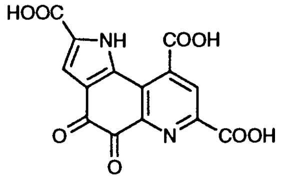
Ruthenium(II) Coordination to a Model for the Topasemiquinone Cofactor of Amine Oxidases. Resolution of ^{1}H and ^{99,101}Ru EPR Hyperfine Structure

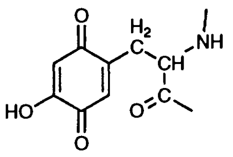
Eberhard Waldhör, Brigitte Schwederski and Wolfgang Kaim*
Institut für Anorganische Chemie der Universität, Pfaffenwaldring 55, D-705 50 Stuttgart 80, Germany

The paramagnetic complex ($L^{2-\bullet}$) Ru^{II} $(bpy)_2$, where $L^{2-\bullet}$ = 5-methyl-2-oxido-1,4-benzosemiquinone and bpy = 2,2′-bipyridine, was studied because the quinonoid form LH is a model of the topaquinone (TPQ) cofactor of certain copper-dependent amine oxidases. For the metal complex the potential *ortho*- and *para*-quinonoid character of the LH_n system was probed by high resolution EPR spectroscopy of the semiquinone state which could be obtained through oxidation of the basic catecholate form.

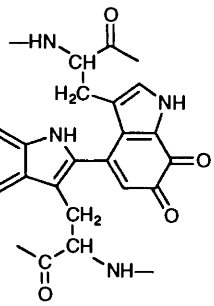
Oxidase enzymes in which one transition metal centre and one organic redox cofactor interact are known in the form of haem (Fe + porphyrin) and molybdenum-dependent systems (Mo + molybdopterin).[1] Recently, another combination has been established for some amine oxidases which exhibit a clear interaction between 'type 2' copper[1] and an *o*-quinonoid cofactor.[2] This cofactor has long been assumed to be exclusively pyrroloquinoline quinone (PQQ, methoxatin), however, recent studies led to a reformulation with topaquinone (TPQ, 6-hydroxydopaquinone)[3,4] or tryptophan tryptophylquinone (TTQ)[5,6] as covalently linked cofactors.[2]



PQQ

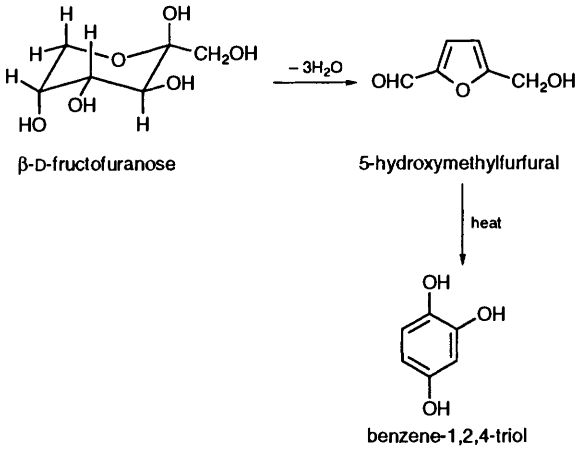


TPQ



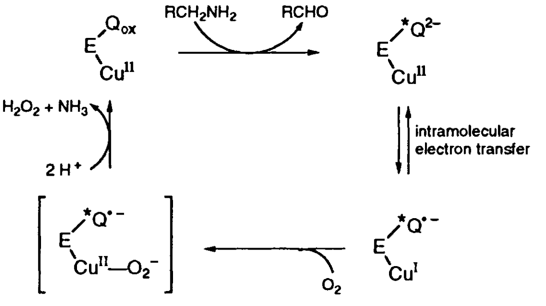
TTQ

All the above cofactors contain a redox-active and potentially metal-chelating *o*-quinonoid function. In addition, PQQ and TPQ exhibit a *p*-quinonoid structure with a blocked fourth *o*,*p*-position. A similar arrangement is also present in the metal coordinating[7] antibiotic streptonigrin. The blocking of the fourth *o*,*p*-position is essential for stability as will be evident from the EPR results presented here; *o*-quinonoid compounds are generally reactive at this position.[8-10] It should also be noted that the underlying benzene-1,2,4-triol structure can be obtained as a result of hydrothermal treatment of carbohydrates such as fructose (β-D-fructofuranose, Scheme 1).[11]



Scheme 1

Although a direct coordination between copper and the organic cofactor has not yet been firmly established for amine oxidases studies using NMR,[12,13] EPR,[4,14,15] spin echo,[15] resonance Raman and MCD spectroscopy[16] have indicated a considerable interaction between the two redox active components. The observation of organic radical intermediates by EPR has led to the formulation of a reaction mechanism in which intramolecular electron transfer between the Cu^{II}/catecholate state and the presumably $^3O_2$-binding Cu^I/semiquinonoid form is a crucial step[14] (Scheme 2).

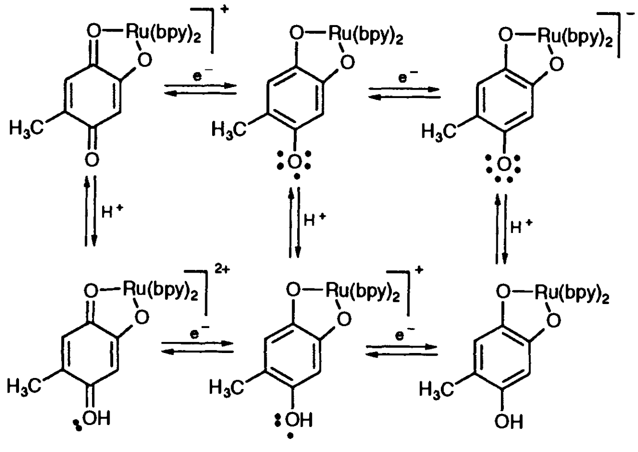


E = enzyme
Q = quinone
$Q^{\bullet-}$ = semiquinone
Q^{2-} = catecholate
$^*Q^{n-}$ = imine/amine form

Scheme 2 Proposed catalytic cycle involving metal and quinonoid cofactor (*Q*) functions in amine oxidases (adapted from ref. 14)

The dissociable cofactor PQQ has been studied in coordinated form with respect to electron and proton transfer behaviour.[17,18] We now report results for a ruthenium(II) complex of a model compound of TPQ, *i.e.*, 2-hydroxy-5-

methyl-1,4-benzoquinone (LH) as derived from 2,4,5-trihydroxytoluene (LH_3). Bis(2,2'-bipyridine)ruthenium complexes of *o*-quinones, semiquinones and catecholates have been studied intensely in recent years because of their internal electron transfer equilibria.[19–22] In contrast with many bis-chelating systems[21–23] the ligand LH_n can only form a mono-chelate complex. However, the free hydroquinonoid hydroxy group offers an interesting functionality. The related ligand 2-methyl-3-hydroxy-1,4-naphthoquinone (phthiocol) was studied as a Mn^{II} complex.[24] Combination of the two-step quinonoid redox equilibria with the single proton transfer equilibrium leads to Scheme 3, of which we were able to characterize three species.



Scheme 3

Experimental

All reactions were carried out under an atmosphere of dry argon. Analytical grade solvents and commercially available reagents were used without further purification, 2,4,5-trihydroxytoluene (LH_3) was obtained according to the literature procedure.[25]

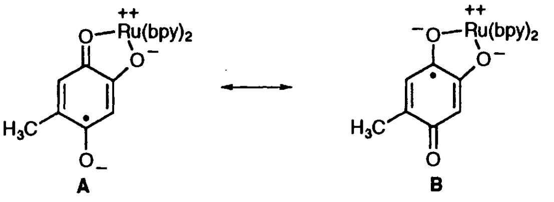
Synthesis of [(L)Ru(bpy)$_2$]Na•2EtOH.—A suspension of *cis*-Ru(bpy)$_2$Cl$_2$•2H$_2$O (57.2 mg, 0.110 mmol) and 2,4,5-trihydroxytoluene (LH_3) (15.4 mg, 0.110 mmol) in ethanol (10 cm^3) was treated with a solution of sodium hydroxide in ethanol (13.2 mg; 0.330 mmol) and heated at reflux for 20 h. On cooling, the dark green solution formed a black precipitate which was collected and washed with ethanol and diethyl ether. Drying *in vacuo* afforded the title compound (47 mg, 64%) (Found: C, 56.05; H, 5.00; N, 8.45%. $C_{31}H_{33}N_4NaO_5Ru$ (*M*, 665.77) requires C, 55.93; H, 4.99; N, 8.41%).

Instrumentation.—EPR spectra were taken on a Bruker ESP 300 system. UV–VIS spectra were recorded on a Shimadzu UV 160 spectrometer. Cyclic voltammetry was carried out in dichloromethane–Bu_4NPF_6 (0.1 mol dm^{-3}) using a three electrode configuration (GCE, Ag/AgCl, Pt) and a PAR 273/175 potentiostat and function generator.

Results and Discussion

The complex was best prepared in the deprotonated catecholate form (Scheme 3, top right) by treating Ru(bpy)$_2$Cl$_2$ with 2,4,5-trihydroxytoluene in the presence of three equivalents of base. As shown by EPR spectroscopy, the precipitated compound already contained small amounts of the partially oxidized complex, *i.e.* in semiquinonoid form. The redox potentials as obtained by cyclic voltammetry in dry CH_2Cl_2–Bu_4NPF_6 (0.1 mol dm^{-3}) at −0.92 V and −0.07 V *vs.* $FeCp_2{}^{+/0}$ are very similar to those of other dioxolene complexes of Ru(bpy)$_2$.[19] In the catecholate form, the metal coordinated ligand L^{3-} is basic as is evident from electrochemical potential shifts ($-0.92\ V \longrightarrow -0.81\ V$) and from effects of protonation as determined by absorption spectroscopy. In agreement with results obtained for the $[Ru(bpy)_2]^{2+}$ complex of the electronically related PQQ molecule[17] the protonation with CF_3COOH–CH_2Cl_2 (1 mmol dm^{-3}) at the free phenolate oxygen causes bathochromic shifts of the charge transfer bands, *i.e.*, from 387 and 589 to 532 and 840 nm (isosbestic points at 465, 550 and 755 nm). The corresponding shifts of the PQQ complex with the more basic (pyrrolic) *N*-function are from 332 and 465 to 375 and 722 nm.[17]

The paramagnetic form of the complex (Scheme 4) exhibits an unusually well resolved EPR spectrum in solution at 300 K [Fig. 1(*a*)] which is dominated by the quartet coupling from the methyl protons. This result is in agreement with results for the radical ligand $LH^{2-\bullet}$ (Table 1)[10] and with the π_{as} nature of the singly occupied MO.[26] Coupling constants from the ring protons in the 3,6-position were too small to be detected (a < 0.05 mT) since a π nodal plane is running through these positions. The rather small linewidth has now allowed us to detect and analyse the ruthenium hyperfine splitting in such a rather simple dioxolene radical complex; insufficiently resolved resonances were reported previously for related compounds.[19–21] Naturally occurring ruthenium has two isotopes with non-zero nuclear spin, *viz.*, ^{99}Ru (12.7%, I = 5/2) and ^{101}Ru (17.0%, I = 5/2). Due to the small difference of only 12% between the nuclear magnetic moments the hyperfine satellite lines from individual isotopes are usually not resolved.[17,27,28] The computer simulated spectrum [Fig. 1(*b*)] shows that the two outermost lines of the sextet satellite splitting of the methyl quartet are well observable at the wings of the spectrum.



Scheme 4

The size of the ^{99,101}Ru isotope coupling (0.225 mT) is

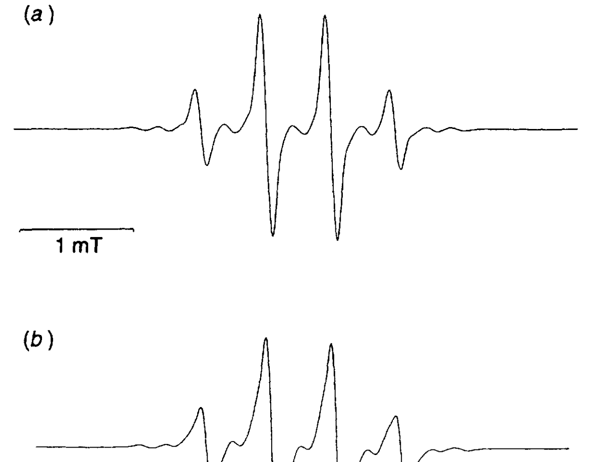


Fig. 1 (*a*) EPR spectrum of (L)Ru(bpy)$_2$ in CH_2Cl_2 (L = dianionic semiquinone form of 2-hydroxy-5-methyl-1,4-benzoquinone, see Scheme 4); (*b*) computer-simulated spectrum with the data from Table 1 and a line width of 0.095 mT

Table 1 EPR data[a] of the ligand and of ruthenium complexes

	$L^{2-\bullet}$ [b]	$(L)Ru(bpy)_2$ [c]	$[(PQQ)Ru(bpy)]^{4-}$ [d]
$a(CH_3)$	0.506	0.588	/
$a(CH)$	0.062 0.067	<0.05	<0.1
$a(^{99,101}Ru)$	/	0.225	0.5
$\langle g \rangle$	n.r.	2.0023	2.0048
g_1		2.0053[e]	2.0210[f]
g_2		2.0053[e]	2.0032[f]
g_3		1.997[e]	1.9851[f]

[a] Coupling constants *a* in mT. [b] In aqueous solution (ref. 10). [c] In CH_2Cl_2. [d] In acetonitrile (ref. 17). [e] In glassy frozen CH_2Cl_2. [f] Adsorbed on Al_2O_3 (ref. 17).

comparable to previously reported values for Ru^{II} radical complexes.[17,27,28] The absence of an OH proton coupling suggests that the semiquinone complex is observed in the neutral deprotonated form. Attempts to protonate the phenolate function in the neutral radical complex with trifluoroacetic acid led to the disappearance of the EPR signal; the PQQ complex which contains a more basic NHR group instead of OH in the *para*-position was studied in the *N*-protonated form and showed a higher $^{99,101}Ru$ coupling of 0.5 mT.[17] Contributions from the two resonance forms of the paramagnetic complex shown in Scheme 4 may be estimated on the basis of the EPR results. The metal hyperfine coupling and the failure to reversibly protonate the neutral radical suggest a higher contribution from form B with the free carbonyl oxygen centre.

In glassy frozen dichloromethane solution at 113 K the neutral radical complex exhibited a hardly resolved axial signal (Table 1) which is in agreement with the rather small metal contribution to the radical ground state. A slightly more pronounced anisotropy was reported for the PQQ complex[17] (which also showed higher metal hyperfine coupling, Table 1) and for the 3,5-di-*tert*-butylsemiquinone complex of $[Ru(bpy)_2]^{2+}$.[19] We attribute this decrease of metal contributions to the doublet ground state in the present case (Scheme 4) to the negatively charged conjugating oxygen substituent which stabilizes the singly occupied π_{as} orbital relative to interacting ruthenium d levels and thus disfavours orbital mixing.[19–21,29]

Summarizing, we have shown that the model system of the TPQ cofactor has similar but not identical redox and coordination properties relative to PQQ. The most crucial difference with respect to the effects studied here is the replacement of basic (and normally protonated) pyrrolic nitrogen by less basic phenolate oxygen in the *para*-position on going from PQQ to TPQ. As a consequence the persistent semiquinone complex of the deprotonated TPQ model shows less metal contribution to the singly occupied MO, resulting in a better EPR resolution, a smaller metal coupling constant, and a *g* factor closer to *g* (electron) = 2.0023.

Acknowledgements

This work was supported by the Fonds der Chemischen Industrie and by a grant from DFG (Schwerpunktprogramm).

References

1 (*a*) J. J. R. Frausto da Silva and R. J. P. Williams, *The Biological Chemistry of the Elements*, Clarendon Press, Oxford, 1991; (*b*) W. Kaim and B. Schwederski, *Bioanorganische Chemie*, Teubner, Stuttgart, 1991.

2 J. A. Duine, *Eur. J. Biochem.*, 1991, **200**, 271.

3 S. M. Janes, D. Mu, D. Wemmer, A. J. Smith, S. Kaur, D. Maltby, A. L. Burlingame and J. P. Klinman, *Science*, 1990, **248**, 981.

4 M. A. McGuirl, D. E. Brown, C. D. McCahon, P. N. Turowski and D. M. Dooley, *J. Inorg. Biochem.*, 1991, **43**, 186.

5 W. S. McIntire, D. E. Wemmer, A. Chistoserdov and M. E. Lindstrom, *Science*, 1991, **252**, 817.

6 S. Itoh, M. Ogino, M. Komatsu and Y. Ohshiro, *J. Am. Chem. Soc.*, 1992, **114**, 7294.

7 J. Hajdu and E. C. Armstrong, *J. Am. Chem. Soc.*, 1981, **103**, 232.

8 W. S. Pierpoint, *Trends Biochem. Sci.*, 1990, **15**, 299.

9 S. Patai and Z. Rappoport, *The Chemistry of the Quinonoid Compounds*, Vol. 2, Part 1; Wiley, New York, 1988.

10 K. A. K. Lott, E. L. Short and D. N. Waters, *J. Chem. Soc. (B)*, 1969, 1232.

11 G. C. A. Luijkx, F. van Rantwijk and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 1991, **110**, 343.

12 T. J. Williams and M. C. Falk, *J. Biol. Chem.*, 1986, **261**, 15949.

13 D. M. Dooley, M. A. McGuirl, C. E. Cote, P. F. Knowles, I. Singh, M. Spiller, R. D. Brown III and S. H. Koenig, *J. Am. Chem. Soc.*, 1991, **113**, 754.

14 D. M. Dooley, M. A. McGuirl, D. E. Brown, P. N. Turowski, W. S. McIntire and P. F. Knowles, *Nature*, 1991, **349**, 262.

15 J. McCracken, J. Peisach, C. E. Cote, M. A. McGuirl and D. M. Dooley, *J. Am. Chem. Soc.*, 1992, **114**, 3715.

16 (*a*) D. M. Dooley, W. S. McIntire, M. A. McGuirl, C. E. Cote and J. L. Bates, *J. Am. Chem. Soc.*, 1990, **112**, 2782; (*b*) D. E. Brown, M. A. McGuirl, D. M. Dooley, S. M. Janes, D. Mu and J. P. Klinman, *J. Biol. Chem.*, 1991, **266**, 4049.

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

18 T. Ishida, M. Doi, K. Tomita, H. Hayashi, M. Inoue and T. Urakami, *J. Am. Chem. Soc.*, 1989, **111**, 6822.

19 M. Haga, E. S. Dodsworth and A. B. P. Lever, *Inorg. Chem.*, 1986, **25**, 447.

20 (*a*) H. Masui, A. B. P. Lever and P. R. Auburn, *Inorg. Chem.*, 1991, **30**, 2402; (*b*) H. Masui, A. B. P. Lever and E. S. Dodsworth, *Inorg. Chem.*, 1993, **32**, 258.

21 S. Ernst, P. Hänel, J. Jordanov, W. Kaim, V. Kasack and E. Roth, *J. Am. Chem. Soc.*, 1989, **111**, 1733.

22 (*a*) A. Dei, D. Gatteschi and L. Pardi, *Inorg. Chem.*, 1990, **29**, 1442; (*b*) A. Dei and D. Gatteschi, *Inorg. Chim. Acta*, 1992, **198–200**, 813.

23 G. G. Sadler and N. R. Gordon, *Inorg. Chim. Acta*, 1991, **180**, 271.

24 M. P. Mulay, P. L. Garge, S. B. Padhye, R. C. Haltiwanger, L. A. deLearie and C. G. Pierpont, *J. Chem. Soc., Chem. Commun.*, 1987, 581.

25 J. Thiele and E. Winter, *Liebigs Ann.*, 1900, **307**, 341.

26 E. Heilbronner and H. Bock, *The HMO-Method and its Application*, Wiley, London, 1976.

27 W. Kaim, S. Ernst and V. Kasack, *J. Am. Chem. Soc.*, 1990, **112**, 173.

28 M. Krejcik, S. Zalis, J. Klima, D. Sykora, W. Matheis, A. Klein and W. Kaim, *Inorg. Chem.*, in the press.

29 W. Kaim, *Coord. Chem. Rev.*, 1987, **76**, 187.

Paper 3/01340E
Received 8*th March* 1993
Accepted 21*st April* 1993