Manganese and iron flavonolates as flavonol 2,4-dioxygenase mimics†

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Mononuclear manganese(II) and iron(III) flavonolates were synthesized as synthetic enzyme-substrate complexes, and their oxygenation reactions as biomimetic functional models with relevance to flavonol 2,4-dioxygenases are briefly described.

Flavonols (flaH) are widely distributed in vascular plants,[1] and flavonoids form active constituents of a number of herbal and traditional medicines.[2] Flavonol 2,4-dioxygenase (FDO), which catalyses the oxidative degradation of flavonols to a depside (phenolic carboxylic acid esters) with concomitant evolution of carbon monoxide, was first recognized more than four decades ago in species of *Aspergillus* grown on rutin, and quercetinases from *Aspergillus flavus*,[3] *Aspergillus niger*,[4] and *Aspergillus japonicus*[5] have been characterized, and the crystal structure of quercetinase from *Aspergillus japonicus* has been reported [eqn. (1)]. [5]

(1)

The diffraction studies showed that the enzyme forms homodimers, and each unit is mononuclear, with a type 2 copper center. With the availability of the sequence and structural parameters for *Aspergillus japonicus* quercetinase, homologous enzymes were sought from other species. A BLAST search conducted against the sequence of *Aspergillus japonicus* identified the *YxaG* protein from *Bacillus subtilis*, as the protein with the highest degree of similarity.[6] Recent studies has been described the protein *YxaG* as an iron-containing flavonol 2,4-dioxygenase,[7] which can function with a number of different divalent metals such as copper, cobalt and manganese, although manganese(II) appeared to be the preferred cofactor for this enzyme.[8] Since flavonol 2,4-dioxygenases are metal-containing enzymes, metal complexes of copper have been used in model reactions.[9] Autooxidation reactions of potassium and zinc flavonolates have also resulted in enzyme-like products, and some efforts have been made to elucidate the mechanism of the reaction.[10] Since there are no manganese- or iron-containing model systems in the literature, in this paper we report details for the synthesis and characterization of $Mn^{II}(fla)_2(py)_2$ and $Fe^{III}(4'OMefla)_3$ complexes in order to obtain information of the possible binding of the substrate to the metal ion and to use them in oxygenation reactions to study the oxidative ring splitting of the heterocyclic ring in the substrate compared to the copper-containing models.

Because of the oxidation state of manganese in the manganese-containing flavonol 2,4-dioxygenase is believed to be two, the preparation and characterization of a manganese(II) flavonolate seemed to be of great interest. Complex $Mn^{II}(fla)_2(py)_2$ (**1**) was isolated as a yellow solid in ~80% yield by the reaction of $Mn(ClO_4)_2 \cdot 6H_2O$, flavonol, and pyridine (py) in the presence of triethylamine at room temperature in methanol under argon. Compound **1** shows a characteristic π–π* transition in the visible region at 432 nm due to the coordinated flavonolate ligand.[11] A strong IR band at 1542 cm^{-1} assigned to ν(CO), showing a decrease of 60 cm^{-1} [ν(CO) = 1602 cm^{-1}] compared to flavonol, arise as a result of the formation of a five-membered ring.[12] A strong band around 1600 cm^{-1} and a weak band at 1003 cm^{-1} are seen due to the coordinated pyridines [ν(C=N)]. The molecular structure and atom numbering scheme is depicted in Fig. 1.‡ The manganese ion, which lies on an inversion center, has a slightly distorted tetragonal-bipyramidal geometry, which possesses high symmetry with *trans* coordination of the flavonolate ligands in the basal plane and the two pyridines in apical positions.

The manganese–oxygen bond distances are in the range of 2.127–2.184 Å, somewhat longer than those in $Cu^{II}(fla)_2$ (1.901–1944 Å).[9] The O2–C1 distance is shorter while the O3–C9 distance is longer than those in the uncoordinated flavonol [1.357(3) and

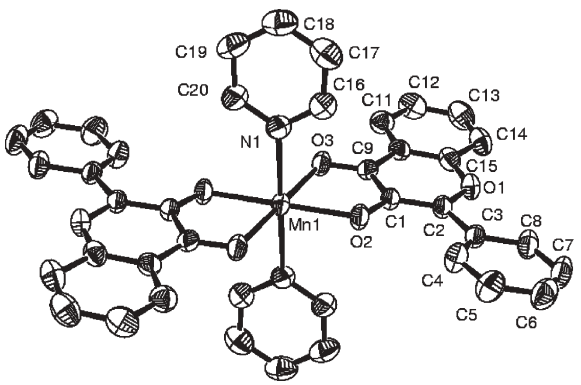

Fig. 1 The molecular structure of **1** with selected bond distances (Å) and angles (°): Mn1–O2 2.1274(16), Mn1–O3 2.1839(18), Mn1–N1 2.348 (2), O1–C15 1.351(3), O1–C2 1.374(3), O2–C1 1.302(3), O3–C9 1.257(3), C1–C9 1.460(3), C1–C2 1.385(3), C10–C15 1.393(3), C9–C10 1.437(4); O2–Mn1–O3 76.81(6), O2–Mn1–N1 90.99(7), N1–Mn1–N1* 180.0. *Symmetry code: (1 − x, 1 − y, 1 − z). Ellipsoids are shown at the 50% probability level.

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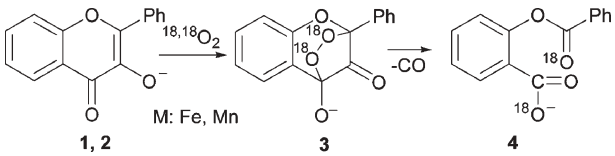
1.232(3) Å].[13] Due to coordination to the manganese ion there are also changes in the bond lengths of the pyranone ring. The O1–C2 [1.374(3) Å] and C10–C15 [1.393(3) Å] bond lengths become longer, and the C1–C9 bond length [1.460(3)] is somewhat shorter, which may be assigned to delocalization of the π-system over the whole molecule.

Tris(ethoxo)iron(III) reacted with 4′-methoxyflavonol (4′MeOflaH) in acetonitrile at room temperature to give pure, homoleptic tris(4′-methoxyflavonolato)iron(III) (**2**) in 85% yield. It shows absorption in the visible region at 411 nm, and *ν*(CO) absorption band at 1547 cm^{-1} in the IR spectrum assignable to the flavonolate ligand, A shoulder at 680 and a maximum at 530 nm characteristic of an octahedral arrangement around the ferric ion, can be assigned to the $^6A_{1g} \rightarrow {}^4T_{1g}$ and $^6A_{1g} \rightarrow {}^4T_{2g}$ transition, respectively. The crystal structure of **2**, shown in Fig. 2‡ together with selected data, shows a distorted octahedral geometry around the iron(III) center, with all coordination sites being occupied by the bidentate 4′-methoxyflavonolate ligands. The iron–oxygen bond distances are in the range of 1.955–2.109 Å, somewhat longer than those in $Cu^{II}(fla)_2$, but somewhat shorter than those in $Mn^{II}(fla)_2(py)_2$. Complex **2** crystallizes as two independent molecules with each Fe cation lying on a three-fold axis.

After the characterization of the prepared complexes, their flavonol 2,4-dioxygenase activity was examined. The manganese and iron flavonolate complexes **1** and **2**, in DMF solutions are stable under anaerobic conditions and oxidized upon addition of dioxygen at 95 °C. The CO content was determined in both cases by GC-MS (80–90%). The formation of *O*-benzoylsalicylic acid from flavonol requires dioxygen but no apparent dioxygen uptake was observed since the absorption of dioxygen and the liberation of carbon monoxide compensate each other. The GLC-MS analysis of the residue of the hydrolyzed complexes, after treatment with ethereal diazomethane, showed the presence of the *O*-benzoylsalicylic acid methylester. Provided that a mixture of $^{18,18}O_2$ and $^{16,16}O_2$ is used in the oxygenation, the labelling of the product permits identification of the place of the dioxygen incorporation. As a result of oxygenations, carried out under an atmosphere containing ~60% $^{18,18}O_2$, the ^{18}O-benzoylsalicylic acid derivative gave a molecular ion at *m/z* 260 (256 + 4), showing that both ^{18}O atoms of $^{18,18}O_2$ are incorporated into the carboxylic acid from the molecular oxygen, and the gas phases showed only the presence of unlabeled CO [eqn. (2)]. The relative abundances of *m/z* 260 to that at *m/z* 256 parallel the $^{18,18}O_2$ enrichments used in the experiments.



(2)

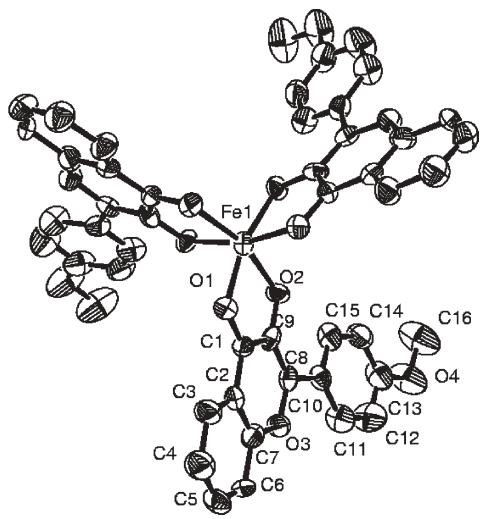


Fig. 2 The molecular structure of one of the two independent co-crystallized molecules of **2** with selected bond distances (Å) and angles (°): Fe1–O1 2.109(8), Fe1–O2 1.955(7), O1–C1 1.306(12), O2–C9 1.299(13), O3–C7 1.302(13), O3–C8 1.373(13), C1–C9 1.367(14), C1–C2 1.490(15), C2–C7 1.454(16), C8–C9 1.404(15); O2–Fe1–O1 80.0(3), O1–C1–C9 120.1(9). Ellipsoids are shown at the 50% probability level.

Table 1 Kinetic data for the oxygenation of metal flavonolates

	k^a/M^{-1} s^{-1}	$\Delta H^{\#}$/ kJ mol^{-1}	$\Delta S^{\#}$/ J mol^{-1} K^{-1}	
2	0.08	49	−137	This work
1	0.50	40	−144	This work
$Cu^{II}(fla)_2$	0.0087	53	−138	9
$Cu^{II}(fla)_2(py)_n$	0.04	—	—	—

[a] In DMF at 100 °C.

Reactions of **1** and **2** with dioxygen were performed in DMF solutions at 85–120 °C, and the concentration change of **1** and **2** was followed by electronic spectroscopy measuring the absorbance of the reaction mixture at 432 and 411 nm. Kinetic studies on the oxygenation of the manganese and iron flavonolate complexes established second-order overall rate expressions $-d[\mathbf{1} \text{ or } \mathbf{2}]/dt = [\mathbf{1} \text{ or } \mathbf{2}][O_2]$, and both reactions were entropy driven (Table 1) indicating that the rate-determining step is bimolecular.

As a conclusion it can be said that in the enzyme-like oxygenation of the coordinated flavonolate ligand of manganese(II) and iron(III) (**1**, **2**), the formation of endoperoxide (**3**) in bimolecular reactions can be assumed, and the unique decomposition of this species accompanied by loss of carbon monoxide results in the corresponding *O*-benzoylsalicylate complexes (**4**) as a good mimic of the enzyme action. On the basis of the *k* values (compared to our earlier copper-containing systems), it can be said that the reactivity order is Fe > Mn > Cu. Work is still in progress on model studies to disclose more details of this cleavage reaction.

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Notes and references

‡ Intensity data were measured on a Bruker-Nonius Kappa CCD single-crystal diffractometer, using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) and ϕ scan technique at 293 K. The structures were solved by direct and difmap methods (SIR92),[14] and refined on F^2 by using full-matrix least-squares methods.[15]

Crystal data: Compound **1**: $C_{40}H_{28}MnN_2O_6$, M_w = 687.58, triclinic, space group $P\bar{1}$, a = 8.310(6), b = 10.095(6), c = 11.167(5) Å, α = 113.361(4), β = 97.542(4), γ = 106.255(4)°, V = 793.82(8) $Å^3$, Z = 1, D_c = 1.438 g cm^{-3}, μ(Mo-Kα) = 4.70 cm^{-1}, 2882 reflections measured, 223 parameters refined on F^2 using 2667 unique reflections to final indices R [$F^2 > 2\sigma(F^2)$] = 0.0444, wR = 0.1354, $w = 1/[\sigma^2(F_o^2) + (0.0912P)^2 + 0.3239P]$, $P = (F_o^2 + 2F_c^2)/3$.

Compound **2**: $C_{48}H_{33}FeO_{12}$, M_w = 857.59, trigonal, space group $P31c$, a = 15.488(5), b = 15.488(5), c = 19.997(5) Å, γ = 120(4)°, V = 4154.2(2) $Å^3$,

Z = 4, D_c = 1.371 g cm^{-3}, μ(Mo-Kα) = 4.29 cm^{-1}, 2081 reflections measured, 367 parameters refined on F^2 using 1820 unique reflections to final indices R [$F^2 > 2\sigma(F^2)$] = 0.0725, wR = 0.1901, $w = 1/[\sigma^2(F_o^2) + (0.1136P)^2 + 9.3277P]$, $P = (F_o^2 + 2F_c^2)/3$.

CCDC 651207 (**1**) and 651208 (**2**). For crystallographic data in CIF or other electronic format see DOI: 10.1039/b711864c

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