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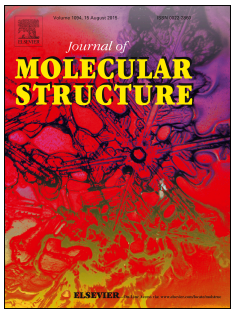

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PII: S0022-2860(19)30208-X

DOI: https://doi.org/10.1016/j.molstruc.2019.02.078

Reference: MOLSTR 26235

To appear in: *Journal of Molecular Structure*

Received Date: 14 September 2018

Revised Date: 31 January 2019

Accepted Date: 20 February 2019

Please cite this article as: W.E. Lynch, D. Nivens, B. Quillian, C.W. Padgett, A. Petrillo, N. Peek, J. Stone, A Copper(II) tris-imidazolylphosphine complex as a functional model of flavonol 2,4-dioxygenase, *Journal of Molecular Structure* (2019), doi: https://doi.org/10.1016/j.molstruc.2019.02.078.

A Copper(II) Tris-imidazolylphosphine Complex as a Functional Model of Flavonol 2,4-Dioxygenase.

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Keywords:

Copper complex

Dioxygenase

X-ray Structure

3-hydroxyflavone

Abstract

Reaction of *tetrakis(*acetonitrile)copper(I) perchlorate ($[Cu(NCCH_3)_4][ClO_4]$), *tris*-1-ethyl-4-methylimidazolylphosphine (T1Et4MeIP) (**1**) and 3-hydroxyflavone (flavH) under ambient conditions produces an *in-situ* generated flavonol bound copper(II) complex, which converts to a stable green complex that formulates to $[Cu(T1Et4MeIP)(flav)][ClO_4]$ (**2**). The crystal structure of **2** was determined by X-ray diffraction and crystallizes in a triclinic system ($P\bar{1}$) with unit cell dimensions of a = 14.537(15) Å, b = 15.794(14) Å, c = 17.044(17) Å, α = 65.58(3)˚, β = 86.80(5)˚, γ = 73.34(4)˚, V = 3376(6) $Å^3$ and Z = 2. While the five-coordinate copper(II) complex is stable under ambient conditions in the solid state, it undergoes oxidative scission of the flavonol pyrone ring under photolytic (300 nm) and/or thermal (120

˚C) conditions in the presence of molecular oxygen. The degradative process produces the corresponding methylated products: methylbenzoate, methyl salicylate and N,N-dimethylbenzamide. In addition, the previously undisclosed single crystal X-ray structure of *tris*-1-ethyl-4-methylimidazolylphosphine (**1**), T1Et4MeIP, is also reported.

Introduction

Dioxygenases are an important class of enzymes capable of inserting two oxygen atoms into a substrate. They play an important biological role in the reduction of aromatic and heteroaromatic compounds to non-aromatic products in biosyntheses, detoxification, and catabolism of various metabolites.[1-4] Early reports on the purification, characterization, and structure of this genre of enzymes, revealed a metal-containing active site bound by three histidine residues as the general motif (Fig. 1).[5-11] A protein structure possessing this motif (quercetin 2,3-dioxygenase), isolated from the *Aspergillus japonicus* fungus without bound substrate, has been reported to have copper in its active site.[9] The copper(II) dependent metalloenzyme catalyzes the conversion of flavonol derivatives to the corresponding carboxylic acid ester (depside) with loss of carbon monoxide (Fig. 2). Corresponding electron paramagnetic resonance spectroscopy studies on the active site of the enzyme suggested a mononuclear type II copper center in two geometric forms.[12] The first geometric form was found to be a distorted tetrahedron with copper bound to three histidine residues and a water molecule. The second form correlated to a trigonal bipyramidal copper center with histidines and a glutamate ligation.[13] Studies have indicated that in the enzyme substrate active site, five coordinate geometry is proposed with glutmate and monodentate coordination of quercetin is observed.[10] Early biomimetic studies showed coordination complexes with similar metal centers were also capable of flavonol oxygenation.[14-22] These reports have shed insight into the substrate metal interaction and have shown that the complexes are capable of degrading 3-hydroxyflavone at elevated temperatures. Recent reports have also expanded the model systems capable of mimicking this chemistry to include alternative metal atoms and extended conjugated flavonol derivatives.[23-26]

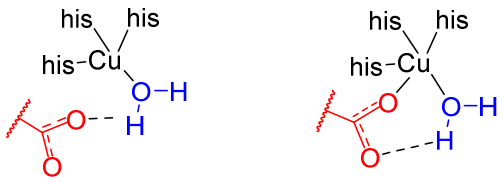


Figure 1. General active site for copper containing dioxygenases showing distorted tetrahedral and trigonal bipyramidal coordination geometries about copper.

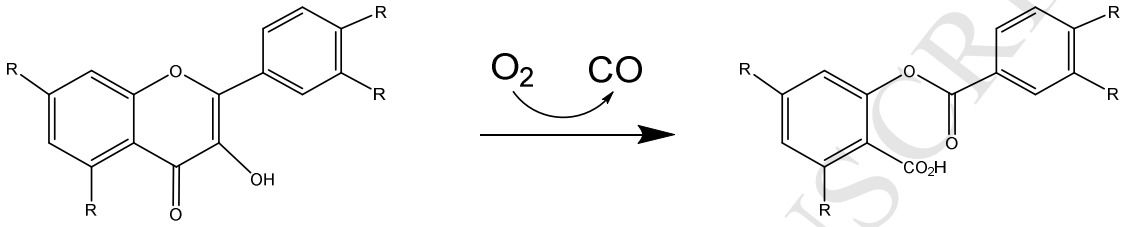


Figure 2. Oxidative decarbonylation reaction of 3-hydroxyflavones.

These reports prompted our investigation into incorporating a biomimetic ligand with three imidazoles to represent the three bound histidine residues in the protein. Herein, we report the synthesis, characterization and single-crystal X-ray structure of a flavonol (flavH) bound model copper(II) complex supported by a facially coordinating *tris*-imidazolylphosphine ligand (*tris*-1-ethyl-4-methylimidazolylphosphine, T1Et4MeIP) and its reaction with dioxygen. The use of imidazolyl ligation instead of other O, N or P donor ligands should more closely model the binding site in flavonol dioxygenase (FDO) than those previously reported.[12-24] To our knowledge, this is the first structurally characterized copper(II) complex supported by a *tris*-imidazolyphosphine ligand and flavonol.

2. Experimental

2.1 Materials.

The ligand *tris*-1-ethyl-4-methylimidazolylphosphine (T1Et4MeIP; **1**) was prepared by a previously described procedure.[27] The *tetrakis*(acetonitrile)copper(I) perchlorate complex was prepared as outlined previously with perchloric acid substituted for hexafluorophosphoric acid.[28] All other

commercial reagents, standards (Aldrich Chemical Company) and solvents (Fischer Scientific) were used without further purification.

2.2 Physical Measurements.

C, H, and N analyses were performed by Atlantic Microlab. Infrared spectra (IR) were recorded on a Perkin Elmer Spectrum One using a diffuse reflectance attachment and KBr mixtures. Electronic spectra were recorded on a Hewlett Packer 8453 Diode Array spectrophotometer. GC-MS measurements were recorded on a Hewlett Packard 6890 Gas Chromatograph coupled with a Hewlett Packard 5973 Mass Selective Detector. Fluorescence spectra were obtained using a Shimadzu RF 5301 PC Spectrofluorophotometer. UV lamp intensity was measured using a Fisher Scientific UV light meter. For excitation spectra, the emission wavelength was 644 nm; while for emission spectra, the excitation wavelength was 430 nm. Single-crystal X-ray structure determinations were performed on a Rigaku XtaLAB mini diffractometer.

2.3 X-ray Diffraction.

Crystals suitable for single-crystal X-ray analysis for **1** were grown by slow evaporation of hexane solution of the complex at room temperature and mounted on a Mitigen micromount. Isolated single crystals appropriate for X-ray analysis of **2** were grown by vapor diffusion of diethyl ether into a dichloromethane solution of the copper complex followed by mounting on a Mitigen micromount. Single-crystal X-ray diffraction data for compounds **1** and **2** were collected at 173 K on a Rigaku XtaLAB mini diffractometer equipped with Mo-Kα radiation (λ = 0.71073 Å) and processed using CrystalClear (Rigaku) software.[29] The structures were solved using direct methods and refined by using full matrix least squares refinement using SHELXT and SHELXL2017.[30, 31] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed in ideal positions and refined as riding atoms with relative isotropic displacement parameters. The crystal data and refinement details for compounds **1** and **2** are listed in Table 1.

Crystallographic data of the structures have been deposited with the Cambridge Crystallographic Data Center as supplementary publication CCDC 1867305 for (**1**) and CCDC 1867306 for (**2**). Copies of

the data are available from CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; email: deposit@ccdc.cam.ac.uc.uk or www: http://www.ccdc.cam.ac.uk).

Table 1. X-ray experimental details for **1** and **2**

	(1)	(2)
Crystal data		
Chemical formula	$C_{18}H_{27}N_6P$	$C_{33}H_{36}CuN_6O_3P\cdot ClO_4$
Mr	358.42	758.65
Crystal system, space group	Monoclinic, P21/c	Triclinic, PT
Temperature (K)	173	173
a, b, c (Å)	8.396 (7), 11.365 (8), 21.088 (17)	14.537 (6), 15.794 (7), 17.044 (8)
α, β, γ (°)	90, 98.858 (11), 90	64.58 (3), 86.80 (5), 73.34 (4)
V (Å3)	1988 (3)	3376 (3)
Z	4	4
Radiation type	Mo Kα	Mo Kα
μ (mm−1)	0.15	0.83
Crystal size (mm)	0.57 × 0.26 × 0.26	0.41 × 0.14 × 0.14
Data collection		
Diffractometer	Rigaku XtaLAB mini	Rigaku XtaLAB mini
Absorption correction	Multi-scan (REQAB; Rigaku, 1998)	Multi-scan (REQAB; Rigaku, 1998)
Tmin, Tmax	0.507, 100	0.720, 1.00
No. of measured, independent and observed [I > 2σ(I)] reflections	8067, 4440, 2725	29925, 12212, 9690
Rint	0.072	0.070
(sin θ/λ)max (Å−1)	0.650	0.600
Refinement		
R[F2 > 2σ(F2)], wR(F2), S	0.076, 0.234, 1.01	0.056, 0.153, 1.06
No. of reflections	4440	12212
No. of parameters	232	923
H-atom treatment	H-atom parameters constrained	H-atom parameters constrained
Δρmax, Δρmin (e Å−3)	0.51, −0.56	1.47, −1.28

Computer programs: [27],[28],[37]

*2.4 Synthesis of [Cu(T1Et4MeIP)(flav)][ClO_4](**2**).*

The compound **2** was prepared by mixing stoichiometric amounts of [$Cu(CH_3CN)_4$][ClO_4] (0.091 g, 0.280 mmol) and *tris*-1-ethyl-4-methylimidzolylphosphine (0.100 g, 0.280 mmol) in a minimal amount of dichloromethane under ambient conditions. The solution gradually turned green over 10 minutes, at which time, 3-hydroxyflavone (0.066 g, 0.280 mmol) was added. After 10 minutes, a green solid was precipitated by dropwise addition of diethyl ether. The solid was collected on a glass frit, washed successively with water and diethyl ether and subsequently dried under vacuum (0.144 g, 68 %). X-ray quality crystals of **2** were grown by vapor diffusion of a saturated dichloromethane solution of the title compound with diethyl ether. IR (KBr): 2982, 2923, 1551, 1095, 623 cm^{-1}. UV-vis (MeCN) λmax / nm (ε/$M^{-1}cm^{-1}$): 257 (50900), 412 (18900), 428 (21400), 676 (150). Anal. Calc'd for $C_{33}H_{36}N_6O_7PClCu$: C, 52.24; H, 4.79; N, 11.08. Found: C, 52.18; H, 4.78; N, 11.08.

*2.5 Reactions of **2** with Dioxygen.*

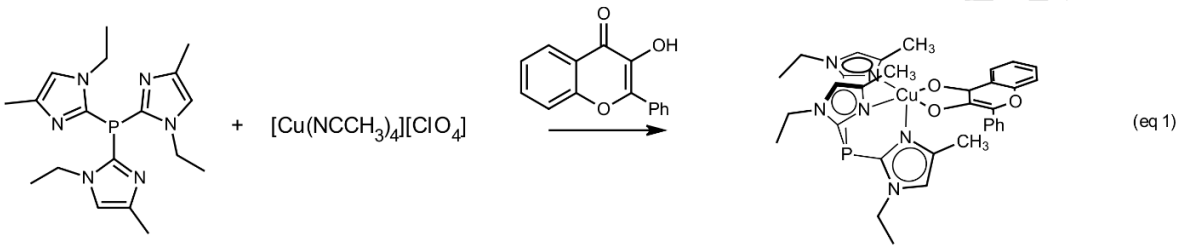
a.) Photolysis. UV-photolysis experiments were carried out with a solution (0.036 mM) of **2** in dichloromethane over 6 hours. While vigorously stirring, the sample was exposed to light at 300 ± 10 nm with a power setting of 2.9 μw/cm^2 from a 150 W Xenon lamp. Periodically, the UV-vis spectrum was recorded over the range of 300 - 550 nm to determine reaction completion. Upon reaction completion, the products were methylated with diazomethane in diethyl ether to lower their boiling points for facile characterization by GC-MS. The products were analyzed *in-situ* via GC-MS and identified by comparing retention times and mass spectra with corresponding standards.

b.) Thermal Degradation. Thermal degradation experiments were carried out in a typical procedure by preparing a solution of **2** in DMF (22 μM). The sample was then heated to 120 ˚C and the reaction progress was monitored every 10 minutes via UV-vis spectroscopy, surveying absorbance changes within the range of 300 - 550 nm. Products were determined by GC-MS and/or GC analysis as outlined above.

3. Results and Discussion.

*3.1 Synthesis and Characterization of **2**.*

The reaction of equimolar quantities of *tetrakis*(acetonitrile)copper(I) perchlorate, **1** and 3-hydroxyflavone in dichloromethane under ambient conditions quantitatively yielded **2** as green crystals (eq 1).



(eq 1)

The IR spectrum of the complex displays peaks at 2982 and 2923 cm^{-1}, which are characteristic of aliphatic C-H stretches of T1Et4MeIP ligand. The band observed at 1551 cm^{-1} is assigned to the $\nu_{C=O}$ stretch of the deprotonated 3-hydroxyflavone ligand, and suggests strong delocalization of the π-bond. This is likely the result of the deprotonated α-hydroxy groups. Other bands assigned to perchlorate are observed at 1195 and 623 cm^{-1}. The electronic spectrum displays transitions at 412 and 428 nm that are assigned as intraligand π–π* processes in the flavonolate ligand.[15] A low energy d-d transition characteristic of a copper(II) center is observed at 676 nm.

*3.2 X-ray Structure of T1Et4MeIP (**1**) .*

The ligand, compound **1**, crystallizes in the monoclinic space group, $P2_1/c$ (Fig. 3). The geometry about the phosphorous atom was found to have strained trigonal pyramid geometry with C-P-C bond angles of 101.90 (15)°, 101.46 (16) ° and 103.37 (15) °. The average C–P–C bond angle in **1** (102.24 °) is slightly larger than those found in other structurally characterized tris(imidazolyl/thiazolyl)phosphines,[32-36] but is substantially smaller than that (107.1 °) of the tri-cationic, *tris*(1,3,4,5-tetramethylimidazolium-2-yl)phosphine. [37] A list of C–P–C bond angles and P–C

lengths, including their averages, of previously reported structurally characterized *tris*(imidazolyl/thiazolyl)phosphines is shown in Table 2. Two of the imidazolyl units in **1** have methyl groups pointed towards the trigonal cavity, whereas the third unit shows the methyl group pointing away from the cavity and towards backside of the molecule. The C–P bond distances in **1** (P1–C1 =1.820 (4) Å; P1–C7 = 1.810 (4) Å; P1–C13 =1.827 (4) Å) compares well with those previously reported for reported *tris*(imidazolyl/thiazolyl)phosphines (see Table 2). There are no hydrogen bonding (< 3.0 Å) or π-stacking interactions present in the packing of compound **1** and only Van der Waals contacts are observed (See Supporting Information for image of packing).

Table 2. Structural data for **1** and previously reported tris(imidazolyl/thiazolyl)phosphines

Ligand	P-C bond Length (Å)	C-P-C Bond angle (°)	Ref.
tris(2-imidazolyl)phosphine hydrate	1.811 (4) 1.814 (4) 1.807 (4)	102.6 (2) 100.9 (2) 101.4 (2)	[32]
Avg.	1.811	101.6	
tris(4-methylimidazol-2-yl)phosphine	1.8289 1.8167 1.8203	104.45 98.66 99.85	[36]
avg.	1.8220	100.57	
tris[2-(vinyl)imidazolyl]phosphine	1.813 (2) 1.825 (2) 1.827 (2)	101.73 (10) 102.24 (10) 98.39 (10)	[34]
avg.	1.822	100.57	
tris[2-methyl-4-tolylimidazolyl)phosphine	1.841 (5) 1.824 (5) 1.813 (5)	96.8 (2) 103.5 (2) 102.2 (2)	[33]
avg.	1.826	100.8	
tris(1,3,4,5-tetramethylimidazolium-2-yl)phosphine	1.813 (2) 1.818 (2) 1.825 (2)	106.8 (2) 107.2 (2) 107.2 (2)	[37]
avg.	1.819	107.1	
tris(benzothiazol-2-yl)phosphine	1.82 (2)	101.87 (7)	[35]
tris-1-ethyl-4-methylimidazolylphosphine (**1**)	1.820 (4)	101.90 (15)	This

			work
	1.810 (4)	101.46 (16)	
	1.827 (4)	103.37 (15)	
avg.	1.819	102.24	

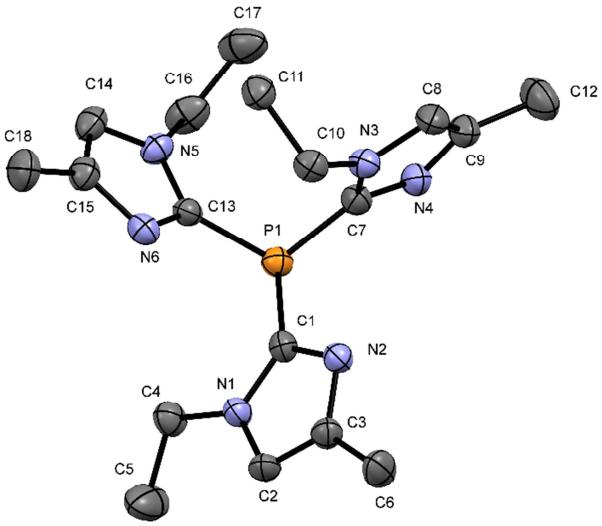


Figure 3. Molecular structure of T1Et4MeIP compound (**1**). Thermal ellipsoids have been drawn at the 50% probability level with hydrogen atoms omitted for clarity. Selected bond lengths (Å): P1–C1 = 1.820 (4); P1–C7 = 1.810 (4); P1–C13 =1.827 (4). Selected bond angles (°):C1–P1–C13 101.90 (15), C7–P1–C1 = 101.46 (16); C7–P1–C13 = 103.37 (15)°.

*3.3 X-ray Structure of [Cu(T1Et4MeIP)(flav)][ClO_4](**2**).*

Compound **2** crystallizes in the triclinic space group ($P\bar{1}$). The structure was refined to a final R value of 5.63%. There was disorder associated with two of the perchlorate oxygen atoms located on Cl2 (53% and 47% occupancy). There are two independent, five-coordinate, copper(II) centers per unit cell with minor variances in the bond distances surrounding the copper atom and a more noticeable difference

in apical Cu-N bond distances (*vide infra*), which is likely due to lattice stacking. The coordination geometry of the two copper centers is best described as square pyramidal with slightly different τ values (Cu(1) = 0.094 and Cu(2) = 0.17). [23] Due to similarities between the two structures, a single unique structure is shown (Fig. 4).

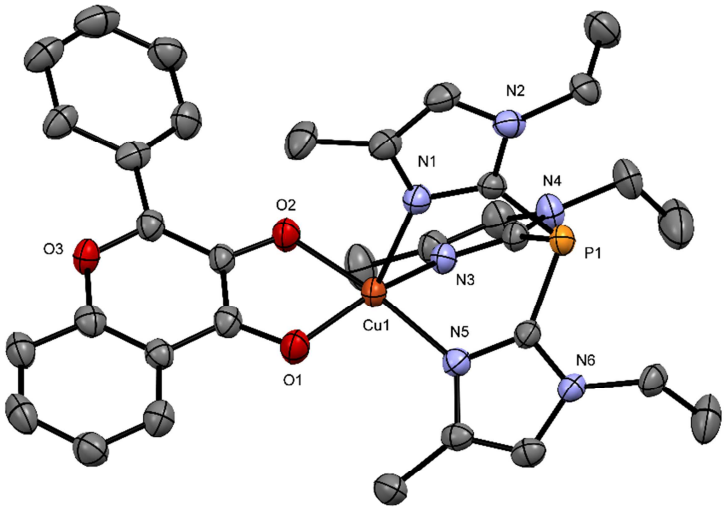


Figure 4. Molecular structure of the cationic portion of **[**Cu(II)(T1Et4MeIP)(flav)]ClO_4, (**2**). Thermal ellipsoids have been drawn at the 50% probability level with hydrogen atoms omitted for clarity.

The five-coordinate copper(II) centers of **2** are supported by a facially coordinated T1Et4MeIP ligand that occupies three coordination sites through nitrogen donors and κ^2-bonding of the two oxygen atoms of the deprotonated flavone ligand. The square planar base of **2** is formed by two of the three nitrogen donor atoms (N(3) and N(5) for Cu(1) and N(9) and N(11) for Cu(2)) of T1Et4MeIP and the oxygen atoms (O(1), O(2) and O(4), O(5) for Cu(1) and Cu(2), respectively) of the deprotonated 3-hydroxyflavone chelate. The third imidazole ring occupies the axial position. The bond distances are very similar as noted in Table 4. The largest bond deviation is 0.052 A from the copper center to N1 and N7 in

the two complexes. The other five bond distances vary by less than 0.021 AA comparison of the coordination spheres in structure of the model complex **2** with the structurally characterized enzymatic active site of *A. Japonicus* reveals a number of similarities and disparities. While the copper coordination spheres in complex **2** and the type II center found in *A. Japonicus* are both five-coordinate, they differ in geometry (complex **2** = square pyramidal [τ values (Cu(1) = 0.094 and Cu(2) = 0.17)}) *A. Japonicus* exhibits a distorted trigonal bipyramidal copper center with three histidine residues, a glutamate and a monodentate substrate binding.[11] This disparity in coordination geometries may be due to the rigid *tris*-imidizolylphosphine ligand in **2**, which maintains acute bond angles and the fact that no other Lewis base ligands are available in our reaction scheme. Moreover, unlike that observed in **2**, which displays κ^2-bidentate binding of flavonolate substrate, utilizing the carbonyl and α-hydroxyl groups, a manually modeled enzyme-substrate (ES) complex of the FDO active site suggests only monodentate binding of quercetin through its α-hydroxyl group. However, models of the FDO-ES complex with kojic acid suggests similar κ^2-bidentate binding as observed in **2**. Bidentate flavonol binding has also been observed with a number of synthetic copper-containing FDO-ES mimics with various ligands supporting copper,[13] and has been suggested the source of their retarded oxidative reactivity as compared to the native enzyme. Previously reported, structurally characterized, synthetic FDO-ES complexes have displayed coordination geometries between distorted square-pyramidal and trigonal-bipyramidal with τ values ranging from 0.12-0.61.[13, 14, 38-40] A list of selected synthetic FDO-ES complexes with geometrical parameters and structural data is shown in Table 3. The apical Cu–N bond distances (N(1)–Cu(1) = 2.244 (4) Å; N(7)–Cu(2) =2.296 (3) Å) in the two independent units of **2** are slightly different and are significantly longer than the Cu–N bonds found in other structurally characterized synthetic FDO-ES complexes (see Table 3), suggesting a very weak bind interaction. The two Cu–O bonds in both units of **2** are asymmetric. In both independent units, the Cu–O bond distance (Cu(1)–O(1) = 1.999 (3) Å and Cu(2)–O(4) =1.998(3)Å) involving the carbonyl is somewhat longer than that of the deprotonated α-alkoxide (Cu(1)–O(2) = 1.944 (3) Å and Cu(2)–O(5) = 1.956 (3) Å). These C–O bond distances in **2**, as

expected, are significantly longer than those reported for either glutamate or water coordination in the type II copper center in the FDO active site of *A. Japonicus* (Glutamate Cu-O bond distance = 2.1 Å; H_2O Cu–O bond distance = 2.4 Å). The Cu–N bond distances of the copper-imidazole interactions in the square pyramid basal plane of **2** are all slightly over 2.0 Å (Cu1-N3 = 2.023(3) Å; Cu1-N5 = 2.019(4) Å; Cu2-N11 = 2.029(3) Å; Cu2-N9 = 2.002(3) Å) for both copper centers and are significant shorter than the apical Cu–N bond distances by more than 0.2 Å. Both unique copper atoms of **2** rest slightly less than 0.2 Å above the N_2O_2 basal plane (Cu(1) is 0.194 Å and Cu(2) is 0.170 Å). Bond distances and angles around the copper atoms for 2 are reported in Table 4.[14, 27, 41] The molecular packing diagram for **2** shows no intermolecular interactions of significant consequence between the molecules (see supporting information).

Table 3. Geometric and structural data for previously reported structurally characterized synthetic FDO-ES complexes.

Structurally Characterized Synthetic FDO-ES Complexes	τ values	Geometry	Basal plane atoms	Bond distance (Å)	Apical atom(s)	Bond Distance (Å)	Ref
Cu(Ind)(mco)	0.38	Distorted square pyramidal	Cu-N	2.0418(16)	$Cu\text{-}O_{(carbonyl)}$	2.2966	[38]
			Cu-N	2.0410(15)			
			Cu-N	1.904(8)			
			$Cu\text{-}O_{(hydroxyl)}$	1.9586(14)			
$[Cu(Fla)\text{-}(idapH)]ClO_4$	0.61	Distorted trigonal pyramidal	Cu-N	2.137(3)	$C\text{-}O_{(hydroxyl)}$	1.918(3)	[39]
			Cu-N	2.112(4)	Cu-N	2.006(4)	
			$Cu\text{-}O_{(carbonyl)}$	2.210(3)			
Cu(Fla)(Bz-TAC)	0.12	Distorted square pyramidal	Cu-N	2.040(30	Cu-N	2.231(3)	[40]
			Cu-N	2.027(3)			
			$Cu\text{-}O_{(hydroxyl)}$	1.917(3)			
			$Cu\text{-}O_{(carbonyl)}$	2.012(3)			
CuII(Fla)(Ind)	0.5	Trigonally distorted bipyramidal	Cu-N	2.039(6)	Cu-N	1.903(6)	[14]
			Cu-N	2.031(7)	$Cu\text{-}O_{(hydroxyl)}$	1.942(5)	
			$Cu\text{-}O_{(carbonyl)}$	2.2065(5)			

Compound **2**	0.094, 0.17	Distorted square pyramidal	$C\text{-}O_{(carbonyl)}$	1.999(3), 1.998(3)	C-N	2.244(4), 2.296	This work
			Cu-O $_{(hydroxyl)}$	1.944(3), 1.956(3)			
			Cu-N	2.023(3), 2.003(3)			
			Cu-N	2.019(4), 2.029(3)			

Ind = 1,3-bis(2-pyridylimino)isoindolide; **mco** = 3-hydroxy-(4H)-benzopyran-4-one; **idpaH** = 3,3'-iminobis(*N*,*N*-dimethylpropylamine ;**Bz-TAC** = *N*,*N*,*N*-tribenzyl-1,4,7, triazacyclononane

Table 4. Geometric parameters (Å, °) for (**2**) at the copper centers.

Bonds	Distances	Bonds	Distances
Cu1—N1	2.244 (4)	Cu2—N7	2.296 (3)
Cu1—O1	1.999 (3)	Cu2—O4	1.998 (3)
Cu1—O2	1.944 (3)	Cu2—O5	1.956 (3)
Cu1—N3	2.023 (3)	Cu2—N9	2.002 (3)
Cu1—N5	2.019 (4)	Cu2—N11	2.029 (3)
Atoms	Angles	Atoms	Angles
O1—Cu1—N1	105.40 (13)	O4—Cu2—N7	96.63 (13)
O1—Cu1—N3	162.97 (12)	O4—Cu2—N9	172.55 (11)
O1—Cu1—N5	91.36 (14)	O4—Cu2—N11	91.14 (13)
O2—Cu1—N1	95.22 (13)	O5—Cu2—O4	82.79 (12)
O2—Cu1—O1	83.20 (13)	O5—Cu2—N7	103.66 (13)
O2—Cu1—N3	94.80 (13)	O5—Cu2—N9	93.92 (13)
O2—Cu1—N5	173.15 (12)	O5—Cu2—N11	166.91 (12)
N3—Cu1—N1	91.62 (14)	N9—Cu2—N7	90.64 (13)
N5—Cu1—N1	90.23 (13)	N9—Cu2—N11	90.69 (14)
N5—Cu1—N3	89.18 (14)	N11—Cu2—N7	88.51 (13)

*3.3 Luminescence of **2**.*

Free and metal ion bound hydroxyflavones are known to be highly fluorescent with a typical blue excitation (300-430 nm) and corresponding green emission of 510-560 nm with λ_{max} and peak shape dependent upon solvation and substitution or degree of deprotonation.[42-46] Compound **2** is luminescent in both the solid and solution states. The photochemistry of flavonoids has been studied extensively and reviewed due to their ubiquity in biological processes.[35] In inert atmosphere, the compounds generally undergo cyclization and rearrangement, whereas in the presence of oxygen, it is commonly observed that ring opening occurs with concomitant depside formation with loss of carbon monoxide.[36]

The excitation and emission spectra of the free flavone (3-hydroxyflavone) and complex **2** are shown in Figure 5. For reference, 3-hydroxyflavone excitation spectrum shows a broad absorbance (dashed gray). Using 430 nm excitation, the emission spectrum (solid gray) is observed. Similarly, the 430 nm excitation observed in **2** (dashed black) results in an emission spectrum observed in solid black with the major green emission observed at 644 nm.

In solution, the complex presumably dissociates to some extent. This slight dissociation of flavonol from the complex results in the weak luminescence of the complex being obscured. This is primarily due to the high quantum efficiency of the flavonol. This observation has been previously reported.[46] In the solid state, compound **2** (Fig. 5) was observed to be luminescent at room temperature with a sharp λ_{ex} = 430 nm and $\lambda_{em's}$ = 644, 690 and 715 nm. The emission is likely due to the ligand based transitions of the flavonol that are lost upon solution formation due to the proposed dissociation of

the flavonol. Additional excitation bands, if present, are obscured by the overlap of the broad 3-hydroxyflavone excitation.

3.4 Reactions of Dioxygen with [Cu(II)(T1Et4MeIP)(flav)]ClO_4.

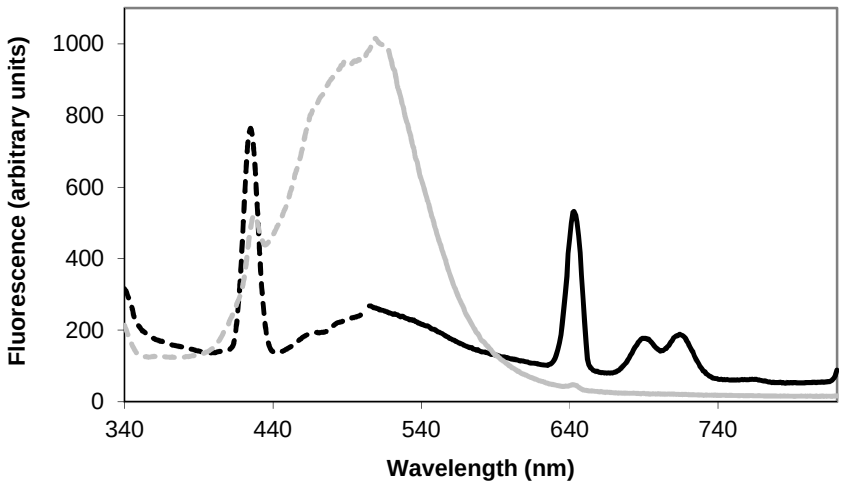


Figure 5. Solid-state luminescence spectra of **[Cu(II)(T1Et4MeIP)(flav)]ClO_4** and **3-hydroxyflavone. [Cu(II)(T1Et4MeIP)(flav)]ClO_4**: excitation spectrum (dashed black); emission spectrum using 430 nm excitation (solid black). **3-hydroxyflavone:** excitation spectrum (dashed gray) using 520 nm emission; 3-hydroxyflavone emission spectrum (solid gray) using 430 nm excitation.

a. Photolysis of 2. For photolytic degradation of the complex, a wavelength range of 300 nm (± 10 nm) was chosen. Dichloromethane samples of the title complex were prepared and illuminated for 6 hours. As shown (Fig. 6), the photolysis was followed via UV-vis at 1-hour intervals of exposure. After the flavonol absorbance at 428 nm decreased to 5 % of the starting absorbance (indicating degradation of the substrate), the solution was methylated using diazomethane. The transition excited by irradiation at 300 nm is assigned as the metal to ligand charge transfer process. This implies that the initiation step of degradation of a bound flavonol is a redox process at the metal center which has been proposed previously in model complexes.[14] The product distribution for the degradation reaction was determined to be 0.25 mole equivalent methylbenzoate (A), 0.24 mole equivalent methyl salicylate (B) (Fig. 7).

These products also correspond to ring opening oxidation products of 3-hydroxyflavone.[14] It is noted here that if the complex is excited with 430 ± 10 nm light, corresponding to an intraligand charge transfer process, the ligand is not degraded but dissociated from the metal.

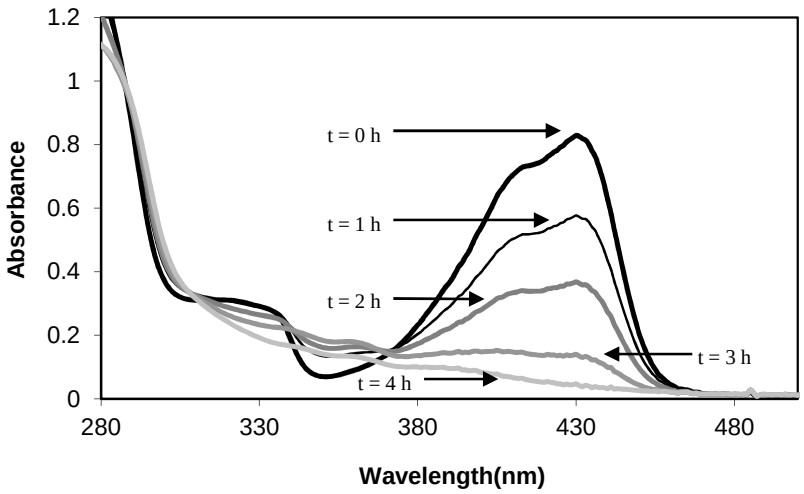


Figure 6. UV-vis spectra of [Cu(II)(T1Et4MeIP)(flav)]ClO_4 in dichloromethane during photolysis. Reaction progress was monitored for 4 hours at 1 hour intervals.

b. Thermal Degradation of 2. Thermal degradation studies involved a DMF solution of the title complex, sealed in a glass vial under ambient conditions. Samples were extracted and monitored via UV-vis. After the reaction reached completion, the solution was methylated in the same manner as in the photolysis studies. The product distribution for the reaction was determined to be 0.26 mole equivalent methylbenzoate (A), 0.37 mole equivalent methyl salicylate (B) and 0.12 mole equivalent N,N-dimethylbenzamide (C) (**See Fig. 7**). These products correspond to ring opening oxidation products of 3-hydroxyflavone.[9] The N,N-dimethylbenzamide product is believed to arise from a reaction with the solvent DMF. This product was not seen in photolysis experiments carried out in dichloromethane.

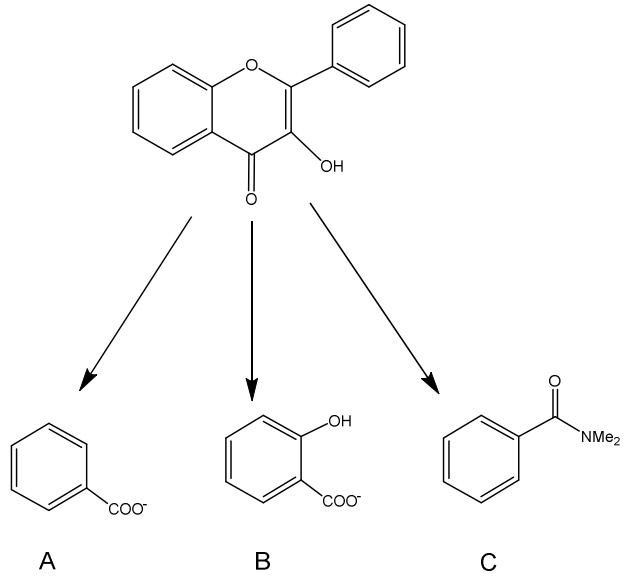


Figure 7. Degradation products observed for oxidation of 3-hydroxyflavone. Note compound C is only observed in degradation studies conducted in dimethylformamide.

4. Conclusion

The tridentate ligand, *tris*-1-ethyl-4-methylimidazolylphosphine supports copper flavonol complex formation and functions analogous to the binding site of 2,3-quercetin dioxygenase. The imidazole ligation is a more accurate structural model for the 3 histidine residues found in the protein. These complexes are also models for examining the reactivity of flavonol bound copper complexes. Ring-opening reactions occur via photolysis at 300 nm or highly elevated temperatures. The products observed are relevant degradation products to that of the metalloenzyme and evidence indicates that these degradation reactions are initiated by a charge transfer process involving the copper atom. Further work is being carried out on the mechanism of the degradation and dissociation processes, the intermediates formed in the reactions as well as electronic and substituent group effects on both the flavonol and trisimidazolylphosphine ligand.

Acknowledgements

Financial Support from the American Chemical Society Petroleum Research Fund (30550-GB3 to W. E. L.) and NSF Improvement in Laboratory Instruction grants (DUE 9451465) for the Gas Chromatograph Mass Spectrometer and (DUE 975103) for the Gas Chromatograph.

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Highlights:

- Structural characterization of a copper(II) tris-imidazolylphosphine ligand/flavonol complex
- New synthetic enzyme-substrate model of flavonol dioxygenase
- Photo and thermal degradation of Flavanol
- Structural characterization of *tris*-1-ethyl-4-methylimidazolylphosphine
- Luminescence assay of a copper(II) tris-imidazolylphosphine ligand/flavonol complex