Binding of Molybdenum–Iron–Sulfur Clusters by Amino Acid Esters

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Reaction of both the double-cubane and monocubane clusters $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]$ **1** and $[NEt_4]_3[MoFe_3S_4(SEt)_3\{Fe(cat)_3\}]$ **2** (H_2cat = catechol) with L-cysteine ethyl ester hydrochloride (HCys-OEt·HCl) gives the common double-cubane product $[NEt_4]_4[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]$ **3**. The cysteinate-bound cluster has been characterised by ^1H NMR, UV/VIS, IR and Mössbauer spectroscopies. The conversion of the ethanethiolate-bound cluster **1** into the cysteinate cluster **3** proceeds through the chloro-intermediate $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]^{3-}$ **4**. Reaction of cluster **1** or **2** with L-tyrosine methyl ester hydrochloride forms only the chloro-substituted cluster **3** or $[NEt_4]_3[MoFe_3S_4Cl_3\{Fe(cat)_3\}]$ **5**, respectively. The monocubane chloro-substituted cluster **5** has been isolated by this and other routes, and fully characterised. On reaction of chloro-substituted dicubane **4** with 6 equivalents of tetraethylammonium L-tyrosinate methyl ester [NEt$_4$][Tyr-OMe] the tyrosinate-bound cluster $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Tyr\text{-}OMe)_6]^{3-}$ **6** is formed. Proton NMR, UV/VIS, IR and Mössbauer parameters are reported.

Over the past fifteen years there has been much interest in the chemistry of iron–molybdenum–sulfur clusters,[1] particularly as possible structural models for the iron–molybdenum cofactor (FeMoco) of the enzyme nitrogenase. Our interest is in the binding of such clusters to proteins. Cysteinyl residues in proteins are the principal ligands to iron–sulfur clusters in biological systems. The residues which bind, for example, Fe_4S_4-ferredoxin cores to the protein backbone are cysteines generally in the sequence -CXXCXXC- (C = cysteine, X = a non-bonding amino acid). The fourth cysteinyl residue is situated some distance away.[2] The larger molybdenum iron protein (MoFe), which contains FeMoco, has more clusters than can be accommodated in the classic sense by cysteinyl residues alone, as judged from the amino acid sequence of nitrogenase proteins from various organisms.[3,4] More recently Kim and Rees[5] have proposed a structure for FeMoco, based on a fit to the electron density corresponding to FeMoco in a 2.8 Å resolution crystal structure of the MoFe protein of *Azotobacter vinelandii* and on a range of spectroscopic data. They identify two kinds of protein residues bound to FeMoco, cysteine and histidine.

As part of a study to investigate the interaction of amino acids, peptides and proteins with iron–sulfur clusters we have recently demonstrated that not only cysteine but also a range of other amino acids are capable of binding to the model cluster core $\{Fe_4S_4\}^{2+}$.[3,4] Here we extend our studies to molybdenum–iron–sulfur clusters. Preliminary results have shown[6] that, in acetonitrile solution, the double-cubane cluster $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]$ **1** (Fig. 1) and the monocubane $[NEt_4]_3[MoFe_3S_4(SEt)_3\{Fe(cat)_3\}]$ (H_2cat = catechol) **2** (Fig. 1) react with L-cysteine ethyl ester hydrochloride (HCys-OEt·HCl) to generate the double-cubane product $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]^{4-}$ **3**.† These studies are described in more detail below, together with the isolation and spectroscopic characterisation of **3** and the preparation of a tyrosinate analogue.

† In ref. 6 the oxidation state of cluster **3** was erroneously assigned as 3 –.

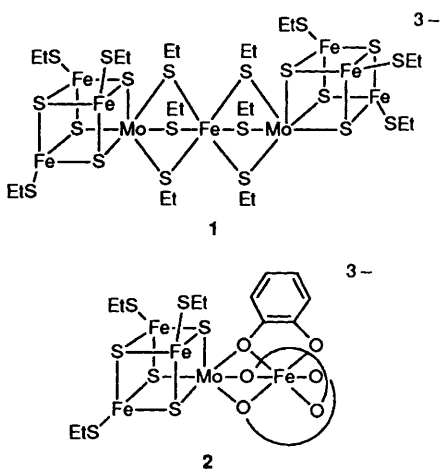

Fig. 1 Molecular structure of the double-cubane $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]^{3-}$ **1** and the monocubane $[MoFe_3S_4(SEt)_3\{Fe(cat)_3\}]^{3-}$ **2**

Results and Discussion

Chloro-derivatives.—The iron–sulfur cluster $[Fe_4S_4(SBu^t)_4]^{2-}$ has been shown[3,4] to react with a range of amino acid ester hydrochlorides. The initial reaction probably produces $[Fe_4S_4Cl_4]^{2-}$, which then reacts further to form the amino acid derivative $[Fe_4S_4(aa)_4]^{2-}$. The chloride cluster can be prepared independently by reaction of $[Fe_4S_4(SBu^t)_4]^{2-}$ with ethanoyl chloride[7] or with 2-aminobutane hydrochloride[6] [equation (1)], the liberated amine and thiol being removed from solution by pumping.

$$[Fe_4S_4(SBu^t)_4]^{2-} + 4CH_3CH(NH_2)C_2H_5\cdot HCl \longrightarrow [Fe_4S_4Cl_4]^{2-} + 4HSBu^t + 4CH_3CH(NH_2)C_2H_5 \quad (1)$$

We have extended our studies to the interaction of amino acid esters with $\{MoFe_3S_4\}$ mono- and double-cubane clusters. We first confirmed that 6 equivalents of ethanoyl chloride convert

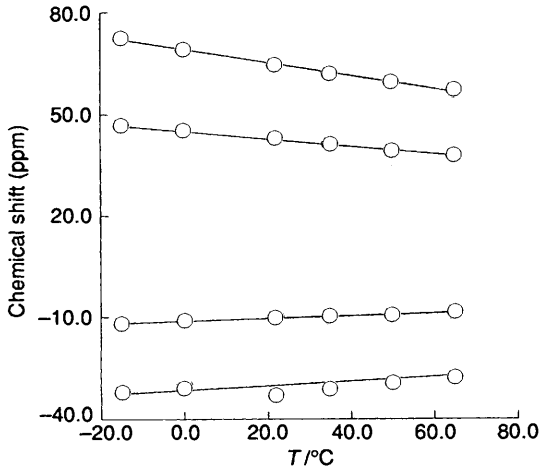

Fig. 2 Variation of chemical shifts with temperature of the catecholate protons in $[MoFe_3S_4Cl_3\{Fe(cat)_3\}]^{3-}$ **5**

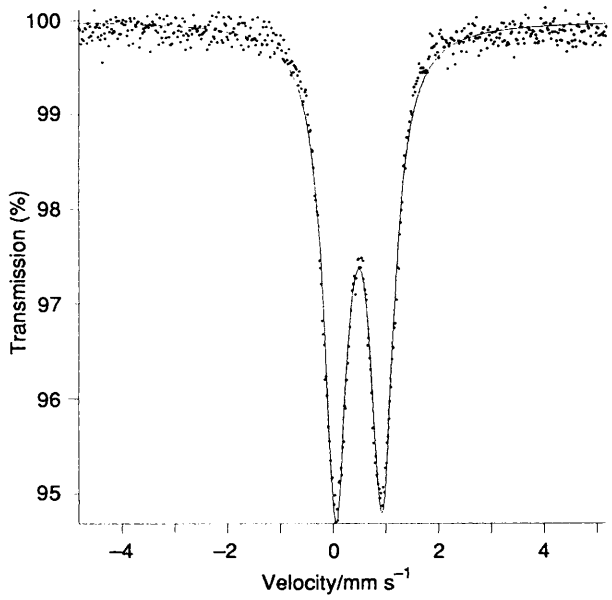

Fig. 3 Mössbauer spectrum of $[NEt_4]_3[MoFe_3S_4Cl_3\{Fe(cat)_3\}]$ **5**

the double-cubane cluster **1**, in acetonitrile, into the terminal chloride substituted cluster $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]$ **4**,[8,9] and have characterised this product by ^1H NMR, UV/VIS and Mössbauer spectroscopies. The same cluster can be prepared by the action of 6 molar equivalents of 2-aminobutane hydrochloride[6] or of L-tyrosine methyl ester hydrochloride (see below). The terminally co-ordinated ethanethiolate groups are labile.

Reaction of monocubane **2** with 3 molar equivalents of trimethylethanoyl chloride, 2-aminobutane hydrochloride or L-tyrosine methyl ester hydrochloride gives the chloro-cluster $[NEt_4]_3[MoFe_3S_4Cl_3\{Fe(cat)_3\}]$ **5**. Crystals suitable for X-ray crystallographic studies were not grown, but the material was unequivocally characterised by spectroscopy and elemental analysis.

The ^1H NMR spectrum of cluster **5** in deuterioacetonitrile at 295 K shows no signals assignable to co-ordinated ethanethiolate. The catecholate proton signals are strongly isotropically shifted (−57.4, −36.2, +16.8, +39.8 ppm).* The sense of the shifts is the same as, and the magnitudes are slightly larger than, those of the analogue **2** with terminal ethanethiolates (−54.2, −33.0, +14.8, +37.3 ppm).[10] Individual proton assignments could not be made. Variable-temperature ^1H NMR spectroscopy shows that all the isotropically shifted catecholate signals move toward the diamagnetic on increasing the temperature (Fig. 2). The shift mechanism cannot be defined from this observation alone.

* Isotropic shifts are calculated relative to the diamagnetic ligand, $(\Delta H/H_0)_{iso} = (\Delta H/H_0)_{dia} - (\Delta H/H_0)_{obs}$.

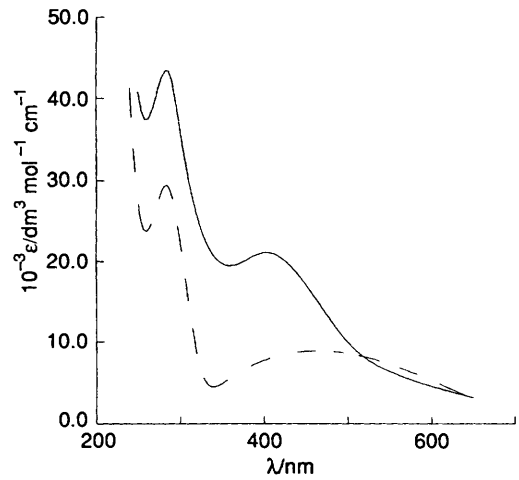

Fig. 4 Electronic absorption spectra of $[NEt_4]_3[MoFe_3S_4(SEt)_3\{Fe(cat)_3\}]$ **2**[10] (——) and $[NEt_4]_3[MoFe_3S_4Cl_3\{Fe(cat)_3\}]$ **5** (— —) in acetonitrile solution

The Mössbauer spectrum of cluster **5** at 77 K exhibits a single symmetric quadrupole doublet with isomer shift (i.s.) 0.49(1), quadrupole splitting (q.s.) 0.89(1) and half width at half maximum (h.w.h.m.) 0.29(1) mm s^{-1}. (Fig. 3). The i.s. is larger than that found[10] for the ethanethiolate analogue **2** (i.s. = 0.43, q.s. = 0.75 mm s^{-1}). A similar difference in i.s. is observed, for example, on substituting ethanethiolate in $[PPh_4]_2[Fe_4S_4(SEt)_4]$ (i.s. = 0.43 mm s^{-1})[11] by chloride to give $[PPh_4]_2[Fe_4S_4Cl_4]$ (i.s. = 0.49 mm s^{-1}).[12] The signal corresponding to the iron of the tris(catecholato)ferrate(III) fragment is obscured by the main doublet. At 77 K $[NEt_4]_3[Fe(cat)_3]$ gives a signal (i.s. = 0.60, q.s. = 0, h.w.h.m. = 1.02 mm s^{-1}),[13] broadened by relaxation effects.

The electronic absorption spectrum of cluster **5** (Fig. 4) shows a bathochromic shift of *ca.* 60 nm of the higher-wavelength maxima compared to the ethanethiolate analogue. A much reduced visible absorption intensity is observed. A similar, though smaller, shift and a decrease in intensity is found when $[Fe_4S_4Cl_4]^{2-}$ (ref. 7) is compared to $[Fe_4S_4(SEt)_4]^{2-}$.[14]

Cysteinate-derivatives.—Addition of 6 molar equivalents of L-cysteine ethyl ester hydrochloride to cluster **1** in deuterioacetonitrile at 295 K initially generates the hexachloro-substituted cluster **4**, as shown by ^1H NMR spectroscopy (Fig. 5). During 3 h the isotropically shifted signals characteristic of the chloro-substituted cluster are lost and signals generated by a new species appear. In a preliminary communication[6] these signals were assigned to the cysteinate-substituted cluster $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]^{3-}$. Now, in the light of further spectroscopic evidence (see below) we reinterpret the assignment and identify the product as $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]^{4-}$ **3** in which the bridging iron is iron-(II) rather than -(III). The signals at δ 81.8 and −20.0 are typical of bridging thiolate methylene and methyl groups respectively. These shifts are similar to those found for a range of related double-cubane clusters in the 4− oxidation level (Table 1). No terminal thiolate signals remain, but the new signals at δ 27.1 and 13.5 are assigned to the β-CH_2 and α-CH protons of terminal cysteinate ethyl ester. The downfield shifts of the β-CH_2 and α-CH are larger than those in $[Fe_4S_4(Cys\text{-}OEt\cdot HCl)_4]^{2-}$.[4] Addition of 9 or 12 molar equivalents of the ester to **1** resulted in the same reactions except that they were

Table 1 Proton NMR chemical shifts (δ) for bridging and terminal groups in $MoFe_3S_4$ double-cubane complexes in CD_3CN solution at ambient temperature[a]

Complex	Bridging		Terminal	
	CH_2	CH_3	CH_2	CH_3
1 $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]^{3-}$	24.4, 17.5	0.81	55.7	4.5
4 $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]^{3-}$[b]	38.2, 26.7	−1.20	—	—
$[W_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]^{4-}$	84.5	−21.4	57.4	9.99
$[Mo_2Fe_7S_8(SCH_2Ph)_{12}]^{4-}$	84.6	—	62.5	—
$[W_2Fe_7S_8(SCH_2Ph)_{12}]^{4-}$	81.8	—	58.8	—
3 $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]^{4-}$[c]	81.8	−20	27.1[d]	13.5[e]
6 $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Tyr\text{-}OMe)_6]^{3-}$[c]	32.4, 27.8	−0.06	48.6[d]	13[e,f]

[a] Data from ref. 8 unless otherwise stated. [b] Ref. 9. [c] This work. [d] β-CH_2. [e] α-CH. [f] Broad, poorly resolved.

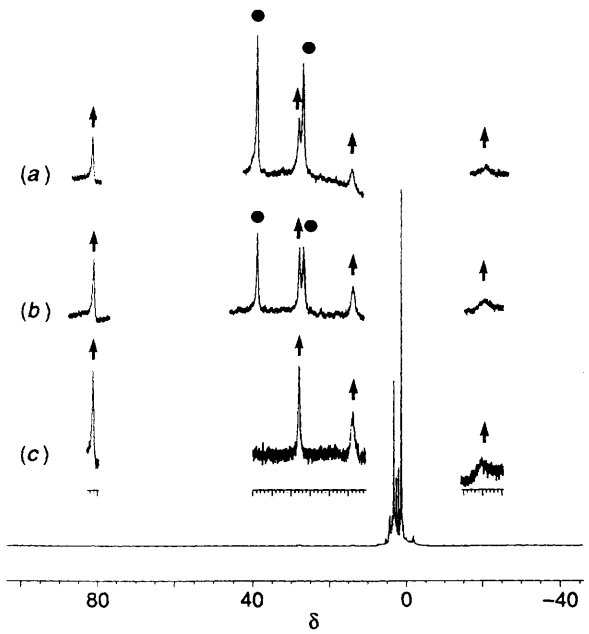

Fig. 5 Proton NMR spectra of the reaction of $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]^{3-}$ with 6 molar equivalents of HCys-OEt·HCl over a period of time (2×10^{-2} mol dm^{-3} cluster in CD_3CN): (*a*) 30, (*b*) 80, and (*c*) 240 min. Peaks: ●, $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]^{3-}$ **4**; ⬆, $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]^{4-}$ **3**

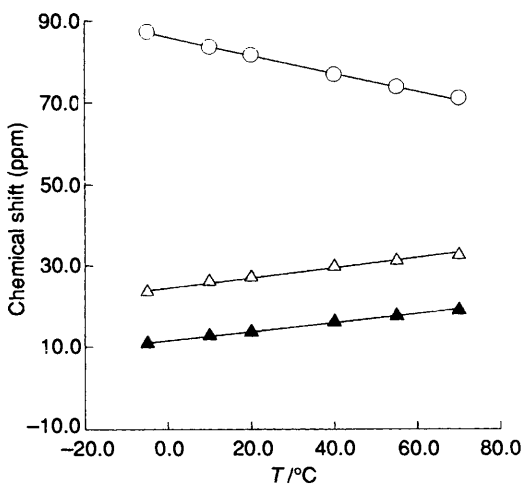

Fig. 6 Temperature dependence of bridging methylene (○) and terminal cysteinate β-CH_2 (△) and α-CH (▲) proton chemical shifts of $[NEt_4]_4[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]$ **3** (2×10^{-2} mol dm^{-3} in CD_3CN)

faster. There is no evidence for bridge cleavage, even when a large excess of ester is employed. Replacement of all six chloro ligands should require 6 equivalents of cysteine ethyl ester hydrochloride, and when only three were used a complex spectrum was obtained, probably resulting from mixed-ligand clusters.

Variable-temperature ^1H NMR studies (Fig. 6) show changes of the isotropic shifts fully consistent with the formulation given for cluster **3**. Cysteinate β-CH_2 and α-CH signals move with increase in temperature away from the diamagnetic, as do those of $[Fe_4S_4(Cys\text{-}OEt\cdot HCl)_4]^{2-}$ (ref. 4) and of the thiolate protons in $[Fe_4S_4(SR)_4]^{2-}$ (R = Me, Et or Bu^t).[15] The bridging thiolate signals move toward the diamagnetic, as expected for clusters of the type $[M_2Fe_7Q_8(SR)_6X_6]^{4-}$ (M = Mo or W, Q = S or Se, X = SEt or Cl).[8,16] Others have concluded that the isotropic shifts of the terminal substituents arise predominantly from contact terms whilst those of the bridge substituents are affected additionally by appreciable dipolar contributions.[8]

Solid samples of the cysteinate-bound cluster **3** can be prepared easily in *ca.* 80% yield by a metathesis reaction of **1**. Solutions of isolated **3** in deuterioacetonitrile show identical ^1H NMR properties to those described above. The amino acid ester carbonyl gives an IR stretch at 1744 cm^{-1}, shifted slightly compared to the unbound ester. The solid-state Mössbauer spectrum of **3** (Fig. 7) can be fitted by Lorentzian line shapes to give the parameters listed in Table 2. The spectrum is composed of two, partially resolved overlapping doublets of relative intensity 5:1. The expected relative intensity ratio of 6:1, for cube iron atoms compared to the iron atom of the $Fe(SEt)_6$ bridge, is close to that observed. The i.s. and q.s. are very different to those found for $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]^{3-}$ **1** and $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]^{3-}$ **4** (Table 2), but are of similar magnitude to those found for $[M_2Fe_7S_8(SCH_2Ph)_{12}]^{4-}$ (M = Mo or W).[8] These observations are consistent with thiolate ligation and confirm the overall oxidation level of 4 – for cluster **3**.

Although elemental analyses for cluster **3** were consistently poor and crystals suitable for X-ray diffraction studies are, as yet, unavailable, spectroscopic studies both in solution and in the solid state confirm that the novel cysteinate-bound cluster, $[NEt_4]_4[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]$, has been prepared.

The intimate mechanism of the reaction of cluster **1** with the ester to give **3** through the hexachloro-intermediate **4** has not been studied in detail. Proton NMR and UV/VIS spectroscopies show that the chloro-cluster **4** is formed rapidly on mixing **1** and the ester, and that ethanethiol is liberated. Over a few hours there is then a concentration-dependent substitution of chloro-groups by cysteine ethyl ester with concomitant cluster reduction (Scheme 1). When ⩾6 equivalents of the ester are used there is no evidence for the intermediate formation of mixed chloride ligated clusters. In the analogous preparation of

Table 2 Mössbauer spectral parameters. Solid state, zero field, 77 K relative to natural iron at 298 K

Complex	i.s.	q.s.	h.w.h.m.	Relative intensity
		mm s^{-1}		
1 $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]$	0.40(1)	1.06(1)	0.26(1)	6
	0.40(1)	2.16(2)	0.21(2)	1
4 $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]$	0.52(1)	0.80(1)	0.30(1)	6
	0.40(1)	2.27(2)	0.20(2)	1
$[NBu^n_4]_4[Mo_2Fe_7S_8(SCH_2Ph)_{12}]$[a]	0.42(2)	0.96(2)	—	6
	0.97(6)	1.90(6)	—	1
$[NBu^n_4]_4[W_2Fe_7S_8(SCH_2Ph)_{12}]$[a]	0.45(2)	1.08(2)	—	*ca.* 8
	0.93(6)	2.02(6)	—	1
3 $[NEt_4]_4[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]$	0.46(1)	1.05(1)	0.34(1)	5
	1.14(1)	3.08(2)	0.37(2)	1
6 $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Tyr\text{-}OMe)_6]$	0.53(1)	1.36(2)	0.25(2)	*b*
	0.40(1)	0.92(2)	0.28(1)	

[a] Ref. 8, i.s. adjusted to be relative to iron at 298 K. [b] Unable to calculate due to overlap of doublets.

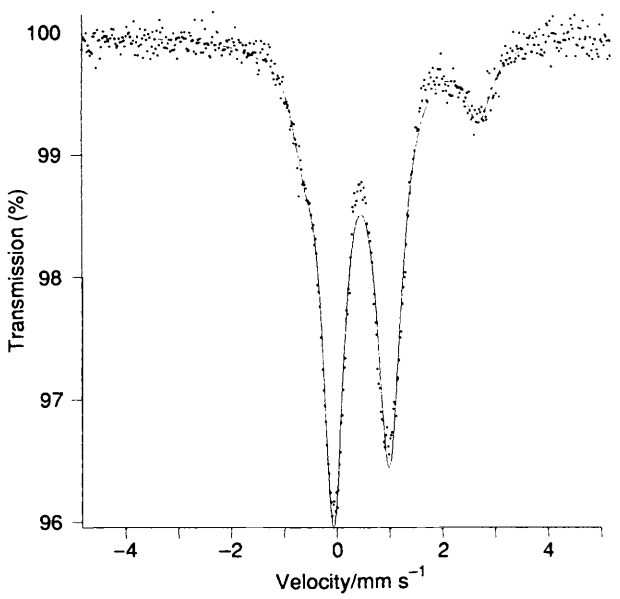

Fig. 7 Mössbauer spectrum of $[NEt_4]_4[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]$ **3** at 77 K

$[Fe_4S_4(Cys\text{-}OEt\cdot HCl)_4]^{2-}$ [equation (2)] the cluster core is not reduced.[4]

$$[Fe_4S_4(SBu^t)_4]^{2-} + 4HCys\text{-}OEt\cdot HCl \longrightarrow [Fe_4S_4(Cys\text{-}OEt\cdot HCl)_4]^{2-} + 4HSBu^t \quad (2)$$

Addition of 3 molar equivalents of the ester to the monocubane cluster **2** was expected to generate the cysteinate-substituted monocubane cluster $[MoFe_3S_4(Cys\text{-}OEt\cdot HCl)_3\{Fe(cat)_3\}]^{3-}$. However, much to our surprise the double-cubane **3** is the only cluster product. The reaction in solution was rapid and complete in less time than required to record a ^1H NMR spectrum (*ca.* 4 min). There was no evidence for a chloro-substituted intermediate. The product generated either in solution or on a preparative scale showed the same ^1H NMR isotropic shifts and temperature dependence and UV/VIS spectrum as found for **3** prepared from the double cubane **1**. In addition, the isolated solid had the same IR and Mössbauer spectra as those of **3** prepared from **1**.

The complex mechanism required for the conversion of cluster **2** into **3** may be regarded as a 'spontaneous disassembly–self-assembly' reaction. Assuming that the cluster core remains intact throughout, the tris(catecholato)ferrate(III) fragment must detach itself from the molybdenum and disintegrate (free

$$[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]^{3-} \quad \mathbf{1}$$

$$\downarrow (i)$$

$$[Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]^{3-} \quad \mathbf{4} + 6HSCH_2CH(CO_2Et)NH_2 + 6HSEt$$

$$\downarrow$$

$$[Mo_2Fe_7S_8(\mu\text{-}SEt)_6\{SCH_2CH(CO_2Et)NH_2\cdot HCl\}_6]^{4-} \quad \mathbf{3}$$

Scheme 1 (*i*) $6HSCH_2CH(CO_2Et)NH_2\cdot HCl$

H_2cat is observed in ^1H NMR solutions), the iron(III) is then available to form the $Fe(SEt)_6$ bridge. As cysteinate exchanges with terminal ethanethiolates, the liberated ethanethiol must insert between the molybdenum and bridging iron to complete the $Fe(SEt)_6$ bridge. Again, a reduction occurs to give the product cluster in the 4− oxidation level.

Tyrosinate-derivatives.—L-Tyrosine methyl ester hydrochloride reacts with the ethanethiolato-clusters **1** and **2** to give chloro-substituted complexes **4** and **5**, respectively. Under the conditions used (see Experimental section) there is no evidence for further reaction to give clusters with terminal tyrosinate. Tyrosine is unable to exchange with the terminal chloro-groups, whereas cysteine is. The tyrosine methyl ester hydrochloride is here acting as a simple amine hydrochloride [equation (3)].

$$[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]^{3-} + 6RNH_2\cdot HCl \longrightarrow [Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]^{3-} + 6RNH_2 + 6HSEt \quad (3)$$

However, we have previously shown that tyrosine methyl ester hydrochloride (HTyr-OMe·HCl) does react with $[Fe_4S_4(SBu^t)_4]^{2-}$ to give the tyrosinate substituted cluster, $[Fe_4S_4(Tyr\text{-}OMe\cdot HCl)^4]^{2-}$.[4] The reason for this difference is not evident.

Nevertheless, a tyrosinate-substituted double-cubane cluster can be prepared by reaction of tetraethylammonium tyrosinate methyl ester $[NEt_4][Tyr\text{-}OMe]$ with **4** [equation (4)]. Identical

$$[Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]^{3-} + 6[NEt_4][Tyr\text{-}OMe] \longrightarrow [Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Tyr\text{-}OMe)_6]^{3-} + 6NEt_4Cl \quad (4)$$

^1H NMR spectra are obtained for samples prepared in solution or isolated as gummy solids. The isotropically shifted signals are listed in Table 1. Bridging methylene and methyl signals are at positions consistent with the formulation $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Tyr\text{-}OMe)_6]^{3-}$ **6**. The tyrosinate β-CH_2 and α-CH resonances are shifted downfield to δ 48.6 and *ca.* 13,

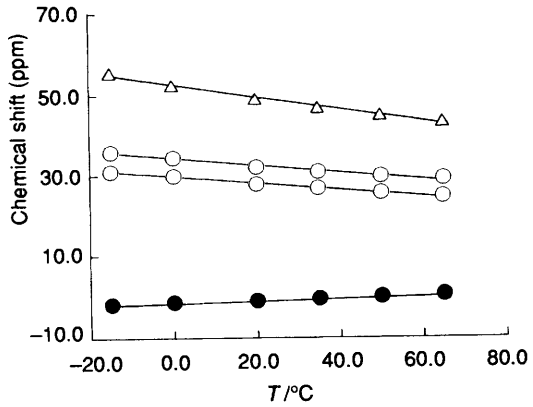

Fig. 8 Temperature dependence of bridging methylene (○) and methyl (●) and terminal tyrosinate β-CH_2 (△) proton chemical shifts of $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Tyr\text{-}OMe)_6]$ **6** (2×10^{-2} mol dm^{-3} in CD_3CN)

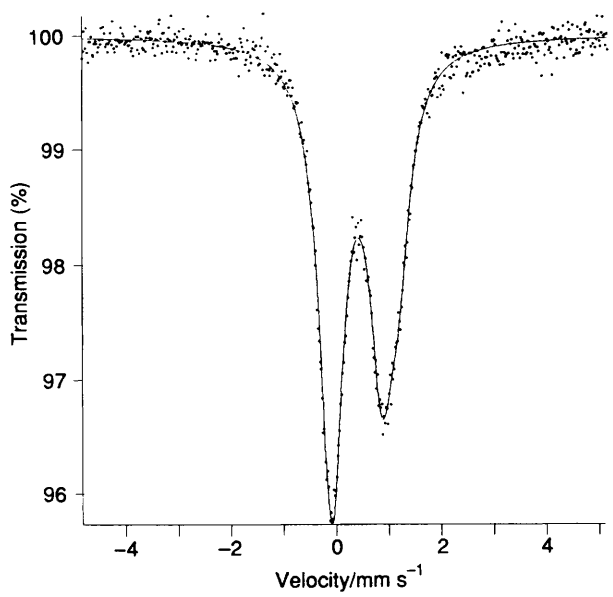

Fig. 9 Mössbauer spectrum of $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Tyr\text{-}OMe)_6]$ **6** at 77 K

respectively, the latter being broad and poorly resolved. The *meta*- and *ortho*-proton signals are observed at δ 10.76 and 5.69 and 5.07. The origin of two signals for the *o*-protons is probably stereochemical, each such proton of a tyrosinate ligand is in a slightly different environment. Variable-temperature data are plotted in Fig. 8. The bridging methylene and methyl groups show the temperature dependence expected for an $Fe^{III}(SEt)_6$ bridging group. The tyrosinate β-CH_2 signals move toward the diamagnetic as the temperature is raised, as has been reported for $[Fe_4S_4(Tyr\text{-}OMe\cdot HCl)_4]^{2-}$.[4] The temperature dependence of the tyrosinate α-CH could not be monitored easily due to its broadness. The *m*-proton resonance moves away from the diamagnetic with increase in temperature, whereas the *o*-protons exhibit the opposite temperature dependence. From this one example alone the mechanism of isotropic shift cannot be defined. It has been shown[4] that tyrosinate exhibits a rather different temperature dependence compared to ordinary phenolate when bound to $\{Fe_4S_4\}^{2+}$ and this may be the case here.

Analytically pure solid samples of cluster **6** could not be prepared. However, a black gum was isolated (>85% pure by ^1H NMR spectroscopy). Solutions of this material gave ^1H NMR and UV/VIS spectra similar to those of **6** prepared in solution. The tyrosinate ester IR carbonyl stretch is seen at 1734 cm^{-1}, shifted slightly compared to that of unbound tyrosinate ester. The Mössbauer spectrum (Fig. 9) is a broad asymmetric doublet which can be fitted by two overlapping doublets (Table 2). The values of i.s. and q.s. support the formulation proposed for **6**.

Reaction of cluster **6** with a slight excess of ethanoyl chloride in acetonitrile quantitatively regenerates the chloro-substituted cluster **4**, confirming that the double-cubane core has remained intact.

The monocubane **4** does not react cleanly with $[NEt_4][Tyr\text{-}OMe]$. A complicated ^1H NMR spectrum suggests either mixed-ligand species or a mixture of products.

Conclusion

In acetonitrile solution, the heterometallic iron–sulfur clusters **1** and **2** react with cysteine ethyl ester hydrochloride to generate the same double-cubane product $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]^{4-}$ **3**. Tyrosinate-substituted analogues cannot be prepared in the same way. Reaction of **1** and **2** with tyrosine methyl ester hydrochloride gives only the corresponding chloro-substituted clusters **4** and **5**. However, reaction of **4** with $[NEt_4][Tyr\text{-}OMe]$ gives the tyrosinate-substituted cluster $[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Tyr\text{-}OMe)_6]^{3-}$ **6**.

Although crystallographic evidence is not available, the extensive spectroscopic studies all support the successful preparation of the novel amino acid substituted clusters **3** and **6**.

Experimental

General Methods and Techniques.—All solvents were distilled from appropriate drying agents and degassed before use. Standard syringe and Schlenk techniques were employed.

The heterometallic iron–sulfur clusters $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]$ **1**,[8] $[NEt_4]_3[MoFe_3S_4(SEt)_3\{Fe(cat)_3\}]$ **2**[10] and $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6Cl_6]$ **4**[9] were prepared by published procedures. Tetraethylammonium tyrosinate methyl ester was prepared from an equimolar mixture of tetraethylammonium hydroxide and tyrosine methyl ester in methanol. All other chemicals were purchased from Aldrich and used as supplied. In all cases the L isomer of the amino acids was used.

Infrared and Mössbauer spectra were recorded on Perkin Elmer 883 and ES-Technology MS105 spectrometers, respectively. Mössbauer parameters were determined at 77 K, using a 925 MBq ^{57}Co source in a rhodium matrix, and were referenced against iron foil at 298 K. Proton NMR spectra were recorded on a JEOL GSX270 spectrometer and chemical shifts were referenced against $SiMe_4$. Typically, reaction solutions for study by NMR spectroscopy were prepared by adding a CD_3CN solution of heterometallic cluster (2×10^{-2} mol dm^{-3}) to an appropriate amount of amino acid ester under dinitrogen. The mixtures were shaken under dynamic vacuum for 3 min and then transferred to NMR tubes. The UV/VIS spectra were measured at 298 K on a Perkin Elmer 550S spectrophotometer. Elemental analyses were performed by Mr. C. J. Macdonald of the Nitrogen Fixation Laboratory, using a Perkin Elmer 2400 CHN elemental analyser.

Syntheses.—$[NEt_4]_3[MoFe_3S_4Cl_3\{Fe(cat)_3\}]$ **5**. (*a*) *Trimethylethanoyl chloride method.* Cluster **2** (0.21 g, 0.16 mmol) in MeCN (10 cm^3) was stirred with trimethylethanoyl chloride (0.06 g, 0.49 mmol) for 1 h. The volume was reduced to *ca.* 5 cm^3 at 10^{-3} mmHg (0.133 Pa), diethyl ether (5 cm^3) added and the mixture stored at −20 °C overnight. The black precipitate was collected and washed with diethyl ether to give cluster **5** (0.11 g, 56%).

(*b*) *Amine hydrochloride method.* Cluster **2** (0.50 g, 0.37 mmol) in MeCN (15 cm^3) was added to solid tyrosine methyl ester hydrochloride (0.26 g, 1.12 mmol). The orange-brown solution was stirred for 6 h, and volatiles were occasionally removed by pumping. The mixture was filtered, the volume of solvent reduced

to *ca.* 10 cm^3 and tetrahydrofuran (15 cm^3) added. After 24 h the black precipitate of cluster **5** was collected by filtration (0.26 g, 55%) (Found: C, 39.9; H, 5.7; N, 3.2. $C_{42}H_{72}Cl_3Fe_4MoN_3O_6S_4$ requires C, 39.8; H, 5.7; N, 3.3%); λ_{max}/nm (MeCN) 290 (ε/dm^3 mol^{-1} cm^{-1} 30 500) and 470 (9600); $\delta_H(CD_3CN)$ 64.2 (H_{cat}), 43.0 (H_{cat}), 3.03 (NEt_4), 1.20 (NEt_4), −10 (H_{cat}) and −33 (H_{cat}).

$[NEt_4]_4[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Cys\text{-}OEt\cdot HCl)_6]$ **3**. (*a*) *From* $[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(SEt)_6]$ **1**. Cluster **1** (0.37 g, 0.19 mmol) was dissolved in MeCN (20 cm^3) and solid cysteine ethyl ester hydrochloride (0.21 g, 1.14 mmol) added against a back-flow of dinitrogen. The solution was stirred for 2 h, volatiles were occasionally removed by pumping. Solvent was removed *in vacuo* to give a black gum, and addition of diethyl ether (20 cm^3) gave the product as a black solid (*ca.* 80%).

(*b*) *From* $[NEt_4]_3[MoFe_3S_4(SEt)_3\{Fe(cat)_3\}]$ **2**. Cluster **2** (0.50 g, 0.37 mmol) was dissolved in MeCN (15 cm^3) and added to solid cysteine ethyl ester hydrochloride (0.20 g, 1.11 mmol). The mixture was stirred for 30 min under partial vacuum, and then for 16 h under dinitrogen. After storage at −20 °C for 24 h the product was collected as a fine black solid (*ca.* 70%); λ_{max}/nm (MeCN) 276 (sh) and 460 (sh); ν_{max}(Nujol) 1744 cm^{-1} (CO).

$[NEt_4]_3[Mo_2Fe_7S_8(\mu\text{-}SEt)_6(Tyr\text{-}OMe)_6]$ **6**. Cluster **4** (0.16 g, 0.09 mmol) was mixed with a solution of tetraethylammonium tyrosinate methyl ester (0.34 g, 1.05 mmol) in MeCN (25 cm^3). The mixture was stirred for 3 h and then solvent removed *in vacuo*. The product was obtained as a sticky black gum. Attempts to solidify or purify further **6** failed. The tyrosinate-substituted cluster **6** was estimated to be >85% pure by ^1H NMR spectroscopy; λ_{max}/nm(MeCN) 284 (sh) and 356 (sh); ν_{max}(Nujol) 1734 cm^{-1} (CO).

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