughout at least one to two half-lives. No significant difference in degree of reaction is apparent for the solution in distilled water compared with pH 7.9 buffer solution (0.2 M). Some difference in reaction rate due to ionic strength was observed for *N*-phenyl-*p*-aminophenol, where the oxidation rate was ~40% faster with no salt ($NaNO_3$) added compared with the 0.25 M salt solution.[3]

Multiple reaction products were observed when 0.1% sodium metabisulfite was added to a 50-μg/mL solution of **1**. The structures of these products are not known. However, sodium

Table I—First-Order Rate Constants of Compound 1 Decomposition at 25 °C

pH	k, min^{-1}
2.5	7.05×10^{-4}
4.6	8.53×10^{-5}
6.1	2.36×10^{-5}
7.4	2.39×10^{-3}
7.9	7.00×10^{-3}
8.3	1.50×10^{-2}

Table II—Solubility of Compound 1 in Lactate (below pH 6) and Phosphate (above pH 6) Buffers at 25 °C

Buffer	pH	Solubility, mg/mL
Phosphate (0.2 M)	8.0	27.1
	7.9	2.3
	7.4	1.1
	6.8	0.38
	6.5	0.32
	6.3	0.37
Lactate (0.2 M)	5.6	2.64
	4.6	2.96

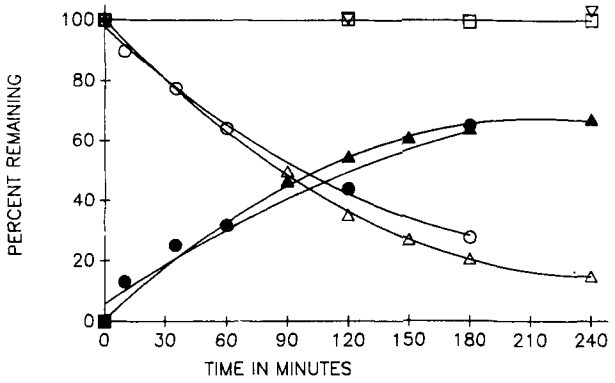

Figure 4—Compound **1** decomposition at 25 °C with and without ascorbic acid. Compound **1** decomposition at pH 7.1 (△) and pH 7.9 (○) in aqueous solution. Compound **2** formation at pH 7.1 (▲) and pH 7.9 (●), respectively. Compound **1** stabilized with 0.1% ascorbic acid at pH 7.1 (□) and pH 7.9 (▽) in aqueous solution.

bisulfite would be expected to react with **2** by 1,4-addition across the iminoquinone moiety.[2]

No change in the concentration of **1** was observed in the presence of 0.1% ascorbic acid. Further, the addition of ascorbic acid to oxidized solutions of **1** and to a solution of **2** resulted in instant reformation of **1**.

Conclusions

The rapid oxidation of **1** at 25 °C in aqueous solution was shown to produce the corresponding iminoquinone (**2**). Separation and quantification of these two compounds were accomplished by using a paired-ion reverse-phase HPLC method. In ethanol or dimethylsulfoxide solutions, the oxidation rate of **1** is considerably slower than in aqueous solutions. As expected, the oxidation rate is highly dependent on the solution pH; above pH 6, the reaction rate increases greatly. For formulating solutions to be used in in vitro testing at body pH, it was necessary to stabilize the compound. Ascorbic acid protects **1** from oxidation, and **2** reduced back to **1** in the presence of ascorbic acid.

References and Notes

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