# First Experimental Structure of a 1:1 Metal Complex with a PQQ Cofactor Derivative outside Dehydrogenase Enzymes

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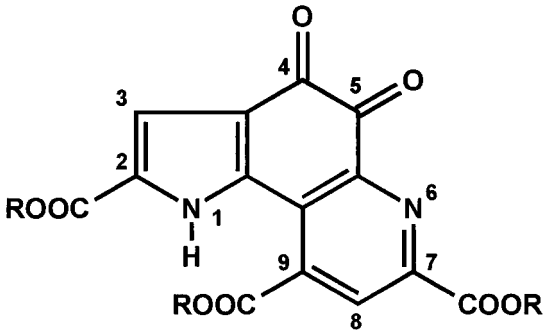
## Introduction

Quinoproteins are a relatively new class of flavin- and NADPH-independent oxidoreductase enzymes that contain single electron transfer-active *ortho*-quinonoid cofactors.[1,2] The interaction of these cofactors with metal ions is now well-established both functionally and structurally for amine oxidases (topaquinone/$Cu^{2+/+}$)[3] and for bacterial dehydrogenases (pyrroloquinoline quinone (PQQ)/$Ca^{2+}$).[4] The dissociable prosthetic group "PQQ"[1,2] is of microbiological, pharmaceutical, and nutritional relevance;[5a] it is a heterocycle with several possible coordination sites for metal ions, such as two quinone O, two heterocyclic N, and three carboxylate O centers (Chart 1). [5−7]

The coordination arrangement found in crystallographically characterized dehydrogenase enzymes[4] involves tridentate PQQ with O(5), N(6), and O(7′) as donors; the $Ca^{2+}$ coordination is complemented by $\eta^2$-glutamate and $\eta^1$-asparagine from the protein backbone.[4] Although the O(5)/N(6)/O(7′) coordination is supported by recent calculations and spectroscopic studies of model systems, such as $[(\mathbf{1})Ca(H_2O)_3]^{2+}$ (**1** = 2′,7′,9′-trimethyl ester of PQQ),[5a,b,6] the question remained whether free PQQ derivatives would undergo the same type of coordination in the absence of the protein support. Structures of *dinuclear* complexes have been reported (Table 1);[5b,c] a spectroscopic study, using $\pi$ electron donating $[(bpy)_2Ru]^{2+}$ as a metal complex fragment (bpy = 2,2′-bipyridine), indicated metal coordination to the quinone oxygen atoms O(4) and O(5),[7] a binding mode which was found less favorable for $Ca^{2+}$ coordination.[6d]

**Chart 1**




R = H : PQQ
R = $CH_3$ : **1**

In the following, we report the first experimental structure determination of a 1:1 metal complex with a PQQ derivative *outside* the protein environment. As isoambialterdentate[8] ligands, PQQ and its derivates tend to form oligonuclear metal compounds with complicated coordination patterns.[5] While a direct Cu−PQQ interaction and triarylphosphine coordination have no immediate relevance for biochemistry, the formation of a five-coordinate copper(I) species and the qualitatively similar but not identical metal binding of **1** to $Cu^{I}$ as compared to the $Ca^{2+}$ ion[4,5a] may render these results useful.

## Experimental Section

**Instrumentation.** EPR spectra were recorded in the X band on a Bruker System ESP 300 equipped with a Bruker ER035M gaussmeter and a HP 5350B microwave counter. $^1$H NMR spectra were taken on a Bruker AC 250 spectrometer. Infrared spectra were obtained using Perkin-Elmer 684 and 283 instruments. UV−vis/NIR absorption spectra were recorded on a Bruins Instruments Omega 10 spectrophotometer. Cyclic voltammetry was carried out at 85 mV/s scan rate in dichloromethane/0.1 M $Bu_4NPF_6$, using a three-electrode configuration (glassy carbon or platinum working electrode, Pt counter electrode, Ag/AgCl reference) and a PAR 273 potentiostat and function generator. The ferrocene/ferrocenium couple served as an internal reference.

**Synthesis.** A deoxygenated solution of 13.0 mg (0.035 mmol) of **1**[6d] and 18.4 mg (0.070 mmol) of $PPh_3$ in 7 mL of $CH_2Cl_2$ was added to 11.0 mg (0.035 mmol) of $[Cu(CH_3CN)_4](BF_4)$. After 15 min of stirring, the dark-brown solution was reduced in volume and a layer of 4 mL of ethyl acetate was added to produce 8.2 mg (23%) of dark, crystalline $[(\mathbf{1})Cu(PPh_3)_2](BF_4)\cdot 0.5CH_3C(O)OC_2H_5$. Drying under vacuum removed most of the solvent of crystallization. Elemental analysis was as expected. $^1$H NMR ($CDCl_3$): $\delta$ = 3.78 (s, 3H, Me), 3.97 (s, 3H, Me), 4.21 (s, 3H, Me), 7.3 (m, 31H, 6Ph + H-3), 9.12 (s, 1H, H-8), 13.03 ppm (br s, 1H, N−H). IR (KBr): $\nu_{CO}$ = 1723, 1700, 1668 $cm^{-1}$. UV−vis ($CH_2Cl_2$): $\lambda_{max}$ ($\epsilon$) = 660 (sh; MLCT), 488 (2100; n → $\pi^*$), 378 nm (9000 $M^{-1}$ $cm^{-1}$; $\pi \rightarrow \pi^*$).

**Crystallographic Studies.** Dark-brown crystals of $[(\mathbf{1})Cu(PPh_3)_2](BF_4)\cdot CH_3C(O)OC_2H_5$ were grown by slow diffusion of ethyl acetate into a dichloromethane solution of the complex. One single crystal

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(1) (a) Duine, J. A. *Eur. J. Biochem.* **1991**, *200*, 271. (b) Duine, J. A.; Jongejan, J. A. In *Bioinorganic Catalysis*; Reedijk, J., Ed.; Marcel Dekker: New York, 1993; p 447.

(2) (a) Davidson, V. L. *Principles and Applications of Quinoproteins*; Marcel Dekker: New York, 1993. (b) Stubbe, J.; van der Donk, W. A. *Chem. Rev.* **1998**, *98*, 705 and literature cited therein.

(3) (a) Klinman, J. P.; Mu, D. *Annu. Rev. Biochem.* **1994**, *63*, 299. (b) Parsons, M. R.; Convery, M. A.; Wilmot, C. M.; Yadav, K. D. S.; Blakeley, V.; Corner, A. S.; Phillips, S. E. V.; McPherson, M. J.; Knowles, P. F. *Structure* **1995**, *3*, 1171. (c) Kumar, V.; Dooley, D. M.; Feeman, H. C.; Guss, J. M.; Harvey, I.; McGuirl, M. A.; Wilce, M. C. J.; Zubak, V. M. *Structure* **1996**, *4*, 943.

(4) (a) White, S.; Boyd, G.; Mathews, F. S.; Xia, Z.-X.; Dai, W.-W.; Zhang, Y.-F.; Davidson, V. L. *Biochemistry* **1993**, *32*, 12955. (b) Xia, Z.-X.; Dai, W.-W.; Zhang, Y.-F.; White, S. A.; Boyd, G. D.; Mathews, F. S. *J. Mol. Biol.* **1996**, *259*, 480. (c) Blake, C. C. F.; Ghosh, M.; Harlos, K.; Avezoux, A.; Anthony, C. *Nat. Struct. Biol.* **1994**, *1*, 102. (d) Cozier, G. E.; Giles, I. G.; Anthony, C. *Biochem. J.* **1995**, *308*, 375. (e) Ghosh, M.; Anthony, C.; Harlos, K.; Goodwin, M. G.; Blake, C. *Structure* **1995**, *3*, 177.

(5) (a) Itoh, S.; Kawakami, H.; Fukuzumi, S. *J. Am. Chem. Soc.* **1998**, *120*, 7271 and references therein. (b) $[(PQQ)Na_2(H_2O)_5]$: Ishida, T.; Doi, M.; Tomita, K.; Hayashi, H.; Inoue, M.; Urakami, T. *J. Am. Chem. Soc.* **1989**, *111*, 6822. (c) $[Cu^{II}(terpy)]_2(\mu$-PQQ): Nakamura, N.; Kohzuma, T.; Kuma, H.; Suzuki, S. *Inorg. Chem.* **1994**, *33*, 1594. (d) Cf. also the structure of an iron(II) complex of a ligand related to PQQ: Tommasi, L.; Shechter-Barloy, L.; Varech, D.; Battioni, J.-P.; Donnadieu, B.; Verelst, M.; Bousseksou, A.; Mansuy, D.; Tuchagues, J.-P. *Inorg. Chem.* **1995**, *34*, 1514. (e) Noar, J. B.; Rodriguez, E. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1985**, *107*, 7198.

(6) (a) Itoh, S.; Huang, X.; Kawakami, H.; Komatsu, M.; Ohshiro, Y.; Fukuzumi, S. *J. Chem. Soc., Chem. Commun.* **1985**, 2077. (b) Itoh, S.; Kawakami, H.; Fukuzumi, S. *J. Am. Chem. Soc.* **1997**, *119*, 439. (c) Itoh, S.; Kawakami, H.; Fukuzumi, S. *J. Chem. Soc., Chem. Commun.* **1997**, 29. (d) Itoh, S.; Kawakami, H.; Fukuzumi, S. *Biochemistry* **1998**, *37*, 6562.

(7) Schwederski, B.; Kasack, V.; Kaim, W.; Roth, E.; Jordanov, J. *Angew. Chem.* **1990**, *102*, 74. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 78.

(8) von Zelewsky, A. *Inorg. Chem.* **1981**, *20*, 4448.

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**Table 1.** Summary of Crystal Data for $[(\mathbf{1})Cu(PPh_3)_2](BF_4)\cdot 0.5CH_3C(O)OC_2H_5$

| | | | |
|---|---|---|---|
| empirical formula | $C_{53}H_{42}BCuF_4N_2O_8P_2\cdot 0.5C_4H_8O_2$ | $V$ (Å$^3$) | 4979(2) |
| | | $Z$ | 4 |
| formula wt (g mol$^{-1}$) | 1091.23 | $T$ (°C) | −100 |
| | | $\lambda$ (Å) | 0.71069 |
| space group | $P2_1/n$ (No. 14) | $\rho_{calcd}$ (g·cm$^{-3}$) | 1.456 |
| $a$ (Å) | 19.565(4) | $\mu$ (mm$^{-1}$) | 0.579 |
| $b$ (Å) | 13.807(3) | R1[a] | 0.0537 |
| $c$ (Å) | 20.528(4) | wR2[a] | 0.1319 |
| $\beta$ (deg) | 116.11(3) | | |

[a] $R1 = \sum|F_o - F_c|/\sum|F_o|$ and $wR2 = [\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2]^{1/2}$.

(0.4 × 0.3 × 0.3 mm$^3$) was immersed in Nujol, transferred into a capillary, and mounted on a Siemens P4 diffractometer ($\omega$ scan): 9349 collected data, 7792 unique and 7790 observed reflections; 697 parameters and 5 restraints. The structure was solved by direct methods using the SHELXTL-PLUS package,[9] based on 3506 reflections with $F_o > 4\sigma(F_o)$ (full matrix, least-squares methods on $|F^2|$). Largest difference peak/hole: +0.451/−0.910 e Å$^{-3}$. Non-hydrogen atoms were refined anisotropically; hydrogen atoms were introduced at ideal positions and refined using riding models except H(1) which was refined without geometrical constraints. Ethyl acetate solvent molecules were found disordered close to an inversion center.

## Results and Discussion

Compound $[(\mathbf{1})Cu(PPh_3)_2](BF_4)$ was prepared from **1**, $[Cu(CH_3CN)_4](BF_4)$, and triphenylphosphine in dichloromethane. Whereas the free ligand **1** exhibits complications in cyclic voltammetry experiments, either because of electrode adsorption phenomena[10] or because of acid/base and disproportionation equilibria involving the pyrrolic proton, the N-methylated PQQ triester exhibits reversible reduction behavior.[5a] The complex $[(\mathbf{1})Cu(PPh_3)_2](BF_4)$ is reduced quasireversibly ($i_a/i_c = 0.75$, $\Delta E_{pp} = 160$ mV) at −0.48 V vs $[Fe(C_5H_5)_2]^{+/0}$ in $CH_2Cl_2$/0.1 M $Bu_4NPF_6$ at 298 K and at −0.43 V ($i_a/i_c = 0.80$, $\Delta E_{pp} = 90$ mV) at 195 K to an EPR-detectable copper(I) semiquinone radical complex (see Figure 1). The less complicated cyclic voltammetric response of the copper complex in comparison to the free ligand **1** is attributed to the steric shielding by the triphenylphosphine groups, which should prevent intermolecular association phenomena or electrode adsorption. In any case, the copper(I) coordination results in a positive shift of the one-electron reduction potential of **1** similar to the one reported after addition of $Ca^{2+}$ in acetonitrile.[5a]

In the infrared spectrum, the coordination of a cationic metal complex fragment at O(5) and N(6) results in a very slight high-energy shift of the broad $\nu(C{=}O_{ester})$ band from 1721 (ligand) to 1723 cm$^{-1}$ (complex; three slightly different ester carbonyl functions) and to a splitting of quinone carbonyl bands from 1684 cm$^{-1}$ (1675, sh) for **1** to 1700 and 1668 cm$^{-1}$ in the complex. This spectroscopic observation already indicates the use of only one quinone carbonyl oxygen atom for copper(I) coordination (see Figure 2).

In the UV−vis spectrum, the main difference between **1** and its bis(triphenylphosphine)copper(I) complex is the appearance of the expected[12] metal-to-ligand charge transfer (MLCT) absorption as a broad long-wavelength shoulder at 660 nm. The intraligand absorption bands ($n \rightarrow \pi^*$ at 488 nm and $\pi \rightarrow \pi^*$ at 378 nm) are hypsochromically shifted relative to those of the free ligand in the same solvent (434 and 364 nm in $CH_2Cl_2$).

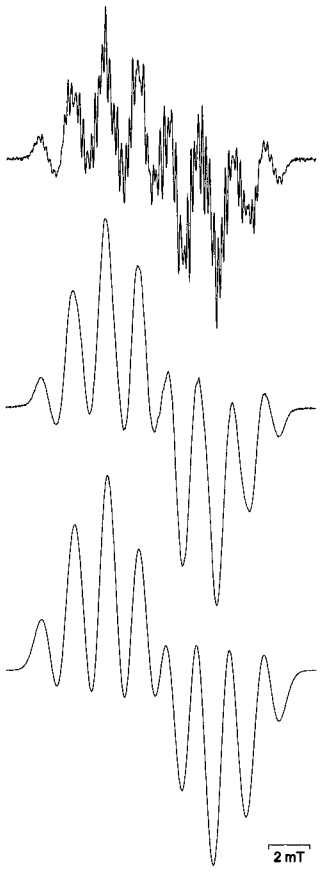



**Figure 1.** Experimental EPR spectrum (top) of $[(\mathbf{1})Cu(PPh_3)_2]^{\bullet}$ as obtained by electrolysis of the precursor at 300 K (top, very dilute solution) and at 280 K (center) in $CH_2Cl_2$/0.1 M $Bu_4NPF_6$. For parameters of simulated spectrum (bottom) see text.

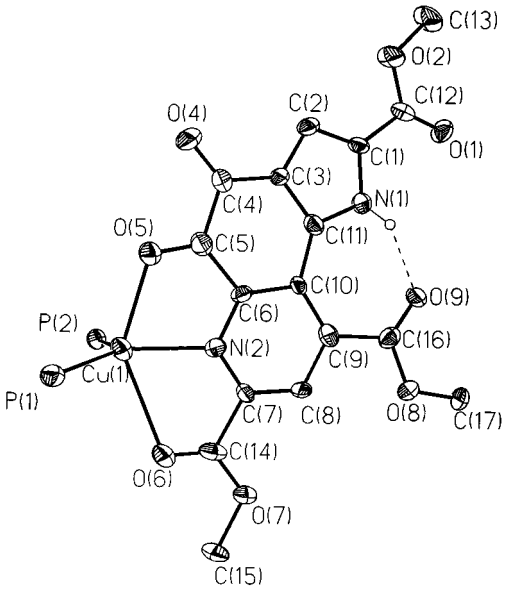
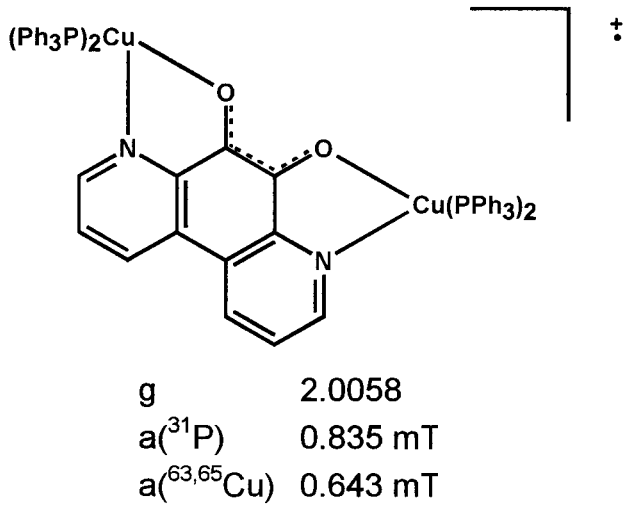



**Figure 2.** Molecular structure of the complex cation in the crystal of $[(\mathbf{1})Cu(PPh_3)_2](BF_4)\cdot 0.5CH_3C(O)OC_2H_5$ (hydrogen atoms and phenyl rings at the P atoms are omitted for clarity).

The EPR spectrum of electrogenerated $[(\mathbf{1})Cu(PPh_3)_2]^{\bullet}$ is much broader at 7.0 mT total spectral width than the spectrum

(9) Sheldrick, G. M. *SHELXTL-PLUS An Integrated System for Solving, Refining and Displaying Structures from Diffraction Data*, Relat. 5.03, Siemens Analytical X-ray Instruments Inc., 1989.
(10) Eckert, T. S.; Bruice, T. C.; Gainor, J. A.; Weinreb, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 2533.
(11) Kaim, W.; Kohlmann, S. *Inorg. Chem.* **1987**, *26*, 1469.
(12) (a) Vogler, C.; Hausen, H.-D.; Kaim, W.; Kohlmann, S.; Kramer, H. E. A.; Rieker, J. *Angew. Chem.* **1989**, *101*, 1734; *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1659. (b) Vogler, C.; Kaim, W.; Hausen, H.-D. *Z. Naturforsch.* **1993**, *48b*, 1470.

**Chart 2**




of free $\mathbf{1}^{\bullet-}$ (2.2 mT[5a]), which reflects considerable spin transfer to the $^{63,65}$Cu ($I = {}^3/_2$) and $^{31}$P ($I = {}^1/_2$) nuclei.[12] The EPR parameters $g = 2.0049$, $a(^{31}\text{P}) = 0.90$ mT (2 P), $a(^{65}\text{Cu}) = 0.71$ mT, $a(^{63}\text{Cu}) = 0.64$ mT (1 Cu), and $a(^{14}\text{N}) = 0.79$ mT (1 N) as obtained from the simulation of a partially resolved spectrum (Figure 1) are comparable to those of a related dinuclear system (Chart 2),[11] reflecting coordination-dependent spin distribution[5a] and increased $^{14}$N hyperfine splitting after copper(I) binding.[12] For instance, the $a(^{14}\text{N})$ value of the 2,2′-bipyrimidine radical anion increases from 0.141 to 0.218 mT on bis(triphenylphosphine)copper(I) coordination.[12] We thus attribute the large $^{14}$N coupling constant to N-6, in agreement with the sizable copper and phosphorus hyperfine splitting; Itoh and co-workers have observed a large $^{14}$N hyperfine value of about 0.5 mT for $\mathbf{1}^{\bullet-}$ (in $CH_2Cl_2$ but not $CH_3CN$ solution) and assigned this to N-1.[5a] In very dilute dichloromethane solution, the EPR spectrum of $[(\mathbf{1})\text{Cu}(\text{PPh}_3)_2]^{\bullet}$ is better resolved (Figure 1); however, the presence of additional coupling from one copper and two $^{31}$P nuclei causes a 12-fold increase in the number of the $\prod(2N_iI+1) = 2 \times 2 \times 2 \times 3 \times 3 \times 4 = 288$ hyperfine lines expected for the free ligand $\mathbf{1}^{\bullet-}$;[5a] the total number of 3456 theoretical lines from eight individual coupling constants of $[(\mathbf{1})\text{Cu}(\text{PPh}_3)_2]^{\bullet}$, therefore, precluded complete analysis by conventional EPR. Attempts to determine $^1$H or $^{14}$N coupling constants through ENDOR experiments failed because of insufficient EPR signal saturation in fluid solution.

Single crystals of $[(\mathbf{1})\text{Cu}(\text{PPh}_3)_2](\text{BF}_4)\cdot 0.5\text{CH}_3\text{C(O)OC}_2\text{H}_5$ for X-ray diffraction were obtained via diffusion of ethyl acetate into a dichloromethane solution of the complex. The structure analysis (Tables 1 and 2) revealed (Figure 2) a qualitatively similar coordination of $[\text{Cu}(\text{PPh}_3)_2]^+$ to **1** as that of $Ca^{2+}$ to PQQ in the enzymes (Table 3).[4]

Apparently, the relatively large ionic radius of $Cu^+$ (0.91 Å; 1.14 Å for $Ca^{2+}$)[13] still allows for the binding via three donor centers O(5), N(6), and O(7′). However, the "softer" characteristics of copper(I) are evident from much shorter bonds to the quinone oxygen and the quinoline nitrogen center; in contrast, the bond to the ester carbonyl atom O(7′) is far longer in the copper(I) complex than the bond between the carboxylate O(7′) and $Ca^{2+}$ in the enzymes (Figure 2).[4,6d] Nevertheless, the Cu−(O(7′)) distance of 2.579(4) Å still indicates "semicoordination" as observed similarly in copper(I) complexes with weakly basic anion ligands.[14a,b]

**Table 2.** Selected Distances (Å) and Angles (deg)[a]

| | | | |
|---|---|---|---|
| Cu−P(1) | 2.250(2) | Cu−P(2) | 2.264(2) |
| Cu−N(2) | 2.118(4) | Cu−O(5) | 2.254(4) |
| Cu−O(6) | 2.579(4) | C(4)−O(4) | 1.212(6) |
| C(5)−O(5) | 1.222(6) | N(1)···O(9) | 2.615(5) |
| P(1)−Cu−P(2) | 123.39(7) | P(1)−Cu−N(2) | 135.16(14) |
| P(2)−Cu−N(2) | 100.00(14) | P(1)−Cu−O(5) | 105.74(11) |
| P(1)−Cu−O(6) | 90.47(10) | P(2)−Cu−O(5) | 99.76(12) |
| P(2)−Cu−O(6) | 100.21(11) | N(2)−Cu−O(5) | 74.30(20) |
| N(2)−Cu−O(6) | 69.44(14) | O(5)−Cu−O(6) | 140.98(14) |

[a] For atom numbering see Figure 2.

**Table 3.** Bond Lengths (Å) in Metal Complexes of PQQ Derivatives

| | distances from M to[a] | | | |
|---|---|---|---|---|
| | O−5 | N-6 | O−7′ | ref |
| enzyme[b] | 2.50 | 2.47 | 2.30[e] | 4b, 6d |
| $[(\mathbf{1})]\text{Ca}(\text{H}_2\text{O})_3]^{2+}$ [c] | 2.43 | 2.46 | 2.36[f] | 6d |
| $[(\text{PQQ})\text{Na}_2(\text{H}_2\text{O})_5]$ | 2.642 | 2.532 | 2.401[e] | 5b |
| $[\text{Cu}^{\text{II}}(\text{terpy})]_2(\mu\text{-PQQ})$[d] | 2.47(1) | 2.03(2) | 2.23(1)[e] | 5c |
| $[(\mathbf{1})\text{Cu}^{\text{I}}(\text{PPh}_3)_2]](\text{BF}_4)$[g] | 2.254(4) | 2.118(4) | 2.579(4)[f] | this work |

[a] Numbering of positions according to heterocyclic nomenclature (Chart 1). [b] $Ca^{2+}$/PQQ-semiquinone interaction in methanol dehydrogenase from *Methylobacterium extorquens*. [c] Calculated using the Spartan program (version 4.1). [d] terpy = 2,2′:6′,2″-terpyridine. [e] Carboxylate oxygen. [f] Ester carbonyl oxygen. [g] Ethyl acetate solvate.

The bonding of $Cu^{\text{I}}$ to the $PPh_3$ groups is typically unsymmetrical,[12] with P(2) occupying a somewhat more apical position than P(1). Considering the angles at the Cu center, the overall coordination geometry is close to a square pyramid with P(2) at the apex that, however, implies essentially identical Cu−P bonds to the axial and equatorial $PPh_3$ ligands. Another view based more on bond distances involves a very distorted trigonal bipyramid with an N(2)/P(1)/P(2) equatorial plane and a skewed O(5)−Cu−O(6) axis (Figure 2) from the restricted "bite" of tridentate **1**. In any case, copper(I) centers are rarely[15] five-coordinate in contrast to $Cu^{\text{II}}$ and the arrangement with a $P_2NO_2$ donor set is unprecedented.[15a] The planarity of **1** and the N(1)−H(1)···O(9) hydrogen bond are as expected.[5b]

Obviously, the PQQ nucleus still prefers the α-iminoketo (O/N)-coordination to the moderately $\pi$ donating copper(I) center in a similar fashion as pterin, lumazine, and flavin ligands.[16] It remains to be studied whether reduced forms of PQQ derivatives and/or more $\pi$ basic metal centers opt for the O(4)/O(5) alternative or whether even dynamic fluctuation between these sites can be observed.

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**Supporting Information Available:** A cyclic voltammogram, an infrared spectrum, and a UV−vis spectrum of the title compound. An X-ray crystallographic file in CIF format is also available. This material is available free of charge via the Internet at http//:pubs.acs.org.

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(13) Values for coordination number 6: *Inorganic Chemistry*, 4th ed.; Huheey, J., Keiter, E. A., Keiter, R. L., Eds.; Harper Collins: New York, 1993.

(14) (a) Ferrara, J. D.; Tessler-Youngs, C.; Youngs, W. J. *Organometallics* **1987**, *6*, 676. (b) Masuda, H.; Machida, K.; Munakata, M.; Kitagawa, S.; Shimono, H. *J. Chem. Soc., Dalton Trans.* **1988**, 1907.

(15) (a) Holloway, C. E.; Melnik, M. *Rev. Inorg. Chem.* **1995**, *15*, 147. (b) Ainscough, E. W.; Brodie, A. M.; Ingham, S. L.; Waters, J. M. *J. Chem. Soc., Dalton Trans.* **1994**, 215. (c) Ainscough, E. W.; Brodie, A. M.; Ingham, S. L.; Waters, J. M. *Inorg. Chim. Acta* **1996**, *249*, 47.

(16) Kaim, W.; Schwederski, B.; Heilmann, O.; Hornung, F. *Coord. Chem. Rev.* **1999**, *182*, 323.