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Complexation and proton transfer by hydroxamic acids in model inhibited metallohydrolases: formation of metal hydroxamate trimers ☆

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Abstract

Reaction between the dimeric metal complexes, $[M_2(\mu\text{-}OAc_F)_2(OAc_F)_2(\mu\text{-}H_2O)(tmen)_2]$, M = Co(II), Ni(II), and aceto- and benzohydroxamic acids (AHA, BHA) gives the novel trimeric complexes, $[M_3(\mu\text{-}OAc_F)_4(\mu\text{-}RA)_2(tmen)_2]$, M = Co(II), Ni(II); RA = AA, BA, in which each hydroxamate bridges two metal centres via its deprotonated hydroxyl and the carbonyl oxygen bonds one metal centre only together with the doubly protonated salt $[(tmen)\cdot 2H][OAc_F]_2$. In contrast, self-assembly from $M(OAc_F)_2\cdot 4H_2O$, tmen and RHA gives the dibridged hydroxamate dimers $[M_2(\mu\text{-}OAc_F)(\mu\text{-}RA)_2(tmen)_2]$ $[OAc_F]$.

Keywords: Crystal structures; Trinuclear cobalt; Nickel hydroxamates; Model inhibited complexes

Dinuclear metal hydrolases which catalyse the hydrolysis of a range of peptide and phosphate ester bonds contain a dinuclear metal site featuring Zn(II), Ni(II), Co(II) and Mn(II) with carboxylate bridges [1]. Hydroxamic acids are ubiquitous bioligands and, through chelation of the zinc centre in the matrix metalloproteinases (MMP) by the hydroxamate anion, form the basis of a wide range of therapeutic inhibitors [2]. In contrast to these chelates, the acetohydroxamate-inhibited C319A variant of *Klebsiella aerogenes* urease (KAU) contains a novel μ^2–η^1:η^2 hydroxamate with the deprotonated hydroxyl oxygen of the hydroxamic acid bridging the two nickel atoms in the active site and the carbonyl oxygen bonding one nickel atom only [3].

Inhibition of enzymes in biological systems by hydroxamic acids is generally assumed to involve initial attack by a neutral hydroxamic acid molecule [4] but it is not clear where the proton bonds on formation of the chelates [2,3]. One suggestion based on the calculation of proton dissociation energies is that amino acid side chains in proteins might serve as proton acceptors [4].

In our previous model studies [5] reaction of the dimeric nickel complex $[Ni_2(\mu\text{-}OAc)_2(OAc)_2(\mu\text{-}H_2O)(tmen)_2]$ with a neutral hydroxamic acid (RHA = MeHA, PhHA) gave attack by RHA at the central water-dicarboxylate bridge with proton transfer to the η^1 acetates and formation of a dibridged hydroxamate complex $[Ni_2(\mu\text{-}OAc)(\mu\text{-}RA)_2(tmen)_2][OAc]\cdot AcOH$, with a very similar structure to that of the C319A

Abbreviations: OAc, CH_3COO^-; OAc_F, CF_3COO^-; tmen, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; AHA, acetohydroxamic acid; AA, acetohydroxamate anion; BHA, benzohydroxamic acid; BA, benzohydroxamate anion.

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variant of KAU(3). The formation of these bridged/chelated hydroxamate model inhibited complexes is a general reaction and occurs also for the imidazole series in which each tmen capping ligand is replaced by two imidazoles and the pivalate series in which the bridging acetates are replaced by bridging pivalates [6]. However, in contrast to the acetate and pivalate series, the present note reports different reactions of the analogous trifluoroacetate bridged dimers $[M_2(\mu\text{-}OAc_F)_2(OAc_F)_2(\mu\text{-}H_2O)(tmen)_2]$, M = Co(II), Ni(II), with aceto- and benzohydroxamic acids (AHA, BHA) which give proton transfer to the tmen nitrogen atoms and formation of the doubly protonated salt, $[(tmen)\cdot 2H][OAc_F]_2$ (**I**) with H-bonding between the protons and the trifluoroacetates as shown in Fig. 1 together with the novel trimeric complexes $[M_3(\mu\text{-}OAc_F)_4(\mu\text{-}RA)_2(tmen)_2]$ (**II**), (M = Co(II), Ni(II); RA = AA, BA) in which the central metal atom is linked to the outer metal atoms by hydroxamates in the bridging/chelating bonding mode and bridging trifluoroacetates with the outer metal atoms retaining their capping tmen ligands (Scheme 1, path c). The structure of the cobalt-acetohydroxamate trimer is shown in Fig. 2 and similar structures occur in the other three trimers (M = Co(II), RA = BA; M = Ni(II), RA = AA, BA).

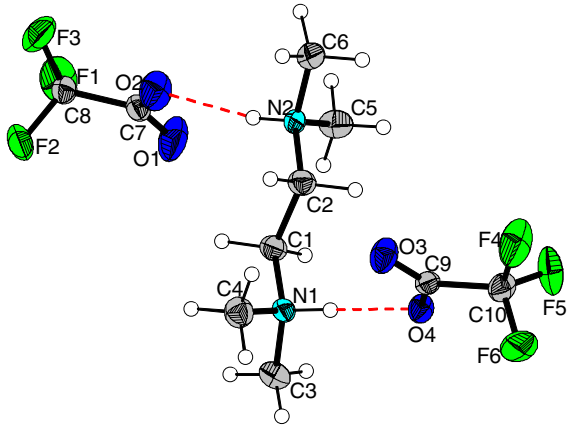
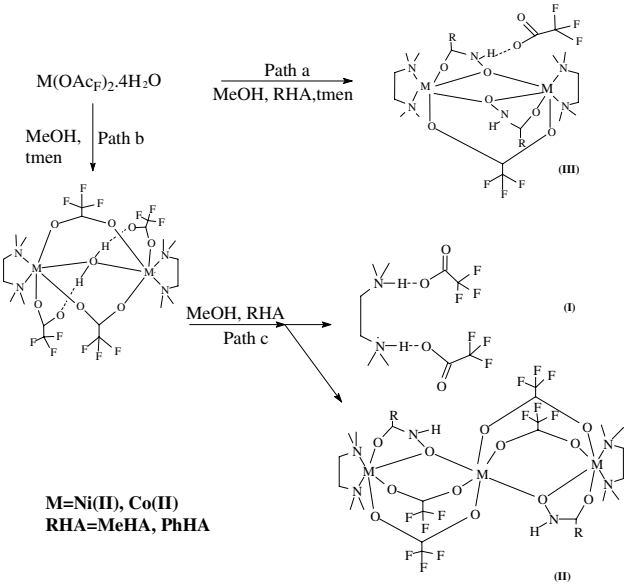


Fig. 1. The molecular structure of $[(tmen)\cdot 2H][OAc_F]_2$, (**I**). Selected bond lengths (Å) and angles (°): N(1)–C(4), 1.474(4); N(1)–C(3), 1.484(4); N(1)–C(1), 1.497(3); N(1)–H(1)···O(4), 2.708(3); C(1)–C(2), 1.501(4), C(9)–O(4), 1.248(3); C(9)–O(3), 1.212(3), C(4)–N(1)–C(3), 110.2(2); C(4)–N(1)–C(1), 115.7(2); O(3)–C(9)–O(4), 129.1(2).



Scheme 1.

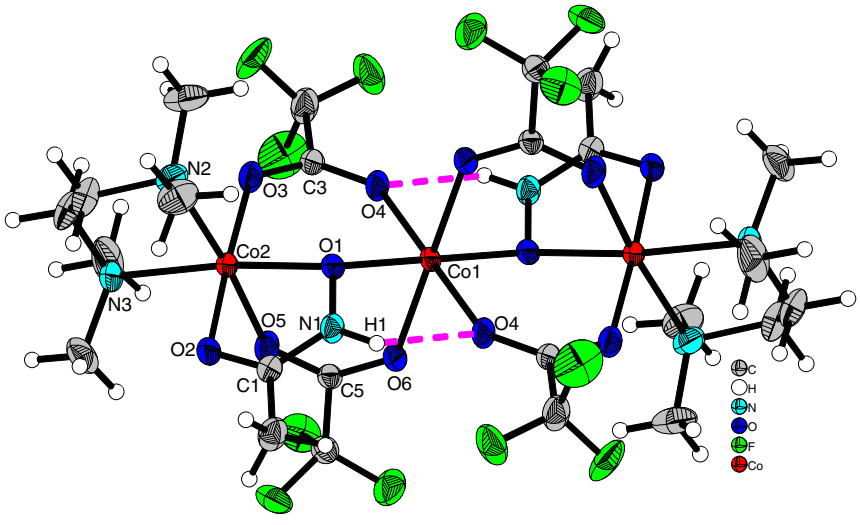


Fig. 2. The molecular structure of $[Co_3(\mu\text{-}OAc_F)_4(\mu\text{-}AA)_2(tmen)_2]$, (**II**). Selected bond lengths (Å) and angles (°): Co(1)–Co(2), 3.550(5); Co(1)–O(1), 2.0481(13); Co(2)–O(1), 2.0655(12); Co(2)–O(5), 2.1121(15); Co(1)–O(6), 2.0956(13); Co(1)–O(4), 2.1605(14); Co(2)–O(3), 2.0795(15); Co(2)–N(3), 2.1796(17); Co(2)–N(2), 2.205(2); N(1)–H(1)···O(4)#1, 2.690(2); Co(1)–O(1)–Co(2), 119.31(6); O(1)#1–Co(1)–O(1), 180.00; O(1)–Co(2)–O(5), 89.83(5); O(1)–Co(1)–O(6), 89.03(5).

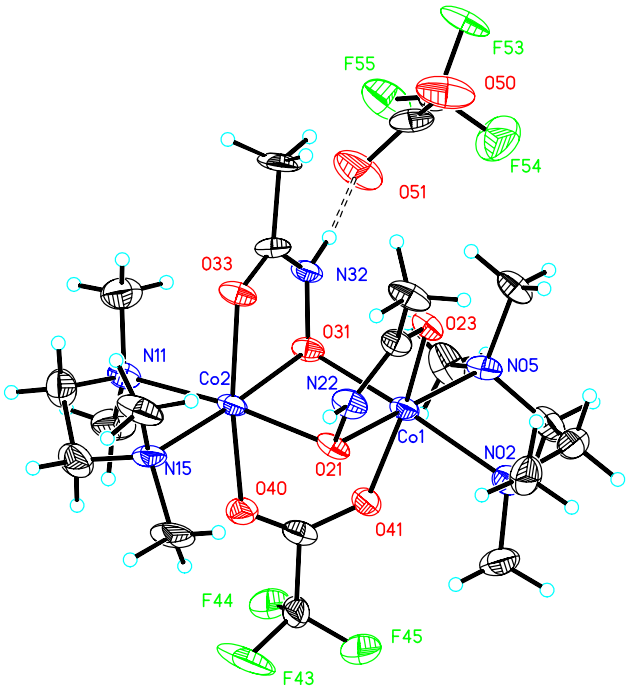


Fig. 3. The molecular structure of $[Co_2(\mu\text{-}OAc_F)(\mu\text{-}AA)_2(tmen)_2][OAc_F]$, (**III**). Selected bond lengths (Å) and angles (°): Co1–Co2, 3.0280(0.0015); Co1–O21, 2.080(5); Co1–O31, 2.102(5); Co1–O23, 2.081(5); Co1–O41, 2.110(5); Co2–O40, 2.121(5); Co2–N15, 2.175(6); Co2–N11, 2.236(7); N(32)–H(32A)···O(51), 2.843(9); O21–Co1–O31, 87.2(2); O23–Co1–O31, 94.33(19); Co1–O21–Co2, 91.74(19); N05–Co1–N02, 82.1(2).

In contrast, self-assembly from $M(OAc_F)_2 \cdot 4H_2O$ (M = Co(II), Ni(II)), tmen and RHA (RHA = AHA, BHA) gives the dibridged hydroxamate dimers $[M_2(\mu\text{-}OAc_F)(\mu\text{-}RA)_2(tmen)_2][OAc_F]$ with very similar structures to those reported previously for the acetate and pivalate series [5,6] (Scheme 1, path a). The structure of the cobalt-acetohydroxamate dimer (**III**) is shown in Fig. 3 with similar structures for the other three dimers.

Experimental details for the preparation of **I**, **II** and **III** are given in the supporting information.

Crystallographic data for **I**, **II** and **III** are given in Table 1 and selected bond angles and distances in Figs. 1–3 respectively. Crystal data for **I** and **II** were collected using a Bruker SMART APEX CCD area detector diffractometer and for **III** using a Siemens SMART CCD area-detector diffractometer. A full sphere of the reciprocal space was scanned by phi–omega scans for **I** and **II** and a full hemisphere for **III**. Numerical absorption correction was performed by the program SADABS [7]. The structures were solved by direct methods using SHELXTL-PC [8] and refined by full matrix least-squares on F^2 for all data using SHELXL-97 [9]. For **I** and **II**, hydrogens attached to disordered carbons were added at calculated positions and refined using a riding model. Their isotropic temperature factors were fixed to 1.2 (1.5 for methyl hydrogens) times the equivalent isotropic displacement parameter of the carbon atom the H-atom is attached to. All other hy-

Table 1
Crystal data and structure refinement for complexes **I**, **II**, **III**

Parameters	I	II	III
Formula	$C_{10}H_{18}F_6N_2O_4$	$C_{24}H_{40}Co_3F_{12}N_6O_{12}$	$C_{20}H_{40}Co_2F_6N_6O_8$
Molecular weight	344.26	1009.41	724.44
Crystal system	Monoclinic	Triclinic	Orthorhombic
Space group	$P2_1/n$	$P\bar{1}$	$P2_12_12_1$
a/Å	9.3892(11)	9.0953(13)	13.269(2)
b/Å	15.8868(18)	9.8345(14)	15.244(2)
c/Å	10.5003(12)	13.0368(18)	15.260(2)
α/°	90	71.571(2)	90
β/°	102.080(2)	75.979(2)	90
γ/°	90	69.309(2)	90
V/Å^3	1531.6(3)	1023.7(3)	3086.7(8)
T/K	293(2)	293(2)	180(2)
Z	4	1	4
θ/Å	0.71073	0.71073	0.71073
$\rho_{(calc)}$/mg m^{-3}	1.493	1.637	1.559
Crystal size (mm^3)	0.70 × 0.40 × 0.16	0.63 × 0.60 × 0.15	0.38 × 0.08 × 0.08
μ/mm^{-1}	0.158	1.315	1.161
hkl Ranges	−11,11; −18,18; −12,12	−11,12; −12,12; −17,16	−15,15; −18,11; −18,18
Number of reflections collected	21373	8800	15961
Independent reflections	2703 (R_{int} = 0.0267)	4604 (R_{int} = 0.0160)	5421 (R_{int} = 0.1148)
Max. and min. transmission	0.9752 and 0.8975	0.8272 and 0.4914	0.93 and 0.64
$R(F)[I > 2\sigma(I)]$/%	6.63	3.18	6.34
$R_w(F^2)$ (all data)/%	7.65	3.77	12.88
Goodness-of-fit on F^2	1.062	1.037	0.973
Largest peak and hole/e Å^{-3}	0.655, −0.294	0.281 and −0.331	0.613 and −0.812
M–M distance (Å)		3.550(5)	3.0280(0.0015)

$[tmen \cdot 2H][OAc_F]_2$, **I**; $[Co_3(AA)_2(OAc_F)_4(tmen)_2]$, **II**; $[Co_2(AA)_2(OAc_F)(tmen)_2][\ OAc_F]$, **III**.

drogens were located in the difference fourier map and allowed to refine freely including isotropic temperature factors. In **III**, all hydrogens were treated as the hydrogens attached to disordered carbons in **I** and **II**. Anisotropic temperature factors were used for all non-hydrogen atoms.

A comparison of the structures of the trimeric and dimeric cobalt hydroxamates **II** and **III** (Figs. 2 and 3, respectively) shows the presence of bridging/chelating hydroxamates in both cases with two bridges in the case of the dimer **III** and single hydroxamate bridges between each pair of cobalt atoms in **II** with the central cobalt occupying an inversion centre with $O(1)^{\#}$ 1–Co(1)–O(1) at 180.000°. The Co(1)–Co(2) distance of 3.028(2) in **III** is shorter than the corresponding distance Co(1)–Co(2) of 3.550(5) in **II**, due to the presence of a double hydroxamate bridge in **III** compared with one hydroxamate bridge for each pair of cobalt atoms in **II**. Small lengthenings are present in some comparable distances e.g. Co(1)–O(1) of 2.048(1) in **II** and Co(1)–O(21) of 2.080(5) in **III** reflecting the linear Co–O–Co moiety in **II** compared with the bent bridging Co–O bonds in **III**, where Co(1)–O(21)–Co(2) is 91.74 (19)°. In both complexes the cobalt atoms are in near octahedral environments e.g. O(21)–Co(1)–O(31) is 87.2(2)° in **III** compared with O(1)–Co(2)–O(5) is 89.83(5)° in **II**.

The different behaviour between the trifluoroacetate and acetate bridged dimers is a consequence of their different electronic properties. Thus OAc_F is a weaker donor then OAc and so the bridging water–carboxylate bonds are more easily broken by the neutral hydroxamic acid ligand for the former series, but the resulting trifluoroacetic acid thereby formed is more acidic than acetic acid and so protonates the tmen nitrogen atoms. This result suggests that in related enzymes, e.g. ureases, protonation may occur at the nitrogen atoms of aminoacid side chains in proteins as suggested previously [4].

Crystallographic data for the structures of **I**, **II** and **III** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC.

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References

[1] D.E. Wilcox, Chem. Rev. 96 (1996) 2435–2458.
[2] E.M.F. Muri, M.J. Nieto, R.D. Sindelar, J.S. Williamson, Curr. Med. Chem. 9 (2002) 1631–1653.
[3] (a) M.A. Pearson, L.O. Nickel, R.P. Hausinger, P.A. Karplus, Biochemistry 36 (1997) 8164–8172;
(b) S. Ciurli, S. Benini, W.R. Rypniewski, K.S. Wilson, S. Miletti, S. Mangani, Coord. Chem. Rev. 190-192 (1999) 331–355.
[4] J. El Yazal, Y.P. Pang, J. Phys. Chem. 104 (2000) 6499–6504.
[5] M. Arnold, D.A. Brown, O. Deeg, W. Errington, W. Haase, K. Herlihy, T.J. Kemp, H. Nimir, R. Werner, Inorg. Chem. 37 (1998) 2920–2925.
[6] D.A. Brown, W.K. Glass, N.J. Fitzpatrick, T.J. Kemp, W. Errington, G. Clarkson, W. Haase, F. Karsten, A. Mahdi, Inorg. Chim. Acta, in press; doi:10.1016/j.ica.2003.08.021.
[7] G.M. Sheldrick, SADABS, Empirical Absorption Corrections Program, University of Gottingen, Germany, 1996.
[8] Siemens SHELXTL-PC Version 5.0 Reference Manual, Siemens Industrial Automation, Inc., Analytical Instrumentation, Madison, WI, 1994.
[9] G.M. Sheldrick, SHELXL97 Program for Crystal Structure Refinement, University of Gottingen, Germany, 1997.