Model Studies of Topaquinone-Dependent Amine Oxidases. 1. Oxidation of Benzylamine by Topaquinone Analogs†

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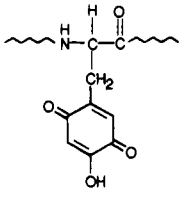
Abstract: The aerobic oxidation of benzylamine by model compounds of topaquinone, the active site organic cofactor in copper-containing amine oxidases, was studied in order to elucidate the chemical function of the cofactor in substrate oxidation. In this study, topaquinone hydantoin ($\mathbf{1}_{ox}$) and a series of 2-hydroxy-5-alkyl-1,4-benzoquinones which differ in the bulk of their alkyl substituent (**5**, **6**, **7**, and **8**) were employed as model compounds of the cofactor. The *p*-quinones (**9**, **10**, **11**, and **12**) and the *o*-quinones (**13** and **14**) were prepared in order to compare them to the topaquinone analogs. Benzylamine was oxidized by the topaquinone analogs ($\mathbf{1}_{ox}$, **5**, **6**, **7**, and **8**) to yield *N*-benzylidenebenzylamine ($PhCH{=}NCH_2Ph$) as a sole product in acetonitrile at room temperature. The quinones bearing a bulky substituent ($\mathbf{1}_{ox}$, **5**, and **6**) were found to be more efficient catalysts than those bearing a small primary alkyl group (**7** and **8**). In the latter case, the dimers (**16** and **17**) of the substrate Schiff base intermediates (**15**, R = methyl, ethyl) were isolated. The *p*-quinones (**9**, **10**, **11**, and **12**) were catalytically inactive. The *o*-quinones (**13** and **14**) had detectable catalytic activity at room temperature. In anaerobic reactions of the *o*-quinones (**13** and **14**) with benzylamine, quantitative formation of the product ($PhCH{=}NCH_2Ph$) was observed. For both *o*-quinones, products and intermediates which support a transamination mechanism were identified by ^1H NMR spectroscopy. The order of reactivity of quinones (**5** > **14** > **13**) reflects their redox potentials, such that regeneration of quinone may be rate-determining with *o*-quinones. These results demonstrate a substantial role of the 2-hydroxyl group of the topaquinone in preventing the formation of Michael adducts with substrate amine and in facilitating the reoxidation of aminoresorcinol intermediates.

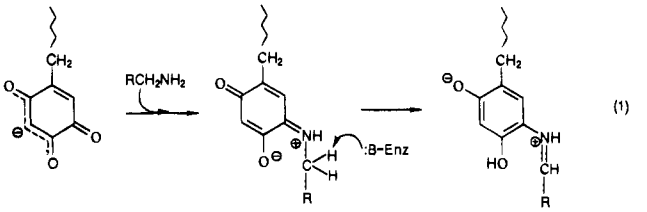
Introduction

Since topaquinone (6-hydroxydopaquinone) was first identified as the covalently bound active site cofactor of bovine serum amine oxidase (BSAO) in 1990,[1] copper-containing amine oxidases from mammals, plants,[2] and micro-organisms[3] have been demonstrated to be topaquinone-dependent enzymes. These



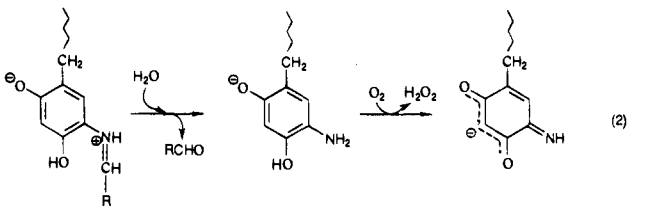
Topaquinone

enzymes catalyze the oxidative deamination of a primary amine to produce the corresponding aldehyde and ammonia, concomitant with a two-electron reduction of dioxygen to hydrogen peroxide. As will be described in greater detail in the accompanying paper, enzyme mechanistic studies[4] have implicated the formation of a covalent Schiff base complex between substrate and cofactor, which undergoes proton abstraction to yield a product Schiff base complex:



(1)

Hydrolysis of this product Schiff base leads to a reduced cofactor (as aminoresorcinol), which undergoes reoxidation coupled to a two-electron reduction of dioxygen to hydrogen peroxide:



(2)

In an earlier study of model compounds, topaquinone hydantoin ($\mathbf{1}_{ox}$), its quinol ($\mathbf{1}_{red}$), and 4-amino-6-ethylresorcinol (**2**) were synthesized to represent different forms of the enzyme-bound cofactor and a dopa analog ($\mathbf{3}_{red}$) was also synthesized for comparative purposes.[5]

Extensive spectroscopic and electrochemical studies have shown that the quinone ($\mathbf{1}_{ox}$) has a pK_a of 4.13 ± 0.01 (3.0 ± 0.2 in the enzyme-bound form) and that its anionic species has a characteristic broad absorption band at 484 nm. The quinol ($\mathbf{1}_{red}$) and the aminoquinol (**2**) have no absorption in this region, comparable to the reduced form of the enzyme.[5] The pK_a values of the reduced cofactors ($\mathbf{1}_{red}$, **2**) show significantly elevated

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(1) Janes, S. M.; Mu, D.; Wemmer, D.; Smith, A. J.; Kaur, S.; Maltby, D.; Burlingame, A. L.; Klinman, J. P. *Science* **1990**, *248*, 981.
(2) Janes, S. M.; Palcic, M. M.; Scaman, C. H.; Smith, A. J.; Brown, D. E.; Dooley, D. M.; Mure, M.; Klinman, J. P. *Biochemistry* **1992**, *31*, 12147.
(3) Mu, D.; Janes, S. M.; Smith, A. J.; Brown, D. E.; Dooley, D. M.; Klinmnan, J. P. *J. Biol. Chem.* **1992**, *267*, 7979.
(4) Hartmann, C.; Brzovic, P.; Klinman, J. P. *Biochemistry* **1993**, *32*, 2234.
(5) Mure, M.; Klinman, J. P. *J. Am. Chem. Soc.* **1993**, *115*, 7117.

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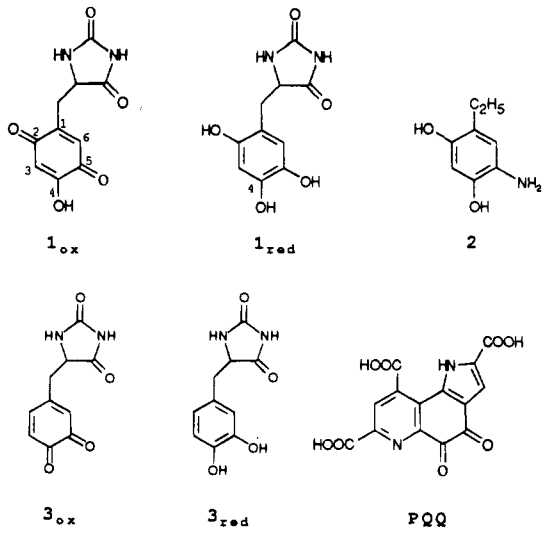
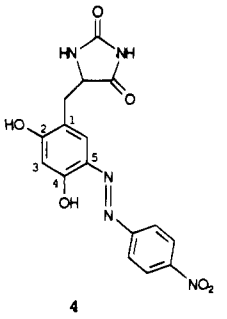


values with an increase of ca. 5 pH units. It has been proposed that the ionization state of the 4-hydroxyl group (see numbering in structures $\mathbf{1}_{ox}$ and $\mathbf{1}_{red}$) controls the differential stability of reaction intermediates. Electrochemical studies show that topaquinone $\mathbf{1}_{ox}$ has a ca. 300 mV less positive redox potential than dopaquinone $\mathbf{3}_{ox}$ but that its redox potential is similar to pyrroloquinoline quinone (**PQQ**) at physiologic pH. In other words, the extra hydroxyl group has a marked effect, increasing the oxidizing capacity of the reduced form of topaquinone by 300 mV. Using (4-nitrophenyl)hydrazine, a potent inhibitor of copper amine oxidases, the position of attack of the nucleophile on topaquinone has been shown by 2D NMR experiments to be at C_5 (see numbering in **4**), the carbonyl carbon next to the

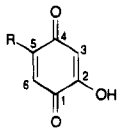


hydroxyl group. Since **4** exhibits spectroscopic properties similar to (4-nitrophenyl)hydrazone derivatives of native enzymes and identical to active site derived peptides, similar chemistry has been inferred at the active site of copper amine oxidases.[2]

The goal of the present study has been to examine the ability of model compounds of the topaquinone cofactor to act as amine oxidants. We have investigated structure–reactivity correlations in the catalytic oxidation of benzylamine using model compounds which include $\mathbf{1}_{ox}$, as well as a series of 2-hydroxy-5-alkyl-1,4-benzoquinones which differ in the bulk of their alkyl substituent [R = *tert*-butyl (**5**), isopropyl (**6**), ethyl (**7**), and methyl (**8**)]. These studies have been carried out in anhydrous acetonitrile due to an observed inhibition of product formation by water. During the course of this study, Sayre and his coworkers reported a catalytic turnover reaction in buffered solutions of a acetonitrile/water mixture.[6] However, we find much higher catalytic efficiency in anhydrous acetonitrile.

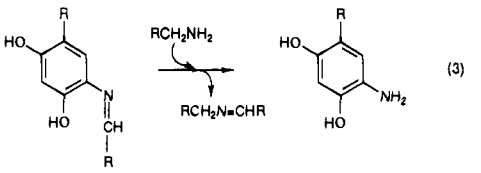
The mechanism of model compounds is expected to differ from that described for the enzyme reaction (in eqs 1 and 2).

(6) Wang, F. J.; Bae, J. Y.; Jacobson, A. R.; Lee, L. M.; Sayre, L. M. *J. Org. Chem.* **1994**, *59* (9), 2409.



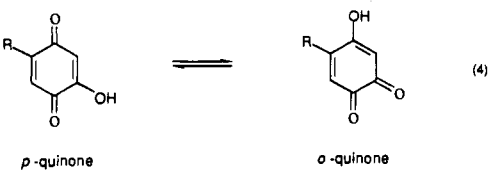
R = $C(CH_3)_3$ **5**
$CH(CH_3)_2$ **6**
CH_2CH_3 **7**
CH_3 **8**

As illustrated below, trapping of the product Schiff base was anticipated to occur with excess amine substrate rather than solvent water:

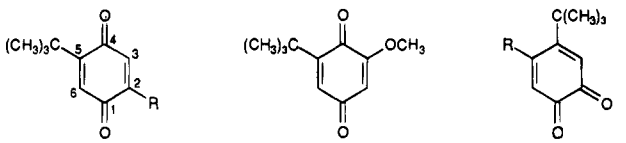


Since model reactions described herein have been largely conducted under an atmosphere of saturating oxygen, reoxidation of the product aminoresorcinol to iminoquinone completes the catalytic cycle (cf. eq 2).

In organic solvents, topaquinone can exist as a *p*- or *o*-quinonoid structure (eq 4). In order to gain insight into which



species contributes to the reactivity of the topaquinone, several *p*-quinones (**9**, **10**, **11**, and **12**) and *o*-quinones (**13** and **14**) were also prepared.



R = OCH_3 **9**
CH_3 **10**
H **11**

12

R = H **13**
OCH_3 **14**

We report the first detailed characterization for the nonenzymatic oxidation of benzylamine by topaquinone analogs, as well as *o*- and *p*-quinones. As demonstrated, *o*-quinones give relatively weak turnover comparable to topaquinone analogs, whereas *p*-quinones (**9**, **10**, **11**, and **12**) are inert. The overall properties of each of these catalysts and their reaction products indicate that the 2-hydroxyl group of topaquinone plays a substantial role in preventing the formation of Michael adducts with substrate amine and in facilitating the reoxidation of aminoresorcinol intermediates.

Experimental Section

Topaquinone hydantoin ($\mathbf{1}_{ox}$) was prepared by the reported method.[5] The ^1H NMR and ^{13}C NMR were performed on Bruker AM-400 MHz and AM-500 MHz spectrophotometers. Mass spectra (MS) were obtained on a VG 70-SE or a VG ZAB 2-EQ instrument. UV–vis absorbance data were obtained on a HP 8450A diode array spectrophotometer equipped with a thermostated cell holder at 25 ± 0.2 °C

(path length 1 cm). Anhydrous acetonitrile (water content of <0.005%) was purchased from Aldrich.

Synthesis of 2-Hydroxy-5-*tert*-butyl-1,4-benzoquinone (5). 2-Hydroxy-5-*tert*-butyl-1,4-benzoquinone (**5**) was prepared from 4-*tert*-butylresorcinol[7] by oxidation with Fremy's salt [$(KSO_3)_2NO$] according to reported methods[8] with some modification. Resorcinol (40 g, 0.36 mol) was dissolved in 150 g of 100% H_3PO_4 (d = 1.87 g/L). *tert*-Butyl alcohol (30 g, 0.40 mol) was added dropwise to the solution over 2 h at 38~40 °C. The reaction mixture was vigorously stirred overnight at 45 °C and quenched with water (200 mL) to form a viscous oil. The oil was dissolved in 300 mL of ether and washed with 200 mL of 12% aqueous NaOH. The remaining ether layer contained *di-tert*-butylresorcinol as a major product and some unreacted starting material. 4,6-*Di-tert*-butylresorcinol: ^{1}H NMR ($CDCl_3$) δ 1.368 (18H, s, tBu × 2), 4.788 (2H, br s, OH × 2), 6.083 (1H, s), 7.121 (1H, s). The aqueous layer was acidified with 6 N HCl to pH 3, saturated with K_2CO_3, and extracted with ether. The ether layer was dried with Na_2SO_4 and concentrated under reduced pressure to give a dark red oil. The oil was purified by silica gel chromatography with a gradient from 15% ethyl acetate and 85% petroleum ether to 25% ethyl acetate and 75% petroleum ether. 4-*tert*-Butylresorcinol was obtained as a slightly discolored oil which slowly crystallized to give opaque needles, 5.69 g (9.5%). 4-*tert*-Butylresorcinol: ^{1}H NMR ($CDCl_3$) δ 1.361 (9H, s, tBu), 4.754 (1H, br s, OH), 4.894 (1H, br s, OH), 6.219 (1H, d, J = 2.6 Hz), 6.321 (1H, dd, J = 2.6, 8.5 Hz), 7.084 (1H, d, J = 8.5 Hz).

To the mixture of 4-*tert*-butylresorcinol (1.0 g, 6 mmol) and $K_2HPO_4{\cdot}3H_2O$ (1.048 g, 4.6 mmol) in 15 mL of water was added 120 mL of water containing 4.0 g (0.015 mol) of Fremy's salt [$(KSO_3)_2NO$] and 1.048 g of $K_2HPO_4{\cdot}3H_2O$. After 2 min, the mixture was acidified with 2 N H_2SO_4 and extracted with ether. The ether layer was dried with Na_2SO_4 and concentrated to give a dark yellow solid. The solid was recrystallized from cyclohexane and further purified by rapid sublimation under reduced pressure. 2-Hydroxy-5-*tert*-butyl-1,4-benzoquinone (**5**) was obtained as golden yellow needles, 980 mg (91%). 2-Hydroxy-5-*tert*-butyl-1,4-benzoquinone (**5**): ^{1}H NMR (acetone-d_6) δ 1.271 (9H, s, tBu), 5.896 (1H, s, exchangeable with D_3O^+), 6.523 (1H, s); ^{1}H NMR (CD_3CN) δ 1.249 (9H, s, tBu), 5.907 (1H, s, exchangeable with D_3O^+), 6.546 (1H, s), 7.80 (1H, br s, OH). Anal. Calcd ($C_{10}H_{12}O_3$): C, 66.67; H, 6.67; O, 26.66. Found: C, 66.93; H, 6.85; O, 26.22.

Fremy's salt oxidation of di-*tert*-butylresorcinol also gave **5**. Di-*tert*-butylresorcinol (770 mg, 2.1 mmol), Fremy's salt (3.84 g, 0.014 mol), and K_2HPO_4 (770 mg) were dissolved in 170 mL of MeOH/H_2O (1/1, v/v). The mixture was brought to pH 8.0 with 2 N NaOH and stirred at 0 °C. After 2 h, the mixture was acidified with 2 N H_2SO_4 and extracted with ether. The ether layer was dried with Na_2SO_4 and concentrated under reduced pressure. **5** was isolated in 70% (267 mg) by flash chromatography on SiO_2 ($CHCl_3$). The preparation of additional topa models (**6**, **7**, and **8**) is described in the supporting information.

NMR Assignment of 8. All NMR spectra were acquired on a Bruker AM-400 spectrometer fitted with an inverse ^{1}H−^{13}C probe operating at a proton frequency of 400.13 MHz. ^{1}H chemical shifts were relative to a residual DMSO-d_5 signal set to 2.490 ppm. ^{13}C chemical shifts were relative to the ^{13}C signal of DMSO-d_6 set to 39.5 ppm. The long-range inverse proton carbon correlation (HMBC) spectra were processed in magnitude mode; 512 t_1 increments of 1K data points were corrected.[9] A delay of 75 ms was used in the pulse sequence to allow for the evolution of long-range couplings. All 2D spectra were zero fitted to twice their size in both dimensions prior to processing.

Synthesis of 2-Methoxy-5-*tert*-butyl-1,4-benzoquinone (9). 2-Methoxy-5-*tert*-butyl-1,4-benzoquinone (**9**) was prepared from **5** according to the reported method[10] with some modification. **5** (128.5 mg, 0.71 mmol) was dissolved in anhydrous methanol (2 mL) containing 40 μL of concentrated sulfuric acid. The mixture was heated under reflux for 2 h. The 2-methoxy quinone (**9**) precipitated during the reaction as pale yellow plates. After cooling the mixture, it was extracted with $CHCl_3$ and dried over Na_2SO_4. The solvent was removed by evaporation, and the crude product was purified by silica gel chromatography. The yellow fraction eluted with $CHCl_3$/cyclohexane = 3/7 (v/v) contained the pure quinone, 55 mg (40%); λ_{max} = 266, 360 nm in this solvent. 2-Methoxy-5-*tert*-butyl-1,4-benzoquinone (**9**): mp 154−155 °C (lit.[11] 162−163 °C); ^{1}H NMR ($CDCl_3$ δ 1.270 (9H, s, tBu), 3.781 (3H, s, OCH_3), 5.840 (1H, s), 6.526 (1H, s); HRMS (EI) $C_{11}H_{14}O_3$ (M^+) calcd 194.0943, obsd 194.0940. The synthesis of additional 1,4-benzoquinones (**10**, **11**, and **12**) is described in the supporting information.

Synthesis of 4-*tert*-Butyl-1,2-benzoquinone (13). Ag_2O (11.6 g, 0.05 mol) was added to a solution of 4-*tert*-butylcatechol (3 g, 0.018 mol) in 80 mL of ether containing a catalytic amount of Na_2SO_4. After 5 min, the ether layer was decanted and the remaining catalyst was washed with ether. The combined ether layer was concentrated to give 4-*tert*-butyl-1,2-benzoquinone (**13**) as a red solid quantitatively. 4-*tert*-Butyl-1,2-benzoquinone (**13**): ^{1}H NMR (CD_3CN) δ 1.198 (9H, s, tBu), 6.194 (1H, d, J = 2.4 Hz), 6.320 (1H, d, J = 10.2 Hz), 7.314 (1H, dd, J = 10.2, 2.4 Hz); HRMS (EI) $C_{10}H_{12}O_2$ (M^+) calcd 164.0837, obsd 164.0837. The synthesis of 4-methoxy-5-*tert*-butyl-1,2-benzoquinone (**14**) is described in the supporting information.

Aerobic Oxidation of Benzylamine Catalyzed by Quinones (1_{ox}, 5, 6, 7, 8, 9, 10, 11, 13, and 14). The O_2 gas used in this study was saturated with acetonitrile by passing it through anhydrous acetonitrile prior to use. The reaction was initiated by injecting a 125 μL aliquot of a quinone stock solution ([quinone] = 2.0 × 10^{-2} M in CH_3CN) into 5 mL of O_2-saturated CH_3CN containing benzylamine ([$BzNH_2$] = 0.05 M). The initial concentration of the quinone was 4.9 × 10^{-4} M. The reaction mixture was stirred at room temperature under an O_2 atmosphere. Benzylamine was oxidized to yield *N*-benzylidenebenzylamine (PhCH=NCH_2Ph) as the sole product. PhCH=NCH_2Ph: ^{1}H NMR (CD_3CN) δ 4.765 (2H, s, CH_2), 7.316~7.349 (8H, m), 7.774 (2H, m), 8.456 (1H, s, PhC**H**=). The formation of benzaldehyde, which was the acid hydrolysis product of *N*-benzylidenebenzylamine, was monitored by HPLC (pump, Shimadzu LC-6A, UV detector, Shimadzu SPD-6A). Benzylamine and benzaldehyde were separated at room temperature on a RAININ-C_{18} column, using 40% CH_3CN−0.1% aqueous trifluoroacetic acid, while monitoring at 254 nm. Benzylamine eluted at 2.8 min and benzaldehyde eluted at 5.5 min using a flow rate of 1.0 mL/min.

Reaction of Benzylamine and 2-Hydroxy-5-methyl-1,4-benzoquinone (8) under Anaerobic Conditions. 8 (15.4 mg, 0.112 mmol) was treated with a slight excess (1.5 equiv) of benzylamine in anhydrous methanol under anaerobic conditions. After 15 min, a white precipitate was formed. The precipitate was separated from the supernatant (a deep red solution) and recrystallized in ethanol to give dimer **16** of imine **15** (R = methyl, cf. Scheme 2) as colorless crystals: 12.5 mg (49%); ^{1}H NMR (DMSO-d_6) δ 1.080 (6H, s, CH_3 × 2), 3.507 (2H, s), 4.121 (4H, m, J = 6.1 Hz, NHC**H**$_2$Ph × 2), 5.215 (2H, s), 5.977 (2H, t, J = 6.1 Hz, N**H**CH_2Ph × 2, exchangeable with D_2O), 7.172~7.341 (10H, m, Ph × 2) λ_{max} (CH_3CN) 330 nm (ϵ 3900). The formation of dimer **17** from 2-hydroxy-5-ethyl-1,4-benzoquinone (**7**) is described in the supporting information.

X-ray Structure Analysis of the Dimer (16). Data were measured on a ENGRAF-NONIUS CAD-4 diffractometer. Mo Kα (λ = 0.710 73 Å) radiation with a graphite crystal monochrometer in the incident beam was used. The unit cell dimensions were obtained by a least-squares fit to the setting angles of the unresolved Mo Kα components of 24 reflections with 2θ between 20° and 28°. Intensity data were collected using the θ−2θ technique in the range 3 < 2θ < 45° ($\pm h,\pm k,l$). The scan width, $\Delta\theta$, for each reflection was 0.55 + 0.35 tan θ with a scan speed 5.49 deg/min. Background was measured over 0.25 × ($\Delta\theta$) added to each end of the scan. Intensity standards were measured every 1 h of X-ray exposure time and showed no significant variations. No correction for crystal decay and absorption was necessary.

Crystallographic parameters: $C_{28}H_{26}N_2O_4$, MW = 454.51, crystal dimensions (mm) 0.20 × 0.28 × 0.45, monoclinic, space group $C2/c$, a = 21.738(5) Å, b = 10.001(4) Å, c = 11.670(4) Å, β = 118.79(2)°,

(7) Tchitchibabine, A. E. *Bull. Soc. Chim. Fr.* **1935**, *5* (2), 497.
(8) Musso, H.; Maassen, D. *Liebigs. Ann. Chem.* **1965**, *689*, 93.
(9) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
(10) Woodward, R. B.; Sondheimer, F.; Taub, D.; Heusler, K.; McLamore, W. M. *J. Am. Chem. Soc.* **1952**, *74*, 4223.
(11) Hewgill, F. R.; Kennedy, B. R.; Kilpin, D. *J. Chem. Soc.* **1965**, 2905.
(12) Hansen, P. E. *Prog. NMR Spectrosc.* **1981**, *14*, 175.

V = 2223.2(25) Å^3, Z = 8, ϱ_{calcd} = 1.35 g cm^{-3}, μ(Mo Kα) = 0,85 cm^{-1}; no. of unique reflections = 1456, no. of reflections with $F^2 > 3\sigma(F^2)$ = 1263, no. of parameters = 69; final $R(F)$, $R(wF)$ = 10.6, 15.7%; final GOF = 7.07, ϱ factor = 0.03, highest peak in difference map = 0.40 e/Å^3.

Reaction of 2-*tert*-Butyl-1,4-benzoquinone (11) with Benzylamine under Anaerobic Conditions. To a solution of **11** (50 mg, 0.31 mmol) in 5 mL of degassed anhydrous acetonitrile was added a 5-fold excess of benzylamine. After being stirred at room temperature for 1 h, the solvent was removed by evaporation to give an orange red oil (63.6 mg). ^{1}H NMR of the oil revealed the formation of three products. The ratio of the products was determined by comparing the integrals of the respective signals (three 9H singlets) due to the *tert*-butyl protons. These products were isolated by silica gel chromatography using a cyclohexane−$CHCl_3$ gradient. Yields are shown in Table 3 together with those under aerobic conditions. The structure of **18** is confirmed by its meta coupling of the ring protons with a typical coupling constant (J = 2.4 Hz) as seen in **12**. A (**18**): ^{1}H NMR ($CDCl_3$) δ 1.257 (9H, s, tBu), 4.258 (2H, d, J = 5.7 Hz, NHC**H**$_2$Ph), 5.472 (1H, d, J = 2.4 Hz), 5.971 (1H, br s, N**H**CH$_2$Ph), 6.454 (1H, d, J = 2.4 Hz), 7.26~7.37 (5H, m, NHCH$_2$**Ph**); HRMS (EI) calcd for $C_{17}H_{19}NO_2$ 269.1416, obsd 269.1412; MS (EI) 269 (M$^+$, 82%), 254 (M$^+$ − 15, 100%), 227 (18%), 91 (62%). B (**19**): ^{1}H NMR ($CDCl_3$) δ 1.291 (9H, s, tBu), 4.258 (2H, d, J = 5.8 Hz, NHC**H**$_2$Ph), 5.446 (1H, s), 5.735 (1H, br s, N**H**CH$_2$Ph), 6.454 (1H, s), 7.260~7.374 (5H, m, NHCH$_2$**Ph**); HRMS (EI) calcd for $C_{17}H_{19}NO_2$ 269.1416, obsd 269.1407; MS (EI) 269 (M$^+$, 82%), 252 (M$^+$ − 17, 22%), 226 (23%), 91 (100%). C (**20**): ^{1}H NMR ($CDCl_3$) δ 1.385 (9H, s, tBu), 6.543 (2H, d, J = 1.6 Hz), 6.773 (1H, t, J = 1.6 Hz); HRMS (EI) calcd for $C_{10}H_{14}O_2$ 166.0994, obsd 166.0991; MS (EI) 166 (M$^+$, 68%), 151 (M$^+$ − 15, 100%), 123 (M$^+$ − 43, 77%).

Reaction of 2-Methyl-5-*tert*-butyl-1,4-benzoquinone (10) with Benzylamine under Anaerobic Conditions. To a solution of 50.7 mg (0.29 mmol) of **10** in anhydrous ethanol (3 mL) was added an equimolar amount of benzylamine. The mixture was refluxed under N_2 for 1 h. The solvent was removed under reduced pressure, and the residue was analyzed by ^{1}H NMR and mass spectroscopy. It was a mixture of the ring-benzylaminated quinone (**21**) in 40% yield and the corresponding amount of the quinol (**22**), and the rest was the starting material (**10**). **21**: ^{1}H NMR ($CDCl_3$) δ 1.241 (9H, s, tBu), 2.048 (1H, s, CH_3), 4.651 (2H, d, J = 6.3 Hz, NHC**H**$_2$Ph), 5.787 (1H, br t, N**H**CH$_2$-Ph), 6.475 (1H, s); MS (EI) 283.0 (M$^+$).

Synthesis of 2-Methyl-5-*tert*-butylhydroquinone (22). To a solution of the quinone (**10**) in 10 mL of anhydrous ethanol/ether (1/1, v/v) was added 5 mol equiv of aqueous $Na_2S_2O_4$ (5 mL). The mixture was vigorously stirred under N_2 at room temperature until the solution became colorless (ca. 3 min). The solution was acidified with 0.1 N HCl, extracted with ether, and dried over Na_2SO_4 to give quinol **22** quantitatively. **22**: ^{1}H NMR ($CDCl_3$) δ 1.362 (9H, s, tBu), 2.147 (3H, s, CH_3), 6.447 (1H, s), 6.700 (1H, s); MS (EI) 180.0 (M$^+$).

Reaction of 2-Methoxy-5-*tert*-butyl-1,4-benzoquinone (9) with Benzylamine under Anaerobic Conditions. To a solution of 33.2 mg (0.17 mmol) of **9** in a 3 mL of anhydrous ethanol was added an equimolar amount of benzylamine. The mixture was refluxed under N_2 for 1 h. The solvent was removed under reduced pressure, and the residue was analyzed by ^{1}H NMR and mass spectroscopy. It was a mixture of the aminophenol (**23**) and a corresponding amount of the product (PhCH=NCh$_2$Ph) in 17% yield, and the rest was the unreacted quinone (**9**). **23**: ^{1}H NMR ($CDCl_3$) δ 1.351 (9H, s, tBu), 3.865 (3H, s, OCH_3), 6.252 (s, 1H), 6.656 (s, 1H); MS (EI) 195 (M$^+$).

Reaction of 3-Methoxy-5-*tert*-butyl-1,4-benzoquinone (12) with Benzylamine under Anaerobic Conditions. To a solution of **12** (57.3 mg, 0.30 mmol) in anhydrous ethanol (3 mL) was added an equimolar amount of benzylamine. The mixture was refluxed under N_2 for 1 h. The solvent was removed under reduced pressure, and the residue was analyzed by ^{1}H NMR and mass spectroscopy. It was a mixture of ring-benzylaminated quinone **18** in 65% and the starting material (**12**).

Anaerobic Reaction of 4-*tert*-Butyl-1,2-benzoquinone (13) with Benzylamine. **13** (4 mg, 0.024 mol) was dissolved in 0.5 mL of CD_3-CN in a NMR tube and was degassed by flushing with Ar for 30 min. Benzylamine (2 mol equiv) was added to the tube, and the reaction was monitored by ^{1}H NMR. At 4 min, the formation of two species, isomers of the product Schiff bases (**26** and **27**), was detected. One was assigned as **26** by comparison with the authentic sample. The other had very close chemical shifts. The ring protons could not be distinguished from those of **26**. The signals of *tert*-butyl protons (at 1.298 ppm) and the iminoproton (N=**C**HPh at 8.765 ppm) were slightly upfield-shifted. At 25 min, the signals of the product Schiff bases completely diminished and were converted to two products. These were isomers of the aminophenols (**28** and **29**) together with the formation of a quantitative amount of the product (PhCH=NCH$_2$Ph). One isomer was identified as **28** by comparison with the authentic sample. The second isomer had very similar chemical shifts, and the ring protons could not be distinguished from those of **28**. The signal of *tert*-butyl protons (at 1.231 ppm) was slightly downfield-shifted.

Table 1. ^{1}H and ^{13}C NMR Data for **8** in DMSO-d_6

carbon no.[a]	δH[b]	H coupled with H	δC[c]	H coupled with C ($^1J_{C-H}$)	H coupled with C ($^3J_{C-H}$) or C ($^2J_{C-H}$)
1			183.7		H_3 (s)[d]
2			157.6		H_3 (w), H_6 (m)
3	5.882		108.5	H_3	
4			187.9		H_7 (m), H_6 (w)
5			146.6		H_3 (m), H_7 (w)
6	6.625	H_7[e]	130.3	H_6	H_7 (m)
7	1.920	H_6[e]	15.3	H_7	H_6 (m)

[a] For numbering system, see structure **8**. [b] Relative to DMSO-d_5 set to δ 2.49. [c] Relative to DMSO-d_6, set to δ 39.5. [d] Relative intensity of the cross peak. [e] J = 1.6 Hz.

Preparation of the Product Schiff Base (26) of 4-*tert*-Butyl-2-aminophenol (28). To 0.5 mL of a CD_3CN solution of **28** (0.024 mmol) in a NMR tube was added an equimolar amount of benzaldehyde. **28**: δ 1.218 (9H, s, tBu), 6.541 (1H, dd, J = 2.2, 8.2 Hz), 6.595 (1H, d, J = 8.2 Hz), 6.729 (1H, d, J = 2.2 Hz). **26**: δ 1.314 (9H, s, tBu), 6.845 (1H, d, J = 8.5 Hz), 7.226 (1H, dd, J = 2.3, 8.5 Hz), 7.398 (1H, d, J = 2.3 Hz), 7.660~7.700 (3H, m), 8.000~8.042 (2H, m, ortho protons of the phenyl group), 8.806 (1H, s, N=**C**HPh).

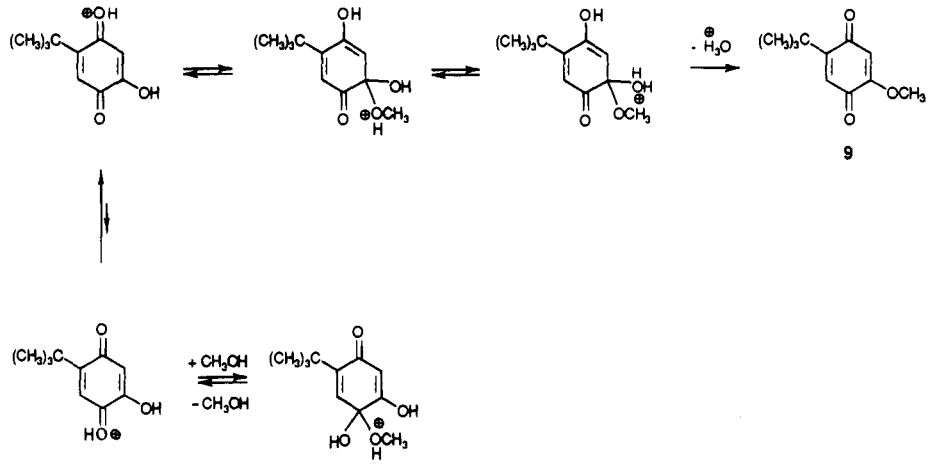
Anaerobic Reaction of 4-Methoxy-5-*tert*-butyl-1,2-benzoquinone (14) with Benzylamine. **14** (4.0 mg, 0.021 mmol) was dissolved in 0.5 mL of CD_3CN in a NMR tube and was degassed by flushing with Ar for 30 min. Benzylamine (2 mol equiv) was added to the tube and the reaction was monitored by ^{1}H NMR. At 10 min, the appearance of the signals assigned as those of the product Schiff base (**31**) was detected. **31**: δ 1.365 (9H, s, tBu), 3.814 (3H, s, OCH_3), 6.565 (1H, s), 7.317 (1H, s), 7.475~7.492 (3H, m), 7.982~8.007 (2H, m, ortho protons of the phenyl group), 8.746 (1H, s, N=**C**HPh). At 1 h, the signals of **31** were still observed. In addition to the signals of **31**, new signals most plausibly corresponding to aminophenol (**32**) and the intermediate (**33**) of the aminolysis reaction (formed by addition of benzylamine to **31**) were observed. The corresponding amount of the product (PhCH=NCH$_2$Ph) was also detected. **32**: δ 1.275 (9H, s, tBu), 3.681 (3H, s, OCH_3), 6.410 (1H, s), 6.628 (1H, s). **33**: δ 1.282 (9H, s, tBu), 3.703 (3H, s, OCH_3), 3.771 (2H, s, C**H**$_2$Ph), 6.472 (1H, s), 6.729 (1H, s), 7.26~7.35 (10H, m, Ph × 2).

Results and Discussion

Synthesis and NMR Assignment of Model Compounds. Quinones (**5**, **6**, **7**, and **8**) were easily synthesized from the corresponding 4-alkylresorcinols by oxidation with Fremy's salt [$(KSO_3)_2NO$] based on the reported method[7,8] and purified by rapid sublimation under reduced pressure. ^{1}H and ^{13}C NMR signal assignments of these quinones were based on those of 2-hydroxy-5-methyl-1,4-benzoquinone (**8**) which were assigned by 1D and 2D NMR experiments such as HMQC (heteronuclear multiple-quantum correlation) and HMBC (heteronuclear multiple-bond correlation) (Table 1).

The proton resonance at 6.625 ppm showed a coupling to the methyl proton (H_7) and thus was assigned to H_6 (J = 1.6 Hz). H_3 (5.882 ppm) was assigned on the basis of its upfield chemical shift and small intensity in CD_3OD due to the proton−deuterium exchange as seen in $\mathbf{1}_{ox}$.[5] This exchange reaction was catalyzed by D_3O^+ (DCl). Carbons were assigned by

Scheme 1. Acid-Catalyzed Formation of **9** from **5**



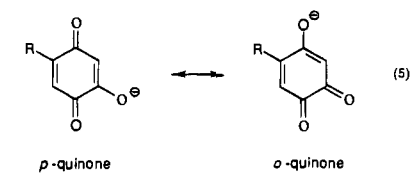
HMQC and HMBC experiments. An HMQC experiment establishes the connectivity of a proton directly bound to carbon; connectivities between C_3 (108.5 ppm) and H_3 (5.882 ppm), C_6 (130.3 ppm) and H_6 (6.625 ppm), and C_7 (15.3 ppm) and H_7 (1.920 ppm) were observed. The D−H exchange reaction decreased the intensity of the C_3 signal (108.5 ppm), which became a triplet in CD_3OD. In an HMBC experiment, correlations can be observed between a proton and a carbon two or three bonds away, where $^3J_{C-H}$ (three-bond coupling) is larger than $^2J_{C-H}$ (two-bond coupling) in *p*-quinone systems.[12] There were two resonances with the typical chemical shift of a quinonoid carbonyl carbon, 183.7 and 187.9 ppm, respectively. The long-range correlation *via* a $^3J_{C-H}$ of H_7 to the carbonyl resonance at 187.9 ppm led to its assignment as C_4. A $^3J_{C-H}$ correlation of H_6 and C_4 was also observed. The carbon resonance at 183.7 ppm showed a $^3J_{C-H}$ correlation to H_3 (5.882 ppm) and hence was assigned as C_1. C_2 (157.6 ppm) shows a $^3J_{C-H}$ coupling to H_6 and a $^2J_{C-H}$ coupling to H_3, with the typical chemical shift for an aromatic carbon attached to a hydroxyl group.[5] C_5 (146.6 ppm) was assigned from a $^2J_{C-H}$ coupling to H_7 and a $^3J_{C-H}$ coupling to H_3.

2-Methoxy-5-*tert*-butyl-1,4-benzoquinone (**9**) was prepared from topaquinone analog **5** on the basis of the method by Woodward et al.[10] A methanol solution of **5** was refluxed for 2 h in the presence of a catalytic amount of concentrated sulfuric acid to yield the 2-methoxy quinone **9** in 40% yield. It is interesting to note that the 2-hydroxyl group of **5** can be substituted with methanol in the presence of acid catalyst. The fact that the reaction is catalyzed by an acid indicates the 1,4-addition−elimination mechanism as shown in Scheme 1. There may also be a competetive 1,2-addition reaction leading to the nonproductive equilibrium. Fraig et al.[13] reported that the *tert*-butyl group of **5** was cleaved off when HCl was used as an acid catalyst. In our hands, the *tert*-butyl group was retained on the ring.

Ashley prepared 2-methoxy-5-methyl-1,4-benzoquinone from 2-methyl-1,4-benzoquinone and methanol in the presence of $ZnCl_2$ as a catalyst.[14] However, we obtained only the 2-methoxy-6-*tert*-butyl-1,4-benzoquinone (**12**) from 2-*tert*-butyl-1,4-benzoquinone (**11**) under similar conditions. Without $ZnCl_2$, a mixture of **9** and **12** was obtained in poor yield. Similar results were reported by Hewgill et al.[11] In comparison to **12**, **9** had a higher mp (ca. 80 °C) and was easily crystallized. ^1H NMR showed two singlets for the ring protons of **9**, whereas those of **12** showed a coupling of 2.4 Hz. 2-Methyl quinone **10** was prepared from 2-*tert*-butyl-5-methylphenol by oxidation with CrO_3 according to the method by Carpenter et al.[15]

4-*tert*-Butyl-1,2-benzoquinone (**13**) was prepared by oxidation of 4-*tert*-butylcatechol with Ag_2O. An initial attempt to make **13** by oxidation of the catechol with $NaIO_4$ in methanol failed, and methoxy derivative **14** was obtained as the major product. The position of the methoxy group of **14** was confirmed in NOE experiments. A 39.4% NOE was observed between a ring proton at 6.282 ppm and the *tert*-butyl group (1.305 ppm). A 29.4% NOE was observed between a ring proton at 5.766 ppm and the methoxy group (3.877 ppm).

UV−Vis Spectra of Model Compounds. The UV−vis spectra of the model compounds (**5**, **9**, **10**, **11**, **12**, **13**, and **14**) and the anionic form of **5** in CH_3CN are shown in Figure 1, and their absorption maxima and ϵ values are summarized in Table 2. For *p*-benzoquinones, it is well-known that there are three absorptions: a π to π^* transition band of strongest intensity at 240 to 300 nm, a medium π to π^* transition band at 285 to 440 nm, and a weak n to π^* transition band in the visible region.[16] A more extended π-electron system is responsible for a red shift of the second π to π^* transition band but does not affect either the first or the visible band.[16] Wavelength shifts (Δ nms) for the substituents are as follows: + 60 (OH), + 52 (OMe). The methoxyquinones (**9** and **12**) have almost identical absorption maxima. The methyl group (**10**) does not have a significant resonance effect. The *o*-quinones also show three absorption bands: a π to π^* transition band at 250 to 290 nm, a second π to π^* transition band at around 370 to 470 nm, and a weak band in the visible region.[16] The intensity of the first π to π^* band is lower than that of *p*-quinones. As in *p*-quinones, substitution causes a red shift in the second band; the change in λ_{max} (Δ nm) for MeO is +34. It should be noted that the protonated form of topaquinone analog **5** shows a *p*-quinone-type absorption spectrum. Addition of *tert*-butylamine as a base causes a significant red shift of the second band (Δ nm is +126 nm) and some loss in the intensity of the first π to π^* band, attributed to a resonance hybrid between an *o*- and a *p*-quinone (eq 5).



(5)

Aerobic Oxidation of Benzylamine by Model Compounds in Acetonitrile. To an acetonitrile solution containing benzyl-

(13) Flaig, V. W.; Ploetz, T.; Biergans, H. *Annalen* **1956**, *597*, 196.
(14) Ashley, J. N. *J. Chem. Soc.* **1937**, 1471.

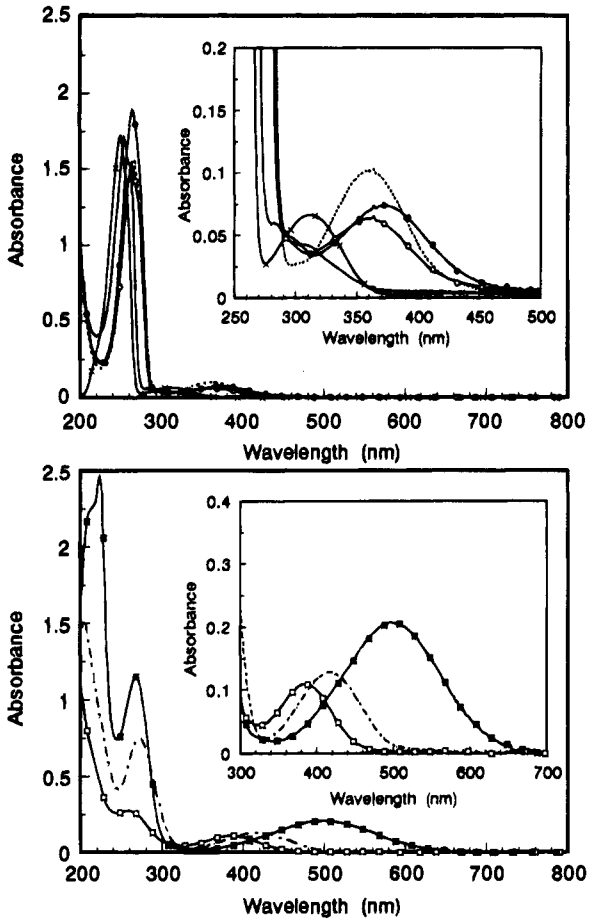


Figure 1. Absorption spectra of model compounds. ([quinone] = 1.0 × 10^{-4} M in anhydrous CH_3CN): (●) **5**, (■) anionic form of **5**, (○) **9**, (—) **10**, (×) **11**, (- - -) **12**, (□) **13**, (— - —) **14**. Inset: expansion of the spectra.

Table 2. UV–Vis Spectral Data of the Model Quinones

quinone[a]	λ_{max} (ϵ)
5	264 (19 200), 372 (700)
anionic form of **5**[b]	268 (11 600), 498 (2100)
p-quinones	
9	264 (15 400), 364 (700)
10	254 (17 300), 262 (15 200), ~310[c] (400)
11	250 (17 300), 312 (700)
12	266 (15 700), 364 (1000)
o-quinones	
13	258 (2800), 386 (1100)
14	272 (7600), 420 (1300)

[a] [quinone] = 1.00 × 10^{-4} M in CH_3CN. [b] Generated by addition of 100-fold excess of *tert*-butylamine to the solution of **5**. [c] Shoulder.

amine (50 mM) was added a catalytic amount (1 mol%) of a quinone, and the mixture was stirred at room temperature under 100% O_2 atmosphere. Benzylamine was oxidized to yield *N*-benzylidenebenzylamine ($PhCH{=}NCH_2Ph$) as a sole product. The time course of the reaction was monitored by following the formation of benzaldehyde, which was the acid hydrolysis product of *N*-benzylidenebenzylamine, on HPLC. Although the reaction proceeded slowly, ca. 70% of total benzylamine was oxidized by **5** based on an expected total yield of 25 mM benzaldehyde (Figure 2). For the 2-hydroxy quinones $\mathbf{1}_{ox}$, **5**,

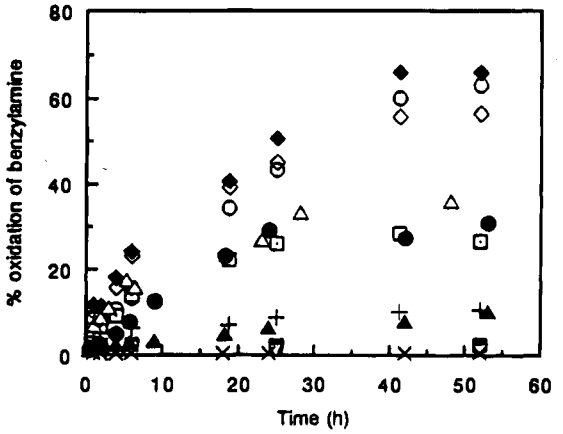


Figure 2. Time course of the oxidation of benzylamine (50 mM) catalyzed by model compounds (0.5 mM) in CH_3CN under O_2 atmosphere: (○) $\mathbf{1}_{ox}$, (◆) **5**, (◇) **6**, (△) **7**, (⊡) **8**, (■) **9**, (×) **10**, (▽) **11**, (▲) **13**, (●) **14**, (+) **5** in H_2O/CH_3CN = 1/1 (v/v).

6, **7**, and **8**, the reactivity of the quinone was dependent on the steric bulk of the alkyl substituent on the quinone ring. The bulky substituents such as hydantoin ($\mathbf{1}_{ox}$), *tert*-butyl (**5**), and isopropyl (**6**) were more favorable than the small primary alkyl substituents such as ethyl (**7**) and methyl (**8**) for the catalytic reaction. Changing the solvent from CH_3CN (polar aprotic) to either a polar protic solvent (MeOH) or a nonpolar aprotic solvent (CH_2Cl_2) did not have a significant effect on the reaction (data are not shown). Water, on the other hand, led to a marked decrease in the catalytic efficiency.

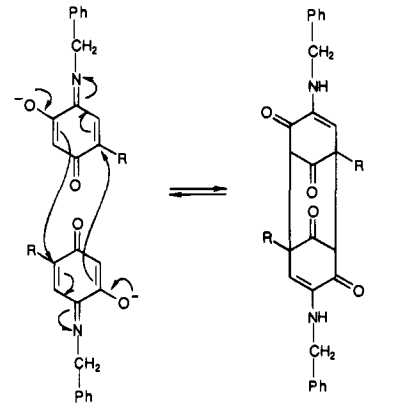
In order to study the role of the 2-hydroxyl substituent in catalysis, derivatives such as 2-methoxy- (**9**), 2-methyl- (**10**), and 2-unsubstituted (hydrogen)- (**11**) *p*-quinones were employed as catalysts. None of these *p*-quinones had a significant catalytic ability to oxidize benzylamine (Figure 2). These results clearly demonstrate the substantial effect of the 2-hydroxyl group upon the catalytic oxidation of benzylamine. The *o*-quinones (**13** and **14**) possessed some activity (Figure 2). Especially, the 4-methoxy *o*-quinone (**14**) was found to oxidize benzylamine at a rate approaching those of topaquinone analogs bearing small alkyl substituents. In previous studies, Corey and co-workers have shown the ability of *o*-quinones to support *sec*-alkyl primary amine oxidation, presumably *via* a 1,5-sigmatropic rearrangement.[17] The relationship of this process to topaquinone catalysis will be discussed in the context of mechanistic results with topa analogs (see below and the following manuscript).

Reaction of 7 and 8 with Benzylamine. On a preparative scale, these quinones (**7** and **8**) were treated with a slight excess (1.5 equiv) of benzylamine in acetonitrile. After stirring for 15 min, white precipitates were formed. Surprisingly, these precipitates proved to be the dimers (**16** and **17**) of the substrate Schiff bases (**15**, R = methyl, ethyl) (Scheme 2). This could be shown from X-ray crystallographic analysis of **16** (Figure 3). The ^1H NMR data of **17** showed a pattern similar to that for **16** (see the Experimental Section). The nucleophilic addition of benzylamine is revealed to be at C_1, next to the hydroxyl group as in the case of (4-nitrophenyl)hydrazone derivatives (**4**).[5]

It is well-known that a wide variety of quinones undergo cycloaddition with various dienes, and particularly, a quinone Diels–Alder reaction has been well studied and used in synthetic organic chemistry.[18] However, the dimers (**16** and **17**) are not

(15) Carpenter, M. S.; Easter, W. M.; Wood, T. F. *J. Org. Chem.* **1951**, *16*, 586.
(16) (a) Berger, St.; Rieker, A. In *The Chemistry of Quinonoid Compounds*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1988; Vol. II, Chapter 4. (b) Meier, A. R.; Wagniere, G. H. *Chem. Phys.* **1987**, *113*, 287.
(17) Corey, E. J.; Achiwa, K. *J. Am. Chem. Soc.* **1969**, *91*, 1429.
(18) (a) Finley, K. T. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Ed.; Wiley: New York, 1974; Section II.C, Chapter 17. (b) Naruta, Y.; Maruyama, K. In *The Chemistry of Quinonoid Compounds*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1988; Vol. II, Chapter 8.

Scheme 2. Formation of the Dimer (**16**, **17**)



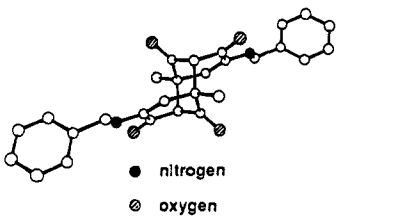


Figure 3. X-ray structure of **16**.

the products of the well-known cycloaddition reaction. A plausible mechanism for the formation of the dimers (**16** and **17**) is provided in Scheme 2. It should be noted that the dimer (**16** or **17**) is not stable in an aqueous solution and easily decomposes to the corresponding quinone (deprotonated form of **7** or **8**). When the dimer (**16**) was treated with a base such as triethylamine, the formation of benzaldehyde (in 70% yield) was confirmed by HPLC. In the catalytic system, the substrate Schiff base (**15**, R = methyl or ethyl) appears to be in an equilibrium with its dimer (**16** or **17**), and this most possibly reflects the decreased rate of the catalytic oxidation with **7** or **8**. For the substrate Schiff base with a bulky substituent (**15**, R = *tert*-butyl, isopropyl, hydantoin), no such equilibrium exists due to steric hindrance by a bulky substituent, which appears to prevent the dimerization and results in higher catalytic turnovers.

Reaction of *p*-Quinones (9, 10, 11, and 12) with Benzylamine. These *p*-quinones lacking the 2-hydroxyl group (**9**, **10**, and **11**) were found to be catalytic inactive as shown in Figure 2. It is well-known that a *p*-quinone undergoes nucleophilic 1,4-addition of an amine to form an aminoquinol which upon oxidation yields the corresponding aminoquinone.[18] The system of 2-methyl-1,4-benzoquinone and *n*-butylamine gave 2,5-bis-(butylamino)-6-methyl-1,4-benzoquinone (32%), 2,5-bis(butylamino)-1,4-benzoquinone (8%), and a large number of byproducts.[19]

We found that the reaction of *tert*-butyl-1,4-benzoquinone (**11**) and a 5-fold excess of benzylamine yielded two isomers of mono-ring-benzylaminated quinones (**18** and **19**) and the corresponding quinol (**20**) in a stoichiometric fashion (Table 3). Clearly the bulk of the *tert*-butyl group inhibits the further quinone nucleus amination. Under anaerobic conditions, the formation of 3-benzylaminated quinone **18** predominated the 2-benzylaminated one (**19**) and the corresponding amount of quinol **20** was obtained. This indicates that the quinone was

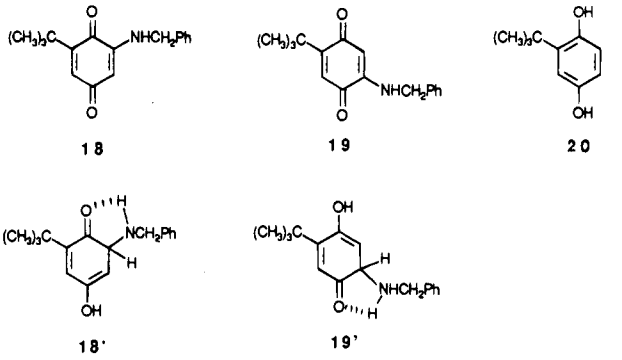
(19) Kumanotani, J.; Kagawa, F.; Hikosaka, A.; Sugita, K. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 2118–2123.

Table 3. Reaction of **11** and Benzylamine

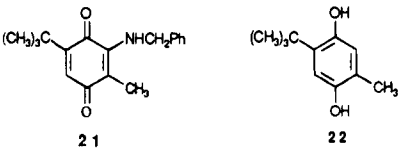
	yield		
reaction conditions[a]	**18**	**19**	**20**
Ar	40.6%	6.6%	52.8%
O_2	65.6%	18.8%	15.6%

[a] **11** (0.31 mmol) and benzylamine (1.53 mmol) in 5 mL of CH_3CN for 1 h at room temperature.

used to oxidize the aminoquinol intermediates. Under aerobic conditions, quinol **20** was reoxidized to quinone **11** and reacted further with the amine. It should be noted that **18** is the preferred product under both anaerobic and aerobic conditions, indicating that the *tert*-butyl group has some effect on the reaction. The failure to find any reaction other than ring monoamination of **11** explains the inability of this quinone to oxidize benzylamine. It should be noted that 16% of the quinol (**20**) was isolated under aerobic conditions. This indicates that the quinol (**20**) is not as redox active as $\mathbf{1}_{red}$ or **2**, consistent with the previously reported difference of 300 mV in the redox potential of topaquinone ($\mathbf{1}_{ox}/\mathbf{1}_{red}$ couple) and dopaquinone ($\mathbf{3}_{ox}/\mathbf{3}_{red}$ couple).[4] The dominant 1,4-addition over 1,2-addition is consistent with the high reactivity of unprotected *p*-quinones toward Michael attack by nucleophiles. The addition intermediate may be stabilized by hydrogen bonding between the amino group and the carbonyl group (**18'**, **19'**).



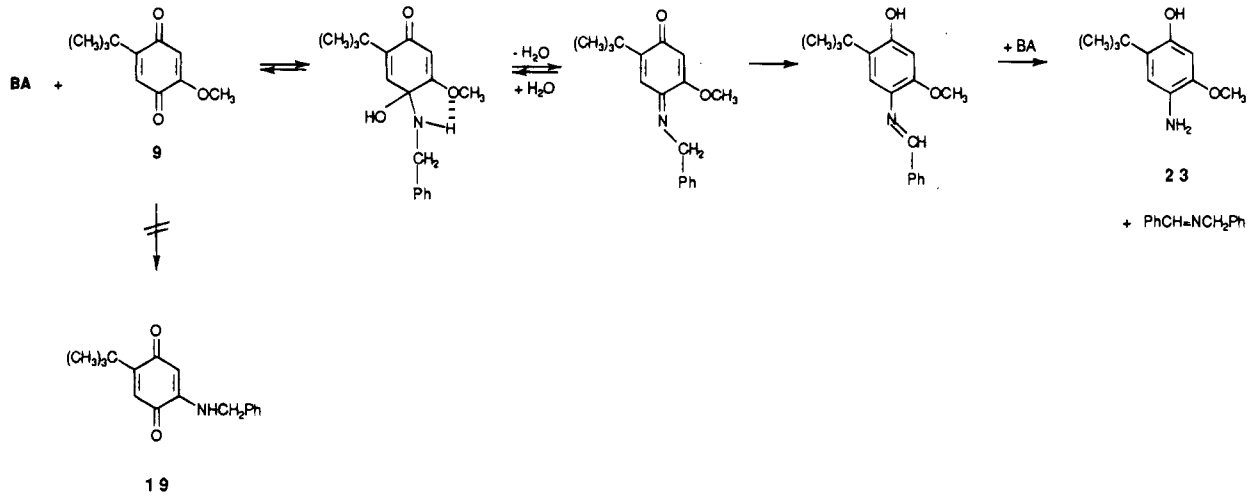
The 2-methyl quinone **10** (0.05 M) was treated with 2-fold excess benzylamine in CD_3CN at room temperature, but no reaction was detected for 1 day by ^1H NMR. When it was treated with an equimolar amount of benzylamine in ethanol at refluxing temperature under anaerobic conditions for 1 h, 40% of the quinone was converted to the ring-benzylaminated quinone (**21**) and the corresponding amount of the quinol (**22**) was obtained.



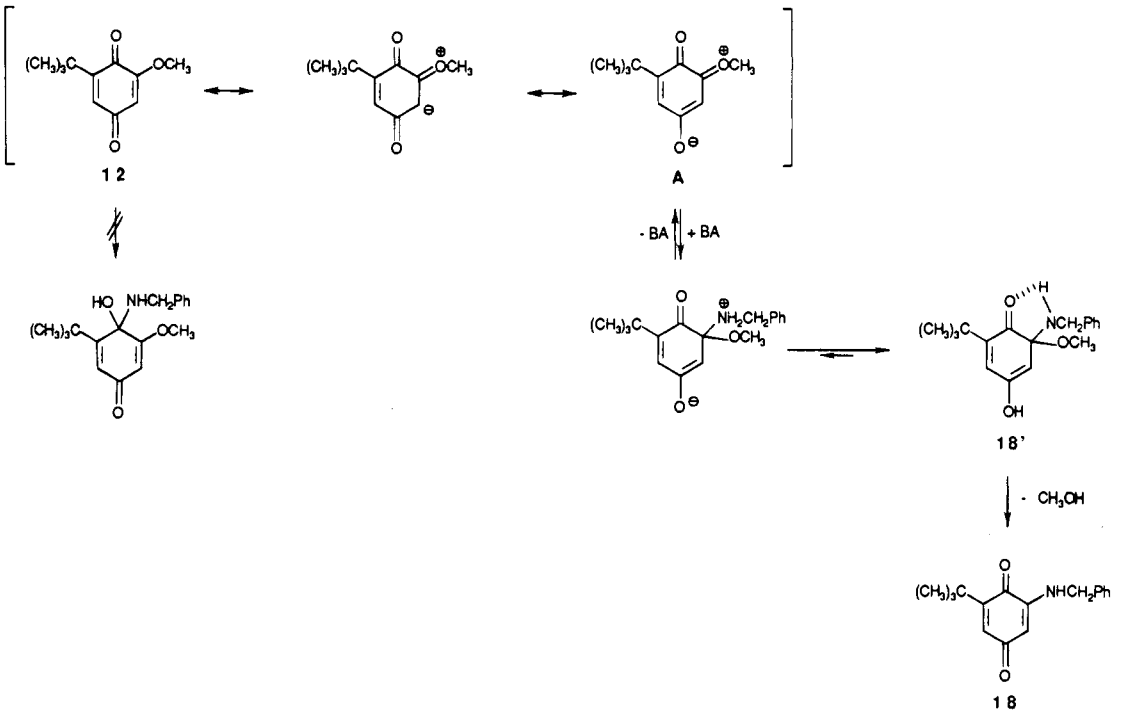
The displacement of the 2-methoxy group of 2-methoxy-5-methyl-1,4-benzoquinone by an amine was first reported by Anslow and Rainstrick in 1939.[20] They attempted the reaction of alcoholic methylamine with 2-methoxy-5-methyl-1,4-benzoquinone and isolated 2,5-bis(methylamino)-1,4-benzoquinone, in which both the methyl and the methoxy groups had been displaced by methylamine. Cameron and Scott reexamined the reaction in 1964[21] and proposed that the displacement of the methoxy group by methylamine would proceed at the initial

(20) Anslow, W. K.; Rainstrick, H. *J. Chem. Soc.* **1939**, 1446–1457.
(21) Carmeron, D. W.; Scott, P. M. *J. Chem. Soc.* **1964**, 5569–5573.

Scheme 3. Reaction of **9** and Benzylamine (BA)



Scheme 4. Reaction of **12** and Benzylamine (BA)

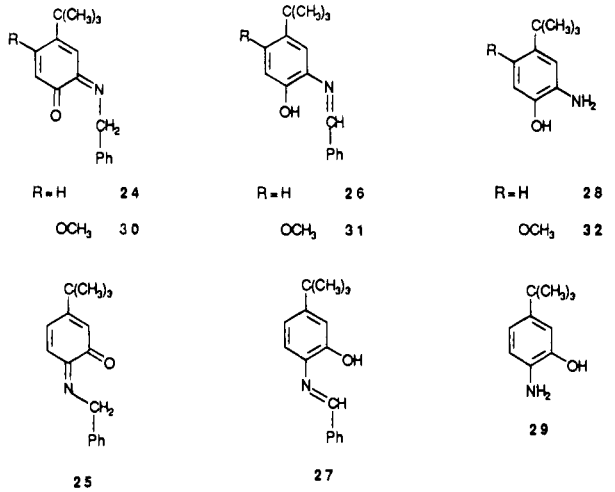


stage, although isolation of the intermediate (2-(methylamino)-5-methyl-1,4-benzoquinone) was unsuccessful. A quinone methide tautomer was proposed for the substitution of the 5-methyl group by an amine.[21]

The 2-methoxy quinone **9** was treated with a 5 molar excess of benzylamine in CD_3CN; however, no reaction was observed for 1 day by ^{1}H NMR. When it was treated with an equimolar amount of benzylamine in boiling ethanol under anaerobic conditions for 1 h, 17% of the quinone was converted to aminophenol **23** and the corresponding amount of the product (PhCH=NCH$_2$Ph) (Scheme 3). No substitution of the 2-methoxy group to yield the ring-benzylaminated quinone **19** was detected. On the other hand, 3-methoxy quinone **12** selectively underwent the substitution reaction to yield the ring-benzylaminated quinone **18** in 65% yield (Scheme 4). The absorption spectra shown in Figure 1 indicate that these quinones (**9** and **12**) have very close electronic structures and exist predominantly in their *p*-quinone forms. The substitution reaction with **12** is attributed to the reactivity of resonance forms (e.g., **A** in Scheme 4) with nucleophilic reagents; this resonance effect reduces the electrophilicity of the C_1 carbonyl of **12**. In the case of **9**, the C_1 carbonyl group does not participate in the resonance, such that the 1,2-addition of benzylamine is favored to yield the aminophenol (**23**). Although the C_4 carbonyl group of **12** is not involved in the resonance, the bulk of the adjacent C_5 *tert*-butyl group hinders the 1,2-addition reaction. As a result, the C_3 of **12** is the favored position for the nucleophilic addition. It should be noted that the methoxy group at the C_2 position of **9** could, in principle, direct the nucleophilic addition at the C_1 carbonyl carbon; however, it has no discernible effect on the rate of the reaction (Figure 2).

Reaction of *o*-Quinones (13 and 14) with benzylamine. When 4-*tert*-butyl-1,2-benzoquinone (0.052 M) was treated with 2 mol equiv of benzylamine under anaerobic conditions for 25 min, the formation of two isomers of the aminophenol (**28** in 40% and **29** in 60% yield) and the corresponding amount of the product (PhCH=NCH$_2$Ph) was detected by ^{1}H NMR. One of the isomers was identified as **28** by comparison of its chemical shifts with the authentic 4-*tert*-butyl-2-aminophenol. The other isomer has chemical shifts very close to those of **28** so that it was assigned as **29** (see the Experimental Section). At the early stage of the reaction (1.5 min), the formation of

two isomers of the product Schiff base intermediate (**26** and **27**) was detected. The product Schiff base **26** was assigned by comparison of its chemical shift with the authentic sample, which was generated by treatment of aminophenol **28** with benzaldehyde. The substrate Schiff base intermediates (**24** and **25**) could not be detected. From the product ratio, the C_1 appears to be slightly more electrophilic than C_2, reflecting the electron-donating nature of the *tert*-butyl group.



Under the same conditions, 4-methoxy-5-*tert*-butyl-1,2-benzoquinone (**14**) also gave aminophenol **32** and the product ($PhCH{=}NCH_2Ph$). At the initial stage of the reaction (10 min), the conversion of the quinone to the product Schiff base intermediate **31** was detected by both ^1H NMR and UV−Vis spectroscopy. The substrate Schiff base **30** could not be detected. Considering the electron-donating nature of the 4-methoxy group, the nucleophilic addition of the amine was expected to be at C_1. This is supported by the observation that the signal of H_6 was broadened on addition of amine and that of H_3 was unchanged. No substitution of the methoxy group was detected. The aminolysis of the product Schiff base (**31**) was found to be slower than that of **26** or **27** (see the Experimental Section). This suggests that the electron-donating nature of the 4-methoxy group reduces the electrophilicity of the imino carbon of **31** and increases the basicity of the amino group of **32**. 3,5-Di-*tert*-butylquinone is well-known as "Corey's reagent" which efficiently oxidizes *sec*-alkyl primary amines.[17] This quinone is unsuitable for oxidation of primary amines due to its preferential formation of substituted benzoxazoles.[17] In the reaction of the *o*-quinones (**13** or **14**) with benzylamine, we could not detect any formation of benzoxazole derivatives. When these *o*-quinones (**13** or **14**) were treated with an equimolar amount of benzylamine, the rate of aminolysis of the product Schiff base to the aminophenol step decreased; however, no oxazole formation was detected (for 1 day). Klein et al. studied the mechanism of the oxidative deamination of *sec*-alkyl primary amines with 3,5-di-*tert*-butyl-1,2-benzoquinone.[22] They also could not detect the formation of the substrate Schiff base intermediate, observing rapid conversion of the quinone to the product Schiff base. A non-base-catalyzed spontaneous rearrangement of the substrate Schiff base to the product Schiff base intermediate was proposed for the reaction mechanism. 1,4-Addition reactions and displacement of alkyl groups were reported for 1,2-benzoquinones with a small alkyl group such as methyl or ethyl at C_4 or C_5.[23] The steric hindrance of the *tert*-butyl group of **13** or **14** prevented these reactions.

(22) Klein, R. F. X.; Bargas, L. M.; Horak, V. *J. Org. Chem. Soc.* **1988**, *53*, 5994.

Table 4. Midpoint Potentials for **5**, **13**, and **14**

quinone	E_m, mV (*vs* SCE)[a]
5	−181
14	−32
13	+109

[a] [quinone] = 1 mM in 0.1 M phosphate buffer (pH 6.7), μ = 0.3 with KCl. Working electrode: gold wire (area = 0.2 cm^2). Reference electrode: saturated calomel. Counter electrode: Pt wire. BAS 100A electrochemical analyzer.

As seen in Figure 2, the catalytic activity of *o*-quinones (**13** and **14**) is lower than that of topaquinone analogs. Klein et al. reported that di-*tert*-butyl-1,2-benzoquinone could not be regenerated from its aminophenol by oxidation/hydrolysis in neutral media due to an oxidative coupling of the quinone and the aminophenol to yield phenoxazinones as an intense blue byproduct.[22] As we show in Table 4, the order of the catalytic activity of *o*-quinones (**13** and **14**) and topaquinone (**5**) correlates with their redox potentials. This suggests that regeneration of quinone from aminoquinol may be a rate-limiting step for *o*-quinone turnover.

Conclusions

We report that 2-hydroxy-1,4-benzoquinones, bearing bulky substituents at the C_5 position, can act as efficient catalysts for the oxidation of benzylamine in acetonitrile. In our model system, the bulky substituent is necessary to prevent the dimerization of the substrate Schiff base intermediate. In copper amine oxidases, the cofactor is covalently bound to the peptide backbone at this position. Isolation of the substrate Schiff base dimer (**16** or **17**) indicates the intermediacy of a substrate Schiff base (**15**) in the course of amine oxidation. The 2-hydroxyl group is found to be essential for optimal catalytic activity. *p*-Quinones lacking the 2-hydroxyl group such as **10** and **11** undergo ring amination and have no catalytic activity. The 2-methoxy quinone (**9**) undergoes a very slow 1,2-addition of amine to the C_1 carbonyl to yield a small amount of the aminophenol (**23**), whereas the 3-methoxy quinone (**12**) undergoes only the substitution reaction. This indicates that the methoxy group at C_2 position can direct the reaction to the C_1 position without a significant enhancement of the reaction rate. Turning to *o*-quinones, the 4-methoxy *o*-quinone (**14**) is found to oxidize benzylamine at a rate approaching those of topaquinone analogs bearing small alkyl substituents. However, care must be taken in extrapolating from this finding to the catalytically relevant structure of topaquinone analogs. As we discuss in the following paper, the functional form for topaquinone in the active site of copper amine oxidases is best represented as a 2-hydroxy-1,4-benzoquinone.

Acknowledgment. We acknowledge Dr. Graham Ball for running 2D NMR studies on **8** and his helpful discussions, Dr. Fred Hollander for the crystal structure determination of **16**, and Tetsuo Uno for providing the samples of *tert*-butylresorcinol and isopropylresorcinol and his helpful discussions. We also thank Professor Marcin Majda for use of the instruments for the electrochemical study.

Supporting Information Available: Experimental details (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(23) Horspool, W. M.; Smith, P. I.; Tedder, J. M. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1024.