Synthesis and Characterization of the 6-Deaza Derivative of Coenzyme PQQ, Methyl 4,5-Dihydro-4,5-dioxobenz[g]indole-2-carboxylate

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The synthesis of the 6-deaza derivative of coenzyme PQQ, methyl 4,5-dihydro-4,5-dioxobenz[g]indole-2-carboxylate (4), is described, and its physical and chemical properties are compared to those of the trimethyl ester of PQQ (2) and the methyl ester of 7,9-didecarboxy PQQ (3). The synthesis of 4 was achieved by starting with 1-aminonaphthalene and constructing 2-carbomethoxybenz[g]indole (7) by a Japp–Klingemann reaction with methyl α-methylacetoacetate and a subsequent Fischer indolization reaction. The quinone function was introduced by a Fremy's salt oxidation of 5-aminoindole 9 which was prepared from 7 by regioselective nitration and catalytic hydrogenation. From the physical properties, it can be recognized that the peri pyridine nitrogen and the ester groups at the 7- and 9-positions considerably affect the electronic nature of the molecules. This is reflected on the reactivities of the quinones in the acetone-adduct formation, the reaction with phenylhydrazine, and the aerobic autorecycling oxidation of benzylamine. The significant roles of the pyridine nitrogen and the ester groups in these reactions are discussed.

Introduction

Since PQQ (1) was first reported to be a prosthetic group of methanol dehydrogenase from methylotrophic bacteria,[1] several non-flavin- or nicotinamide-dependent dehydrogenases have been recognized to be PQQ-containing enzymes, known as quinoproteins.[2] In addition to the enzymological importance, the pharmaceutical activities[3,4] and the nutritional importance[5] of PQQ have recently received much attention in various research fields.

PQQ is an efficient electron-transfer catalyst in the nonenzymatic oxidation of biologically important substances such as amines,[6] amino acids,[7] thiols,[8] glucose,[9] and NADH.[10] In order to know the effect of substituents, decarboxy derivatives of PQQ were synthesized[11,12] and their reactivities in both enzymatic[13] and nonenzymatic systems were investigated.[11] However, little attention has been focused on the structural importance of the *pyrroloquinolinequinone* skeleton. Bruice and Eckert pointed out the significant role of the pyridinyl nitrogen in the oxidation of amines by phenanthrolinequinones.[14] To address this issue, we synthesized and characterized the 6-deaza derivative of PQQ, methyl 4,5-dihydro-4,5-dioxobenz[g]indole-2-carboxylate (4), and investigated its chemical reactivity toward adduct formation and its redox properties. In this study, the trimethyl ester of PQQ (2) and the methyl ester of 7,9-didecarboxy PQQ (3) were employed as reference compounds. So far, 3-carbethoxy-2-methylbenz[g]indole-4,5-dione is known as a structurally related compound,[11a] but its properties have not been studied in detail.

Table I. Comparison of the Physical Properties of 2–4

		2	3	4
^{13}C NMR	C-4	173.5[a]	173.4	174.0[c]
(ppm, DMSO-d_6)	C-5	177.2[b]	179.4	181.0[d]
IR, quinonoid $\nu_{C=O}$ (cm^{-1})		1686	1683	1668
pK_a of the pyrrole proton (CH_3CN)		18.4	18.8	20.5
E_m[e] (mV vs SCE)		−51	−170	−296

[a] Doublet, $^3J_{CH}$ = 1.4 Hz. [b] Single. [c] Doublet, $^3J_{CH}$ = 1.3 Hz. [d] Doublet–doublet, $^3J_{CH}$ = 4.2 Hz, $^4J_{CH}$ = 1.5 Hz. [e] $E_m = {}^1/_2(E_{ap} + E_{cp})$ in 0.1 M phosphate buffer (pH 7.0) containing 5% CH_3CN.

Results and Discussion

Synthesis of Benzindolequinone 4. The benz[g]-indole skeleton having a carbomethoxy group at the 2-position was constructed by applying the combination of a Japp–Klingemann reaction and a Fischer indolization, as in the case of the total synthesis of PQQ.[15] Treatment of 1-aminonaphthalene in aqueous hydrochloric acid at 0 °C with 1.0 equiv of $NaNO_2$ for 10 min produced the diazonium salt, which was added to a solution of 1.0 equiv of methyl α-methylacetoacetate and 1.4 equiv of KOH in CH_3OH–H_2O (1:1) at 0 °C. After 5 h the resulting 1-diazonaphthalene 5 was isolated as a crude product and treated in refluxing H_3PO_4–CH_3OH (1:9) for 15 min to

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(2) *PQQ and Quinoproteins, Proceedings of the First International Symposium on PQQ and Quinoproteins*; Jongejan, J. A., Duine, J. A., Eds., Kluwer Academic: The Netherlands, 1989. The general term "quinoproteins" had been used for enzymes containing PQQ as the coenzyme, but after the discovery of new quinone cofactors, TOPA quinone and TTQ (tryptophan tryptophylquinone), this term was expanded into enzymes which contain the quinonoid cofactors; see: Duine, J. A. *Eur. J. Biochem.* **1991**, *200*, 271. The recent findings raise serious questions regarding the role of PQQ in higher eukaryotes; see: Klinman, J. P.; Dooley, D. M.; Duine, J. A.; Knowles, P. F.; Mondovi, B.; Villafranca, J. J. *FEBS Lett.* **1991**, *282*, 1.
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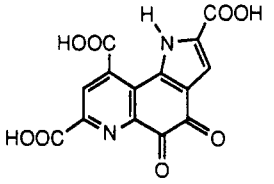
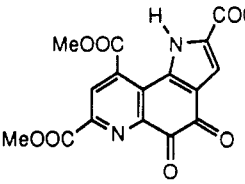


1 PQQ



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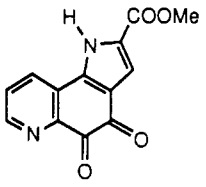

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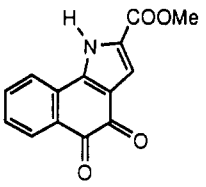
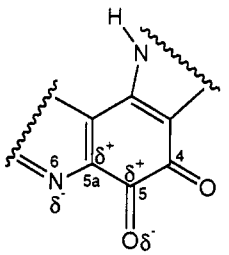


4

produce the hydrazone **6**. The compound **6** exists as a mixture of cis–trans isomers at the hydrazo function or of azo–hydrazone tautomers (see Experimental Section), but was used in the next step without further purification. The treatment of **6** in refluxing methanol saturated with dry HCl for 30 min gave the benzindole **7**, that was nitrated with HNO_3–CH_3COOH at 0 °C. The position of nitration was confirmed to be at C-5 by the NOE observed between H-3 and H-4 of the product. The nitro derivative **8**, thus obtained, was converted into the amino derivative **9** by catalytic hydrogenation (H_2 (4 atm)/PtO_2). Oxidation of **9** by Fremy's salt produced the expected quinone product **4** in 44% yield.

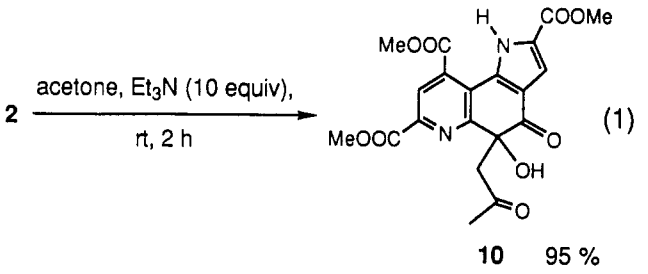
Physical Properties. Some of the physical and spectral data of **2**–**4** are summarized in Table I. ^{13}C-NMR signals for C-4 and C-5 were assigned from the ^1H–^{13}C coupling patterns ($^3J_{CH}$ and $^4J_{CH}$ indicated in the footnote of Table I). As in the case of PQQ itself,[16] C-5 appears at lower field than C-4 in all cases. It is interesting to note that C-5 tends to shift to lower field in going from **2** to **4**, while the chemical shift of C-4 is essentially unchanged. The IR absorption for the quinonoid carbonyl stretching of **4** is about 15–20 cm^{-1} lower than those of **2** and **3**, respectively. From these results, it can be said that polarization of the C-5 carbonyl function of the pyrroloquinolinequinone molecule is diminished to some extent by the electronegativity of the peri pyridine nitrogen. In other words, the electropositive character of the C-5 carbonyl carbons of **2** and **3** decrease to some extent, as compared with that of the C-5 of **4**, due to the adjacent electron deficient C-5a carbon as illustrated below.



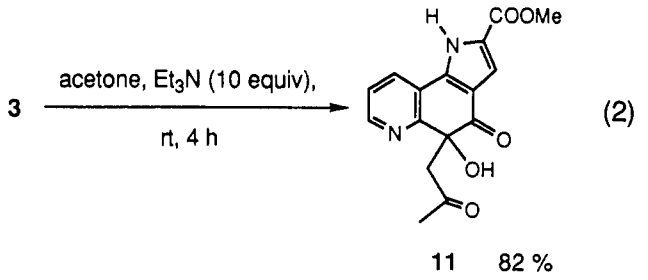
The pK_a values of the pyrrole proton (H-1) were determined by an ordinary spectrophotometric titration in *acetonitrile* by using triethylamine as a base. Because of the electron-attracting nature of both the pyridine nucleus and the ester groups, the negative charge in the pyrrole ring, generated by the dissociation of the pyrrole proton, is considerably stabilized, and this is reflected on the order of the pK_a values: **2** < **3** ≪ **4**. Such an electronic effect of the pyridine nucleus and the ester groups is also detected in the redox potentials. Namely, the E_m of **2** is about 120 mV more positive than that of **3** and is about 250 mV more positive than that of **4**. In the UV–vis spectrum (Figure 1), each compound has a very broad and weak absorption at around 400–550 nm that corresponds to the quinonoid n–π* transition, but the absorption at the lower wavelength region is largely different among these quinones. Similar spectral change has been reported between PQQ and 7,9-didecarboxy methoxatin (carboxylic acid derivative of **3**) in an aqueous solution at pH 7.[11d]

In general, the electronic effect of the peri pyridine nitrogen is much larger than that of the ester groups. This tendency will be demonstrated further in the following reactions.

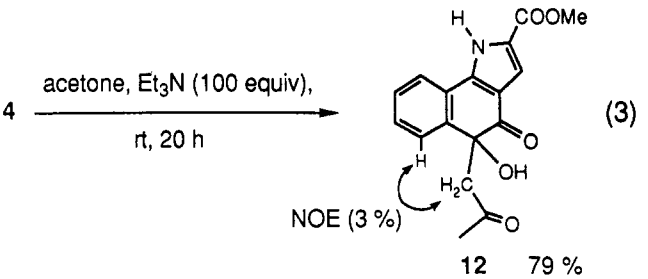
Acetone Adduct Formation. Nucleophilic addition to the quinone carbonyl carbon has been proposed to be a key step in the redox reactions of PQQ with amines,[6] amino acids,[7] thiols,[8] and hydrazines.[17] In order to estimate the reactivity of the quinone function toward nucleophiles, reaction with acetone was examined in the presence of triethylamine in acetonitrile (eqs 1–3). Each



10 95 % (1)



11 82 % (2)



12 79 % (3)

quinone compound formed a stable aldol-type adduct as in the cases of PQQ.[1] The position of nucleophilic addition has been shown to be at C-5 by X-ray crystallographic analysis in the case of PQQ itself[1] and is thus assumed to be at C-5 for the present model compounds. This was confirmed by measuring the NOE on each adduct. About a 3% NOE was observed between the methylene protons of the acetonyl chain and the aromatic proton at the 6-position of **12**, but no NOE on the methylene protons was observed in the case of **10** and **11**. If the addition occurred at C-4, the NOE should be detected between the methy-

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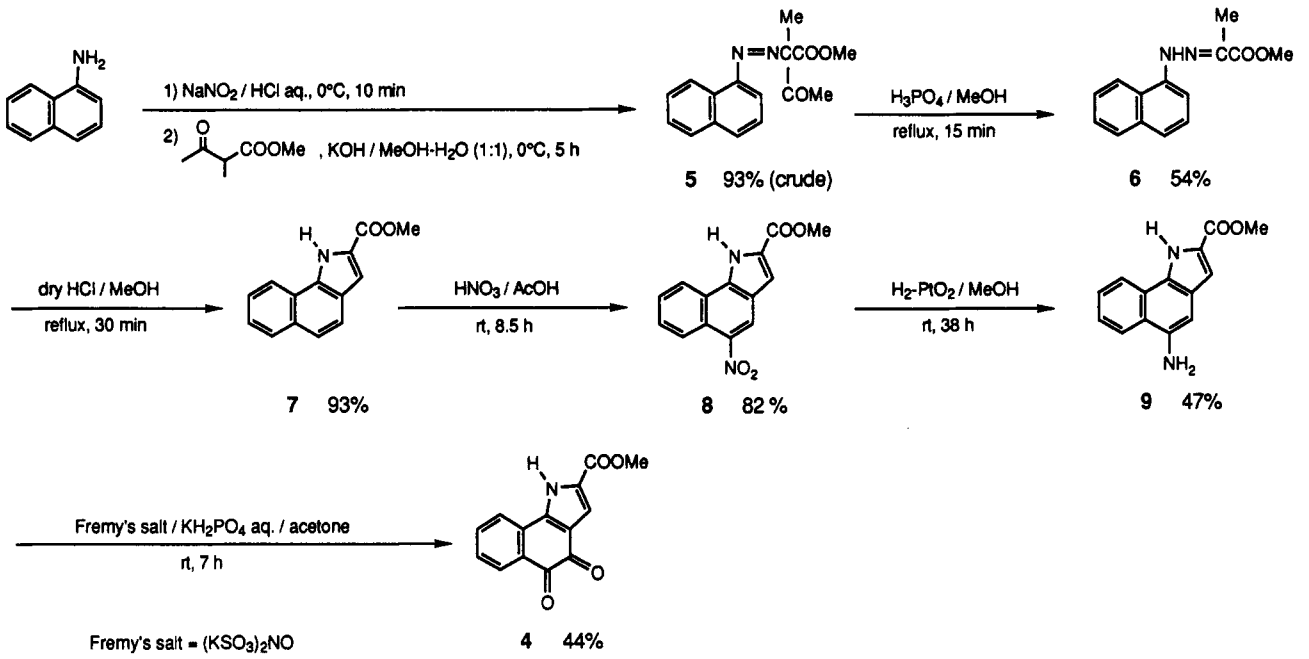
Scheme I



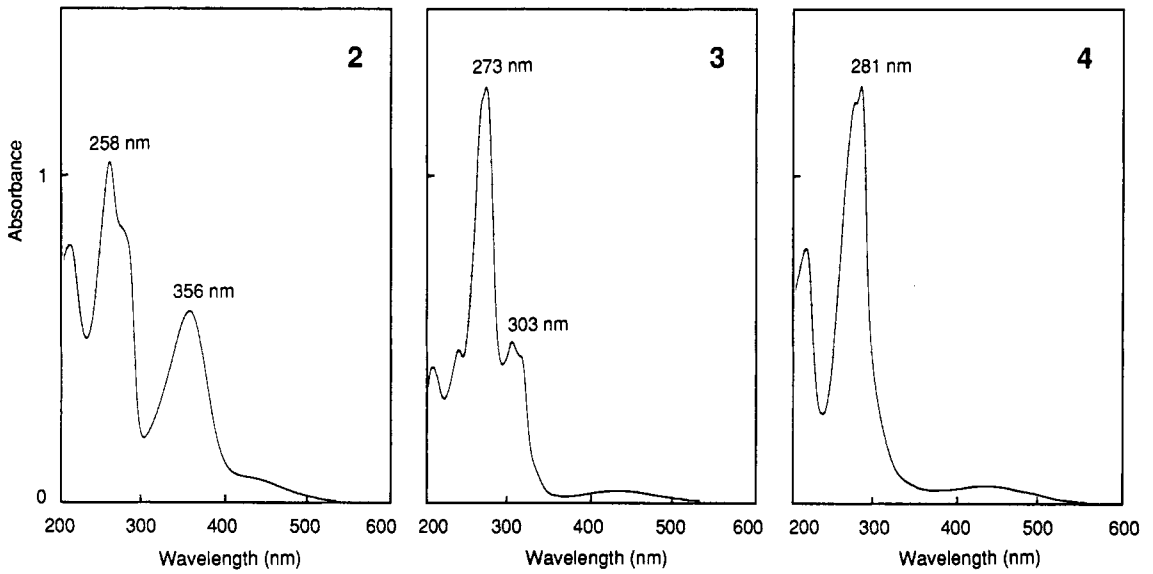


Figure 1. UV–vis spectra of **2–4** in acetonitrile (4.0×10^{-5} M).

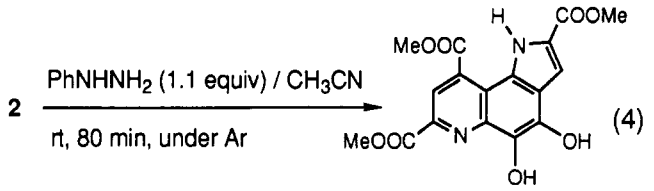
lene protons and H-3 of each product.

The UV–vis spectrum of the adduct **10** in acetonitrile is shown in Figure 2. Characteristics of the C-5 adduct is that the absorption band at around 356 nm shifts toward longer wavelength by about 15 nm, and the quinonoid n–π^* transition at 400–500 nm and a shoulder around 270 nm disappear. In the ^{1}H NMR, the aromatic proton at the 8-position shifts toward upfield larger than the proton at the 3-position as being the C-5 adduct (**2**: H-3, δ 7.48, H-8, 8.89, **10**: H-3, δ 7.44, H-8, 8.67 in $CDCl_3$). This may be attributed to the disappearance of the mesomeric effect between the C-5 quinone carbonyl and the 8-position.

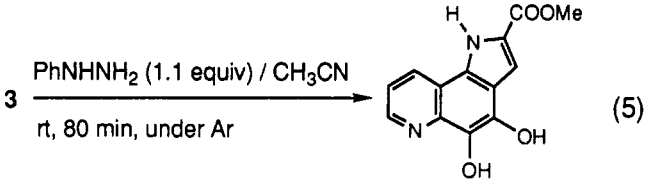
It should be noted that the reactivities of the three quinones are relatively different with an order of **2** > **3** ≫ **4**. Considering the electron-withdrawing nature of the pyridine nucleus and the electron-releasing nature of the pyrrole nucleus under the basic conditions, the addition site of C-5 and that order of the reactivity seem to be very reasonable.

Reaction with Phenylhydrazine. The reaction of PQQ with hydrazines has been investigated in connection with the mechanism of amine oxidation by PQQ and also with the inhibitory action of hydrazines on quinoproteins.[17] The compounds **2** and **3** were quantitatively reduced to the corresponding quinol, $\mathbf{2H_2}$ and $\mathbf{3H_2}$, respectively, by phenylhydrazine (eqs 4 and 5). In the case of **4**, however, no redox reaction was observed, but the hydrazone derivative **13** was obtained as the major product (eq 6). Furthermore, **4** was less reactive than **2** and **3**. An excess of phenylhydrazine was required to promote the reaction with **4** under the same conditions.

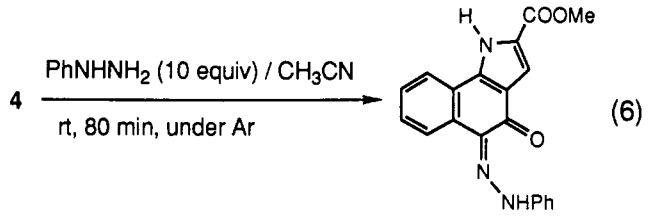
We have already proposed an ionic mechanism that involves a carbinolamine-type intermediate **14** for the reaction of PQQ and hydrazines (Scheme II). From the intermediate, the redox reaction (quinol formation) occurs via electron migration from the hydrazino nitrogen to the quinone (indicated by the arrows in Scheme II). If this is not fast enough, dehydration from the intermediate predominates to give the hydrazone. The present results strongly support the proposed mechanism. In the case of **2** and **3**, the pyridine nucleus may facilitate the nucleophilic addition of the hydrazine to the quinone and sta-



$2H_2$ quant.



$3H_2$ quant.



13 76 %

bilize the carbinolamine-type intermediate thus formed through intramolecular hydrogen bonding between the peri pyridine nitrogen and the hydrazino-NH. The electron-attracting nature of the pyridine nucleus may also facilitate the electron migration from the hydrazino nitrogen to the quinone function. Therefore, quinol formation predominates. A similar role for the peri pyridine nitrogen was suggested in the oxidation of amines by phenanthroline-quinones.[14] In the case of **4**, however, such electronic effects could not be expected, so dehydration predominates over the redox reaction to afford the hydrazone **13**.

Aerobic Oxidation of Benzylamine. Similar effects of the pyridine nucleus would be expected in the oxidation of amines. Thus, the catalytic efficiency of the quinones was examined in the *aerobic autorecycling oxidation* of benzylamine in acetonitrile (Scheme III). Figure 3 shows the time course of the reaction that was obtained by monitoring the formation of benzaldehyde by HPLC. Compound **2** has been already demonstrated to be a very efficient turnover catalyst in this system.[18] In this study, compound **3** was also found to be a good catalyst, though its catalytic efficiency is lower than that of **2**. However, no catalytic reaction occurred in the case of **4**.

A similar ionic mechanism that involves a carbinolamine intermediate has been proposed for the amine oxidation.[18] There may be several factors that control catalytic activity, but at least the similar reasons, argued in the reaction with phenylhydrazine, could be attributed to such a big difference in reactivity between **2** or **3** and **4**. In the reaction of **2** and benzylamine under *anaerobic* conditions, the former was converted into the corresponding reduced species, quinol and aminophenol, within 2 h.[18] On the contrary, about 85% of **4** was recovered in the same reaction with benzylamine even after 46 h, indicating that the *addition step* is very slow. Deactivation of **4** may also take place by forming a redox-inactive adduct through an *imine intermediate* that is produced by *dehydration* from

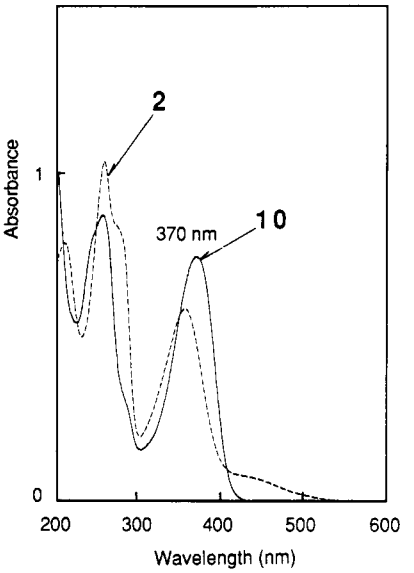


Figure 2. UV–vis spectra of **2** and **10** in acetonitrile (4.0×10^{-5} M).

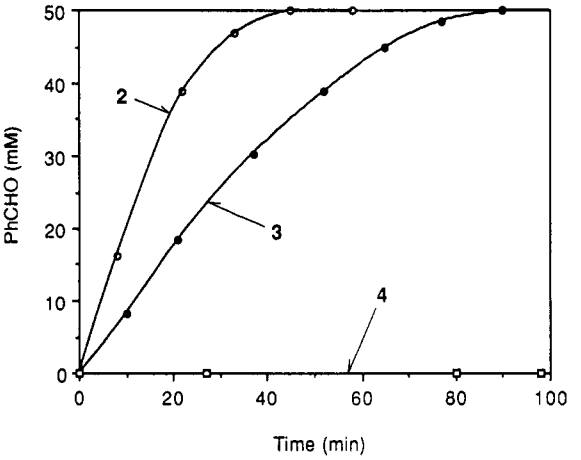
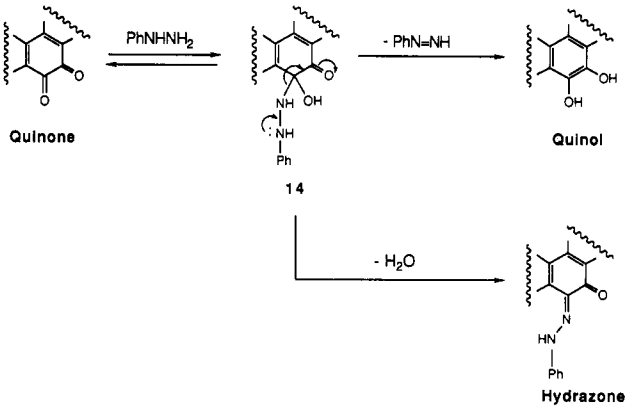


Figure 3. Time course of the oxidation of benzylamine (100 mM) in the presence of **2**–**4** (1.0 mM) at rt in CH_3CN under O_2 atmosphere.

Scheme II



the carbinolamine intermediate as the hydrazone **13** was formed.

PQQ or a closely related compound was thought to be a second organic cofactor of mammalian copper-containing amine oxidases and bacterial methylamine dehydrogenase.[19] But very recently, it has been reported that the new redox cofactors, TOPA quinone and TTQ

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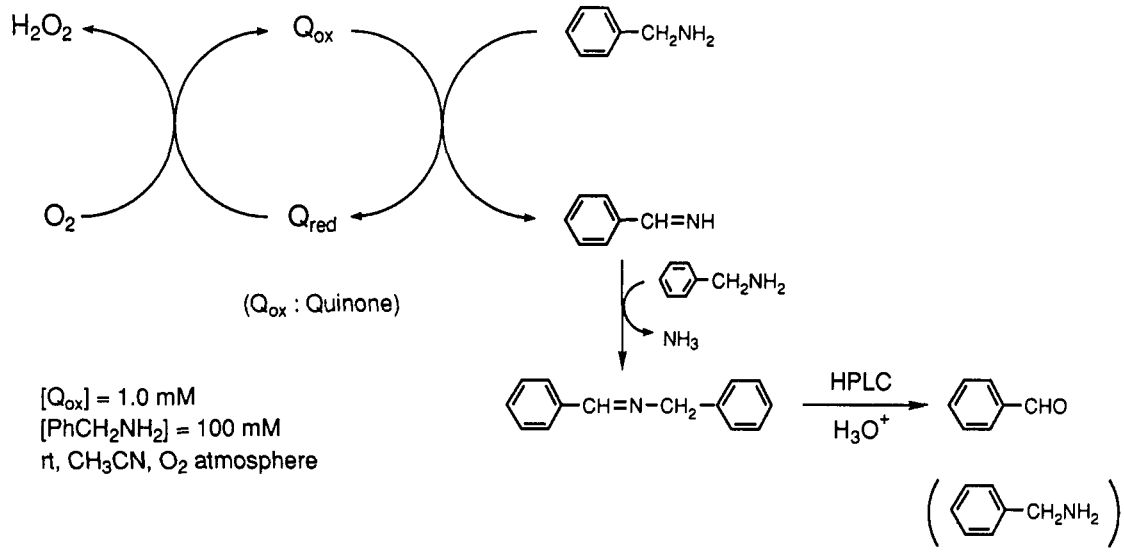
Scheme III



(tryptophan tryptophylquinone), are involved in bovine serum amine oxidase and methylamine dehydrogenase, respectively.[20,21] It is interesting to note, however, that the pyrroloquinolinequinone derivative is the most efficient amine-oxidation catalyst in vitro ever reported. Comparison of the chemical characteristics of these new cofactors to that of PQQ will undoubtedly provide another interesting avenue of research.

Experimental Section

The trimethyl ester of PQQ (**2**)[15] and the methyl ester of 7,9-didecarboxy PQQ (**3**)[12] were prepared by the reported methods. The chemicals used in this study were purified by the standard methods,[22] if necessary. Melting points are uncorrected. The pK_a value of the pyrrole proton (H-1) was determined by an ordinary spectrophotometric titration in acetonitrile by using triethylamine as a base. The pK_a value of 18.6 for triethylamine in acetonitrile was taken from the literature.[23]

1-[[1-(Methoxycarbonyl)-1-(methylcarbonyl)ethyl]azo]-naphthalene (5). An aqueous solution (30 mL) of $NaNO_2$ (1.47 g, 21.3 mmol) was added dropwise to a stirred solution of 1-aminonaphthalene (3.0 g, 21.0 mmol) in 350 mL of 0.14 N hydrochloric acid (50 mmol) at 0 °C. The resulting solution of diazonium salt was then added in one portion to a rapidly stirred solution of methyl α-methylacetoacetate (21 mmol) and KOH (1.65 g, 29.4 mmol) in methanol–water (1:1, 25 mL) at 0 °C. The mixture was stirred for 5 h at 0 °C, and the resulting brown solid was isolated by filtration and dried in vacuo, yielding 5.53 g of a crude product, as a mixture with **6**, which was used in the next step without further purification: IR (KBr) 1724 cm^{-1} (C=O); ^1H NMR (270 MHz, $CDCl_3$) δ 1.78 (3 H, s, $-CH_3$), 2.42 (3 H, s, $-COCH_3$), 3.86 (3 H, s, $-COOCH_3$), 7.5–7.7 (m, aromatic protons), 7.8–8.0 (m, aromatic protons) (those aromatic protons of **5** could not be assigned accurately because of the overlap with the aromatic protons of **6**), 8.01 (1 H, d, J = 8.1 Hz), 8.60 (1 H, d, J = 9.2 Hz).

Methyl Pyruvate 1-Naphthylhydrazone (6). The crude product obtained above (5.53 g) was treated with phosphoric acid–methanol (1:9, 1.0 mL) at refluxing temperature for 15 min. Evaporation of the solvent gave a crude product which was recrystallized from methanol; a brown solid (2.53 g, 54%): mp 94–98 °C dec; IR (KBr) 3272 (NH), 1684 (C=O), 1562 cm^{-1} (C=N); ^1H NMR (270 MHz, $CDCl_3$) δ 2.25 and 2.28 (5:2, total 3 H, each s, $-CH_3$), 3.90 and 3.91 (5:2, total 3 H, each s, $-COOCH_3$), 7.42–7.55 (4 H, m), 7.64–7.72 (1 H, m), 7.80–7.98 (2 H, m), 8.24 (1 H, br s, –NH–) (**6** is considered to exist as a mixture of cis–trans isomers at the hydrazo function or azo–hydrazone tautomers); MS (EI) m/z 242 (M^+). Anal. Calcd for $C_{14}H_{14}O_2N_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.22; H, 5.74; N, 11.54.

Methyl Benz[*g*]indole-2-carboxylate (7). The hydrazone **6** (2.5 g, 10.3 mmol) was treated with refluxing methanol (30 mL) saturated with dry HCl for 30 min. Removal of the solvent gave a solid residue which was recrystallized from methanol to give the indole **7** as a pale brown solid in 93% yield: mp 168–170 °C dec; IR (KBr) 3336 (NH), 1698 cm^{-1} (C=O); ^1H NMR (270 MHz, $CDCl_3$) δ 3.98 (3 H, s, $-COOCH_3$), 7.33 (1 H, d, J = 1.6 Hz, H-3), 7.47–7.62 (3 H, m), 7.68 (1 H, d, J = 8.9 Hz), 7.92 (1 H, d, J = 7.3 Hz), 8.12 (1 H, d, J = 8.1 Hz), 9.70 (1 H, br s, NH); MS (EI) m/z 225 (M^+). Anal. Calcd for $C_{14}H_{11}O_2N$: C, 74.65; H, 4.92; N, 6.22. Found: C, 74.51; H, 4.84; N, 6.19.

Methyl 5-Nitrobenz[*g*]indole-2-carboxylate (8). To a mixture of acetic acid (5.3 mL) and concentrated nitric acid (3.2 mL) was added the indole **7** (5.03 g, 22.4 mmol), and the reaction mixture was stirred at room temperature for 8 h. The resulting yellow solid was isolated by filtration and washed with water to give **8** in 82% yield: mp 202–204 °C dec; IR (KBr) 3336 (NH), 1700 (C=O), 1530 and 1322 cm^{-1} (NO_2); ^1H NMR (270 MHz, $CDCl_3$) δ 4.00 (3 H, s, $-COOCH_3$), 7.44 (1 H, d, J = 1.7 Hz, H-3, about a 4% NOE was detected when irradiated at H-4), 7.67–7.75 (2 H, m, H-7 and H-8), 8.24 (1 H, m, H-9), 8.62 (1 H, s, H-4), 8.71 (1 H, m, H-6), 10.17 (1 H, br s, NH); MS (EI) m/z 270 (M^+). Anal. Calcd for $C_{14}H_{10}O_4N_2 \cdot {}^1/_4H_2O$: C, 61.20; H, 3.85; N, 10.20. Found: C, 61.03; H, 3.55; N, 9.94.

Methyl 5-Aminobenz[*g*]indole-2-carboxylate (9). Catalytic hydrogenation of the nitro group of **8** (1.00 g, 3.70 mmol) was carried out in the presence of a catalytic amount of PtO_2 (83 mg) in methanol under hydrogen (4 atm) at room temperature for 38 h. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The resulting crude product was purified by flash chromatography ($SiO_2/CHCl_3$) to afford a brown solid in 47% yield: mp 158–161 °C dec; IR (KBr) 3348 (NH, NH_2), 1696 cm^{-1} (C=O); ^1H NMR (270 MHz, $CDCl_3$) δ 3.8–4.1 (2 H, br s, $-NH_2$), 3.96 (3 H, s, $-COOCH_3$), 6.98 (1 H, s, H-4), 7.15 (1 H, d, J = 2.2 Hz, H-3), 7.5–7.6 (2 H, m, H-7 and H-8), 7.97 (1 H, d, J = 8.3 Hz, H-6), 8.11 (1 H, d, J = 8.3 Hz, H-9), 9.64 (1 H, br s, NH); MS (EI) m/z 240 (M^+).

The amino derivative **9** is not so stable in a solution under aerobic conditions that its purification was very difficult. An undesirable dimer was readily formed during the purification procedure: mp 203–206 °C dec; IR (KBr) 3450, 3344 (–NH–), 1694 cm^{-1} (C=O); ^1H NMR (270 MHz, DMSO-d_6) δ 3.87 (3 H, s, $-COOCH_3$), 3.91 (3 H, s, $-COOCH_3$), 5.2 (1 H, br s, –NH), 5.4 (2 H, br s, $-NH_2$), 6.74 (1 H, s), 6.82 (1 H, s), 7.02 (1 H, d, J = 2.2 Hz), 7.42–7.62 (4 H, m), 8.05–8.16 (2 H, m), 8.64–8.73 (2 H, m), 12.32 (1 H, br s), 12.51 (1 H, br s). The parent peak could not be detected in mass spectra, although some of the ionization modes

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(EI, FAB, FD) were examined. The monomer peak (m/z 240) was always observed as a major one. The lower yield of this step could be attributed to the instability of **9** under aerobic conditions. Details about the mechanism are now under investigation, but it is assumed that the dimer is formed by dehydrogenation of **9**, forming a quinonoid intermediate, and following Michael addition to the 4-position by another molecule of **9**.

Methyl 4,5-Dihydro-4,5-dioxobenz[*g*]indole-2-carboxylate (4). To a solution of **9** (420 mg, 1.75 mmol) in acetone (340 mL) was added dropwise an aqueous solution of KH_2PO_4 (2.76 g) and Fremy's salt ($(KSO_3)_2NO$, 5.72 g, 21.3 mmol) at 0–5 °C. The reaction mixture was stirred at room temperature for 7 h, and the resulting red solid was isolated by filtration and washed with ether (44% yield): mp 285–288 °C dec; IR (KBr) 3468 (NH), 1700 (ester C=O), 1668 cm^{-1} (quinonoid C=O); UV–vis (CH_3CN) λ_{max} 215 (ϵ 20 300), 281 nm (33 200 M^{-1} cm^{-1}); ^1H NMR (270 MHz, DMSO-d_6) δ 3.86 (3 H, s, $-COOCH_3$), 7.15 (1 H, s, H-3), 7.48 (1 H, t, J = 7.3 Hz, H-7 or H-8), 7.72 (1 H, t, J = 7.3 Hz, H-7 or H-8), 7.92 (1 H, d, J = 7.3 Hz, H-9), 8.31 (1 H, d, J = 7.3 Hz, H-6), 13.2 (1 H, br s, NH); ^{13}C NMR (DMSO-d_6) 51.9 ($-CH_3$), 114.5, 121.7, 123.5, 126.7, 129.2, 129.3, 130.1, 134.7, 135.0, 140.6, 160.5 (C-2′), 174.0 (dd, $^3J_{CH}$ = 1.5 Hz, $^4J_{CH}$ = 4.2 Hz, C-4), 181.0 (d, $^3J_{CH}$ = 1.3 Hz) ppm; MS (EI) m/z 255 (M^+); exact mass for $C_{14}H_9O_4N$, calcd 255.0531, found 255.0545. Anal. Calcd for $C_{14}H_9O_4N$: C, 65.88; H, 3.55; N, 5.49. Found: C, 65.41; H, 3.55; N, 5.81.

Acetone Adduct Formation (General Procedure). To a solution of the quinone (10 mM) in acetone was added triethylamine (10–100 equiv) from a microsyringe under N_2. The reaction was monitored by TLC (SiO_2). When the quinine completely disappeared, acetone was removed under reduced pressure. The residue was dissolved in $CHCl_3$ and crystallized by adding n-hexane to the solution.

Trimethyl 4-oxo-5-acetonyl-5-hydroxy-4,5-dihydro-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylate (10): Isolated yield 95%; mp 211–212 °C dec; UV–vis (CH_3CN) λ_{max} 256 (ϵ 21 700), 370 nm (18 500 M^{-1} cm^{-1}); IR (KBr) 3480, 3260 (OH and NH), 1725, 1718, 1680 cm^{-1} (C=O); ^1H NMR (270 MHz, $CDCl_3$) δ 2.15 (3 H, s, $-COCH_3$), 3.48 (1 H, d, J = 15.9 Hz, $-CHHCO-$), 3.62 (1 H, d, J = 15.9 Hz, $-CHHCO-$), 3.96 (3 H, s, $-COOCH_3$), 4.01 (3 H, s, $-COOCH_3$), 4.13 (3 H, s, $-COOCH_3$), 7.43 (1 H, d, J = 2.4 Hz, H-3), 8.67 (1 H, s, H-8), 12.7 (1 H, br s, NH); ^{13}C NMR ($CDCl_3$) 30.8 ($-COCH_3$), 52.3, 53.1, 53.9, 54.4 ($-OCH_3$ × 3, and $-CH_2-$), 113.6, 122.4, 123.4, 126.4, 127.3, 132.6, 133.7, 137.0, 144.9, 160.5 (nine aromatic carbons and C-5), 162.1, 164.3, 167.7 ($(-COOCH_3)$ × 3), 190.4 (C-4), 206.8 ppm (acetonyl C=O); MS (EI) m/z 430 (M^+); exact mass for $C_{20}H_{18}O_9N_2$ calcd 430.1012, found 430.1009. Anal. Calcd for $C_{20}H_{18}O_9N_2$: C, 55.82; H, 4.22; N, 6.51. Found: C, 55.76; H, 4.07; N, 6.41. (For the elemental analysis, **10** was recrystallized again from benzene.)

Methyl 4-oxo-5-acetonyl-5-hydroxy-4,5-dihydro-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylate (11): isolated yield 82%; mp >300 °C, IR (KBr) 3460, 3300 (OH and NH), 1720, 1676 cm^{-1} (C=O); ^1H NMR (270 MHz, DMSO-d_6) δ 1.98 (3 H, s, $-COCH_3$), 3.56 (1 H, d, J = 17.6 Hz, $-CHHCO-$), 3.86 (3 H, s, $-COOCH_3$), 3.89 (1 H, d, J = 17.6 Hz, $-CHHCO-$), 6.05 (1 H, s, -OH), 7.13 (1 H, s, H-3), 7.42 (1 H, dd, J = 8.1 and 4.9 Hz, H-8), 8.48 (1 H, dd, J = 4.9 and 1.3 Hz, H-9), 8.60 (1 H, dd, J = 8.1 and 1.3 Hz, H-7), 13.12 (1 H, br s, NH); MS (EI) m/z 314 (M^+).

Methyl 4-oxo-5-acetonyl-5-hydroxy-4,5-dihydrobenz[*g*]indole-2-carboxylate (12): isolated yield 79%; mp 129–131 °C dec; IR (KBr) 3440, 3304 (OH and NH) 1698, 1670 cm^{-1} (C=O); ^1H NMR (270 MHz, DMSO-d_6) δ 1.94 (3 H, s, $-COCH_3$), 3.23 (1 H, d, J = 15.7 Hz, $-CHHCO-$), 3.31 (1 H, d, J = 15.7 Hz, $-CHHCO-$), 3.86 (3 H, s, $-COOCH_3$), 5.97 (1 H, s, -OH), 7.09 (1 H, s, H-3), 7.33–7.42 (2 H, m, H-7 and H-8), 7.62 (1 H, m, H-6, a 3% NOE was detected when irradiated at the acetonyl methylene proton), 8.26 (1 H, m, H-9), 12.9 (1 H, br s, NH); MS (EI) m/z 313 (M^+).

Reaction with Phenylhydrazine. To a solution of the quinone (4 mM) in CH_3CN was added phenylhydrazine (1.1–10 equiv) from a microsyringe under anaerobic conditions (Ar). The reaction mixture was stirred at room temperature for 80 min. In the case of **2** and **3**, removal of the solvent gave the corresponding quinol, $\mathbf{2H_2}$ and $\mathbf{3H_2}$, quantitatively. The ^1H NMR spectra of those products were identical to that of authentic samples prepared by the reported method.[24] $\mathbf{2H_2}$: ^1H NMR (270 MHz, DMSO-d_6) δ 3.94 (3 H, s, $-COOCH_3$), 4.01 (3 H, s, $-COOCH_3$), 4.11 (3 H, s, $-COOCH_3$), 7.45 (1 H, s, H-3), 8.55 (1 H, s, H-8). $\mathbf{3H_2}$: ^1H NMR (270 MHz, DMSO-d_6) δ 3.94 (3 H, s, $-COOCH_3$), 7.64 (1 H, s, H-3), 7.84 (1 H, dd, J = 8.2 and 5.8 Hz, H-8), 8.85 (1 H, dd, J = 5.8 and 1.7 Hz, H-9), 9.63 (1 H, dd, J = 8.2 and 1.7 Hz, H-7), 11.4 (1 H, br s, NH).

In the case of **4**, the reaction mixture was extracted with CH_2Cl_2 and washed with 1 N HCl and water. After drying over $MgSO_4$, evaporation of the solvent gave a crude product which was purified by thin-layer chromatography (SiO_2: $CHCl_3$/AcOEt = 3/1) to give the hydrazone **13** in 76% yield: mp 259–262 °C dec; IR (KBr) 3304 (NH), 1694 cm^{-1} (C=O); ^1H NMR (270 MHz, DMSO-d_6) δ 3.89 (3 H, s, $-COOCH_3$), 7.20 (1 H, t, J = 7.7 Hz), 7.33 (1 H, d, J = 1.8 Hz, H-3), 7.42–7.55 (4 H, m), 7.68 (2 H, d, J = 7.7 Hz, H-7 and H-8), 8.40 (1 H, dd, J = 7.7 and 1.8 Hz, H-9), 8.46 (1 H, dd, J = 7.7 and 1.8 Hz, H-6), 13.14 (1 H, br s, NH), 15.82 (1 H, br s, NH); MS (EI) m/z 345 (M^+).

Oxidation of Benzylamines was performed in the same manner as reported elsewhere.[18]

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Supplementary Material Available: ^1H NMR spectra for the products **9** (and its dimer), **11**, **12**, and **13** (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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