Novel elimination of hydroxylamine and formation of a nickel tetramer on reactions of glutarodihydroxamic acid with model dinickel hydrolases‡

David A. Brown,*[a] Laurence P. Cuffe,[a] Oliver Deeg,[b] William Errington,[c] Noel J. Fitzpatrick,[a] William K. Glass,[a] Kara Herlihy,[a]† Terence J. Kemp[c] and Hassan Nimir[a]

[a] *Department of Chemistry, University College, Belfield, Dublin 4, Ireland. E-mail: Noel.Fitzpatrick@ucd.ie*
[b] *Erasmus student, University of Wurzburg, Germany*
[c] *Department of Chemistry, University of Warwick, Coventry, UK CV4 7AL*

Received (in Cambridge, UK) 4th September 1998, Accepted 6th October 1998

Reactions of glutarodihydroxamic acid with the hydrolase enzyme urease models, $[Ni_2(\mu\text{-}H_2O)(OAc)_4(tmen)_2]$ and $[Ni_2(OAc)_3(urea)(tmen)_2][OTf]$, lead to novel hydroxylamine elimination and formation of $[Ni_2(OAc)_2\{\mu\text{-}O(N)(OC)_2(CH_2)_3\}(tmen)_2][OTf]$ and the tetramer $[Ni_4(OAc)_2(gluA_2)_2(tmen)_4][OTf]_2$, respectively, both of which are structurally characterised by X-ray crystallography.

Urease is a hydrolytic metalloenzyme[1] which catalyses the hydrolysis of urea and contains a dinickel active site with a Ni–Ni distance of 3.5 Å.[2] Hydroxamic acids inhibit a number of enzymes including urease.[3] The urea complex, $[Ni_2(OAc)_3(urea)(tmen)_2][OTf]$ **A**,[4] reacts rapidly with acetohydroxamic acid (AHA) to give the monobridged hydroxamate complex $[Ni_2(OAc)_2(AA)(urea)(tmen)_2][OTf]$[5] with a very similar structure to that of the acetohydroxamate inhibited C319A variant of *Klebsiella aerogenes* urease.[6] Similarly, reaction of the hydrolase model $[Ni_2(\mu\text{-}H_2O)(OAc)_4(tmen)_2]$ **B**[7] with AHA gives the dibridged complex $[Ni_2(OAc)(AA)_2(tmen)_2][OAc]$.[5] We now report the reactions of **A** and **B** with glutarodihydroxamic acid, $(CH_2)_3(CONHOH)_2$, $gluH_2A_2$, with novel results. **B** reacts rapidly with $gluH_2A_2$ at room temperature in both methanol and dichloromethane in the presence of the triflate ion to give $[Ni_2(OAc)_2\{\mu\text{-}O(N)(OC)_2(CH_2)_3\}(tmen)_2][OTf]$ **I**,§ which contains a deprotonated bridging *N*-hydroxyglutarimide with the deprotonated N–OH oxygen O6 bridging the two divalent nickel ions Ni1 and Ni2 and the two carbonyl oxygens O5 and O7, each coordinated to their respective nickel atoms Ni1 and Ni2 (Fig. 1). Formation of **I** involves the novel elimination of hydroxylamine. We suggest that the two nickel centres in **B** act as Lewis acids polarising both carbonyl groups of $gluH_2A_2$ (**C**, Scheme 1). Subsequent protonation of one nitrogen (**D**, Scheme 1) and attack by the other nucleophilic nitrogen occurs with loss of NH_2OH, ring closure and formation of the tetrahedral intermediate (**E**, Scheme 1) which on deprotonation forms **I**. In this mechanism the dinickel centre probably prearranges the electrophilic and nucleophilic reaction centres similar to the prearrangement of water and urea in urease with the base OH^- formed by deprotonation of a coordinated water molecule replaced by the nitrogen nucleophile. The dinickel centre is essential, since reaction of $gluH_2A_2$ with nickel acetate gives simply $Ni(gluA_2)$ with properties similar to those reported previously for analogous complexes.[8] In contrast, the longer chain dihydroxamic acids, $(CH_2)_n(CONHOH)_2$, n = 4 (adipodihdroxamic acid) and n = 8 (sebacodihydroxamic acid), reacted with **B** to give $[Ni_2(OAc)\{(CH_2)_n(CONHO)_2\}(tmen)_2][X]$, n = 4 **II** and n = 8 **III**, X = OTf or BF_4^-, with no loss of NH_2OH and retention of the characteristic ν(NH) infrared absorption at 3246 cm^{-1}.§ Unfortunately, crystals of **II** and **III** were not suitable for X-ray crystallography, but most likely **II** and **III** are the longer chain analogues of **IV** described below. Molecular modelling calculations, using SPARTAN PM3(tm), predict **I** to be a stable structure for glutardihydroxamic acid but not for the longer chain sebacodihydroxamic acid, confirming the importance of steric factors.

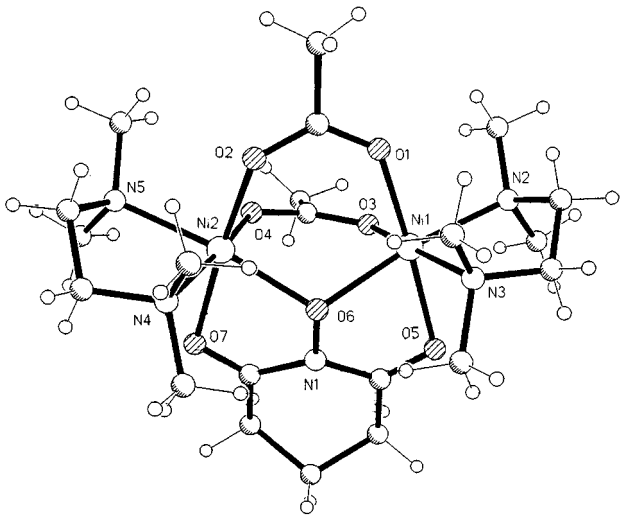
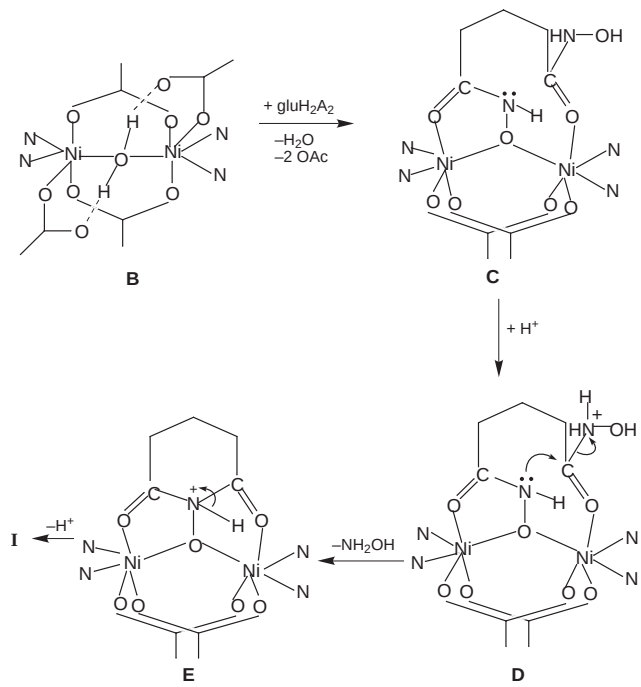


Fig. 1 Molecular structure of the cation of complex **I**. Selected bond distances (Å) and angles(°): Ni1–O6 2.059(2), Ni2–O6 2.040(3), Ni1–O5 2.155(3), Ni2–O7 2.128(3), Ni1–O1 2.019(3), Ni2–O2 2.007(3), O6–Ni1–O5 76.50(10), O7–Ni2–O6 77.97(10), Ni–Ni 3.414(1) and 3.427(1).



Scheme 1

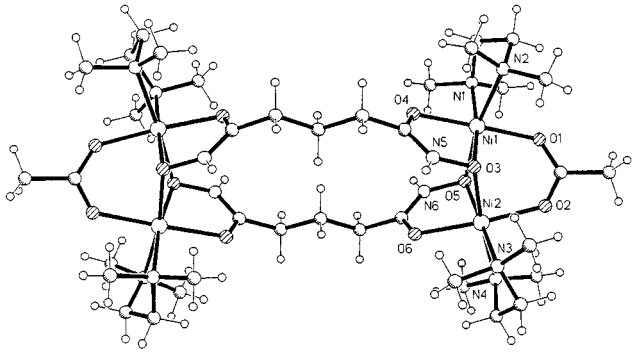


Fig. 2 Molecular structure of the cation of complex **IV**. Selected bond distances (Å) and angles (°): Ni1–O3 2.061(2), Ni2–O3 2.087(2), Ni1–O5 2.121(2), Ni2–O5 2.096(2) Ni1–O4 2.065(2), Ni2–O6 2.062(2), Ni1–Ni2 3.032(1), O4–Ni1–O3 80.27(6), O4–Ni1–O5 97.90(6), O5–Ni1–O3 84.62(6), O5–Ni2–O3 84.62(6).

In contrast to the above reaction of $gluH_2A_2$ with the hydrolase model **B**, reaction with the urease model **A** gives the tetrameric nickel hydroxamate complex **IV**§ $[Ni_4(OAc)_2(gluA_2)_2(tmen)_4][OTf]_2$ (Fig. 2) with accompanying loss of urea. The tetramer contains two sets of nickels atoms Ni1 and Ni2 and their symmetry equivalents, each set bridged by one hydroxamate group of one ligand and one hydroxamate group of the other ligand. As in the corresponding dibridged monohydroxamate (**II** in ref. 5), the deprotonated OH groups O3 and O5 bridge the two nickel centres Ni1 and Ni2, while the carbonyl oxygens O4 and O6 each coordinate to their respective nickel centres Ni1 and Ni2. The bond angles and distances in the dinickel hydroxamate bridges in **IV** and in **II** (ref. 5) are very similar. The structure is duplicated in the other part of the tetramer through the centre of inversion (Fig. 2) with opposite nickel centres, Ni1 and Ni2 being 9.742(1) Å apart. In **I** the Ni–O(bridging) distances Ni2–O6 and Ni1–O6 of 2.040(3) and 2.059(2) Å, respectively, are slightly shorter than the hydroxamate bridging Ni–O distances Ni1–O3 and Ni2–O3 of 2.061(2) and 2.087(2) Å respectively, in the tetramer **IV** (Fig. 2) which may be a factor promoting cyclisation in **I**.

Replacement of bridging water and carboxylates by the deprotonated OH group of the hydroxamic acid is probably part of the driving force of these facile reactions since the resulting bridging oxygen is a feature of all of the complexes which we have studied so far as well as in the dinickel complex containing two salicylhydroxamate bridges.[9] This structural feature also occurs in biological systems such as the acetohydroxamate complex with the C319A variant of urease[6] and the *p*-iodo-D-phenylalanine hydroxamate complex with *Aeromonas proteolytica* aminopeptidase(AAP).[10]

Finally, the displacement of coordinated urea from **A** by $gluH_2A_2$ but not by AHA suggests that inhibition of urease by dihydroxamic acids may also involve displacement of the urea substrate as well as the water molecules (which act as a base source) which is probably the mode of their inhibition by acetohydroxamic acid.

We thank Professor K. Nolan, Royal College of Surgeons in Ireland, for helpful discussions and the EUCOST D8 programme, Project D8/0010/97 for support.

Notes and references

† Present address: Department of Physics, Emory University, Atlanta, GA, USA

‡ Satisfactory microanalyses were obtained for compounds **I**–**IV**. *Preparations* of **I**–**III**: to a solution of **B** (1 mM) in CH_2Cl_2 under nitrogen was added 1 mM of triflate (OTf) (or tetrafluoroborate BF_4^-) and stirred for 1 h followed by 1 mM of $gluH_2A_2$ in methanol. The reaction was monitored by the appearance of IR peaks at 1710, 1750 and 1590 cm^{-1} due to acetic acid and co-ordinated hydroxamate respectively. After work-up, a solution in CH_2Cl_2 was layered with diethyl ether, pentane and 2,2-dimethoxypropane to give blue–green crystals of **I** suitable for X-ray crystallography, **II** and **III** were prepared similarly.

Preparation of **IV**: as for **I**–**III** above but replacing **B** by the urea complex **A** and omitting the addition of triflate ion. Suitable crystals were obtained in this case by vapour diffusion of diethyl ether into a solution of **IV** in methanol/CH_2Cl_2 (1 : 2).

Crystallography: Crystal data: for **I**: $C_{22}H_{44}F_3N_5Ni_2O_{10}S$, M = 745.10, triclinic, space group $P\bar{1}$, a = 10.7655(9), b = 15.7266(13), c = 20.8115(18) Å, α = 73.612(3)°, β = 84.827(3) °, γ = 81.397(3) ° U = 3338.0(5) Å^3 , Z = 4 , λ = 0.71073 Å, μ = 1.262 mm^{-1}. 11303 independent reflections were measured. Final $R1$ = 0.0435 and $wR2$ = 0.1158.

For **IV**: $C_{20}H_{45}F_3N_6Ni_2O_{10}S$, M = 736.10, monoclinic, space group $P2_1/c$, a = 12.4205(7), b = 12.6790(7), c = 20.8914(12) Å, β = 107.1940(10)°, U = 3142.9(3) Å^3 Z = 4 , λ = 0.71073 Å, μ = 1.340 mm^{-1}, 7412 independent reflections were measured. Final $R1$ = 0.0355 and $wR2$ = 0.0867.

Data were collected using a Siemens SMART CCD area-detector diffractometer. Refinement was by full-matrix least squares on F^2 for all data using SHELXL-97.[11] Hydrogen atoms were added at calculated positions and refined using a riding model.

§ Abbreviations: OTf = $CF_3SO_3^-$, OAc = $CH_3CO_2^-$, AHA = acetohydroxamic acid, AA = deprotonated acetohydroxamic acid, $gluH_2A_2$ = glutarodihydroxamic acid, $gluA_2$ = deprotonated glutarodihydroxamic acid.

1 N. Sträter, W. N. Lipscomb, T. Klabunde and B. Krebs, *Angew. Chem. Int. Ed. Engl.*, 1996, **35**, 2024.
2 E. Jabri, M. B. Carr, R. P. Hausinger and P. A. Karplus, *Science*,1995, **268**, 998.
3 H. Kehl, *Chemistry and Biology of Hydroxamic Acids*, ed. S. Karger, Basel, 1982.
4 H. E. Wages, K. L. Taft and S. J. Lippard, *Inorg. Chem.*, 1993, **32**, 4985.
5 M. Arnold, D. A. Brown, O. Deeg, W. Errington, W. Haase, K. Herlihy, T. J. Kemp, H. Nimir and R. Werner, *Inorg. Chem.*, 1998, **37**, 2920.
6 M. A. Pearson, L. O. Michel, R. P. Hausinger and P. A. Karplus, *Biochemistry*, 1997, **36**, 8164.
7 U. Turpeinen, R. Hämäläinen and J. Reedijk, *Polyhedron*, 1987, **6**, 1603.
8 D. A. Brown, N. Ni Choileain, R. Geraty, and J. D. Glennon, *Inorg. Chem.*, 1986, **25**, 3792.
9 A. J. Stemmler, J. W. Kampf, M. L. Kirk and V. L. Pecoraro, *J. Am. Chem. Soc.*, 1995, **117**, 6368.
10 B. Chevrier, H. D'orchymoant, C. Schalk, C. Tarnus and D. Moras, *Eur. J. Biochem.*, 1996, **237**, 393.
11 G. M. Sheldrick, *Shelxl 97, Program for the Refinement of Crystal Structures*, University of Göttingen, Germany, 1997.

Communication 8/06911E