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(O-Benzoylsalicylato)copper(II) complexes as synthetic enzyme-product models for flavonol 2,4-dioxygenase

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Abstract

Mononuclear complexes of the type $[Cu^{II}(BPEA)(O-bs)]$, $[Cu^{II}(BnBPA)(O-bs)]ClO_4$, and $[Cu^{II}(Bn-6Me_2BPA)(O-bs)]ClO_4$ $[O-bsH = O-benzoylsalicylic acid, BPEAH = N, N-bis(2-pyridylmethyl)amino-2-ethanoic acid, BnBPA = N-benzyl-N, N-bis(2-pyridylmethyl)amine, Bn-6Me_2BPA = N-benzyl-N, N-bis(6-methyl-2-pyridylmethyl)amine] were synthesized as synthetic enzyme-depside complexes, and characterized by spectroscopic measurements and X-ray crystal analysis. The oxygenation of flavonol using the above-mentioned complexes as catalyst results in the oxidative cleavage of the heterocyclic ring to give a O-benzoylsalicylic acid and CO as a mimic of quercetinase action.$

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To date, four prokaryotic dioxygenases are known, which catalyze an oxidative C-C bond cleavage with incorporation of two O atoms into, and release of CO from their substrate [1-4]. Flavonol 2,4-dioxygenase (FDO), which catalyzes the cleavage of flavonol (1) to phenolic carboxylic acid ester (2) and carbon monoxide was produced by the copper-containing fungi like Aspergillus flavus [5], Aspergillus niger [6], Aspergillus japonicus [7] and the iron-containing protein Yxag from *Bacillus subtilis* [8] (Eq. (1)). The crystal structure from A. japonicus has recently been solved. The native structure shows an active site with a copper atom coordinated by three histidine residues at 2.09-2.16 Å and a water molecule at 2.21 Å (major conformation); an additional coordination is present, in which a glutamate residue is coordinated at 2.10 Å, while the water molecule is found at a larger distance of 2.41 Å (minor conformation). The co-existence of these two conformations of the native enzyme is also present in the EPR spectrum, which exhibits a mixed signal [9]. Upon anaerobically binding the substrate, the structure (*major* conformation) changes to a pentacoordinated square pyramidal geometry by replacement of the water and additional binding of a glutamate and the substrate. Two histidines, glutamate, and the O3 atom of the substrate form the equatorial base, and the third histidine is the apical ligand. In the course of model studies of FDO numerous copper complexes of flavonol and *O*-benzoylsalicylic acid have been prepared and characterized [10–14].



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In this communication, we present the synthesis and structural characterization of new (O-benzoylsalicylato)copper(II) complexes with a tetradentate ligand N,Nbis(2-pyridylmethyl)amino-2-ethanoic acid (BPEAH), and tridentate ligands N-benzyl-N,N-bis(2-pyridylmethyl)amine (BnBPA) and N-benzyl-N,N-bis(6-methyl-2-pyridylmethyl)amine (Bn-6Me₂BPA) which can serve a good mimics for the enzyme-depside complexes (Fig. 1). These are formulated as Cu^{II}(BPEA)(O-bs) (3), [Cu^{II}(BnBPA)(O-bs)]ClO₄ (4), and $[Cu^{II}(Bn-6Me_2BPA)(O-bs)]ClO_4$ (5). The determination of their structure in most cases was carried out by X-ray single crystal study. In addition, their IR and UV-Vis analysis and dioxygenase-like activity in the oxygenation of flavonol was also examined in order to see whether the carboxylate coordinate to the copper(II) ion or enhance the reactivity of the complex.

Complex 3 isolated as a blue solid is stable in air and analyzed satisfactorily for C, H, N [15]. The infrared (IR) spectrum of the complex shows bands corresponding to the coordinated O-benzoylsalicylate at 1731 v(CO), and 1571, 1344 $v(CO_2)$ cm⁻¹. The difference between the asymmetric and symmetric stretching frequencies of this carboxylato group $[\Delta v = v_{as}(CO_2) - v_s(CO_2)]$ is 227 cm⁻¹, rendering these to a monodentate carboxylate bonding mode [16,17]. The absorption bands at 1601, 1480 and 1421 cm^{-1} can be rendered to the pyridine skeleton of the N,N-bis(2-pyridylmethyl)amino-2-ethanoic acetate. Bands corresponding to $v(CO_2)$ for the BPEAH ligand appear at 1629 and 1445 cm⁻¹ [18]. The electronic spectrum of complex 3 shows an absorption band at 914 nm due to d-d transition. A higher energy band at 290 nm is associated with the ligand-to-metal charge transfer transition.

Suitable crystals of 3 were obtained by slow diffusion of diethyl ether into acetonitrile solution of the complex at 20 °C. The molecular structure of $Cu^{II}(BPEA)(O-bs)$ [19] as well as selected bond lengths and angles is shown in Fig. 2. The molecule is monomeric in the solid state. The overall geometry around the five-coordinate copper ion is described as a distorted square planar geometry as judged by the method of Addison and co-workers [22] ($\tau = 0.51$). Two nitrogen atoms of the tetradentate ligand BPEAH and two oxygen atoms of the monodentate carboxylate groups occupy basal positions. The Cu(1)–O(3) bond is 0.043 Å longer than the Cu(1)–O(1) bond. The third nitrogen atom of N,N-bis(2-pyridylmethyl)amino-2-ethanoic acid ligand is in the apical position. The Cu-N and Cu-O distances in the basal plane are shorter than the Cu-N distance in the apical position.



Fig. 2. Molecular structure of $Cu^{II}(BPEA)(O-bs)(3)$ with crystallographic numbering. Relevant bond lengths (Å) and angles (°): Cu(1)–O(1) 1.972(2), Cu(1)–O(3) 2.015(2), Cu(1)–N(2) 2.016(3), Cu(1)–N(3) 2.047(2), Cu(1)–N(1) 2.264(3), C(14)–O(1) 1.274(4), O(2)–C(14) 1.228(4), O(3)–C(15) 1.225(4), O(4)–C(15) 1.238(4), O(5)–C(22) 1.348(4), O(5)–C(21) 1.405(4), O(6)–C(22) 1.192(4), O(1)–Cu(1)–O(3) 91.41(8), O(1)–Cu(1)–N(2) 145.68(10), O(3)–Cu(1)–N(2) 100.91(9), O(1)–Cu(1)–N(3) 84.80(9), O(3)–Cu(1)–N(3) 176.17(9), N(2)–Cu(1)–N(3) 81.98(10), O(1)–Cu(1)–N(1) 94.10(10), O(3)–Cu(1)–N(1) 102.50(9), N(2)–Cu(1)–N(1) 113.86(10), N(3)–Cu(1)–N(1) 78.42(9), C(14)–O(1)–Cu(1) 115.33(18), C(15)–O(3)–Cu(1) 118.79(19).

The IR spectra of the complexes $[Cu^{II}(BnBPA)(O-bs)]ClO_4$ and $[Cu^{II}(Bn-6Me_2BPA)(O-bs)]ClO_4$ show principal bands corresponding to v(CO) at 1731 and 1735 cm⁻¹, respectively [23,24]. Bands corresponding to $v(CO_2)$ for the two complexes appear at 1559 and 1374, and 1570 and 1375 cm⁻¹. The differences between the asymmetric and symmetric stretching frequencies of the carboxylato groups are 185 and 195 cm⁻¹, rendering these to a bidentate carboxylate bonding mode. Absorptions at 1589, 1474, 1449 (4) and 1592, 1472, 1450 cm⁻¹ (5) can be assigned to the pyridine skeleton. The infrared spectra of the complexes show v(Cl-O) absorptions around 1100 cm⁻¹. Bands at 682 (4) and 736 nm (5) are associated with d–d transitions of the complexes.

Suitable crystals of **5** were obtained by slow diffusion of diethyl ether into ethanol solution of the complex at 20 °C. The molecular structure of $[Cu^{II}(Bn-6Me_2BPA)(O-bs)]ClO_4$ [25] as well as selected bond lengths and angles is shown in Fig. 3.



Fig. 1. Structures of the used ligands.



Fig. 3. Molecular structure of $[Cu^{II}(Bn-6Me_2BPA)(O-bs)]ClO_4$ (5) with crystallographic numbering. Hydrogen atoms are omitted for clarity. Relevant bond lengths (Å) and angles (°): Cu(1)–O(1) 1.9835(19), Cu(1)–N(3) 1.995(2), Cu(1)–N(2) 2.004(2), Cu(1)–N(1) 2.041(2), O(1)–C(22) 1.266(3), C(22)–O(2) 1.242(3), O(4)–C(29) 1.213(5), O(1)–Cu(1)–N(3) 98.03(9), O(1)–Cu(1)–N(2) 98.62(9), N(3)–Cu(1)–N(2) 163.34(9), O(1)–Cu(1)–N(1) 82.83(9), N(2)–Cu(1)–N(1) 82.83(9), C(22)–O(1)–Cu(1) 101.16(17).

The molecule is monomeric in the solid state. The overall geometry around the six-coordinate copper ion is described as a distorted elongated octahedral geometry. Three nitrogen atoms of the tridentate ligand Bn-6Me₂ BPA and one of the oxygen atom of the bidentate carboxylate group occupy basal positions. The another oxygen atom of the depside |Cu(1)-O(2), 2.488 Å| and one of the oxygen of the perchlorate anion |Cu(1)-O(5), 2.496 Å| are in the apical positions.

The reaction between flavonol and dioxygen in the presence of catalytic amounts of copper(II) carboxylates (3, 4, and 5) were performed in DMF solutions and examined at 100 °C with a ratio of 1:20 between initial concentration of copper complexes and flavonol (1). We found that the oxygenation of flavonol results in oxidative cleavage of the heterocyclic ring to give *O*-benzoylsalicylic acid (2) and CO as products [26]. The oxygenation reaction in all cases were selective, no other products were obtained (Table 1). A comparison of their activities was made through the determination of the conversion of the reactions (Table 1). The

Table 1

Cu-catalyzed oxygenation of flavonol

Catalyst	[Cu] ^a (mmol)	[FlaH] ^a (mmol)	Conversion ^b (%)	TN
Cu ^{II} (BPEA)(O-bs) (3)	0.05	1	90	9.0
$[Cu^{II}(BnBPA)(O-bs)]ClO_4$ (4)	0.05	1	91	9.1
$[Cu^{II}(Bn-6Me_2BPA)(\mathit{O}\text{-}bs)]ClO_4 (\textbf{5})$	0.05	1	69	6.9

^a In 15 ml DMF under O_2 at 100 °C.

^b By glc (benzoin as an internal standard).

fact that there is no difference on the catalytic activity of the complexes $Cu^{II}(BPEA)(O-bs)$ (3) and $[Cu^{II}(BnB PA)(O-bs)]ClO_4$ (4) suggests a role for the glutamate in the enzyme and the glioxylate linkage in the model reaction in the deprotonation of the substrate and protonation of the forming depside. It can be seen that in the case of $[Cu^{II}(Bn-6Me_2BPA)(O-bs)]ClO_4$ (5) the conversion is relatively low, which can be explained by steric effect of the methyl-substitution. These reactions resemble the enzyme action on the flavonol to give the cleavage product as shown earlier (Eq. (1)).

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- [19] Crystal data for 3: $C_{28}H_{23}CuN_3O_6$, 561.03 g mol⁻¹, monoclinic, P21/c, a = 9.0410(1) Å, b = 17.5690(3) Å, c = 16.0680(3) Å, $\beta = 103.3540(7)^\circ$, Z = 4, V = 2483.25(7) Å³, μ (MoK α) = 0.929 mm⁻¹, $d_{calc} = 1.501$ g cm⁻³, T = 293(2) K, F(000) = 1156. The intensity data were collected with a Nonius Kappa CCD single-crystal diffractometer using Mo K α radiation ($\lambda = 0.71073$). Reflections collected = 4735, reflections total = 4735, reflections unique = 4275. The structure was refined to R = 0.0461 (0.0520) and $wR_2 = 0.1240$ for the reflections with $I > 2\sigma(I)$ (all data) and max. resd. density = 0.611 e Å⁻³. The computer program used was SHELXL97 [20]. The structure was solved by direct and difmap methods (SIR92) [21]. CCDC reference number: 288962.
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- [23] Synthesis of [Cu^{II}(BnBpa)(*O*-bs)]ClO₄ (4): Cu(ClO₄)₂ · 6H₂O (0.371 g, 1 mmol), *O*-benzoylsalicylic acid (0.242 g, 1 mmol) and *N*-benzyl-*N*,*N*-bis(2-pyridylmethyl)amine (0.289 g, 1 mmol) were dissolved in 10 cm³ of methanol under argon and one equivalent of NEt₃ (0.101 g, 1 mmol) was dropped slowly into the solution. After 8 h stirring, the product was collected by filtration, washed with diethyl ether, dried in vacuum and then recrystallized from ethanol by ether diffusion (0.28 g, 40%). M.p. 189 °C. IR (KBr) cm⁻¹: 3067 vw, 2921 vw, 2820 vw, 1731 s, 1610 vs, 1589 w, 1559 m, 1474 m, 1449 m, 1374 s, 1344 vs, 1258 s, 1201 m, 1193 vs, 1092 vs, 1029 m, 753 m, 706 m, 625 m. UV–Vis (DMF) λ_{max} (log ϵ/dm^3 mol⁻¹ cm⁻¹): 270 (3.85); 682 (2.41). Anal. Calc. for C₃₃H₂₈N₃O₈ClCu: C, 57.15; H, 4.07; N, 6.06%. Found: C, 56.86; H, 4.01; N, 6.12%.
- [24] Synthesis of $[Cu^{II}(Bn-6Me_2BPA)(O-bs)]ClO_4$ (5): $Cu(ClO_4)_2 \cdot 6H_2O$ (0.371 g, 1 mmol), *O*-benzoylsalicylic acid (0.242 g, 1 mmol) and *N*benzyl-*N*,*N*-bis(6-methyl-2-pyridylmethyl)amine (0.381 g, 1 mmol) were dissolved in 10 cm³ of methanol under argon and one equivalent of NEt₃ (0.101 g, 1 mmol) was dropped slowly into the solution. After 8 h stirring, the product was collected by filtration, washed with diethyl ether, dried in vacuum and then recrystallized from ethanol by ether diffusion (0.40 g, 55%). M.p. 119 °C. IR (KBr) cm⁻¹: 3140 vw, 2970 vw, 2921 vw, 2880 vw, 1735 s, 1612 vs, 1592 w, 1570 m, 1472 m, 1450 m, 1375 s, 1344 vs, 1269 s, 1258 s, 1201 m, 1117 vs, 1094 vs, 1025 m, 851 w, 791 w, 750 m, 707 m, 625 s. UV–Vis (DMF) $\lambda_{max} (log \varepsilon/dm^3)$ mol⁻¹ cm⁻¹): 271 (3.93); 736 (2.16). Anal. Calc. for C₃₅H₃₂-N₃O₈ClCu: C, 58.25; H, 4.47; N, 5.82%. Found: C, 58.46; H, 4.51; N, 5.75%.
- [25] Crystal data for $\mathbf{5} \cdot 0.5(C_2H_5)O$: $C_{37}H_{37}ClCuN_3O_{8.5}$, 758.69 g mol⁻¹, monoclinic, P21/c, a = 8.6330(2) Å, b = 15.4140(2) Å, c = 29.1940(4) Å, $\beta = 95.7240(6)^\circ$, Z = 4, V = 3865.45(12) Å³, μ (Mo K α) = 0.687 mm⁻¹, $d_{calc} = 1.304$ g cm⁻³, T = 293(2) K, F(000) = 1576. The intensity data was collected with a Nonius Kappa CCD single-crystal diffractometer using Mo K α radiation ($\lambda = 0.71073$). Reflections collected = 14692, reflections total = 7906, reflections unique = 5614. The structure was refined to R = 0.0506 (0.0819) and $wR_2 = 0.1415$ for the reflections with $I > 2\sigma(I)$ (all data) and max. resd. density=0.332 e Å⁻³. The computer program used were SHELXL97 [20]. The structure was solved by direct and difmap methods (SIR92) [21]. CCDC reference number: 288963.
- [26] Copper-catalyzed oxygenation of flavonol: flavonol (0.238 g, 1 mmol) and the corresponding copper(II) *O*-benzoylsalicylate (0.05 mmol) were dissolved and stirred at 100 °C in 15 cm³ DMF for 20 h under dioxygen atmosphere. Diazomethane solution (2 cm³) (in diethyl ether) was added to 0.5 cm³ of the reaction mixture at r.t. and the conversion of flavonol into *O*-benzoylsalicylic acid was determined by GC as the methylated derivative in the presence of benzoin (internal standard).