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PREPARATION AND CHARACTERIZATION OF IMINOQUINONE AND AMINOPHENOL DERIVATIVES OF COENZYME PQQ

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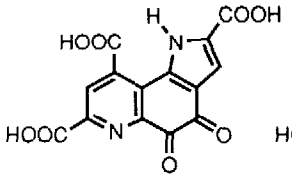
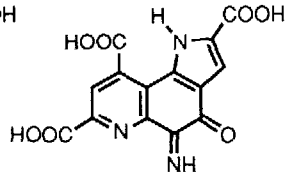
Summary: 5-Iminoquinone derivative of PQQ was easily prepared by treatment with ammonia. Reduction of the iminoquinone with methylhydrazine gave aminophenol derivative of reduced PQQ. Spectral characteristics of them are presented.

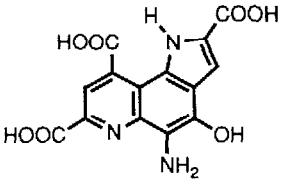
PQQ (4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid) has been given much attention as a novel coenzyme of various important oxidoreductases.[1,2] Ammonia and primary amines are known to be activators for the alcohol dehydrogenases, but it is not clear whether they interact with the coenzyme or with an amino acid residue in the active site.[3,4,5] On the other hand, model studies on the amine-oxidation by PQQ suggested that the mechanism involves a covalent adduct (carbinolamine intermediate) at the initial stage of the reaction followed by α-proton removal to yield $PQQH_2$ (quinol) directly or a Schiff base (imine intermediate) formation followed by rearrangement and hydrolysis to yield aminophenol of reduced PQQ.[6,7] However, such intermediates and reduced products have not been isolated and well characterized yet.[8] In this paper we would like to demonstrate the preparation and characterization of the iminoquinone and aminophenol derivatives of coenzyme PQQ which are very important compounds in connection with the interaction of PQQ and amines in enzymatic systems mentioned above.



PQQ



Iminoquinone

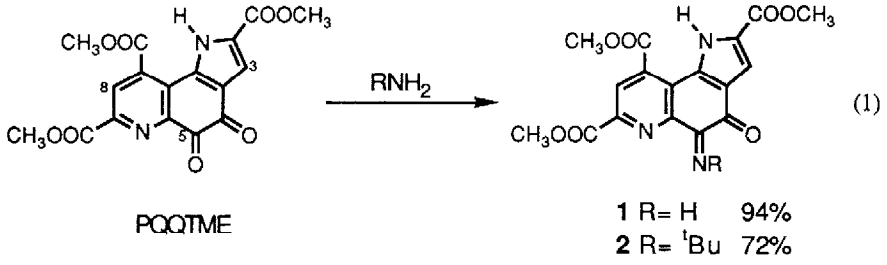


Aminophenol

The reaction of PQQ with ammonia was studied in an ammonia buffer solution at pH 9.6 (μ = 0.2 with KCl). When PQQ (4.0×10^{-5}M) was titrated with ammonia at 30 °C, absorptions at 265 and 335 nm slightly increased with isosbestic points at 344 and 404 nm as increasing the concentration of ammonia (0 - 5.5×10^{-2}M). The apparent equilibrium constant (K_{app}) was calculated to be 88 M^{-1}.

To identify the product, the ammonia buffer solution of PQQ ([PQQ] = 8.0 x 10^{-5}M, [NH_3] = 5.5 x 10^{-2} M) was anaerobically treated with $NaBH_4$. HPLC analysis[9] revealed the formation of two-types of reduced PQQ. One of them, minor product, was identical with $PQQH_2$ (quinol)[10] and the major product showed a same retention time with one of the products in the reaction of PQQ with benzylamine, which was presumed to be aminophenol.[7] Thus, the iminoquinone of PQQ is considered to be formed mainly in the ammonia buffer solution. In a preparative scale experiment, the iminoquinone was easily isolated in the reaction of PQQ and dry ammonia gas in methanol as a brown solid[11] which could be converted into the aminophenol[12] by treatment with $MeNHNH_2$ in methanol.

To obtain further information of the iminoquinone and the aminophenol, trimethyl ester of PQQ (PQQTME) was employed, because it is easier to handle and to analyze than PQQ itself. Dry ammonia gas was passed through a solution of PQQTME (44.7 mg, 1.20 x 10^{-4}mol) in CH_3CN. The corresponding 5-iminoquinone **1** was readily precipitated as a reddish purple solid (41.7 mg, 94 %).[13] The same type of 5-iminoquinone **2**[14] was also obtained in the reaction with *tert*-$BuNH_2$ (Eq. 1).



PQQTME

1 R= H 94%
2 R= ^{t}Bu 72%

(1)

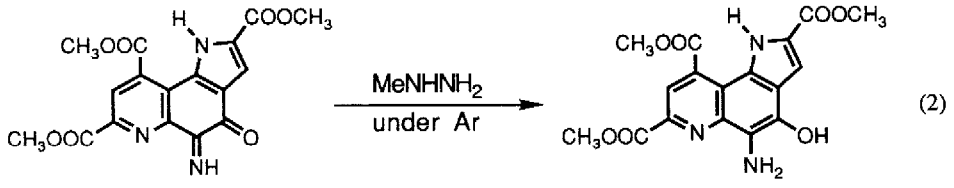
The typical spectral data of **1** and **2** are summarized in Table 1 together with those of PQQTME. The addition position of C-5 is estimated by the fact that the ^{1}H-NMR signal of 8-H shifts toward up-field larger than that of 3-H. It is well known that the C-5 position of PQQ shows unusually high reactivity toward nucleophilic attack.[8] As in fact, phenanthrenequinone was less reactive than PQQ and required severer conditions to yield the corresponding iminoquinones.

Table 1. Spectral data of **1**, **2**, and PQQTME.

	PQQTME	**1**	**2**
^{1}H-NMR	7.28 (3-H)	7.08 (3-H)	6.99 (3-H)
(d_6-DMSO, ppm)	8.61 (8-H)	7.94 (8-H)	7.94 (8-H)
IR (KBr, cm^{-1})	1686 (C=O)	1648 (C=N)	1646 (C=N)
Mass (EI, pos.)	374 (M^++2)[a]	373 (M^++2)[a]	372 (M^++2-^{t}Bu)[a]

a) characteristic peak for *o*-quinones.[15]

The corresponding aminophenol was isolated easily in the reduction of **1** (9.8 mg, 2.63 x 10^{-5}mol) with methylhydrazine (10 eq of **1**). The aminophenol **3** was precipitated as a dark red solid (6.2 mg, 63%, Eq. 2).[16] The UV-vis spectra of **1** and PQQTME are shown in Figure 1a) and those of **3** and $PQQTMEH_2$ (quinol) are also shown in Figure 1b). There is little difference between λ_{max} of the spectrum of **1** and that of PQQTME, while **3** has λ_{max} at 337 nm which is about 10-nm red shifted to compare with that of $PQQTMEH_2$.



1 **3** (2)

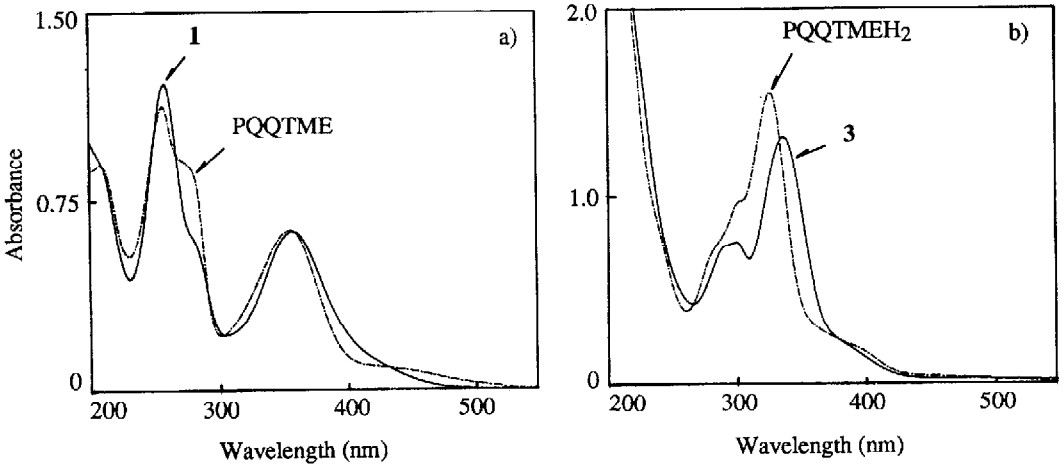


Fig. 1. UV-vis spectra of **1**, **3** , PQQTME and $PQQTMEH_2$ in CH_3CN
a) **1**: λ_{max} 259 nm (ε 29900 $M^{-1}cm^{-1}$), 358 nm (ε 15400 $M^{-1}cm^{-1}$); PQQTME: λ_{max} 258 nm (ε 27700$M^{-1}cm^{-1}$), 356 nm (15500$M^{-1}cm^{-1}$), b) **3** and $PQQTMEH_2$ were generated *in situ* by reduction of **1** and PQQTME with $MeNHNH_2$, respectively. **3** : λ_{max} 337 nm (32600 $M^{-1}cm^{-1}$); $PQQTMEH_2$: λ_{max} 328 nm (ε 38400 $M^{-1}cm^{-1}$)

Finally, the reactivity of iminoquinone **1** was preliminary investigated in the reaction with benzylamine.[17] Interestingly iminoquinone **1** reacts 130 times faster than PQQTME to yield the aminophenol **3**. Although mechanistic details are now being investigated, such an activation of PQQTME by ammonia is very interesting in connection with the role of ammonia as an activator in enzymatic systems.

Acknowledgement

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References and Notes

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9) HPLC system; column: radial pack cartridge C_{18} (Waters), solvent: MeOH / H_2O / 85% H_3PO_4, 45 / 54.5 / 0.5, v / v / v, flow rate : 1.0 ml / min, detection: UV at 300nm, retention time: 14.8 min ($PQQH_2$), 20.8 min (aminophenol).
10) S. Itoh, Y. Ohshiro, and T. Agawa, *Bull. Chem. Soc. Jpn.*, **1986**, *59*, 1911-1914.
11) ^{1}H-NMR (d_6-DMSO, ppm): 6.96 (s, 1H, 3-H), 8.49 (s, 1H, 8-H).
12) ^{1}H-NMR (d_6-DMSO, ppm): 7.20 (s, 1H, 3-H), 8.30 (s, 1H, 8-H).
13) ^{1}H-NMR (d_6-DMSO, ppm): 3.69, 3.90, 3.93 (each s, 3H, -OCH_3), 7.08 (s, 1H, 3-H), 7.17 (br s, exchangeable), 7.94 (s, 1H, 8-H), 11.88, 12.34 (each br s, total 1H (3: 1), exchangeable); ^{13}C-NMR (d_6-DMSO, ppm, by Bruker AM 600): 50.61, 52.58, 52.98 (ester -OCH_3 x 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 x 3), 168.25, 169.36 (C=N, C=O); IR (KBr, cm^{-1}): 3256 (NH), 1726, 1712 (ester C=O), 1700 (C=O), 1648 (C=N); MS (EI, pos., by JEOL JMX-DX 303): 373 (M^++2), 371 (M^+); mp > 300 °C.
14) ^{1}H-NMR (d_6-DMSO, ppm): 1.24 (s, 9H, -^{t}Bu), 3.68, 3.89, 3.92 (each s, 3H, -OCH_3), 6.99 (s, 1H, 3-H), 7.71 (br s, exchangeable), 7.94 (s, 1H, 8-H); ^{13}C-NMR (d_6-DMSO, ppm): 27.29 (-CH_3) 50.60, 51.19, 52.59, 52.97 (ester -OCH_3 x 3, -$\underline{C}(CH_3)_3$) 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 x 3), 171.74, 183.19 (C=N, C=O); IR (KBr, cm^{-1}): 1730, 1718 (ester C=O), 1680 (C=O), 1646 (C=N); MS (EI pos): 372 (M^++2-^{t}Bu); mp >300 °C.
15) K. P. Zeller, " The Chemistry of the Quinonoid Compounds, Part 2", S. Patai, Ed.; John Wiley & Sons, N. Y. 1974.
16) ^{1}H-NMR (d_6-DMSO, ppm): 3.93, 3.99, 4.10 (each s, 3H, -OCH_3), 7.51(s, 1H, 3-H), 8.51 (s, 1H, 8-H), 12.02 (br s, exchangeable); IR (KBr, cm^{-1}): 3364 (NH, OH), 1720 (ester C=O); MS (EI, pos.): 373 (M^+); mp > 300 °C.
17) [**1**] = 4.0 x 10^{-4}M, [$PhCH_2NH_2$] = 5.60 x 10^{-3}M in CH_3CN at 30 °C under anaerobic conditions (Ar).

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