Reaction of the Trimethyl Ester of Coenzyme PQQ (PQQTME) and Amines in Organic Media. Products and Mechanism

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The reaction of PQQTME (1, the trimethyl ester of coenzyme PQQ) and various amines in organic media was investigated in order to gain information on the chemistry of PQQ in vitro. The iminoquinone derivatives **2**, **3**, and **5** were isolated from the reaction of 1 with ammonia, *tert*-butylamine, and cyclopropylamine, respectively, in CH_3CN. The reduction of **2** by methylhydrazine proceeded smoothly to give the aminophenol $\mathbf{2H_2}$ in moderate yield. Detailed analysis of the products from the reaction of 1 (and its 1-methyl derivative **6**) with *n*-propylamine, *N*-methylpropylamine, triethylamine, and several benzylamines established that the oxidation of amines by PQQTME proceeds via an ionic mechanism (addition–elimination and transamination) that involves a carbinolamine intermediate. PQQTME was also found to be an efficient turnover catalyst of the aerobic oxidation of benzylamines in CH_3CN solution.

Introduction

Recently, much attention has focused on the chemistry of a novel coenzyme, PQQ, which is associated with various bacterial dehydrogenases.[1] In particular, how PQQ reacts with amines would be of interest because PQQ, or a structurally related compound, has been believed to be the organic cofactor of various amine oxidases,[2] bacterial methylamine dehydrogenase,[3] and methylamine oxidase.[4] During the last few years, studies of the action of such enzymes have attempted to take into account the *o*-quinone structure of the presumed coenzyme and have provided much information on the structure of the active sites of the enzymes.[5] Very recently Vellieux et al.[6] and Janes et al.[7] independently reported the existence of two new redox cofactors, pro-PQQ (6-hydroxy-4,5-indolequinone derivative) and TOPA quinone, in methylamine dehydrogenase and bovine serum amine oxidase, respectively, thus stimulating new interest in PQQ and structurally similar quinones. Furthermore, the nutritional importance of PQQ has been recognized. PQQ apparently plays a fundamental role in the cross-linking of collagen and elastin during connective tissue biogenesis and in the regulation of intracellular spermine and spermidine levels.[8]

From the twin viewpoints of organic chemistry and biomimetic chemistry, studies on the mechanism of the PQQ-catalyzed oxidation of amines would also be interesting because PQQ possesses a unique heterocyclic *o*-quinone structure. It is well-known that the quinone-catalyzed oxidation of amines proceeds via electron-transfer mechanism within a charge-transfer complex in the case of quinones of high oxidation potential and via a transamination that involves imine intermediates in the case of quinones of moderate oxidation potential.[9] Thus, it would be of value to know by which mechanism the oxidation of amines by PQQ proceeds. Furthermore, in contrast to "ordinary" quinones, PQQ has been shown to be an efficient turnover catalyst of the aerobic oxidation of amines.[10] An investigation of such a catalytic reaction would be worthwhile because information that would be useful for the development of an even more efficient oxidation catalyst would be gained.

Studies of the reactions of PQQ in aqueous media suggested that the oxidation of primary amines by PQQ proceeds via an ionic mechanism that involves a C(5) aminated carbinolamine intermediate. Once formed, the carbinolamne intermediate is converted via two competing reaction pathways, i.e., α-deprotonation and transamination, to give a quinol and an aminophenol, respectively (Scheme I).[10c,11] The latter process very closely resembles the well-known PLP-catalyzed transamination and is also a characteristic reaction of 3,5-di-*tert*-butyl-1,2-benzoquinone[12] and phenanthrolinequinones.[11a]

(1) For example, see: Duine, J. A.; Jongejan, J. A. *Vitam. Horm. (NY)* **1989**, *45*, 223.

(2) (a) Lobenstein-Verbeek, C. L.; Jongejan, J. A.; Frank, J.; Duine, J. A. *FEBS Lett.* **1984**, *170*, 305. (b) Ameyama, M.; Hayashi, M.; Matsushita, K.; Shinagawa, E.; Adachi, O. *Agric. Biol. Chem.* **1984**, *48*, 561. (c) Moog, R. S.; McGuirl, M. A.; Cote, C. E.; Dooley, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8435. (d) Williamson, P. R.; Moog, R. S.; Dooley, D. M.; Kagan, H. M. *J. Biol. Chem.* **1986**, *261*, 16302. (e) van der Meer, R. A.; Duine, J. A. *Biochem. J.* **1986**, *239*, 789. (f) Knowles, P. F.; Pandeya, K. B.; Rius, F. X.; Spencer, C. M.; Moog, R. S.; McGuirl, M. A.; Dooley, D. M. *Biochem. J.* **1987**, *241*, 603. (g) Glatz, Z.; Kovar, J.; Macholan, L.; Pec, P. *Biochem. J.* **1987**, *242*, 603. (h) Paz, M. A.; Gallop, P. M.; Torrelio, B. M.; Fluckiger, R. *Biochem. Biophys. Res. Commun.* **1988**, *154*, 1330. (i) van der Meer, R. A.; van Wassenaar, P. D.; van Brouwershaven, J. H.; Duine, J. A. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 726. (j) Citro, G.; Verdina, A.; Galati, R.; Floris, G.; Sabatini, S.; Finazzi-Agro, A. *FEBS Lett.* **1989**, *247*, 201.

(3) McIntire, W. S.; Stults, J. T. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 562.

(4) van Iersel, J.; van der Meer, R. A.; Duine, J. A. *Eur. J. Biochem.* **1986**, *161*, 415.

(5) (a) Farnum, M. F.; Palcic, M.; Klinman, J. P. *Biochemistry* **1986**, *25*, 1898. (b) Farnum, M. F.; Klinman, J. P. *Ibid.* **1986**, *25*, 6028. (c) Hartmann, C.; Klinman, J. P. *J. Biol. Chem.* **1987**, *262*, 962. (d) Williamson, P. R.; Kagan, H. M. *J. Biol. Chem.* **1987**, *262*, 8196. (e) Williamson, P. R.; Kagan, H. M. *Ibid.* **1987**, *262*, 14520. (f) Dooley, D. M.; McGuirl, M. A.; Peisach, J.; McCracken, J. *FEBS Lett.* **1987**, *214*, 274. (g) Husain, M.; Davidson, V. L.; Gray, K. A.; Knaff, D. B. *Biochemistry* **1987**, *26*, 4139. (h) Collison, D.; Knowles, P. F.; Mabbs, F. E.; Rius, F. X.; Singh, I.; Dooley, D. M.; Cote, C. E.; McGuirl, M. *Biochem. J.* **1989**, *264*, 663. (i) Gacheru, S. N., Trackman, P. C.; Calaman, S. D.; Greenaway, F. T.; Kagan, H. M. *J. Biol. Chem.* **1989**, *264*, 12963. (j) Davidoson, V. L. *Biochem. J.* **1989**, *261*, 107. (k) Dooley, D. M.; McIntire, W. S.; McGuirl, M. A.; Cote, C. E.; Bates, J. L. *J. Am. Chem. Soc.* **1990**, *112*, 2782.

(6) Vellieux, F. M. D.; Hol, W. G. J. *FEBS Lett.* **1989**, *255*, 460.

(7) Janes, S. M.; Mu, D.; Wemmer, D.; Smith, A. J.; Kaur, S.; Maltby, D.; Burlingame, A. J.; Klinman, J. P. *Science* **1990**, *248*, 981.

(8) Killgore, J.; Smidt, C.; Duich, L.; Romero-Chapman, N.; Tinker, D.; Reiser, K.; Melko, M.; Hyde, D.; Rucker, R. B. *Science* **1989**, *245*, 850.

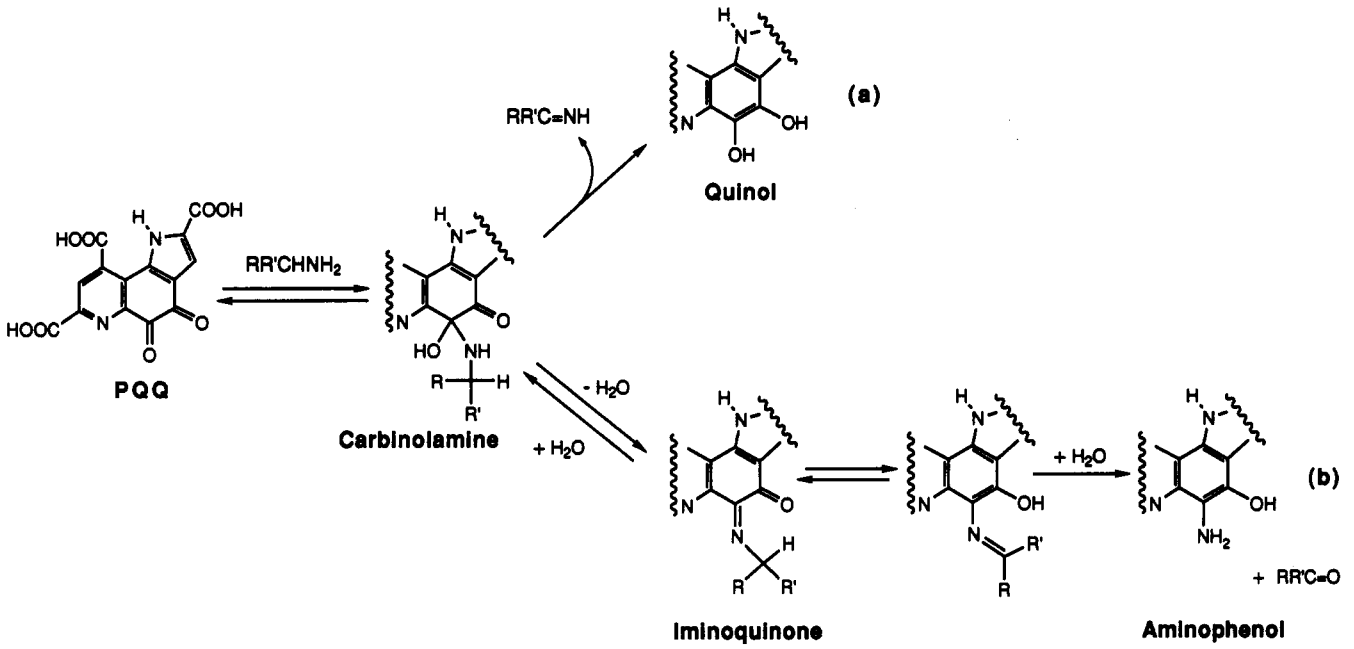
(9) Decker, H.-D. *The Chemistry of the quinonoid compounds*; Patai, S., Ed.; John Wiley & Sons: New York, 1974; pp 335–423.

(10) (a) Ohshiro, Y.; Itoh, S.; Kurokawa, K.; Kato, J.; Hirao, T.; Agawa, T. *Tetrahedron Lett.* **1983**, *24*, 3465. (b) Itoh, S.; Kato, N.; Ohshiro, Y.; Agawa, T. *Ibid.* **1984**, *25*, 4753. (c) Itoh, S.; Kitamura, Y.; Ohshiro, Y.; Agawa, T. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1907. (d) Mure, M.; Itoh, S.; Ohshiro, Y. *Chem. Lett.* **1989**, 1491. (e) Mure, M.; Suzuki, A.; Itoh, S.; Ohshiro, Y. *J. Chem. Soc., Chem. Commun.* **1990**, 1608.

(11) (a) Eckert, T. S.; Bruice, T. C. *J. Am. Chem. Soc.*, **1983**, *105*, 4431. (b) Sleath, P. R.; Noar, J. B.; Eberlein, G. A.; Bruice, T. C. *Ibid.* **1985**, *107*, 3328. (c) Rodriguez, E. J.; Bruice, T. C. *Ibid.* **1989**, *111*, 7947.

0022-3263/91/1956-6857$02.50/0

Scheme I



However, some problems are encountered in studying the reactions of PQQ in aqueous media. First, the reduced species, i.e., the quinol and the aminophenol, are easily oxidized by molecular oxygen in aqueous media,[13] and even under anaerobic conditions the aminophenol is readily converted into the quinol in the presence of a small amount of the quinone (autocatalytic conversion of the aminophenol into the quinol).[11c] This behavior of the reduced PQQ species makes quantitative analysis of the products very difficult. Second, it is well-known that the quinone moiety of PQQ can be hydrated to a relatively great extent even in neutral or weakly alkaline aqueous solution, which also complicates the kinetics of the reaction.[11,14] Third, the imine intermediates are insufficiently stable in aqueous media to permit their isolation and characterization. In order to avoid such problems and thereby obtain useful information, the reaction of a model compound, the trimethyl ester of PQQ (PQQTME, trimethyl 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylate, 1), and amines in organic media was investigated. Because the active sites of the enzymes are generally considered to consist of a hydrophobic environment and also because PQQ is known to be covalently bound to the active sites through an amide or ester linkage,[15] it is believed that PQQTME in organic media represents a better model of enzyme-bound PQQ than does free PQQ in aqueous media.

Experimental Section

PQQTME (1) and 1-Me-PQQTME (6) were prepared by reported methods.[16,17] All the amine substrates and methylhydrazine were commercial products of the highest available purity and were further purified by fractional distillation from CaH_2 under N_2. All the solvents were dried and purified by standard methods.[18] Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. IR spectra were recorded with a Hitachi 270-30 spectrophotometer and UV–vis spectra with a Shimadzu UV-265 spectrophotometer equipped with a Shimadzu TCC-260 thermostated cell holder. Mass spectra were recorded with a JEOL JNX DX 303 HF mass spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded with a JEOL FT-NMR GSX-270 or a Brucker AM 600 spectrometer.

Preparation of Trimethyl 4,5-Dihydro-4-oxo-5-imino-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylate (2). Dry NH_3 gas was passed through a solution of 1 (44.7 mg, 0.120 mmol) in CH_3CN (30 mL). Compound 2 precipitated as a reddish purple solid, which was collected by centrifugation, washed with CH_3CN and Et_2O, and dried in vacuo (41.7 mg, 94%): mp >300 °C; IR (KBr) 3256 (NH), 1726, 1712 (ester C=O), 1700 (C=O), 1648 (C=N) cm^{-1}; UV–vis (CH_3CN) λ_{max} 259 (ϵ 29 900 M^{-1} cm^{-1}), 358 (15 400) nm; MS *m/e* 373 (M^+ + 2), 371 (M^+); ^1H NMR (DMSO-d_6) δ 3.69, 3.90, 3.93 (each 3 H, s, $-OCH_3$ × 3), 7.08 (1 H, s, 3-H), 7.17 (br s, exchangeable with D_2O), 7.94 (1 H, s, 8-H), 11.88, 12.34 (total 1 H (3:1), each br s, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) 50.61, 52.58, 52.98 (ester-OCH_3 × 3) 116.67, 121.43, 123.91, 130.11, 136.43, 139.77, 143.63, 144.21, 148.07 (9 aromatic carbons), 164.46, 164.79, 166.12 (ester C=O × 3), 168.25, 169.36 (C=N and C=O) ppm.

Preparation of Trimethyl 4,5-Dihydro-4-oxo-5-(*tert*-butylimino)-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylate (3). *tert*-Butylamine (2.7 mmol) was added to a solution of 1 (20.2 mg, 0.0543 mmol) in CH_3CN (10 mL) under Ar. The solution was stirred for 4 h. Concentration of the solution under reduced pressure to ca. 3 mL gave a brown solid, which was collected by centrifugation, washed with Et_2O, and dried in vacuo (19.5 mg, 84%): mp >300 °C; IR (KBr) 1730, 1718 (ester C=O), 1680 (C=O), 1646 (C=N) cm^{-1}. UV–vis (CH_3CN) λ_{max} 258 (ϵ 18 900 M^{-1} cm^{-1}), 306 (7500), 358 (13 100) nm; MS, *m/e* 372 (M^+ + 2 - ^{t}Bu); ^1H NMR (DMSO-d_6) δ 1.24 (9 H, s, $-^t$Bu), 3.68, 3.89, 3.92 (each 3 H, s, $-OCH_3$ × 3), 6.99 (1 H, s, 3-H), 7.71 (br s, exchangeable with D_2O), 7.94 (1 H, s, 8-H); ^{13}C NMR (DMSO-d_6) 27.29 ($-CH_3$), 50.60, 51.19, 52.59, 52.97 (ester $-OCH_3$ × 3 and $-C(CH_3)_3$) 117.25, 124.84, 125.53, 132.86, 136.81, 139.56, 143.25, 143.77, 146.31 (9 aromatic carbons), 164.33, 164.48, 167.88 (ester C=O × 3), 171.74, 183.19 (C=N and C=O) ppm. The possibility existed that compound 3 was present as a mixture of syn and anti stereoisomers. However, the ^1H and ^{13}C NMR spectra indicated that 3 was present as only one isomer. This may be a consequence

(12) (a) Corey, E. J.; Achiwa, K. *J. Am. Chem. Soc.*, **1969**, *91*, 1429. (b) Klein, R. F. X.; Bargas, L. M.; Horak, V. *J. Org. Chem.* **1988**, *53*, 5994.
(13) Itoh, S.; Ohshiro, Y.; Agawa, T. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1911.
(14) Dekker, R. H.; Duine, J. A.; Frank, J.; Verwiel, P. E. J.; Westerling, J. *Eur. J. Biochem.*, **1982**, *125*, 69.
(15) Duine, J. A.; Frank, J.; Jongejan, J. A. *Adv. Enzymol.* **1987**, *59*, 170.
(16) Corey, E. J.; Tramontano, A. *J. Am. Chem. Soc.* **1981**, *103*, 5599.
(17) Itoh, S.; Kato, J.; Inoue, T.; Kitamura, Y.; Komatsu, M.; Ohshiro, Y. *Synthesis* **1987**, 1067.
(18) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon Press: Elmsford, New York, 1966.

of the bulkiness of the *tert*-butyl group.

Preparation of Trimethyl 4-Hydroxy-5-amino-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylate ($2H_2$). Methylhydrazine (0.26 mmol) was added to a solution of **2** (9.8 mg, 0.0263 mmol) in CH_3CN (10 mL). The mixture was stirred for 1 h under anaerobic conditions (Ar atmosphere). The dark red solid that precipitated was collected by centrifugation, washed with CH_3CN and Et_2O, and dried in vacuo (6.2 mg, 63%): mp >300 °C; IR (KBr) 3364 (NH, OH), 1720 (ester C=O) cm^{-1}; UV-vis (CH_3CN) λ_{max} 337 (ϵ 32 600 M^{-1} cm^{-1}) nm; MS *m/e* 373 (M^+); ^1H NMR (DMSO-d_6) δ 3.93, 3.99, 4.10 (each 3 H, s, $-OCH_3 \times 3$), 7.51 (1 H, s, 3-H), 8.51 (1 H, s, 8-H), 12.02 (br s, exchangeable with D_2O).

Reaction of PQQTME (1) with Propylamines under Anaerobic Conditions. The reaction of **1** (2.0 mM) with *n*-propylamine (3.7–200 mM) was performed in CH_3CN solution under anaerobic conditions for 2 h. The solid that precipitated was collected by centrifugation, washed with Et_2O, and dried in vacuo. The composition of the product mixture was determined by ^1H NMR. The product ratio was determined by comparing the integrals of the respective signals due to the protons at C(3) and C(8). The reaction in the presence of a large excess of the amine (200 mM) gave trimethyl 4-hydroxy-5-(*n*-propylamino)-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylate (**4**) as the sole isolable product (88%): mp 186–188 °C; IR (KBr) 3300 (NH), 1740 and 1718 (ester C=O) cm^{-1}; UV-vis (CH_3CN) λ_{max} 305 (ϵ 28 000 M^{-1} cm^{-1}), 315 (28 300) nm; HRMS *m/e* 415.1384 (M^+), calcd for $C_{20}H_{21}O_7N_3$ 415.1380; ^1H NMR (DMSO-d_6) δ 0.95 (3 H, t, J = 7.4 Hz, $-CH_3$), 1.52 (2 H, m, $-CH_2-$), 3.36 (2 H, m, $-CH_2NH-$), 3.94, 3.99, 4.10 (each 3 H, s, $-OCH_3 \times 3$), 7.59 (1 H, d, J = 1.4 Hz, 3-H), 8.50 (1 H, s, 8-H), 12.11 (1 H, br, 1-H, exchangeable with D_2O).

The reaction of **1** (2.0 mM) with *N*-methylpropylamine was performed in a similar manner.

The reaction of **1** (2.0 mM) with cyclopropylamine (2.0–200 mM) was performed in the same manner, but in the absence of light. Evaporation of the solvent after 2 h gave a brown solid (**5**) in almost quantitative yield: mp >300 °C; IR (KBr) 1724 (ester C=O), 1668 (C=O and C=N) cm^{-1}; UV-vis (CH_3CN) λ_{max} 274 (ϵ 26 100 M^{-1} cm^{-1}), 355 (14 000) nm; HRMS *m/e* 411.1050 (M^+), calcd for $C_{20}H_{17}O_7N_3$ 411.1067. The ^1H NMR indicated that two isomers were present: ^1H NMR (DMSO-d_6) δ 1.23 and 1.42 (total 4 H, each m), 3.89, 3.93, 4.04 (each 3 H, s, $-OCH_3 \times 3$), 5.20 and 5.39 (1.8:1, total 1 H, each m), 7.23 and 7.30 (1:1.8, total 1 H, each s, 3-H), 8.47 (1 H, br s, 8-H), 12.45 and 12.53 (1:1.8, each br s, 1-H); (in $CDCl_3$) δ 1.50 (m, overlapping with H_2O (δ 1.59) in $CDCl_3$), 3.96 and 3.97 (1:3.2, total 3 H, s, $-OCH_3$), 4.03 (3 H, s, $-OCH_3$), 4.14 and 4.15 (1:3.2, total 3 H, s, $-OCH_3$), 5.36 (1 H, m), 7.49 (1 H, d, J = 2.2 Hz, 3-H), 8.69 and 8.75 (1:3.2, total 1 H, each s, 8-H), 12.50 and 12.68 (1:3.2, each br s, 1-H); (in CD_3CN) δ 1.25 and 1.42 (total 4 H, each m), 3.92, 3.97, 4.09 (each 3 H, s, $-OCH_3 \times 3$), 5.26 and 5.38 (3:1, total 1 H, each m), 7.27 and 7.34 (1:3, total 1 H, each s, 3-H), 8.57 and 8.60 (1:3, total 1 H, each s, 8-H), 12.3 (br, 1-H).

The two components of the mixture are believed to be syn–anti stereoisomers because (i) the isomeric ratio is different in different solvents (DMSO-d_6, $CDCl_3$, and CD_3CN), (ii) acid hydrolysis of the mixture regenerated PQQTME, and (iii) reduction of the mixture by treatment with methylhydrazine afforded a single product, $5H_2$: mp 220–222 °C; IR (KBr) 3200–3600 (-OH and -NH-), 1724 (ester C=O) cm^{-1}; UV-vis (CH_3CN) λ_{max} 303 (ϵ 2690 M^{-1} cm^{-1}) nm; HRMS *m/e* 413.1201 (M^+), calcd for $C_{20}H_{19}O_7N_3$ 413.1223; ^1H NMR ($CDCl_3$) δ 0.66, 0.77 (total 4 H, each m), 2.90 (1 H, m), 4.01 (3 H, s, $-OCH_3$), 4.08 (3 H, s, $-OCH_3$), 4.17 (3 H, s, $-OCH_3$), 7.52 (1 H, s, 3-H), 8.72 (1 H, s, 8-H), 12.50 (br s, 1-H).

Reaction of PQQTME (1) or 1-Me-PQQTME (6) with Benzylamines under Anaerobic Conditions. The reactions of **1** (2.0 mM) with benzylamines (2–200 mM) were performed in a manner similar to that described above. The ratio of the quinol to the aminophenol was determined by ^1H NMR by comparing the integrals of the respective signals due to the C(3) and C(8) protons.

For kinetic studies in CH_3CN solution at 30 °C under anaerobic conditions (Ar atmosphere), a 1-mm path length UV cell, which could be sealed tightly with a silicon rubber cap, was used. Typically, a CH_3CN solution of the quinone (4.0×10^{-4} M) and Et_3N (4.0×10^{-2} M) was placed in the cell and was degassed by bubbling Ar through it for ca. 20 min. Then the amine was added, via microsyringe, to start the reaction ([amine] = 4.0×10^{-2} M). The pseudo-first-order rate constant (k_{obsd}) was calculated from the rate of the increase in intensity of an absorption band at ca. 320 nm due to the products of reduction.

Table I. Spectroscopic Features Characteristic of 1–3

	1	2	3
^1H NMR (DMSO-d_6, δ)	7.28 (3-H) 8.61 (8-H)	7.08 (3-H) 7.94 (8-H)	6.99 (3-H) 7.94 (8-H)
IR (KBr, cm^{-1})	1686 (C=O)	1648 (C=N)	1646 (C=N)
mass (*m/e*)	374 ($M^+ + 2$)[a]	373 ($M^+ + 2$)[a]	372 ($M^+ + 2 - {}^tBu$)[a]

[a] A peak characteristic of the *o*-quinone structure (Zeller, K. P. *The chemistry of the quinonoid compounds*; Patai, S., Ed.; John Wiley & Sons: New York, 1974; pp 231–256.

Aerobic Oxidation of Amines Catalyzed by PQQTME (1). The catalytic reaction was initiated by adding the amine (1 mmol), via microsyringe, to an O_2-saturated CH_3CN solution (10 mL) of **1** (1 mM). The initial amine concentration was thus 100 mM. The mixture was stirred at room temperature under O_2 atmosphere. The rate of formation of benzaldehyde was monitored by HPLC [pump, Waters Model 510 (pump); UV detector, Lambda-Max model; column, radial compression separation system (C_{18}); eluent, $CH_3OH/H_2O/H_3PO_4$ (45:54.5:0.5)]. The ^1H NMR and IR spectra of the concentrated final reaction mixture indicated that *N*-benzylidenebenzylamine ($PhCH_2N=CHPh$) had been formed: ^1H NMR ($CDCl_3$) δ 4.80 (2 H, s, $-CH_2-$), 7.20–7.50 (8 H, m, aromatic protons), 7.70–7.85 (2 H, m, aromatic protons), 8.38 (1 H, br s, -CH=); IR (neat) 1648 (C=N) cm^{-1}. Acidification of the final reaction mixture with 1 N aqueous HCl gave the oxazole derivative **8**, a yellow solid (35%): mp 201–203 °C; MS *m/e* 459 (M^+); IR (KBr) 3300 (NH), 1724 (ester C=O), 1520, 1442 cm^{-1}, no quinone absorption; ^1H NMR (DMSO-d_6) δ 3.97, 4.04, 4.14 (each 3 H, s, $-OCH_3 \times 3$), 7.50–7.63 (4 H, m, 3-H and phenyl protons), 8.36–8.39 (2 H, m, phenyl protons), 8.63 (1 H, s, 8-H), 12.40 (1 H, br s, pyrrole proton).

The aerobic oxidation of other amines listed in Table IV were performed in the same manner. The products and yields were also determined by HPLC.

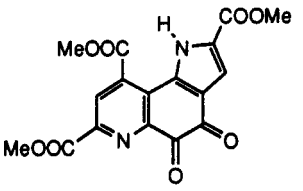
Results

Preparation and Characterization of Iminoquinone Derivatives of PQQTME.[19] The iminoquinone derivatives **2** and **3**, produced by the reaction of **1** with ammonia and *tert*-butylamine, respectively, in CH_3CN, were easily isolated (Chart I). Spectroscopic features characteristic of the iminoquinones and compound **1** are shown in Table I. An absorption band characteristic of the imine function was observed at ca. 1650 cm^{-1} in the IR spectrum of each product. Although further investigation is required to unequivocally establish to which carbonyl group the amine adds, it is believed that addition occurs at C(5) because the ^1H NMR signal due to the proton at C(8) is shifted further upfield than is the signal due to the proton at C(3). This interpretation is reasonable because the C(5) carbonyl group of PQQ is well-known to display unusually high reactivity toward nucleophilic addition.[20] In Figure 1 are shown the UV-vis spectra of the iminoquinone **2** and the quinone **1**. Basically there is no significant difference in λ_{max} of the two compounds. However, in the spectrum of the iminoquinone, the absorption band due to the n–π^* transition of the quinone function at ca. 450 nm is less intense. Introduction of aqueous HCl into a solution of

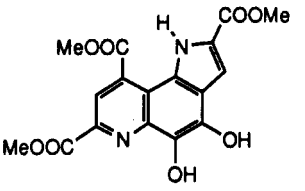
(19) For a preliminary report, see: Mure, M.; Itoh, S.; Ohshiro, Y. *Tetrahedron Lett.* **1989**, *30*, 6875.

(20) The position at which acetone and 2,4-dinitrophenylhydrazine add to PQQ has been confirmed by X-ray crystallographic analysis of the products. See: Salisbury, S. A.; Forrest, H. S.; Cruse, W. B. T.; Kennard, O. *Nature (London)* **1979**, *289*, 844. van Koningsveld, H.; Jansen, J. C.; Jongejan, J. A.; Frank, J.; Duine, J. A. *Acta Crystallogr.* **1985**, *C41*, 89.

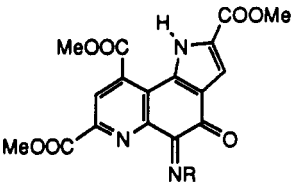
Chart I



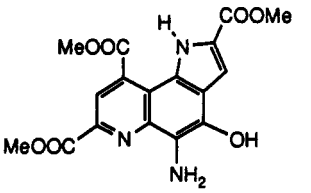
1: PQQTME



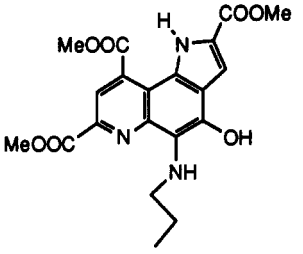
$1H_2$



2 : R = H
3 : R = *t*-Bu



$2H_2$



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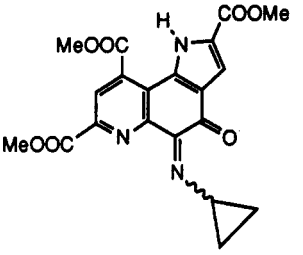

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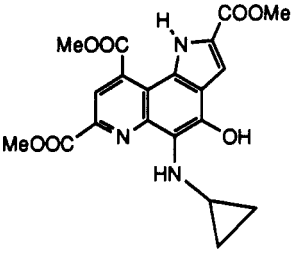
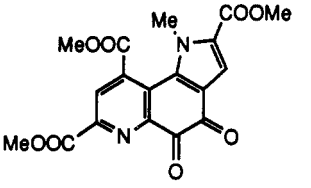
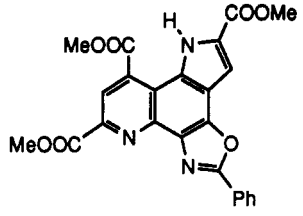


$5H_2$



6 : 1-Me-PQQTME



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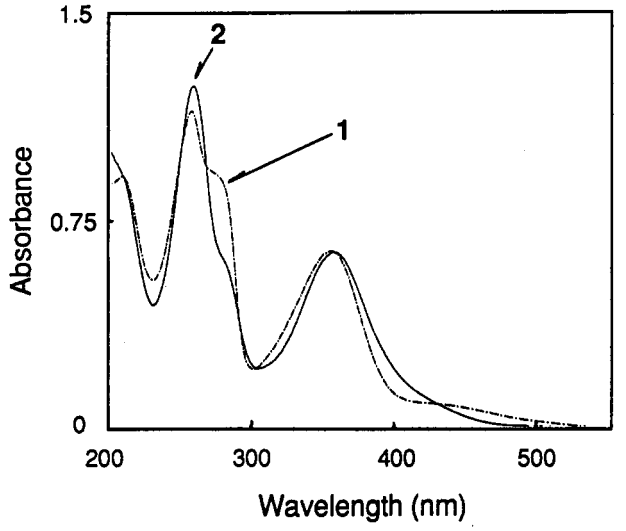


Figure 1. UV–vis spectra of PQQTME (1) and iminoquinone 2 [1: λ_{max} = 258 (ϵ = 27 700 M^{-1} cm^{-1}), 356 (ϵ = 15 500) nm. 2: λ_{max} = 259 (ϵ = 29 900 M^{-1} cm^{-1}), 358 (ϵ = 15 400) nm].

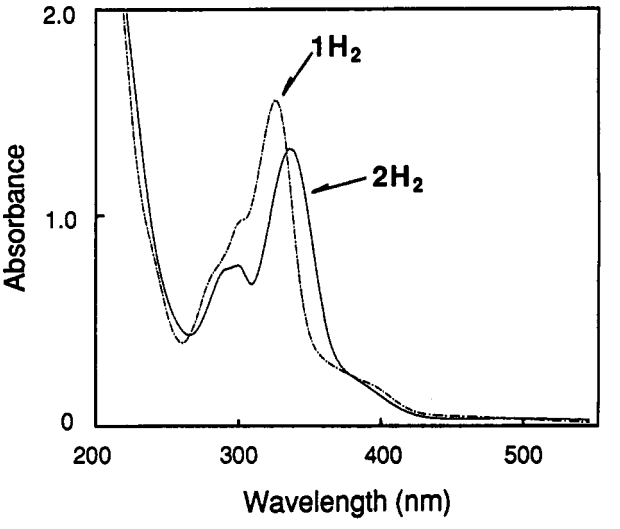


Figure 2. UV–vis spectra of quinol $1H_2$ and aminophenol $2H_2$. $1H_2$ and $2H_2$ were generated in situ by the reduction of 1 and 2, respectively, by treatment with methylhydrazine [$1H_2$: λ_{max} = 328 (ϵ = 38 400 M^{-1} cm^{-1}) nm. $2H_2$: λ_{max} = 337 (ϵ = 32 600 M^{-1} cm^{-1}) nm].

2 immediately regenerated 1, which indicated that the iminoquinone is unstable in acidic aqueous media.

Preparation and Characterization of the Aminophenol Derivative of PQQTME.[19] The aminophenol derivative $2H_2$ was readily formed by the reduction of 2 (1 equiv) with methylhydrazine (10 equiv) and precipitated as a dark red solid (63%). The UV–vis spectra of both $2H_2$ and $1H_2$ (the quinol) are shown in Figure 2. The spectrum of the aminophenol $2H_2$ shows λ_{max} at 337 nm, which is about 10 nm red-shifted compared with λ_{max} of the quinol.

As expected, aeration of an CH_3CN solution of $2H_2$ readily regenerated the iminoquinone 2. Compound 1 could be similarly generated from $2H_2$. In subsequent experiments, product analysis was performed by ^1H NMR, as described below.

Reaction with Propylamines. The reactions of PQQTME with propylamines in CH_3CN solution under anaerobic conditions (Ar atmosphere) was examined. Thus, treatment of 1 with *n*-propylamine readily gave a

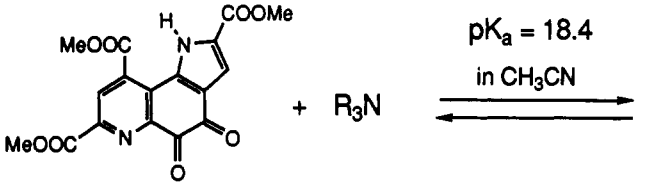
Table II. Products of the Reaction between 1 and Propylamines[a]

amine (mM)	time (h)	total yield (%)	product $1H_2$:$2H_2$:4
n-propylamine (3.7)	2	94	88:12:0
n-propylamine (5.1)	2	98	69:31:0
n-propylamine (20)	2	100	53:33:14
n-propylamine (100)	2	97	0:7:93
n-propylamine (200)	2	88	0:0:100
N-methylpropylamine (200)	24	100	100:0:0

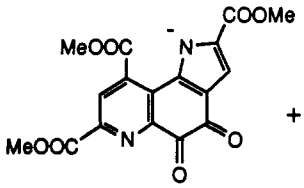
[a] $[1]_0$ = 2.0 mM; CH_3CN solution; room temperature; Ar atmosphere.

mixture of the products of reduction as a brown solid. The ^1H NMR spectrum of the crude mixture indicated that it contained three reduced species: the quinol $\mathbf{1H_2}$, the aminophenol $\mathbf{2H_2}$, and the (propylamino)phenol **4**. Their proportions varied greatly, depending upon the initial *n*-propylamine concentration (Table II). Thus, the quinol $\mathbf{1H_2}$ was the major product when a low initial concentration of amine (3.7 mM, 1.9 equiv) was used. The proportion of $\mathbf{1H_2}$ decreased as the initial amine concentration increased. The highest proportion of $\mathbf{2H_2}$ was produced when the initial *n*-propylamine concentration was 20 mM (10 equiv). On the other hand, **4** was formed predominantly when a large excess of amine (50–100 equiv) was initially present. Interestingly, the reaction of **1** with cyclopropylamine gave only the iminoquinone **5**, as a mixture of syn and anti stereoisomers, over a wide range of initial cyclopropylamine concentration (see Experimental Section).

N-Methylpropylamine was considerably less reactive than *n*-propylamine (Table II), and only $\mathbf{1H_2}$ was obtained as a product of reduction. *N,N*-Dimethylpropylamine did not react at all. However, when *N,N*-dimethylpropylamine was used, dissociation of the pyrrole proton (eq 1) was



1: PQQTME



(1)

detected by UV–vis spectroscopy. The spectrophotometric titration of **1** in CH_3CN solution with triethylamine (pK_a = 18.6 in CH_3CN)[21] afforded a pK_a of 18.4 for **1** (Figure 3). Although it is well-known that triethylamine forms charge-transfer complexes with quinones,[9] the changes in the spectrum of **1** (Figure 3) during titration could not be attributed to the formation of a charge-transfer complex because similar changes in the spectrum of **6** were not observed when that compound was titrated with triethylamine.

Reaction with Benzylamines. Analysis of the products from the reaction between PQQTME and variously substituted benzylamines in CH_3CN solution under anaerobic conditions (Ar atmosphere) was also performed. In all cases, only the corresponding quinol $\mathbf{1H_2}$ and aminophenol $\mathbf{2H_2}$ were present. No (alkylamino)phenol was detected. In these cases also, the ratio of $\mathbf{1H_2}$ to $\mathbf{2H_2}$ depended on the initial benzylamine concentration. However, the quinol was always the major product, even in the presence of a large excess of the amine (Table III). The electronic nature of the para substituent of the benzylamines hardly influenced the product ratio, whereas replacing the α-protons of benzylamine with deuterons led to a slight decrease in the amount of quinol that was formed.

The reaction of 1-Me-PQQTME (**6**) and benzylamine was also investigated in an attempt to elucidate the role played by the acidic pyrrole proton of PQQTME. The rate of reaction of **6** (k_{obsd} = 2.2 × 10^{-5} s^{-1}) was about 2 orders of magnitude lower than that of PQQTME (k_{obsd} = 2.0 × 10^{-3} s^{-1}). The final UV–vis spectrum of the reaction mixture did not resemble that of the quinol $\mathbf{6H_2}$, but did rather closely resemble the spectrum of the aminophenol $\mathbf{2H_2}$ (Figure 4b). A detailed analysis of the products was not possible because of the instability and the high solubility of the products. However, the ^1H NMR spectrum of the final reaction mixture indicated that the mixture did not contain the quinol $\mathbf{6H_2}$. Furthermore, changes in the UV–vis spectrum were observed during the initial

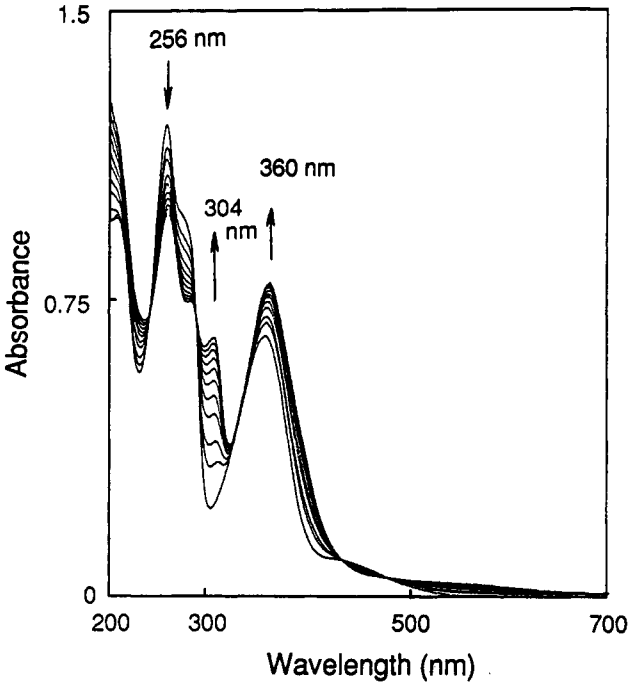


Figure 3. Spectrophotometric titration of **1** (4.1 × 10^{-4} M) with Et_3N (4.27 × 10^{-5}–3.88 × 10^{-3} M) in CH_3CN. All spectra are of solutions contained in a 1-mm path length UV cell.

Table III. Products of the Reaction between 1 and Benzylamines[a]

substrate (p-X-$C_6H_4CH_2NH_2$)	concn (mM)	total yield (%)	product $1H_2$:$2H_2$
X = H	2.1	60[b]	74:26
X = H	20	95	67:33
X = H	100	91	57:43
X = H	200	78	54:46
X = OMe	20	98	62:38
X = Cl	20	87	64:36
X = Me	20	83	67:33
$PhCD_2NH_2$	20	68[b]	60:40

[a] $[1]_0$ = 2.0 mM; CH_3CN solution; room temperature; Ar atmosphere; 2 h. [b] Some **1** was recovered.

(21) *Kagaku Binran, Kiso-hen II (The Chemical Data Book)*; Chemical Society of Japan, Ed.; Maruzen: Tokyo, 1984; p 343.

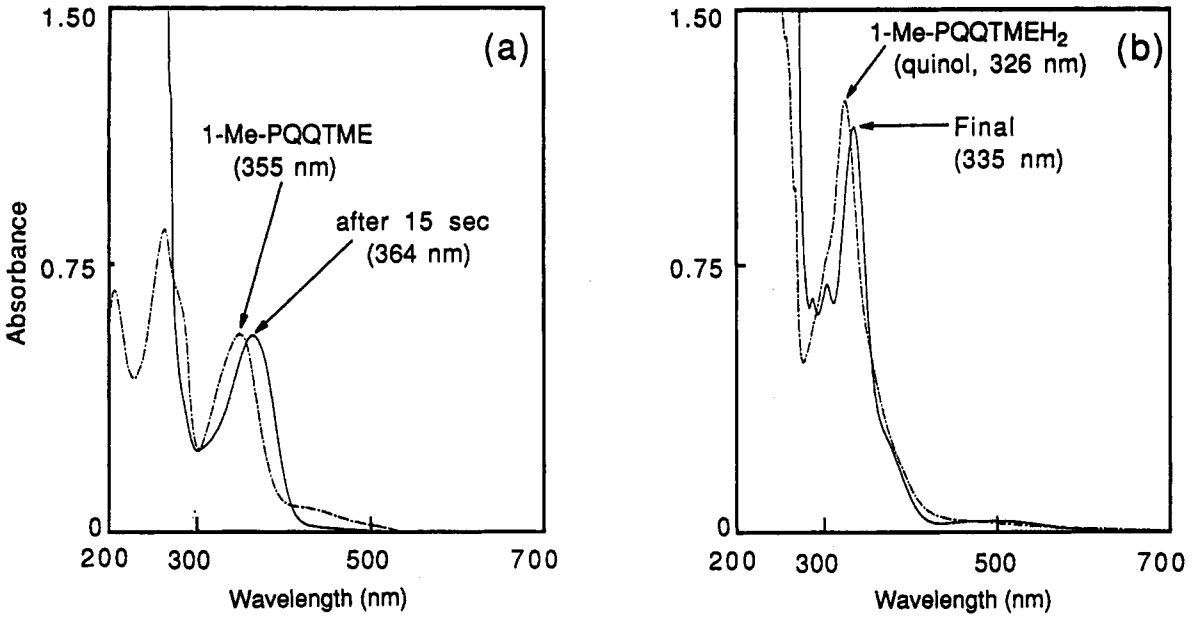


Figure 4. Changes in the spectrum on the reaction mixture containing **6** (4.0×10^{-4} M) and benzylamine (4.0×10^{-2} M) in the presence of Et_3N (4.0×10^{-2} M) in CH_3CN solution at 30 °C under anaerobic conditions (Ar): (a) **6** (-·-), after 15 s (—), (b) the final spectrum (—), $\mathbf{6H_2}$ (4.0×10^{-4} M, generated by the reduction of **6** by treatment with methylhydrazine in situ, -·-). All spectra are of solutions contained in a 1-mm path length UV cell.

stages of the reaction (Figure 4a). The changes could be attributed to the formation of the intermediate carbinolamine, because the characteristic absorption at 400–500 nm due to the quinonoid n–π^* transition coincidentally disappeared.

Aerobic Oxidation of Benzylamines Catalyzed by PQQTME. It has already reported that PQQ is an efficient turnover catalyst of the aerobic oxidation of amines.[10a] PQQTME also catalyzes the oxidation of benzylamine in CH_3CN solution under aerobic conditions. Thus, treatment of benzylamine (100 mM) in CH_3CN solution with a catalytic amount (1 mol %) of **1** under O_2 atmosphere gave *N*-benzylidenebenzylamine as the sole detectable product. Figure 5 shows the time course of the catalytic reaction which was monitored by following the formation of benzaldehyde by HPLC. Because benzaldehyde is spontaneously formed by the hydrolysis of *N*-benzylidenebenzylamine and because 1 mol % of the catalyst was used, the maximum yield of benzaldehyde must be 50 mM. Thus it can be said that this catalytic oxidation of benzylamine was essentially complete within 1 h. At that point, introduction of the same amount of substrate that was present initially (100 mM) reinitiated the catalytic reaction. This indicated that some PQQTME was still catalytically active. However, repeated introductions of substrate led to a decrease in the observed rate of reaction, and it was subsequently found that some of the catalyst had been converted into the oxazole derivative **7**.

Table IV shows the effects of varying both some reaction conditions and the structure of the amine substrate. The reaction was unaffected by the absence of light. CH_3CN was found to be a good solvent, whereas, in CH_3OH, a solvent with nucleophilic character, the reaction rate was slower. The use of a nonpolar solvent, CH_2Cl_2, drastically reduced the activity of the catalyst. α-Substituted primary benzylamines were efficiently oxidized to the corresponding ketones, but the reactivities of the secondary and tertiary benzylamines were extremely low. These results suggest that the reaction proceeds via an ionic rather than a free radical mechanism. Interestingly, alkylamines were not oxidized at all under the conditions employed. Why

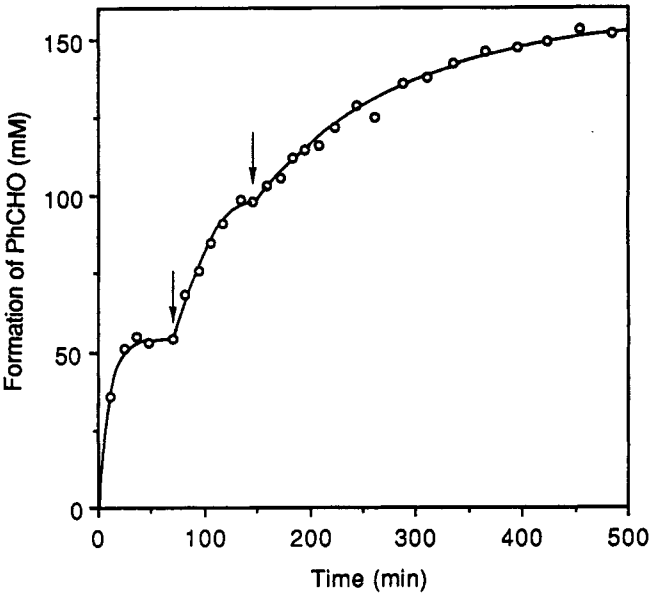


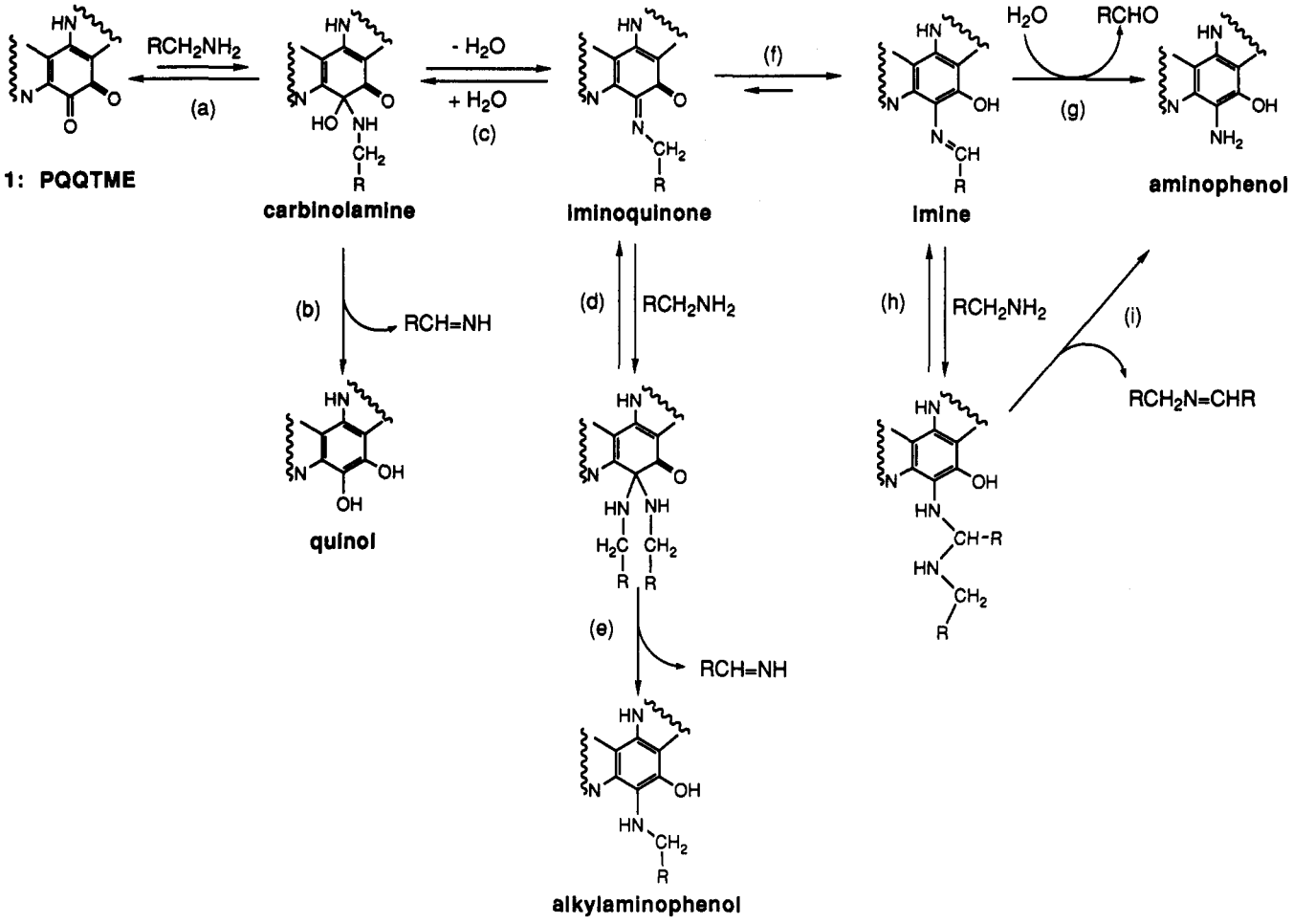
Figure 5. Time course of the oxidation of benzylamine (100 mM) catalyzed by **1** (1.0 mM) in CH_3CN solution under aerobic conditions. The rate of formation of benzaldehyde was monitored by HPLC (see Experimental Section). Benzylamine (100 mM) was added after 70 and 160 min (at the points indicated by arrows).

Table IV. PQQTME-Catalyzed Oxidation of Amines under Aerobic Conditions[a]

amine	solvent	product	time (h)	yield (%)
$PhCH_2NH_2$	CH_3CN	PhCHO	1	52.7
$PhCH_2NH_2$	CH_3CN	PhCHO	1	52.9[b]
$PhCH_2NH_2$	CH_3OH	PhCHO	3	53.4
$PhCH_2NH_2$	CH_2Cl_2	PhCHO	24	4.8
$PhCH_2NHMe$	CH_3CN	PhCHO	24	8.1
$PhCH_2NMe_2$	CH_3CN	PhCHO	24	0.9
$PhCH(Me)NH_2$	CH_3CN	$PhC(O)CH_3$	24	21.6
$PhCH(Ph)NH_2$	CH_3CN	Ph_2CO	24	48.5
n-hexylamine	CH_3CN		24	0
cyclohexylamine	CH_3CN		24	0

[a] $[1]_0$ = 1.0 mM, $[amine]_0$ = 100 mM; room temperature; O_2 atmosphere. [b] Reaction performed in the absence of light.

Scheme II



this was so is discussed later. However, acidity of the α-proton of the substrates seems to be an important factor in the oxidation.

Discussion

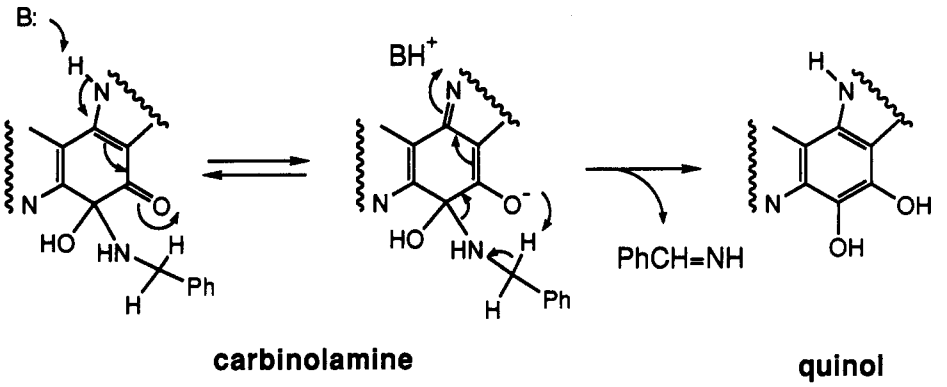
The oxidation of amines by PQQ is believed to proceed via an ionic mechanism that involves a carbinolamine intermediate (Scheme I).[10,11] Additional evidence in support of such a mechanism is provided by the results of the study described here; i.e., (i) the C(5)-aminated iminoquinone adducts **2**, **3**, and **5** were obtained in almost quantitative yield from the reaction of **1** with ammonia, *tert*-butylamine, and cyclopropylamine, respectively; (ii) transamination occurred to give the aminophenol $\mathbf{2H_2}$ and the (propylamino)phenol **4**; (iii) the reactivity of *N*-methylpropylamine was considerably less than that of *n*-propylamine (Table III), and no redox reaction was observed in the case of tertiary alkylamines (eq 1); (iv) the carbinolamine intermediate was detected spectrophotometrically during the initial stages of the reaction between **6** and benzylamine (Figure 4); and (v) in the *aerobic* autorecycling oxidation reaction, the redox-inactive oxazole derivative **7** was gradually formed during the catalytic cycles.

The formation of the quinol could be explained in terms of an electron-transfer mechanism, which is believed to operate in the oxidation of amines by quinones of high oxidation potential like DDQ or chloranil.[9] However, the low reactivity displayed by secondary and tertiary amines argues against such a possibility. As Bruice and co-workers have reported,[11a] in oxidation via an electron-transfer mechanism, the order of the reactivity of amines is tertiary > secondary > primary. Furthermore, the cyclopropyl ring of cyclopropylamine remained intact during the reaction with **1**. If the reaction of PQQTME and amines did proceed via an electron-transfer mechanism, a ring-opened product should have been formed. For example, it has been demonstrated that the radical cation produced by the one-electron oxidation of cyclopropylamine readily gives a ring-opened product.[22] A plausible mechanism for the reaction of PQQTME and amines in CH_3CN solution is presented in Scheme II.

In the reaction of **1** with *n*-propylamine, varying the amine concentration significantly altered the product composition. Why this was so can be explained as follows: At a low amine concentration, α-deprotonation of the carbinolamine intermediate (addition–elimination) predominates to give the quinol (path b). On the other hand, when a relatively large amount of amine is present, the position of the equilibrium of step c is shifted to the right and the iminoquinone intermediate, from which the (propylamino)phenol product **4** is derived via a similar addition–elimination sequence (paths d and e), accumulates. In a process of minor importance, the iminoquinone intermediate rearranges to an imine intermediate (path f), which is eventually converted into the aminophenol $\mathbf{2H_2}$ by hydrolysis or by imine-exchange reaction (path g or paths h and i). In the case of *N*-methylpropylamine, only the addition–elimination pathway (paths a and b) is allowed, so only the quinol $\mathbf{1H_2}$ is formed. However, the reactivity displayed by *N*-methylpropylamine is considerably low than that of *n*-propylamine due to steric hin-

(22) Suckling, C. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 537.

Scheme III



drance during the addition step.

The oxidation of benzylamine appears to proceed in a similar manner. However, in this case, the quinol $\mathbf{1H_2}$ was the major product, even in the presence of a large excess of amine. No (alkylamino)phenol was obtained at all. These results can be explained in terms of the acidity of the α-proton of the carbinolamine intermediate. That is, in the case of the benzylamine adduct, because the α-proton is more acidic than that of a carbinolamine derived from an alkylamine, both α-deprotonation (path b) and dehydration followed by rearrangement of the iminoquinone so formed to the imine (path f) proceed with especial efficiency to give the quinol and the aminophenol, respectively. Therefore attack by a second molecule of amine on the iminoquinone intermediate is prevented. That $PhCD_2NH_2$ was less reactive than $PhCH_2NH_2$ and yielded a slightly smaller amount of quinol (relative to aminophenol) indicates (Table III) that α-deprotonation is involved, at least in part, in the rate-determining step. On the other hand, the lower acidity of the α-proton[22] may be responsible to the different behavior observed in the case of cyclopropylamine. Here, dehydration of the carbinolamine intermediate to form **5** predominates over the α-deprotonation. The rearrangement of **5** to the imine intermediate (path f) is also slow. Furthermore, the addition of a second molecule of amine to C(5) of the iminoquinone intermediate is also sterically hindered by the presence of the cyclopropyl group in the vicinity of the C(5) position. Consequently, the iminoquinone **5** is obtained as the sole product.

The product yields and the ratio of $\mathbf{1H_2}$ to $\mathbf{2H_2}$ were little affected by changing the electronic nature of the para substituent of benzylamines. These observations also seem to support the belief that an ionic mechanism operates. The presence of an electron-withdrawing group would increase the acidity of the α-proton of the carbinolamine intermediate, but would also inhibit the migration of electrons from the α-position to the quinone moiety. On the other hand, the opposite effects, decreased acidity and enhanced electron migration, would be expected if an electron-donating group were present. In fact, the electronic nature of the para substituent had little effect. If the quinol were formed via an electron-transfer mechanism, the presence of and electron-donating group in the benzylamine would lead to an increase in the relative amount of quinol that was formed.

The results in Table IV clearly indicate that the PQQTME-catalyzed aerobic oxidation is also ionic in nature.[23] The lower reactivities of the secondary and the tertiary benzylamines can be explained by the ionic mechanism as was discussed above. The lower reactivity displayed by benzylamine in CH_3OH may be due to a competing addition of CH_3OH to the quinone carbonyl group of PQQTME. The extremely low reactivity displayed in nonpolar CH_2Cl_2 also argues in support of an ionic mechanism. In the case of alkylamines, no evidence of a catalytic reaction was detected. That this was so may reflect stability of the (alkylamino)phenol product toward oxidation by O_2 (Table II). The oxazole **7** is formed gradually during the catalytic cycles by the intramolecular cyclization of the imine intermediate and subsequent aromatization. It appears that the reaction described here is the first example of an efficient quinone-catalyzed autoxidation of amines in organic media. It should be possible to apply such a reaction to organic synthesis.

It should also be mentioned that the reaction to form an aminophenol (transamination) is the only pathway followed by 3,5-di-*tert*-butyl-1,2-benzoquinone, even in its reaction with benzylamines.[12] Thus, the question is why α-deprotonation of the carbinolamine intermediate (path b and path e) occurs efficiently in the case of PQQ. One of the most attractive explanations is that intramolecular general base catalysis operates and aids the α-deprotonation of the carbinolamine intermediate (Scheme III). The observation that 1-Me-PQQTME is less reactive than PQQTME and yields a different product (an aminophenol) strongly support such a possibility.

Because knowledge of the identity of the coenzyme is lacking, the mechanism of the enzymatic oxidation of amines has not been clearly elucidated. Some of the earliest investigations focused on the possible roles of pyridoxal phosphate[24] and a ring-modified flavin.[25] Recently, Klinman et al.[26] investigated the mechanism of the oxidation of amines catalyzed by bovine plasma amine oxidase by taking into account the *o*-quinone structure of the presumed coenzyme and proposed a transamination mechanism that involves the formation of a Schiff base (via the reaction of one of the quinone carbonyl group and the amine) followed by a 1,3-prototrophic shift to give the aminophenol derivative of the coenzyme. Kagan et al.[5d] proposed a similar transamination mechanism for oxidations catalyzed by lysyl oxidase and demonstrated the existence of a carbanionic intermediate by trapping that species with nitromethane.

The results presented here clearly indicate that, in nonenzymatic systems, addition–elimination (the forma-

(23) The oxidation of the reduced species of PQQTME by molecular oxygen was much faster than the oxidation of amines catalyzed by PQQTME under the reaction conditions employed.

(24) Petterson, G. *Structure and Functions of Amine Oxidases*; Mondovi, B., Ed.; CRC Press: Boca Raton, FL, 1985; pp 105–117.

(25) Hammilton, G. A. *Copper Proteins*; Spiro, T. G., Ed.; Wiley-Interscience: New York, 1981; pp 193–218.

(26) Hartmann, C.; Klinman, J. P. *BioFactors* 1988, *1*, 41 and references cited therein.

tion of a quinol and an (alkylamino)phenol) is energetically more favorable than is transamination. The mechanistic discrepancy between the enzymatic reaction and the model reaction may arise from the fact that the *o*-quinone cofactor in *mammalian copper-containing amine oxidases* is TOPA and not PQQ. But in any case, it can be said that the novel structure of PQQ, *pyrroloquinoline quinone*, contributes to the catalytic efficiency in the amine oxidation. Simple *o*-quinones, like phenanthrenequinone, do not display any catalytic activity at all in the related aerobic oxidation of amines. As Bruice et al. have reported,[11a] the presence of a pyridine nucleus in PQQ facilitates the nucleophilic addition of amines to the C(5) quinone carbon atom and stabilizes the carbinolamine intermediate thus formed by intramolecular hydrogen bonding.[11a] The presence of an acidic pyrrole proton is also very important for intramolecular general base catalysis to operate, as is indicated in Scheme III. The significance of the *pyrroloquinoline quinone* structure is now being further investigated by using indolequinone and quinolinequinone analogues of PQQ.

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Registry No. 1, 74447-88-4; 1 H_2, 102408-71-9; 2, 128031-21-0; 2 H_2, 128031-23-2; 3, 128031-22-1; 4, 136342-75-1; 5 (isomer 1), 136342-76-2; 5 (isomer 2), 136342-77-3; 5 H_2, 136342-78-4; 6, 116451-41-3; 7, 136342-79-5; $PhCH_2NH_2$, 100-46-9; $PhCH_2NMe_2$, 103-83-3; $PhCH(Me)NH_2$, 98-84-0; $PhCH_2NHMe$, 103-67-3; $PhCH(Ph)NH_2$, 91-00-9; PhCHO, 100-52-7; $PhC(O)CH_3$, 98-86-2; Ph_2CO, 119-61-9; *p*-$MeOC_6H_4CH_2NH_2$, 2393-23-9; *p*-$ClC_6H_4CH_2NH_2$, 104-86-9; *p*-$MeC_6H_4CH_2NH_2$, 104-84-7; $PhCD_2NH_2$, 15185-02-1; $PhCH_2N{=}CHPh$, 780-25-6; *tert*-butylamine, 75-64-9; *n*-propylamine, 107-10-8; *N*-methylpropylamine, 627-35-0; cyclopropylamine, 765-30-0; *n*-hexylamine, 111-26-2; cyclohexylamine, 108-91-8; methylhydrazine, 60-34-4; *N,N*-dimethylpropylamine, 926-63-6; triethylamine, 121-44-8.

Synthesis of Racemic and Optically Active Δ^9-Tetrahydrocannabinol (THC) Metabolites

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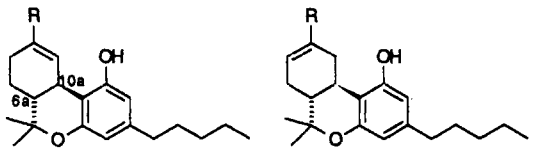
The preparation of racemic and optically active Δ^9-THC metabolites is described from synthon **13**. Racemic synthon **13** is prepared in four steps (46%) from Danishefsky's diene. Optically active synthon **13** is prepared from perillaldehyde via the enone **22** in six steps (23% yield). Alternatively, nopinone can be converted to **13** in three steps (50% yield) via a cyclobutane ring cleavage. The acid-catalyzed condensation of **13** with olivetol (**6a**) and subsequent conversion to 11-hydroxy and 9-carboxyl Δ^9-THC metabolites **2a** and **4a** is described, as well as the preparation of 1′,1′-dimethylheptyl THC analogues **2b**, **3b**, and **4b** from 5-(1′,1′-dimethylheptyl)resorcinol (**6c**).

Introduction

Although *Cannabis Sativa* (marijuana) has been used for centuries and its active constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC, **1a**, Chart I),[1] was identified and synthesized many years ago, until recently very little was known about its mode of action. The recent reports of isolations of possible cannabinoid receptors,[2] as well as cannabinoid-like activity in "nonclassical" compounds (e.g., H-CP-55940),[3] have lead to a renewed interest in Δ^9-THC's structure–activity relationships (SAR) as well as receptor(s) identification. Because of the extremely active pharmacology of some THC metabolites,[4] they seem ideal targets for radiolabelled binding and SAR studies.

Chart I



Δ^9-THCs	Δ^8-THCs
1a; R = CH_3	**1b**; R = CH_3
2a; R = CH_2OH	**2b**; R = CH_2OH
3a; R = CHO	**3b**; R = CHO
4a; R = COOH	**4b**; R = COOH

In addition, the continued illicit use of marijuana, as well as present concern over drug abuse, have lead to the development of methods to determine accurately marijuana use by individuals. One of the main metabolic pathways of Δ^9-THC (**1a**) is hydroxylation at the allylic C-11 position followed by oxidation to the 11-nor-9-carboxy-Δ^9-THC (**4a**). This compound is then excreted as the glucuronide in urine.[5] Metabolite **4a** is used as an internal standard in various analytical procedures to unequivocally confirm its presence in biological fluids. It has, therefore, gained

(1) For a dibenzopyran numbering system used in this paper and a review of cannabinoid synthesis, see: (a) Razdan, R. K. In *Total Synthesis of Natural Products*; ApSimon, J., Ed.; John Wiley: New York, 1981; Vol. 4, pp 186–262. (b) Mechoulam, R.; McCallum, N. K.; Burstein, S. *Chem. Rev.* **1976**, *76*, 75.

(2) (a) Devane, W. A.; Dysarz, F. A., III; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. *Mol. Pharmacol.* **1988**, *34*, 605. (b) Matsuda, L. A.; Lolait, J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. *Nature* **1990**, *346*, 561. (c) Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; DeCosta, B. R.; Rice, K. C. *Proc. Natl. Acad. Sci.* **1990**, *87*, 1932.

(3) (a) Little, P. J.; Compton, D. R.; Johnson, M. R.; Melvin, L. S.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 1046. (b) Martin, B. R.; Compton, D. R.; Thomas, B. F.; Prescott, W. R.; Little, P. J.; Razdan, R. K.; Johnson, M. R.; Melvin, L. S.; Mechoulam, R.; Ward, S. *J. Neuroscience and Biochemical Rev.*, in press.

(4) Razdan, R. K. *J. Pharmacol. Rev.* **1986**, *38*, 75.

(5) Agurell, S.; Halldin, M.; Lindgren, J.; Ohlsson, A.; Widman, M. *Ibid.* **1986**, *38*, 21.

0022-3263/91/1956-6865$02.50/0