# CENTENARY LECTURE*

Metal Clusters in Biology: Quest for a Synthetic Representation of the Catalytic Site of Nitrogenase

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1 Introduction

It is now well established that there occur, in certain proteins and enzymes, aggregates of two or more metal atoms that are bridged by oxygen or sulphur atoms and terminally co-ordinated mainly or entirely by functional side-groups of amino-acid residues comprising polypeptide chains.[1] In those instances where the collective properties of an individual metal site depart significantly from those of an isolated co-ordination unit of comparable constitution and geometry, the aggregate – by the usual definition of the inorganic chemist – may be considered a cluster. Such is the case for the 2-Fe and 4-Fe prosthetic groups (1) and (2) of

(1)

(2)
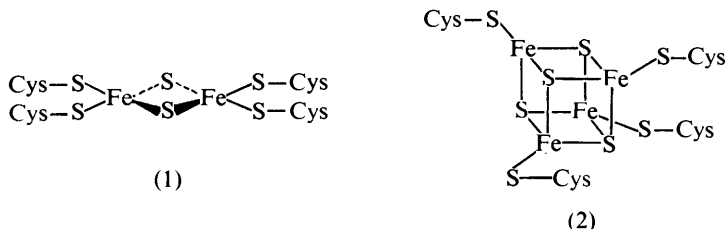
iron–sulphur electron-transfer proteins, the ferredoxins (Fd). These entities, which are the most thoroughly investigated biological clusters,[2-6] contain tetrahedrally co-ordinated iron atoms which are antiferromagnetically spin-coupled in all oxidation levels *in vitro*. Accurate synthetic representations (3) and

[1] J. A. Ibers and R. H. Holm, *Science*, 1980, **209**, 223.
[2] 'Iron–Sulfur Proteins', ed. W. Lovenberg, Academic Press, New York; (*a*) 1973, Vols. 1 and 2; (*b*) 1977, Vol. 3.
[3] G. Palmer, in 'The Enzymes', (3rd edn.), Vol. XII, Part B, ed. P. D. Boyer, Academic Press, New York, 1975, pp. 1—56.
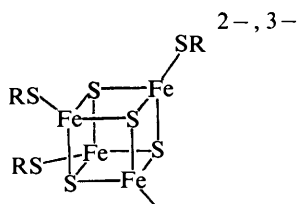[4] R. H. Sands and W. R. Dunham, *Q. Rev. Biophys.*, 1975, **7**, 443.
[5] B. A. Averill and W. H. Orme-Johnson, *Met. Ions Biol. Syst.*, 1978, **7**, 127.
[6] W. V. Sweeney and J. C. Rabinowitz, *Annu. Rev. Biochem.*, 1980, **49**, 139.
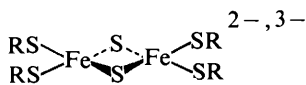
(3)

(4)

(4) of these centres can be readily assembled in simple preparative systems (see below), and their structural and electronic properties and reaction chemistry have been elucidated in considerable detail.[1,7-13] In these species, Fe–Fe distances of ~ 2.7 Å allow direct orbital overlap, which, in addition to interactions through intermediary sulphur atoms, leads to electronic coupling between or amongst Fe sites. The spectroscopic and magnetic properties of clusters (3) and (4) closely approach those of protein groups (1) and (2), respectively, in equivalent oxidation levels, and thus serve as synthetic analogues of the latter. Among the noteworthy features of (1)—(4) is the electronically delocalized nature of the cubane-type clusters (2) and (4) in those oxidation levels that require formally inequivalent oxidation states of iron, a matter most clearly revealed by Mössbauer spectroscopy of Fd proteins[14] and their redox-group analogues.[9,10] Both the Mössbauer spectral and bulk magnetic properties[15] of these clusters are accountable in terms of a description involving antiferromagnetic spin-coupling of iron atoms.

Over the past decade, a new type of biological cluster, as yet undefined in detail, and not anticipated by serendipitous inorganic cluster chemistry, has emerged. This is the Fe–Mo–S entity that is present in the native FeMo protein of the nitrogenase enzyme complex, and which is removable from it in the form of a cofactor (FeMo-co).[16] The present extensive body of enzymological and physical evidence, while not decisive, is suggestive in respect of FeMo-co (in a reduced oxidation level) being the catalytic site for reduction of dinitrogen to ammonia. Stimulated by this possibility, and abetted by increasing physicochemical definition of the biological cluster and our experience in Fe–S

[7] R. H. Holm and J. A. Ibers, in ref. 2*b*, Ch. 7.
[8] R. H. Holm, *Acc. Chem. Res.*, 1977, **10**, 427.
[9] E. J. Laskowski, R. B. Frankel, W. O. Gillum, G. C. Papaefthymiou, J. Renaud, J. A. Ibers, and R. H. Holm, *J. Am. Chem. Soc.*, 1978, **100**, 5322.
[10] E. J. Laskowski, J. G. Reynolds, R. B. Frankel, S. Foner, G. C. Papaefthymiou, and R. H. Holm, *J. Am. Chem. Soc.*, 1979, **101**, 6562.
[11] J. G. Reynolds, C. L. Coyle, and R. H. Holm, *J. Am. Chem. Soc.*, 1980, **102**, 4350.
[12] P. K. Mascharak, G. C. Papaefthymiou, R. B. Frankel, and R. H. Holm, *J. Am. Chem. Soc.* 1981, **103**, 6110.
[13] J. M. Berg and R. H. Holm, in 'Iron–Sulfur Biochemistry', ed. T. G. Spiro, Wiley, New York, in the press.
[14] P. Middleton, D. P. E. Dickson, C. E. Johnson, and J. D. Rush, *Eur. J. Biochem.*, 1978, **88**, 135; *ibid.*, 1980, **104**, 289.
[15] G. C. Papaefthymiou, E. J. Laskowski, S. Frota-Pessoa, R. B. Frankel, and R. H. Holm, *Inorg. Chem.*, in the press.
[16] V. K. Shah and W. J. Brill, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 3249.

analogue chemistry, we have initiated research directed toward the synthesis of this cluster. Unlike the cases of prosthetic groups of Fd proteins, for which synthetic analogues have been prepared,[7-10,13,17] the target cluster enjoys only a modicum of direct structural characterization.[18,19] Consequently, our endeavour is, in Hill's description,[20] a venture in speculative rather than corroborative modelling of a prosthetic group. The leading results of the endeavour form the substance of this report. For summaries or reviews of the biochemistry of nitrogenase,[21-27] of FeMo-co,[26-29] of relevant Fe–Mo–S chemistry,[27,29,30] and of chemical approaches to the complexation and reduction of dinitrogen,[21,22,25,27,29,31,32] other sources should be consulted.

2 Metal Clusters of Nitrogenase

A. The Native Enzyme.—The nitrogenase enzyme complex consists of two types of proteins: the Fe protein, which transfers electrons that are supplied by an external reductant, with concomitant hydrolysis of ATP; and the FeMo protein, which is reduced by the Fe protein, contains the catalytic site(s) for reduction of substrate, and is the source of FeMo-co. The sequence shown in Scheme 1 is an abbreviated representation of the current view of coupled reactions in a reconstituted enzyme system, resulting in the reduction of physiological (N_2, H^+) and a number of non-physiological substrates, including acetylene.

The Fe protein from all organisms has four iron and 'inorganic' sulphur (S^*) atoms in an α_2 subunit structure of molecular weight ~ 57 000. The structural

[17] D. Coucouvanis, D. Swenson, N. C. Baenziger, C. Murphy, D. G. Holah, N. Sfarnas, A. Simopoulos, and A. Kostikas, *J. Am. Chem. Soc.*, 1981, **103**, 3350.
[18] S. P. Cramer, K. O. Hodgson, W. O. Gillum, and L. E. Mortenson, *J. Am. Chem. Soc.*, 1978, **100**, 3398.
[19] S. P. Cramer, W. O. Gillum, K. O. Hodgson, L. E. Mortenson, E. I. Stiefel, J. R. Chisnell, W. J. Brill, and V. K. Shah, *J. Am. Chem. Soc.*, 1978, **100**, 3814.
[20] H. A. O. Hill, *Chem. Br.*, 1976, **12**, 119.
[21] 'Recent Developments in Nitrogen Fixation', ed. W. Newton, J. R. Postgate, and C. Rodriguez-Barrueco, Academic Press, New York, 1977.
[22] 'A Treatise on Dinitrogen Fixation, Sections I and II: Inorganic and Physical Chemistry and Biochemistry', ed. R. W. F. Hardy, F. Bottomley, and R. C. Burns, Wiley, New York, 1977.
[23] W. H. Orme-Johnson and L. C. Davis, in ref. 2b, Ch. 2.
[24] L. E. Mortenson and R. N. F. Thorneley, *Annu. Rev. Biochem.*, 1979, **48**, 387.
[25] 'Nitrogen Fixation', ed. W. E. Newton and W. H. Orme-Johnson, University Park Press, Baltimore, 1980, Vols. I and II.
[26] 'Molybdenum and Molybdenum-Containing Enzymes', ed. M. P. Coughlan, Pergamon Press, New York, 1980.
[27] 'Current Perspectives in Nitrogen Fixation', ed. A. H. Gibson and W. E. Newton, Australian Academy of Science, Canberra, 1981.
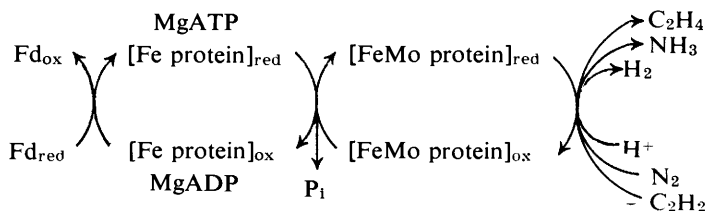[28] B. K. Burgess and W. E. Newton, in 'Nitrogen Fixation: Chemical/Biochemical/Genetics Interface', ed. A. Müller and W. E. Newton, Plenum Press, New York, in the press.
[29] 'Molybdenum Chemistry of Biological Significance', ed. W. E. Newton and S. Otsuka, Plenum Press, New York, 1980.
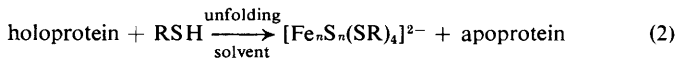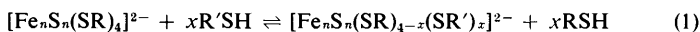[30] D. Coucouvanis, *Acc. Chem. Res.*, 1981, **14**, 201.
[31] J. Chatt, J. R. Dilworth, and R. L. Richards, *Chem. Rev.*, 1978, **6**, 589.
[32] W. E. Newton, *Adv. Chem. Ser.*, 1980, **191**, 351.

$$\text{Fd}_{ox} \quad \overset{}{\underset{}{\bigtimes}} \quad [\text{Fe protein}]_{red} \quad \overset{}{\underset{}{\bigtimes}} \quad [\text{FeMo protein}]_{red}$$

(Scheme diagram)

MgATP

Fd_{ox} — [Fe protein]_{red} — [FeMo protein]_{red} → C₂H₄, NH₃, H₂

Fd_{red} — [Fe protein]_{ox} — [FeMo protein]_{ox} → H⁺, N₂, C₂H₂

MgADP   P_i

Scheme 1

organization of the Fe–S content of this protein has been examined by the core extrusion method, which has been described at length elsewhere.[13,33-37] The method is based on the reversible ligand-substitution reaction (1) (where $n = 2$ or 4 and $x = 1$—4), first established with the analogue dianion complexes (3)[38] and (4).[39] Subsequently, a demonstration of the extrusion reaction [reaction (2)]

$$[\text{Fe}_n\text{S}_n(\text{SR})_4]^{2-} + x\text{R}'\text{SH} \rightleftharpoons [\text{Fe}_n\text{S}_n(\text{SR})_{4-x}(\text{SR}')_x]^{2-} + x\text{RSH} \tag{1}$$

$$\text{holoprotein} + \text{RSH} \xrightarrow[\text{solvent}]{\text{unfolding}} [\text{Fe}_n\text{S}_n(\text{SR})_4]^{2-} + \text{apoprotein} \tag{2}$$

was provided.[33,36,37,40] Here the treatment of a small Fd protein, unfolded in an aqueous/organic solvent that contains a large excess of the thiol which acts as an extrusion reagent, results in the quantitative removal of $[\text{Fe}_n\text{S}_n]^{2+}$ cores of (1) and (2) in the form of their analogue complexes $[\text{Fe}_n\text{S}_n(\text{SR})_4]^{2-}$ ($n = 2$ or 4). The latter, which can be independently synthesized, are assayed by an appropriate spectroscopic technique (u.v.–visible spectra, ^{19}F n.m.r., or e.p.r. of reduced clusters). Application of the method to a clostridial Fe protein has shown the presence of one Fe_4S_4 cluster (2),[23,33] possibly bridging the two subunits.

The FeMo proteins have an $\alpha_2\beta_2$ subunit structure (the range of molecular weights is 220 000—240 000) with two molybdenum atoms and about equal amounts of iron and inorganic sulphur. More recent preparations contain $\sim$30—32 iron atoms. Our approach to the investigation of the structural

[33] W. O. Gillum, L. E. Mortenson, J.-S. Chen, and R. H. Holm, *J. Am. Chem. Soc.*, 1977, **99**, 584.

[34] R. H. Holm, in 'Biological Aspects of Inorganic Chemistry', ed. A. W. Addison, W. R. Cullen, and D. Dolphin, Wiley, New York, 1977, pp. 71—111.

[35] W. H. Orme-Johnson and R. H. Holm, *Methods Enzymol.*, 1978, **53**, 268.

[36] G. B. Wong, D. M. Kurtz, Jr., R. H. Holm, L. E. Mortenson, and R. G. Upchurch, *J. Am. Chem. Soc.*, 1979, **101**, 3078.
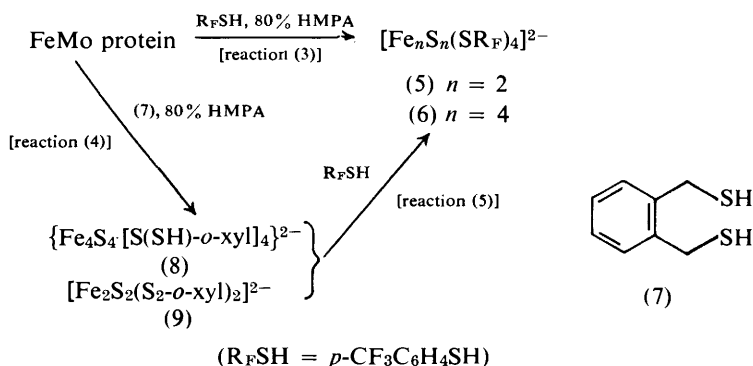
[37] B. A. Averill, J. R. Bale, and W. H. Orme-Johnson, *J. Am. Chem. Soc.*, 1978, **100**, 3034.

[38] L. Que, Jr., M. A. Bobrik, J. A. Ibers, and R. H. Holm, *J. Am. Chem. Soc.*, 1974, **96**, 4168.

[39] J. J. Mayerle, S. E. Denmark, B. V. DePamphilis, J. A. Ibers, and R. H. Holm, *J. Am. Chem. Soc.*, 1975, **97**, 1032.

[40] L. Que, Jr., R. H. Holm, and L. E. Mortenson, *J. Am. Chem. Soc.*, 1975, **97**, 463.

organization of this formidable metal content has utilized core extrusion. Initial experiments[36] were based on reaction (3) in Scheme 2. Here, semi-reduced

FeMo protein $\xrightarrow[\text{[reaction (3)]}]{\text{R}_\text{F}\text{SH, 80\% HMPA}}$ $[\text{Fe}_n\text{S}_n(\text{SR}_\text{F})_4]^{2-}$

(5) $n = 2$
(6) $n = 4$

(7), 80% HMPA

[reaction (4)]

R$_\text{F}$SH

[reaction (5)]

$\{\text{Fe}_4\text{S}_4\text{[S(SH)-}o\text{-xyl]}_4\}^{2-}$
(8)
$[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-}$
(9)

(7)

$(\text{R}_\text{F}\text{SH} = p\text{-CF}_3\text{C}_6\text{H}_4\text{SH})$

Scheme 2

(as isolated) proteins were placed in the unfolding medium 4:1 v/v hexamethyl-phosphoramide (HMPA):aqueous buffer containing p-CF$_3$C$_6$H$_4$SH. Extrusion products, assayed by ^{19}F n.m.r. spectroscopy, consisted of small amounts of the Fe$_2$S$_2$ complex (5) and much larger quantities of the Fe$_4$S$_4$ complex (6). However, $\lesssim 25\%$ of the total iron content was removed in these reactions, suggestive of incomplete extrusion. In subsequent experiments,[41] using the same protein samples, reaction conditions, and method of assay of the product, o-xylyl-α,α'-dithiol (7) was introduced as the extrusion reagent in reaction (4). The species that were liberated were subjected to the ligand-substitution reaction of reaction (5), which is a specific example of reaction (1).

Only cluster (6) was detected, indicating removal of protein Fe/S* in the form of (8) rather than (9). Of the predetermined iron content of the protein, 49—56% was liberated. When normalized to 30—32 iron atoms, the results indicate about four Fe$_4$S$_4$ clusters per $\alpha_2\beta_2$ unit. Under the same conditions, separate samples of FeMo-co afforded no evidence of formation of (5) and (6), consistent with an earlier report that benzenethiol does not disrupt FeMo-co.[42] These findings lead to the conclusion that only non-cofactor iron is extruded, and that the large majority of this iron content is organized in the form of Fe$_4$S$_4$ clusters. The first of these conclusions is supported by chemical analyses of FeMo-co,

[41] D. M. Kurtz, Jr., R. S. McMillan, B. K. Burgess, L. E. Mortenson, and R. H. Holm, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4986.
[42] J. Rawlings, V. K. Shah, J. R. Chisnell, W. J. Brill, R. Zimmermann, E. Münck, and W. H. Orme-Johnson, *J. Biol. Chem.*, 1978, **253**, 1001.

which show that about half of the iron content of an FeMo protein (7—8 iron atoms per molybdenum[16,43-45]) is associated with the cofactor.

Extrusion results gained from the sequential application of reactions (4) and (5) are encouragingly consistent with the deduction from Mössbauer spectroscopy that FeMo proteins contain four Fe_4S_4 ('P') clusters.[46,47] These may form the electron-transfer conduit that couples the Fe protein to the catalytic site(s). However, the nature of these clusters differs from that of conventional Fd-type groups (2). Mössbauer[46,47] and m.c.d.[48] spectral features in particular are unusual. Further, except for transient signals, these clusters are e.p.r.-silent in isolated, oxidized, and re-reduced protein samples,[46,49] despite the fact that sufficient electrons have been subtracted or added to change the oxidation level of P-clusters by one electron. A minority population of iron atoms, accounting for $\sim 6\%$ of the total, and of unknown structure, has also been detected in the Mössbauer spectra of FeMo proteins.[46,47] Although the properties of P-clusters might be subject to some clarification by application of the synthetic analogue approach, the main concern here is with synthetic clusters that are possibly related to FeMo-co, whose properties are considered next.

B. FeMo-co.—If, as seems likely, the essential catalytic machinery (excluding electron-transfer apparatus) for the reduction of substrates of nitrogenase is contained in FeMo-co, its isolation by Shah and Brill[16] in 1977 has opened the way for the eventual elucidation of the structure of the catalytic site and its mechanism at an atomic level of resolution. In the original procedure,[16] FeMo-co was extracted with *N*-methylformamide (NMF) from a pellet of FeMo protein that had previously been denatured with citric acid. Modified and improved procedures are now available.[43-45] In NMF, FeMo-co forms a brown, oxygen-sensitive solution. It is not as yet a fully characterized entity; selected properties, at the current stage of definition, are collected in Table 1.[50-57] In particular, neither the ratios of constituent atoms nor the molecular weight[45] are precisely

[43] B. K. Burgess, D. B. Jacobs, and E. I. Stiefel, *Biochim. Biophys. Acta*, 1980, **614**, 196.
[44] S.-S. Yang, W.-H. Pan, G. D. Friesen, B. K. Burgess, J. L. Corbin, E. I. Stiefel, and W. E. Newton, submitted for publication.
[45] B. E. Smith, in ref. 29, pp. 179—190.
[46] R. Zimmermann, E. Münck, W. J. Brill, V. K. Shah, M. T. Henzl, J. Rawlings, and W. H. Orme-Johnson, *Biochim. Biophys. Acta*, 1978, **537**, 185.
[47] B. H. Huynh, M. T. Henzl, J. A. Christner, R. Zimmermann, W. H. Orme-Johnson, and E. Münck, *Biochim. Biophys. Acta*, 1980, **623**, 124.
[48] P. J. Stephens, C. E. McKenna, M. C. McKenna, H. T. Nguyen, and F. Devlin, *Biochemistry*, 1981, **20**, 2857.
[49] G. D. Watt, A. Burns, S. Lough, and D. L. Tennent, *Biochemistry*, 1980, **21**, 4926.
[50] B. H. Hunyh, E. Münck, and W. H. Orme-Johnson, *Biochim. Biophys. Acta*, 1979, **527**, 192.
[51] W. E. Newton, B. K. Burgess, and E. I. Stiefel, in ref. 29, pp. 191—202.
[52] E. Münck, H. Rhodes, W. H. Orme-Johnson, L. C. Davis, W. J. Brill, and V. K. Shah, *Biochim. Biophys. Acta*, 1975, **400**, 32.
[53] B. K. Burgess, S.-S. Yang, C.-B. You, J.-G. Li, G. D. Friesen, W.-H. Pan, E. I. Stiefel, W. E. Newton, S. D. Conradson, and K. O. Hodgson, in ref. 27, pp. 71—74.
[54] G. D. Watt, in ref. 29, pp. 3—21.
[55] B. E. Smith, D. J. Lowe, and R. C. Bray, *Biochem. J.*, 1979, **135**, 331.
[56] B. K. Burgess, E. I. Stiefel, and W. E. Newton, *J. Biol. Chem.*, 1980, **255**, 353.
[57] V. K. Shah, in ref. 25, Vol. I, pp. 237–247.

Table 1 *Selected properties of FeMo-co*

Property	*References*
(1) Atom ratio: (6—8)Fe:(4—6)S*:Mo	16, 42—45, 50
(2) No amino-acids or identified endogenous organic components	44, 45
(3) Anionic (possible ligands: citrate, phosphate, Cl^-, $S_2O_4^{2-}$, SO_3^{2-}, H_2O, OH^-, or NMF)	28, 51
(4) E.p.r. + Mössbauer spectral properties:[a] iron atoms are spin-coupled; $S = \frac{3}{2}$ per Mo; $g \approx$ 4.6, 3.3, < 2.0 $\lvert\pm\frac{1}{2}\rangle$, 5.8—5.9 (weak) $\lvert\pm\frac{3}{2}\rangle$; zero-field splitting $\approx$ + 6 cm^{-1}; e.p.r. hyperfine broadening with ^{57}Fe but not ^{95}Mo enrichment	42, 50, 52
(5) Molybdenum XAS: 3 or 4 S at 2.36 Å, 2 or 3 Fe at 2.68 Å, 2 or 3 N,O at 2.10 Å (EXAFS)	19, 53
(6) Redox:[a] $[\text{FeMo-co}]_{\text{red}}$ $S > 1$ (e.p.r.-silent) $\longleftarrow$ [FeMo-co] $\sim -300\,\text{mV}$ $S = \frac{3}{2}$ $\longrightarrow$ $[\text{FeMo-co}]_{\text{ox}}$ (e.p.r.-silent)	42, 46, 49, 51 54—56
(7) Activity: $\sim$ 250—300 nmole of C_2H_4 are formed per minute per nano (gram atom) of molybdenum. Reconstitutes cofactor-deficient FeMo protein from a mutant organism (*Azotobacter vinelandii* UW45) to full activity	43—45, 51, 53, 57 16

(a) These results were obtained in part from studies of native FeMo proteins.

known. However, the near-identity of spectroscopic properties and reconstitution activities of samples of FeMo-co from five different organisms points to an identical cofactor from all sources. FeMo-co is distinct from a molybdenum cofactor (Mo-co) that is common to all other molybdo-enzymes.[58] While some nitrogen-fixing organisms produce both cofactors, FeMo-co and Mo-co are not genetically related,[28] and their activation of protein extracts from mutant organisms is mutually exclusive.[59]

Very recently, a second species, designated 'FeMo-cluster', and having the atom ratio (6.0 ± 0.5) iron per molybdenum atom, has been isolated.[60] It is obtained by extraction of HCl-treated FeMo-protein with butan-2-one, in which solvent it displays an e.p.r. spectrum quite different from that of FeMo-co. After transfer to NMF, the distinctive $S = \frac{3}{2}$ e.p.r. spectrum, although weak, appears. In NMF, FeMo-cluster does not activate *Azotobacter vinelandii* UW45, but, as

[58] J. L. Johnson, in ref. 26, Ch. 10.
[59] P. T. Pienkos, V. K. Shah, and W. J. Brill, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 5468.
[60] V. K. Shah and W. J. Brill, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 3438.

FeMo-co,[61] it reduces acetylene to ethylene in the presence of sodium boro-hydride. Other evidence[60] indicates that FeMo-cluster is derived from FeMo-co. Thus it appears that a content of > 6 iron atoms per molybdenum atom is required for activity, but not for the e.p.r. spectrum; this spectrum is unique in biology. FeMo-cluster may be the centre 'M_{EPR}', containing the spin system of $S = \frac{3}{2}$ and which was earlier deduced to contain, most probably, 6Fe per Mo atom from a detailed Mössbauer and e.p.r. spectral analysis of native proteins[46,47,52] and FeMo-co.[42,50]

The only direct structural information for the Fe–Mo–S cluster of nitrogenase follows from X-ray absorption spectra (XAS) of molybdenum and associated extended X-ray absorption fine structure (EXAFS) of several FeMo proteins[18] and the cofactor.[19,53] Analysis of the EXAFS by the Stanford group has led to the conclusions in Table 1 and the proposal of structures (10) and (11) as models

(10)   (11)
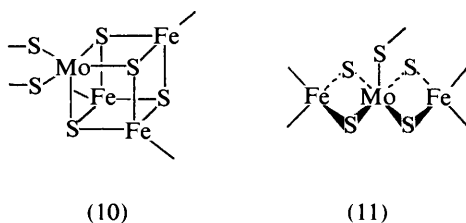
for the co-ordination unit of molybdenum in the proteins. Inasmuch as the technique[62] does not reliably sense atoms that are $\gtrsim 3.5$ Å from the atom whose X-ray spectrum is excited, the presence of the sulphur atom that is diagonally opposite the molybdenum atom in (10) is conjectural. The similarity of the cubane-type $MoFe_3S_4$ core unit of (10) to the Fe_4S_4 core in (2) and (4) is obvious. Ligands of the molybdenum atom that are external to the core framework are less well defined, but may consist in part of one or two sulphur atoms at about 2.5 Å. Oxo ligands (as in Mo=O) are absent. No Mo $\cdots$ Mo interactions of $\lesssim 3.5$ Å have been detected, suggesting (together with spectroscopic[46,47] and molecular-weight[45] evidence) that two cofactors, each containing one molybdenum atom, are present rather than one cofactor, containing two molybdenum atoms. Upon removal from the protein, FeMo-co retains the 2 or 3 iron and 3 or 4 sulphur atoms around the molybdenum atom. A recent EXAFS analysis[53] suggests the presence of 2 or 3 oxygen or nitrogen atoms, which presumably arise from exogenous ligands that are introduced in the isolation procedure. Likely candidates are given in Table 1. A schematic representation of the current view of the clusters that are present in FeMo proteins is given in Figure 1.
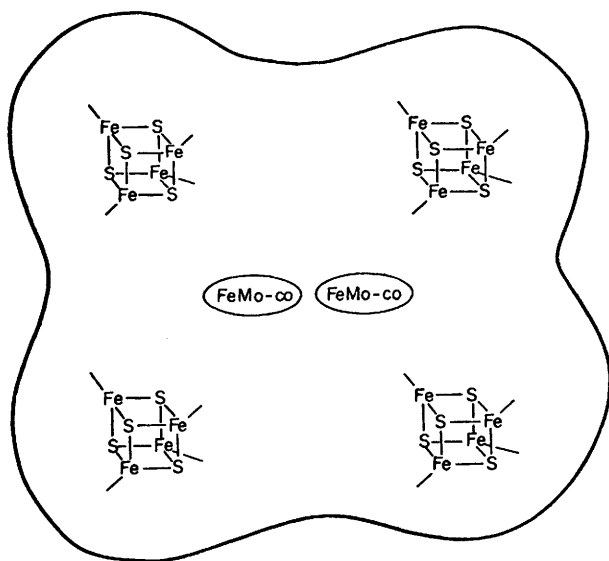
Based on the ratios of atoms and results from EXAFS, including a somewhat different interpretation of the latter,[63] various structural models for the Mo site in

[61] V. K. Shah, J. R. Chisnell, and W. J. Brill, *Biochem. Biophys. Res. Commun.*, 1978, **81**, 232.
[62] S. P. Cramer and K. O. Hodgson, *Prog. Inorg. Chem.*, 1979, **25**, 1.
[63] B.-K. Teo and B. A. Averill, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 1454.

Figure 1 *Clusters in the FeMo protein of nitrogenase, according to current evidence*

nitrogenase have been proposed[63-66] subsequent to the suggestions of (10) and (11). While some are of arresting conception, none of these models has been synthesized. It has proven possible to prepare clusters that contain the core unit in (10). The developing chemistry of these species is described in the following sections. Because they are the first mixed-metal $M_nM'_{4-n}S_4$ cubane-type clusters to be isolated, $MoFe_3S_4$ species are of interest in the context of abiological cluster chemistry. In addition, sufficient information has been accumulated to suggest the appropriateness of these clusters as *preliminary* models of the co-ordination unit of molybdenum in the enzyme.

3 The Assembly of Fe_4S_4 and $MoFe_3S_4$ Clusters
Initial syntheses of the vast majority of clusters that are now known were not deliberate, in the sense that the reactants and conditions employed could not necessarily have afforded molecules of uniquely predictable composition and structure. This was the case in our original synthesis of $[Fe_4S_4(SR)_4]^{2-}$

[64] Lu Jiaxi, in ref. 25, Vol. I, pp. 343—371.
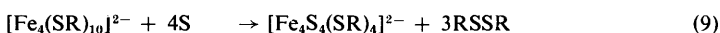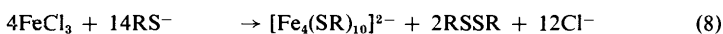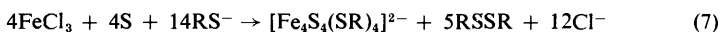[65] K. R. Tsai, in ref. 25, Vol. I, pp. 373—387.
[66] W. E. Newton, J. W. McDonald, G. D. Friesen, B. K. Burgess, S. D. Conradson, and K. O. Hodgson, in ref. 27, pp. 30—39.

clusters,[67,68] where structure (4) was only one of the conceivable possibilities. It is now abundantly clear that these clusters, derived from substitutionally labile iron(II, III) reactants, form as a consequence of being the thermodynamically most stable, soluble reaction products. The term 'spontaneous self-assembly' has been introduced[1] as a reminder of the thermodynamic origin of cluster synthesis. Before proceeding to the preparation of MoFe$_3$S$_4$ species, an examination of the course of assembly reactions that afford [Fe$_4$S$_4$(SR)$_4$]$^{2-}$ will prove instructive.
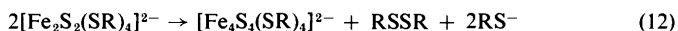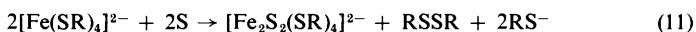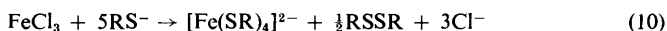
A. Fe$_4$S$_4$ Clusters.—The [Fe$_4$S$_4$(SR)$_4$]$^{2-}$ clusters are assembled in a series of reactions, dependent upon mole ratios of reactants. In recent work,[69] component reactions have been identified by the use of spectrophotometric and ^{1}H n.m.r. spectral observations, and products of these reactions have been isolated. The original assembly reaction, which is applicable to virtually all thiolates, and which affords yields of $\sim 80\%$ *in situ*, has the limiting stoicheiometry of reaction (6). However, this reaction proved too rapid for convenient study. Attention was

$$4FeCl_3 + 4HS^- \qquad [Fe_4S_4(SR)_4]^{2-} + RSSR$$
$$+ \longrightarrow + \qquad\qquad (6)$$
$$6RS^- + 4OMe^- \qquad 12Cl^- + 4MeOH$$
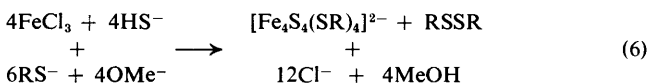
turned to the system in reaction (7),[70] utilizing R = Ph and elemental sulphur as the source of cluster sulphide. With the mole ratio RS$^-$:Fe = 3.5:1, in methanol or acetonitrile as solvent, the rapid reaction (8) ensues, affording, the complex [Fe$_4$(SR)$_{10}$]$^{2-}$ (12) in high yield. Subsequent introduction of sulphur affords [Fe$_4$S$_4$(SR)$_4$]$^{2-}$. The overall reaction is {(8) + (9)} = (7). When the mole ratio RS$^-$:Fe $\gtrsim$ 5:1 is employed, in acetonitrile, the initial identifiable product is the

$$4FeCl_3 + 4S + 14RS^- \rightarrow [Fe_4S_4(SR)_4]^{2-} + 5RSSR + 12Cl^- \qquad (7)$$

$$4FeCl_3 + 14RS^- \qquad \rightarrow [Fe_4(SR)_{10}]^{2-} + 2RSSR + 12Cl^- \qquad (8)$$

$$[Fe_4(SR)_{10}]^{2-} + 4S \qquad \rightarrow [Fe_4S_4(SR)_4]^{2-} + 3RSSR \qquad (9)$$

mononuclear tetrahedral complex [Fe(SR)$_4$]$^{2-}$ (13) that is produced in reaction (10). Addition of sulphur results in reaction (11), by which the well-characterized binuclear clusters [Fe$_2$S$_2$(SR)$_4$]$^{2-}$ (3) are formed. No further reaction occurs in acetonitrile, but addition of methanol results in the slow formation of the final cluster product by means of reaction (12). The sum of reactions {[4 × (10)] + [2 × (11)] + (12)} is reaction (7). The course of reactions in the two assembly

$$FeCl_3 + 5RS^- \rightarrow [Fe(SR)_4]^{2-} + \tfrac{1}{2}RSSR + 3Cl^- \qquad (10)$$

$$2[Fe(SR)_4]^{2-} + 2S \rightarrow [Fe_2S_2(SR)_4]^{2-} + RSSR + 2RS^- \qquad (11)$$

$$2[Fe_2S_2(SR)_4]^{2-} \rightarrow [Fe_4S_4(SR)_4]^{2-} + RSSR + 2RS^- \qquad (12)$$

[67] T. Herskovitz, B. A. Averill, R. H Holm, J. A. Ibers, W. D. Phillips, and J. F. Weiher, *Proc. Natl. Acad. Sci. USA*, 1972, **69**, 2437.

[68] B. A. Averill, T. Herskovitz, R. H. Holm, and J. A. Ibers, *J. Am. Chem. Soc.*, 1973, **95**, 3523.

[69] K. S. Hagen, J. G. Reynolds, and R. H. Holm, *J. Am. Chem. Soc.*, 1981, **103**, 4054.

[70] G. Christou and C. D. Garner, *J. Chem. Soc., Dalton Trans.*, 1979, 1093.

systems is summarized in Scheme 3. These reaction schemes appear to apply just as well to those alkylthiolate systems in which insoluble polymers are not formed prior to the introduction of sulphur. In addition, iron(II) salts may be used as reactants, resulting in obvious changes in the stoicheiometries of reactions (7), (8), and (10).
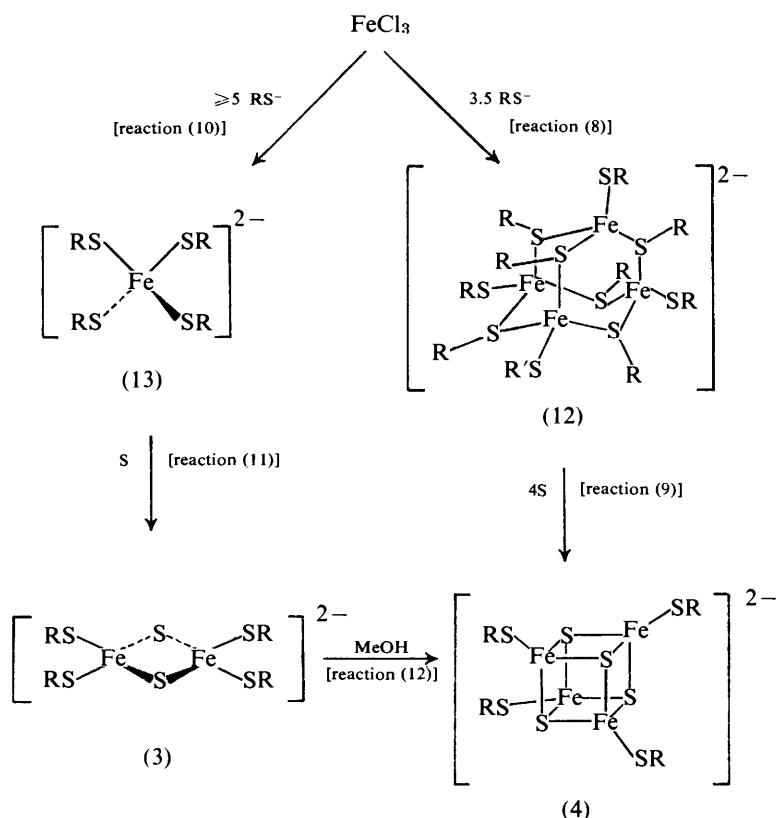
Scheme 3

The reaction sequences depicted in Scheme 3 are notable in several respects. When conducted in methanol solutions, reactions {(8) + (9)} and {(10) + (11) + (12)} afford the cluster (4) in quantitative (> 99 %) yields *in situ*. In the former sequence, the adamantane-like structure (12) has been demonstrated crystallo-graphically for $[Fe_4(SPh)_{10}]^{2-}$,[71] and it is very similar to that of $[Co_4(SPh)_{10}]^{2-}$,

[71] K. S. Hagen, J. M. Berg, and R. H. Holm, *Inorg. Chim. Acta*, 1980, **45**, L17.

prepared earlier.[72] The cluster $[Fe_4(SPh)_{10}]^{2-}$ reacts with elemental sulphur in an 'all-or-nothing' fashion, forming (4) (and no other product) in proportion to the amount of sulphur present. As seen from the stoicheiometry of reaction (9), the reducing equivalents that are required to form the requisite amount of sulphide are contained within the molecule. The quantitative nature of this reaction is impressive upon the observation that all twelve skeletal bonds of (12) must be ruptured in the course of formation of the product cluster. The other reaction sequence is of significance because it provides the demonstration that tetra-nuclear clusters can be elaborated by a series of spontaneous irreversible reactions, commencing with trivial reagents and passing through successive mononuclear and binuclear intermediates. In this sequence, the structures of the intermediates $[Fe(SPh)_4]^{2-}$ (13)[17] and $[Fe_2S_2(SPh)_4]^{2-}$ (3)[39] have been established. Several implications concerning the biosynthesis of Fe_4S_4 protein groups (2) may be drawn from these results. Chief among these are that no protein is required to guide the assembly of a cluster from simple reactants and, given the occurrence of reaction (1) and the reverse of reaction (2) when protein is re-folded, the insertion of a cluster into a peptide environment may be a non-enzymatic process.

B. $MoFe_3S_4$ Clusters.—Identification of reaction sequences that terminate in the formation of $[Fe_4S_4(SR)_4]^{2-}$ has provided a reasonably satisfactory (albeit non-mechanistic) picture of the modes of self-assembly of these clusters. Similar orderly chemistry may ultimately be shown to yield $MoFe_3S_4$ clusters. However, our initial synthetic approaches have relied on the assumption that such clusters might spontaneously assemble from appropriate reactants, as has been shown for the clusters (4). Purposeful synthetic pursuit then reduces to elementary considerations of the choice of reactants and reaction stoicheiometry. Because it is the simplest soluble source of molybdenum and sulphide, and in view of its demonstrated prowess as a ligand,[73] MoS_4^{2-} was employed, together with $FeCl_3$ and thiolate, in the successful reaction system that is shown in Scheme 4. Yields of the three cluster assembly products, *i.e.* $[Mo_2Fe_6S_8(\mu_2-S)(\mu_2-SR)_2(SR)_6]^{3-}$ (14), $[Mo_2Fe_6S_8(\mu_2-SR)_3(SR)_6]^{3-}$ (15), and $[Mo_2Fe_7S_8(\mu_2-SR)_6(SR)_6]^{3-,4-}$ (16), can be optimized by the adjustment of reaction conditions.[74–77] Clusters are readily isolated in the form of quaternary ammonium salts. The indicated bridged 'double-cubane' structures have been demonstrated by X-ray diffraction.[75,78] The analogous tungsten clusters can be prepared from WS_4^{2-}.

[72] I. G. Dance, *J. Am. Chem. Soc.*, 1979, **101**, 6264.
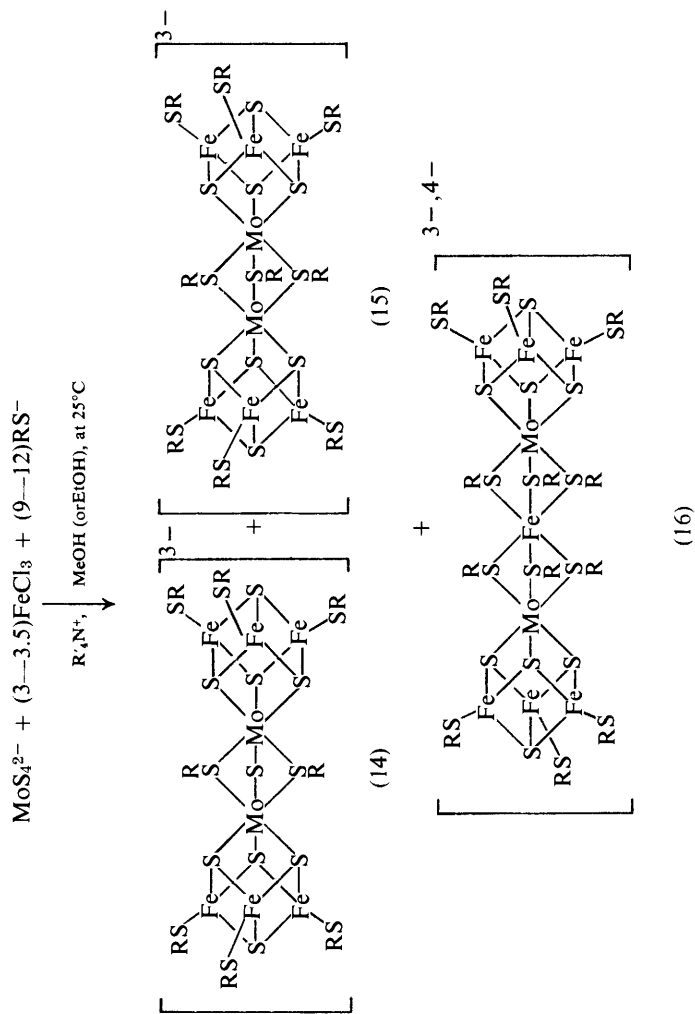[73] E. Diemann and A. Müller, *Coord. Chem. Rev.*, 1973, **10**, 79.
[74] T. E. Wolff, J. M. Berg, C. Warrick, K. O. Hodgson, R. H. Holm, and R. B. Frankel, *J. Am. Chem. Soc.*, 1978, **100**, 4630.
[75] T. E. Wolff, J. M. Berg, K. O. Hodgson, R. B. Frankel, and R. H. Holm, *J. Am. Chem. Soc.*, 1979, **101**, 4140.
[76] T. E. Wolff, J. M. Berg, P. P. Power, K. O. Hodgson, R. H. Holm, and R. B. Frankel, *J. Am. Chem. Soc.*, 1979, **101**, 5454.
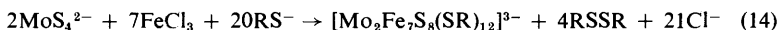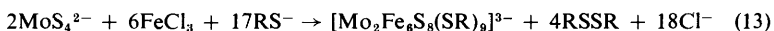[77] T. E. Wolff, P. P. Power, R. B. Frankel, and R. H. Holm, *J. Am. Chem. Soc.*, 1980, **102**, 4694.
[78] T. E. Wolff, J. M. Berg, P. P. Power, K. O. Hodgson, and R. H. Holm. *Inorg. Chem.*, 1980, **19**, 430.

$$MoS_4^{2-} + (3\text{---}3.5)FeCl_3 + (9\text{---}12)RS^-$$

R'$_4$N$^+$, MeOH (orEtOH), at 25°C

(14)

(15)

(16)

Scheme 4

Simultaneous with our initial report of a Fe–Mo–S cluster, *i.e.* [Mo$_2$Fe$_6$S$_9$-(SEt)$_8$]$^{3-}$ (14; R = Et), in 1978,[74] the structure and several properties of [Mo$_2$Fe$_6$S$_8$(SPh)$_9$]$^{3-}$ (15; R = Ph) were described by Christou, Garner, and co-workers.[79,80] Thereafter this group, using essentially the same reaction system as that shown in Scheme 4, has reported the synthesis, structures, and electronic properties of a number of clusters (15) and their tungsten analogues.[81-85]

Clusters (15) and (16) have been the most thoroughly investigated. Their formation is accountable in terms of the limiting stoicheiometries of reactions (13) and (14). Purified yields of cluster salts that exceed 50% from both reactions,

$$2MoS_4^{2-} + 6FeCl_3 + 17RS^- \rightarrow [Mo_2Fe_6S_8(SR)_9]^{3-} + 4RSSR + 18Cl^- \quad (13)$$

$$2MoS_4^{2-} + 7FeCl_3 + 20RS^- \rightarrow [Mo_2Fe_7S_8(SR)_{12}]^{3-} + 4RSSR + 21Cl^- \quad (14)$$

are usual in the author's laboratory. A different type of bridged double-cubane, [Mo$_2$Fe$_6$S$_8$(μ_2-OMe)$_3$(SR)$_6$]$^{3-}$ (17), is isolated when reaction (13) (if R = Ph) is

(17)
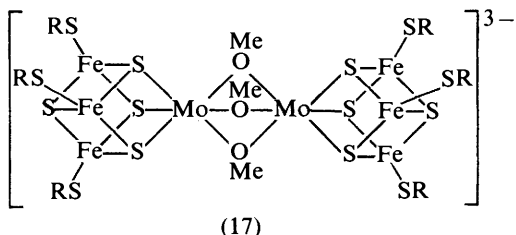
conducted in the presence of excess methoxide ion.[84] The tungsten analogue is formed in methanol in the absence of methoxide.[84,86] The use of Bu^{t}S$^-$ in reaction (13), with no methoxide ion, affords the corresponding cluster (17; R = Bu^t).[87] In this case the Mo(μ_2-SBu^t)$_3$Mo bridge unit is presumably destabilized by steric interactions.

The reaction system given in Scheme 4 has been shown to lead to the assembly of a new family of clusters, the double-cubanes (14)—(17). Each of these contains

[79] G. Christou, C. D. Garner, and F. E. Mabbs. *Inorg. Chim. Acta*, 1978, **28**, L189.
[80] G. Christou, C. D. Garner, F. E. Mabbs. and T. J. King, *J. Chem. Soc., Chem. Commun.*, 1978, 740.
[81] S. R. Acott, G. Christou, C. D. Garner, T. J. King, F. E. Mabbs, and R. M. Miller, *Inorg. Chim. Acta*, 1979, **35**, L337.
[82] G. Christou, C. D. Garner, and R. M. Miller, *J. Inorg. Biochem.*, 1979, **11**, 349.
[83] G. Christou, C. D. Garner, F. E. Mabbs, and M. G. B. Drew, *J. Chem. Soc., Chem. Commun.*, 1979, 91.
[84] G. Christou and C. D. Garner, *J. Chem. Soc., Dalton Trans.*, 1980, 2354.
[85] G. Christou, C. D. Garner, R. M. Miller, C. E. Johnson, and J. D. Rush, *J. Chem. Soc., Dalton Trans.*, 1980, 2363.
[86] G. Christou, C. D. Garner, T. J. King, C. E. Johnson, and J. D. Rush, *J. Chem. Soc., Chem. Commun.*, 1979, 503.
[87] G. Christou, P. K. Mascharak, W. H. Armstrong, G. C. Papaefthymiou, R. B. Frankel, and R. H. Holm, *J. Am. Chem. Soc.*, 1982, **104**, in the press.

the desired $MoFe_3S_4$ unit, incorporated in the structural proposal (10) for the Mo site in nitrogenase. As yet, no individual $MoFe_3S_4$ cluster has been identified among the products of this system, whose initial composition does not evidently preclude the formation of species such as $[MoFe_3S_4(SR)_6]^{2-,3-}$. The reaction steps in the assembly system shown in Scheme 4 are currently under investigation. In reaction (13) the first identifiable species formed is (18; R = Ph), which has been prepared separately.[88,89] This is one member of an expanding group of recently synthesized, smaller Fe–Mo–S complexes, also including $[Cl_2FeMoS_4]^{2-}$ (19),[89-91] $[(PhS)_2Fe_2MoS_6]^{3-}$ (20),[92] $[Cl_4Fe_2MoS_4]^{2-}$ (21),[90,93] $[FeMo_2S_8]^{3-}$ (22),[94,95] $[(C_2H_4S_2)_2FeMo_2S_6]^{3-}$ (23),[96] and $[(NO)_2FeMoS_4]^{2-}$.[97] Certain of these complexes may represent fragments of the Mo site in the enzyme and FeMo-co. For example, there is a resemblance between (21) and the structural

(18) L = RS
(19) L = Cl

(20)

(21)

(22)

(23)
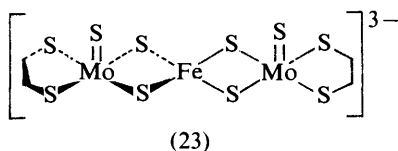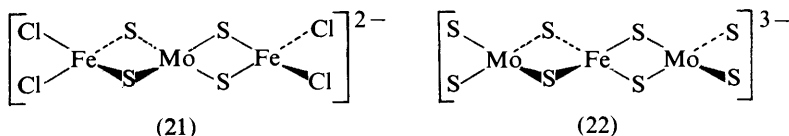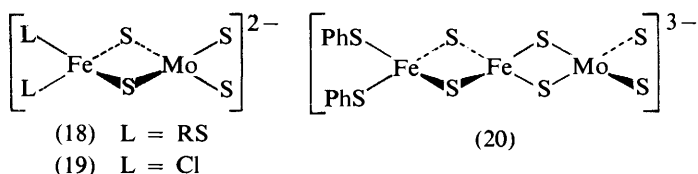
[88] D. Coucouvanis, E. D. Simhon, D. Swenson, and N. C. Baenziger, *J. Chem. Soc., Chem. Commun.*, 1979, 361.

[89] R.-H. Tieckelmann, H. C. Silvis, T. A. Kent, B. H. Huynh, J. V. Waszczak, B.-K. Teo, and B. A. Averill, *J. Am. Chem. Soc.*, 1980, **102**, 5550.

[90] D. Coucouvanis, N. C. Baenziger, E. D. Simhon, P. Stremple, D. Swenson, A. Simopoulos, A. Kostikas, V. Petrouleas, and V. Papaefthymiou, *J. Am. Chem. Soc.*, 1980, **102**, 1732.

[91] A. Müller, H.-G. Tölle, and H. Bögge, *Z. Anorg. Allg. Chem.*, 1980, **471**, 115.

[92] R. H. Tieckelmann and B. A. Averill, *Inorg. Chim. Acta*, 1980, **46**, L35.

[93] A. Müller, S. Sarkar, A.-M. Dommröse, and R. Filgueira, *Z. Nautrforsch., Teil. B*, 1980, **35**, 1592.

[94] J. W. McDonald, G. D. Friesen, and W. E. Newton, *Inorg. Chim. Acta*, 1980, **46**, L79.

[95] D. Coucouvanis, E. D. Simhon, and N. C. Baenziger, *J. Am. Chem. Soc.*, 1980, **102**, 6644.

[96] P. L. Dahlstrom, S. Kumar, and J. Zubieta, *J. Chem. Soc., Chem. Commun.*, 1981, 411.

[97] D. Coucouvanis, E. D. Simhon, P. Stremple, and N. C. Baenziger, *Inorg. Chim. Acta*, 1981, **53**, L135.

proposal (11), and (22) exhibits an $S = \frac{3}{2}$-type e.p.r. spectrum.[66,94] The synthesis and properties of a number of these complexes and their tungsten analogues have been reviewed by Coucouvanis.[30]

4 Properties of MoFe$_3$S$_4$ Clusters

A. Structures.—Single-crystal *X*-ray determinations have proven indispensable in the recognition of these clusters. Thus far, structures of [Mo$_2$Fe$_6$S$_9$(SEt)$_8$]$^{3-}$ (14; R = Et),[75] [Mo$_2$Fe$_6$S$_8$(SEt)$_9$]$^{3-}$ (15; R = Et),[75,81] [Mo$_2$Fe$_6$S$_8$(SPh)$_9$]$^{3-}$ (15; R = Ph),[80] [Mo$_2$Fe$_7$S$_8$(SEt)$_{12}$]$^{3-}$ (16; R = Et),[78] and [Mo$_2$Fe$_7$S$_8$(SCH$_2$Ph)$_{12}$]$^{4-}$ (16; R = CH$_2$Ph)[78] have been solved. In addition, the structure of a cluster that is described as [Mo$_2$Fe$_6$S$_8$(SCH$_2$CH$_2$OH)$_9$]$^{3-}$ has been reported.[83] Our results indicate that this species is the mixed-ligand cluster [Mo$_2$Fe$_6$S$_8$(μ_2-SEt)$_3$-(SCH$_2$CH$_2$OH)$_6$]$^{3-}$. Structures of the tungsten analogues, *i.e.* [W$_2$Fe$_6$S$_8$-
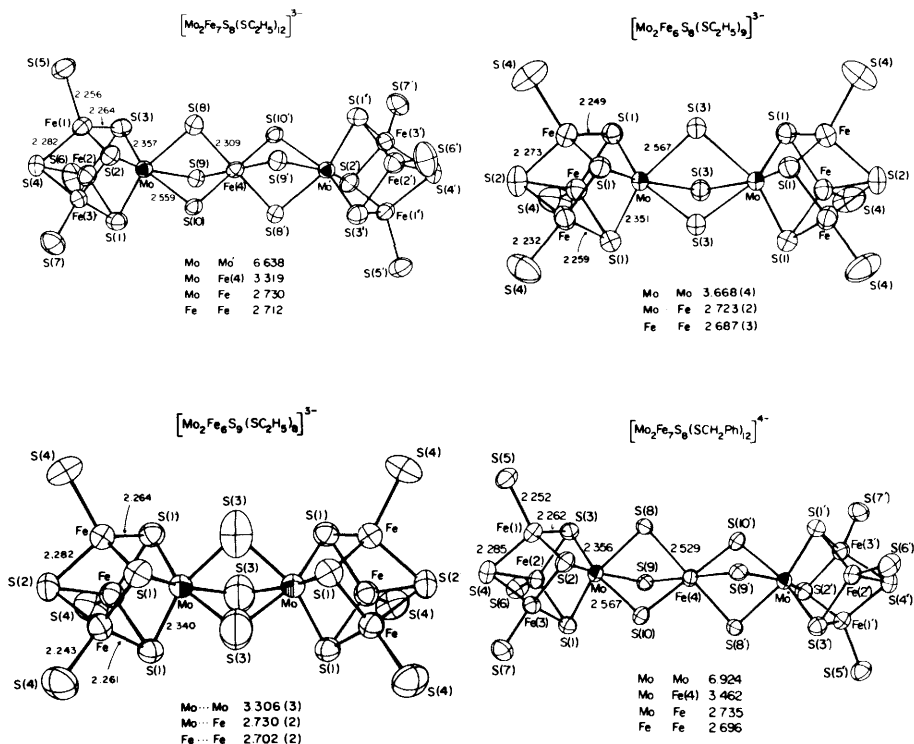
Figure 2 *Structures of* [Mo$_2$Fe$_6$S$_9$(SEt)$_8$]$^{3-}$–(14; R = Et), [Mo$_2$Fe$_6$S$_8$(SEt)$_9$]$^{3-}$ (15; R = Et), [Mo$_2$Fe$_7$S$_8$(SEt)$_{12}$]$^{3-}$ (16; R = Et) *and* [Mo$_2$Fe$_7$S$_8$(SCH$_2$Ph)$_{12}$]$^{4-}$ (16; R = CH$_2$Ph), *excluding carbon atoms and protons*
(Reproduced by permission from *J. Am. Chem. Soc.*, 1979, **101**, 4140 and *Inorg. Chem.*, 1980, **19**, 430)

$(SEt)_9]^{3-}$,[82] $[W_2Fe_7S_8(SCH_2Ph)_{12}]^{4-}$,[78] and $[W_2Fe_6S_8(OMe)_3(SPh)_6]^{3-}$,[86] have also been determined. The latter provides a characterized example of the $M(\mu_2\text{-OMe})_3M$ bridge unit, assuring its presence in the molybdenum clusters (17), which are of corresponding composition and closely related physicochemical properties. The replacement of molybdenum for tungsten effects only small changes in the dimensions of the cluster, as expected from the difference of ~ 0.01 Å in the six-co-ordinate radii of these elements.[98]

Four detailed structures of specific examples of clusters (14)—(16) are set out in Figure 2, and a stereo-view of a cluster of type (15) is shown in Figure 3. Each
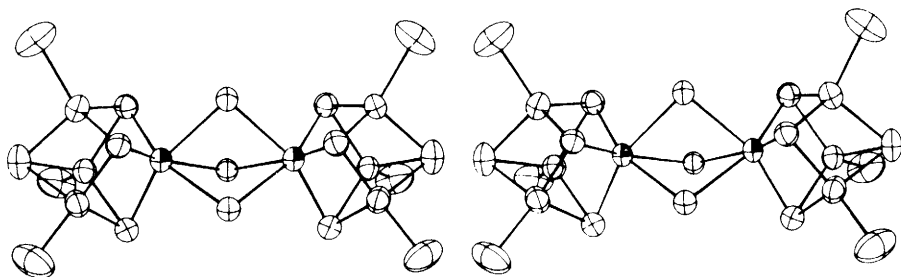

Figure 3 *A stereo-view of the structure of* $[Mo_2Fe_6S_8(SEt)_9]^{3-}$ *(15; R = Et), excluding ethyl groups*
(Reproduced by permission from *J. Am. Chem. Soc.*, 1979, **101**, 4140)

structure consists of two $MoFe_3S_4$ sub-clusters, related by a symmetry centre or plane and connected by different types of bridging units to afford the overall double-cubane arrangements. The sub-clusters, which resemble the cubane-like Fe_4S_4 clusters (4), consist of interpenetrating, nearly concentric, and imperfect $MoFe_3$ and S_4 tetrahedra that afford $MoFe_3S_4$ cores closely approaching idealized C_{3v} symmetry. Each iron atom is terminally co-ordinated by one thiolate ligand. Although all clusters are necessarily mixed-valence in iron, no structure shows any evidence for localized iron(II,III) sites, a property in common with the clusters (4).[13] Six-co-ordination at the molybdenum sites in (14)—(17) is completed by ligation to three bridging atoms. Because of disorder the *bridge* structure of cluster (14) has not been unambiguously established, but there is no doubt as to the presence of three bridging atoms. Chemical analyses[75,77] are consistent with the S:Fe:Mo atom ratio in the formulation (14), which is employed in the following sections. This type of bridge is readily distinguished from that in (15), which is ~ 0.4 Å longer and not disordered. The clusters (16) are bridged by a central iron atom, with trigonally distorted octahedral co-ordination furnished by the two sub-clusters acting as terdentate ligands. The Fe—S bond distances in the bridge[78] and the [57]Fe isomer shifts[77] clearly show the presence of iron(III) and iron(II) in the cluster trianion and tetra-anion, respectively.

[98] R. D. Shannon, *Acta Crystallogr., Sect. A*, 1976, **32**, 751.

Whereas $MoFe_3S_4$ and WFe_3S_4 clusters and the bridging arrangement in (16) are new structural components that have thus far been formed only in the reaction system shown in Scheme 4, the bridge units in (14), (15), and (17) are found in other compounds. Recent structural work has demonstrated the presence of $M(\mu_2\text{-}SR)_3M$ in $[(\eta^7\text{-}C_7H_7)Mo_2(SR)_3(CO)_2L]$, where L is CO[99] or $P(OMe)_3$,[100] $[(\eta^5\text{-}C_5H_5)_2Mo_2(SMe)_3Cl_3]$,[101] and $[W_2(SEt)_3Cl_4(SMe_2)_2]$ (24).[102] Similarly, $W(\mu_2\text{-}S)(\mu_2\text{-}SEt)_2W$ occurs in $[W_2S(SEt)_2Cl_4(C_4H_8S)_2]$ (25)[103] and $M(\mu_2\text{-}OMe)_3M$ in $[(\eta^7\text{-}C_7H_7)(\eta^3\text{-}C_7H_7)Mo_2(OMe)_3(CO)_2]$[104] and in $[(\eta^3\text{-}C_7H_7)(\eta^4\text{-}C_7H_8)W_2\text{-}(OMe)_3(CO)_4]$.[104] With the exception of the last two compounds, M $\cdots$ M separations are substantially shorter than those in (14), (15), and (17) (M $\cdots$ M $\gtrsim 3.2$ Å), where direct metal–metal interactions between sub-clusters contribute no appreciable stability to the double-cubane structures. The W–W distances in (24) [2.505(1) Å] and (25) [2.524(1) Å] are very short, and nearly the same, in

(24)   (25)
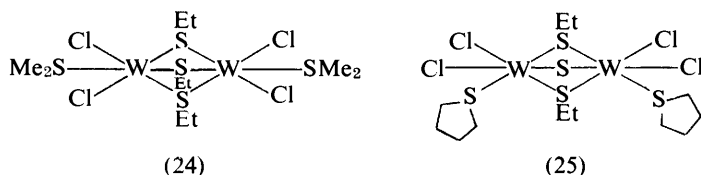
contrast to the corresponding distances in the molybdenum clusters (15) and (14). Strong metal–metal bonding occurs in the two tungsten complexes, which contain unexceptional terminal ligands and whose oxidation states of tungsten atoms are the same or one unit higher than those that are considered most probable for molybdenum atoms in (14) and (15) (see below). Hence it appears that the incorporation of molybdenum (or tungsten) atoms into MFe_3S_4 clusters markedly attenuates the metal–metal interactions that are intrinsic to the $M^{IV}(\mu_2\text{-}S)(\mu_2\text{-}SR)_2M^{IV}$ and $M^{III}(\mu_2\text{-}SR)_3M^{IV}$ bridge units in classical complexes.

The double-cubanes (15)—(17) contain sub-clusters with core $[MoFe_3S_4]^{3+}$ oxidation levels. In (14) the sub-clusters are formally inequivalent, having $[MoFe_3S_4]^{3+,4+}$ oxidation levels, but are crystallographically indistinguishable in the particular salt investigated.[75] The newer clusters $[MoFe_4S_4(SEt)_3\text{-}(cat)_3]^{3-}$ (26)[105] and $[Mo_2Fe_6S_8(\mu_2\text{-}SEt)_2(SEt)_4(3,6\text{-}Pr^n_2cat)_2]^{4-}$ (27)[106] (cat = catecholate), whose syntheses and properties are described in a later section, also contain cores with a net charge of $3+$. Their detailed structures are presented in

[99] K. Weidenhammer and M. L. Ziegler, *Z. Anorg. Allg. Chem.*, 1979, **455**, 29.
[100] I. B. Benson, S. A. R. Knox, P. J. Naish, and A. J. Welch, *J. Chem. Soc., Chem. Commun.*, 1978, 878.
[101] C. Couldwell, B. Meunier, and K. Prout, *Acta Crystallogr., Sect. B*, 1979, **35**, 603.
[102] P. M. Boorman, V. D. Patel, K. A. Kerr, P. W. Codding, and P. Van Roey, *Inorg. Chem.*, 1980, **19**, 3508.
[103] P. M. Boorman, K. A. Kerr, and V. D. Patel, *J. Chem. Soc., Dalton Trans.*, 1981, 506.
[104] K. Weidenhammer and M. L. Ziegler, *Z. Anorg. Allg. Chem.*, 1979, **455**, 43.
[105] T. E. Wolff, J. M. Berg, and R. H. Holm, *Inorg. Chem.*, 1981, **20**, 174.
[106] W. H. Armstrong and R. H. Holm, *J. Am. Chem. Soc.*, 1981, **103**, 6246; and research in progress.

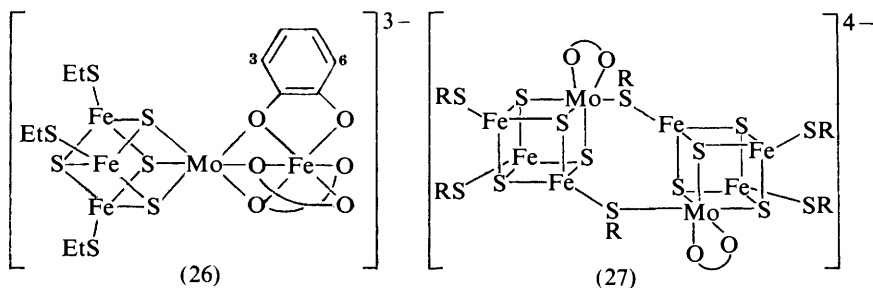(26)   (27)

Figure 4. Consistent with the identity or near-identity of oxidation levels, (sub) cluster Mo–S and Mo–Fe distances occur in the narrow intervals 2.34—2.36 and 2.69—2.73 Å, respectively. These values are in good agreement with those deduced from EXAFS analysis of native FeMo proteins[18] and of FeMo-co (see Table 1). Further, the cubane-type MoFe₃S₄ configuration provides shells of three sulphur and three iron atoms around the molybdenum atom, a configuration that is compatible with the ranges of EXAFS occupancy numbers. Complex (26), with a mean Mo–O distance of 2.15(2) Å,[105] affords an EXAFS spectrum qualitatively resembling that of FeMo-co, in which the molybdenum atom presumably experiences O/N ligand-atom interactions in NMF solution.[53] Other conceivable representations of the Mo site in the native enzyme and FeMo-co have not yet been adequately examined, including an EXAFS test of proposal (11) with a complex such as (21). In the absence of other Mo–Fe–S cluster species of demonstrably more faithful structural features, those containing MoFe₃S₄ units have been pursued as preliminary models on the basis that they contain some structural elements in common with Mo sites in the native enzyme and cofactor.

B. Ligand-substitution Reactions.—The chemistry of the Fe–S clusters (3) and (4) has been significantly expanded by the occurrence of reactions resulting in substitution of thiolate ligands. Effective reactants include electrophiles such as thiols [reaction (1)], acid halides,[107,108] and, more recently, phenol, which affords an isolable salt of $[Fe_4S_4(OPh)_4]^{2-}$.[109] With benzoyl chloride, the fully substituted clusters $[Fe_nS_nCl_4]^{2-}$ ($n = 2$ or 4) have been isolated and structurally characterized.[107,110] The reactions of $[Mo_2Fe_6S_8(SEt)_9]^{3-}$ (15; R = Et) and $[M_2Fe_7S_8(SEt)_{12}]^{3-}$ [M = Mo, *i.e.* (16; R = Et), or W] with thiols and acetyl chloride at ambient temperature have been examined in some detail in the systems shown in Scheme 5 [reactions (15)—(18)].[111] Of the two types of sulphur atoms that are potentially subject to attack by electrophiles, only those of terminal thiolates proved reactive under conditions in which (sub) cluster

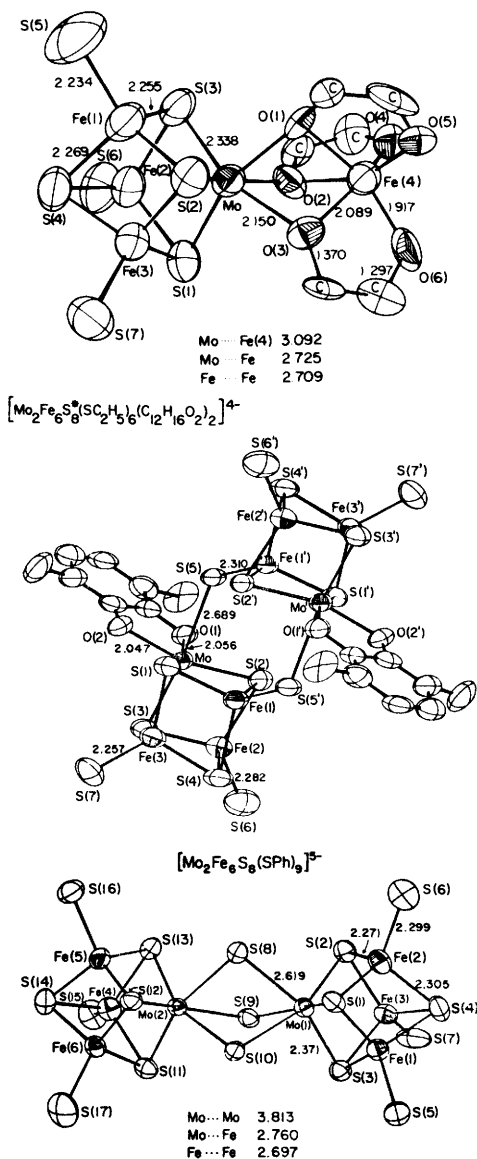[107] G. B. Wong, M. A. Bobrik, and R. H. Holm, *Inorg. Chem.*, 1978, **17**, 578.
[108] R. W. Johnson and R. H. Holm, *J. Am. Chem. Soc.*, 1978, **100**, 5338.
[109] W. E. Cleland and B. A. Averill, *Inorg. Chim. Acta*, 1981, **56**, L9.
[110] M. A. Bobrik, K. O. Hodgson, and R. H. Holm, *Inorg. Chem.*, 1977, **16**, 1851
[111] R. E. Palermo, P. P. Power, and R. H. Holm, *Inorg. Chem.*, 1981, **20**, in the press.

$$\left[Mo_2 Fe_6 S_8^{*}(SC_2H_5)_6(C_{12}H_{16}O_2)_2 \right]^{4-}$$

$$[Mo_2Fe_6S_8(SPh)_9]^{5-}$$

Figure 4 *Structures of* $[MoFe_4S_4(SEt)_3(cat)_3]^{3-}$ (26), $[Mo_2Fe_6S_8(SEt)_6(3,6\text{-}Pr^n_2cat)_2]^{4-}$ (27; $R = Et$), *and* $[Mo_2Fe_6S_8(SPh)_9]^{5-}$. *Carbon atoms of thiolate ligands and a portion of the catecholate* (cat) *rings are omitted*

structures were left intact. Formation of the products $[Mo_2Fe_6S_8(\mu_2\text{-}SR)_3(SR')_6]^{3-}$ (28), $[Mo_2Fe_6S_8(\mu_2\text{-}SR)_3Cl_6]^{3-}$ (29), $[M_2Fe_7S_8(\mu_2\text{-}SR)_6(SR')_6]^{3-}$ (30), and $[M_2Fe_7S_8(\mu_2\text{-}SR)_6Cl_6]^{3-}$ (31) was monitored by spectroscopic and electrochemical methods. Examples of each type of mixed-ligand cluster were isolated. With one exception, our findings are in agreement with results described by other investigators.[83,85,112] Treatment of $[Mo_2Fe_6S_8(SEt)_9]^{3-}$ with excess 2-hydroxyethanethiol affords (28; R = Et, R' = CH_2CH_2OH), and not the fully substituted cluster (15; R = CH_2CH_2OH). Identity of the reaction products obtained in different laboratories[83,111] is assured by the essentially indistinguishable cell constants of their Et_4N^+ salts, which differ from those of authentic $(Et_4N)_3[Mo_2Fe_6S_8(SCH_2CH_2OH)_9]$ prepared by reaction (13).[111] Resolution of this matter presents a consistent picture of the reactivity of the clusters (15) and (16) with thiols and acetyl chloride. Evidently the adjacent metal centres reduce the nucleophilicity of bridging sulphur atoms such that they are not protonated by thiol acids as strong as benzenethiol ($pK_a \approx 6.5$) nor attacked by the strong electrophile acetyl chloride. Kinetic studies of reaction (1) indicate that protonation of bound thiolate is the rate-determining step in substitution,[113] and detection of thioester products in the reaction of (3) or (4) with acid halides[107,108] demonstrates direct attack of electrophile on thiolate. These processes are likely to occur when (15) or (16) react with these reagents. The reaction systems shown in Scheme 5 do not offer a route to individual $MoFe_3S_4$ clusters nor to single- or double-cubane species carrying a labile substituent at the molybdenum site, which is a possible requisite to the binding and activation of substrates of nitrogenase (excluding H^+; see below) by this series of compounds. Other experiments have shown that the iron(II)-bridged form of (16) is more reactive than its iron(III) counterpart, and that the bridge structure of the latter may be disrupted with catechols. Some results from the second approach are described subsequently.

C. Electron-transfer Reactions.—Electrochemical studies have established the electron-transfer series shown in equilibria (19)—(21), based on the trianionic clusters (14)—(17).[77,85,87,111] Evidence for these series, in the form of cyclic voltammograms of clusters in which R is ethyl, is presented in Figure 5. In each, the steps with potentials E_1 and E_2 correspond to one-electron reactions of subclusters, resulting in changes in their oxidation levels and in the internal

$$(14): [(\alpha + \gamma)]^{3-} \underset{\text{or } [(\alpha + \delta)]^{4-}}{\overset{E_1}{\rightleftharpoons} [(\beta + \gamma)]^{4-}} \overset{E_2}{\rightleftharpoons} [(\beta + \delta)]^{5-} \qquad (19)$$

$$(15), (17): [(2\alpha)]^{3-} \overset{E_1}{\rightleftharpoons} [(\alpha + \beta)]^{4-} \overset{E_2}{\rightleftharpoons} [(2\beta)]^{5-} \qquad (20)$$

$$(16): [(Fe^{3+} + 2\alpha)]^{3-} \rightleftharpoons [(Fe^{2+} + 2\alpha)]^{4-} \overset{E_1}{\rightleftharpoons} [(Fe^{2+} + \alpha + \beta)]^{5-}$$
$$\overset{E_2}{\rightleftharpoons} [(Fe^{2+} + 2\beta)]^{6-} \qquad (21)$$

$$\alpha = [Mo^{3+}Fe^{2.67+}{}_3S^{2-}{}_4]^{3+} \quad , \quad \gamma = [Mo^{4+}Fe^{2.67+}{}_3S^{2-}{}_4]^{4+}$$
$$\beta = [Mo^{3+}Fe^{2.33+}{}_3S^{2-}{}_4]^{2+} \quad , \quad \delta = [Mo^{4+}Fe^{2.33+}{}_3S^{2-}{}_4]^{3+}$$
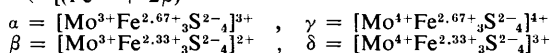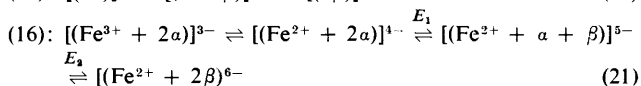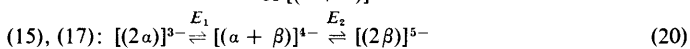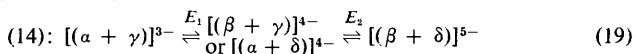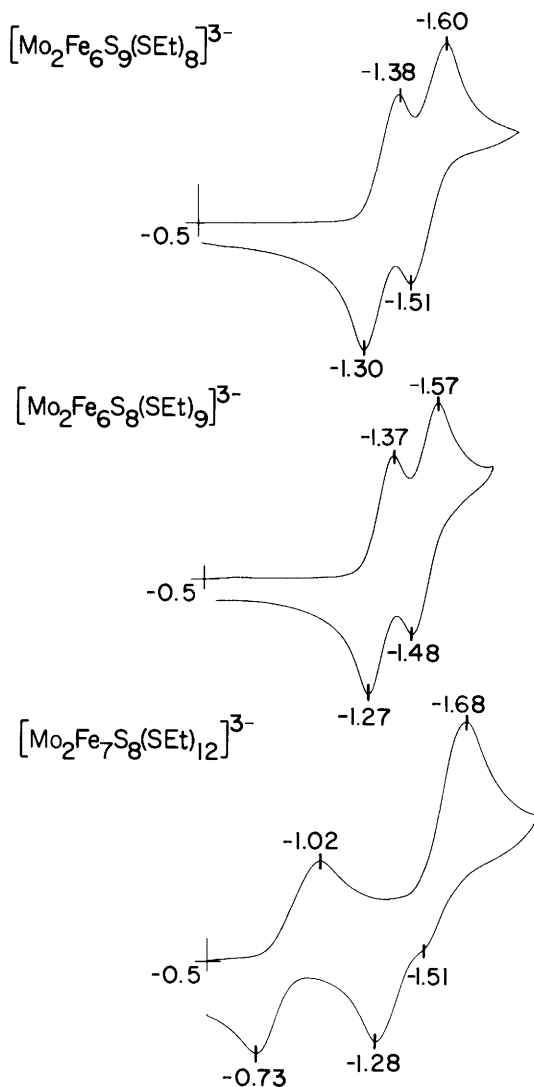
[112] G. Christou and C. D. Garner, *J. Chem. Soc., Chem. Commun.*, 1980, 613.
[113] G. R. Dukes and R. H. Holm, *J. Am. Chem. Soc.*, 1975, **97**, 528.

Scheme 5

$$\left[Mo_2Fe_6S_9(SEt)_8\right]^{3-}$$

$$\left[Mo_2Fe_6S_8(SEt)_9\right]^{3-}$$
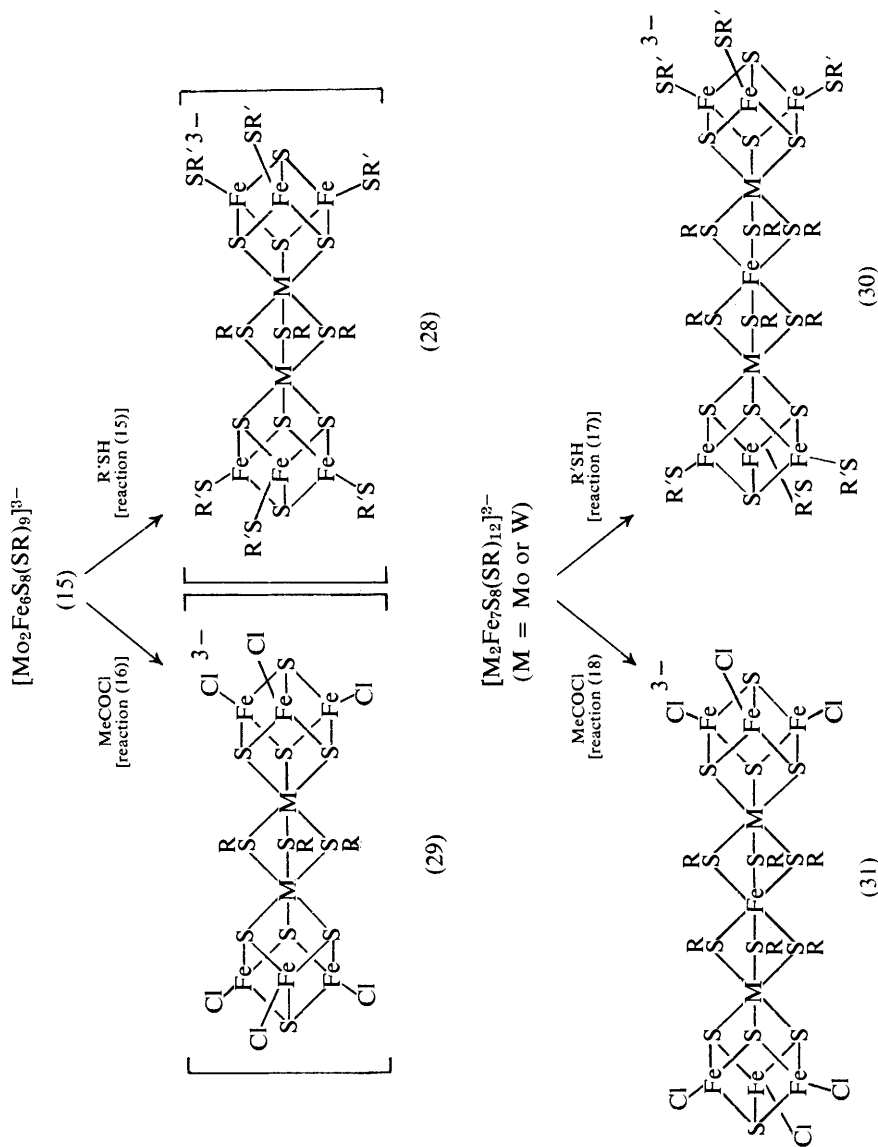
$$\left[Mo_2Fe_7S_8(SEt)_{12}\right]^{3-}$$

Figure 5 *Cyclic voltammograms of* $[Mo_2Fe_6S_9(SEt)_8]^{3-}$ (14; R = Et), $[Mo_2Fe_6S_8\text{-}(SEt)_9]^{3-}$ (15; R = Et), *and* $[Mo_2Fe_7S_8(SEt)_{12}]^{3-}$ (16; R = Et), *recorded in DMF solutions at* 100 mV s^{-1}. *Peak potentials/V versus the saturated calomel electrode (s.c.e.) are indicated. A platinum working electrode was used*

distribution of electrons, as represented by formulations α—δ. The series (21) also contains the Fe^{3+}/Fe^{2+} step of the bridging iron atom, which occurs at potentials that are less negative than E_1 and E_2. In addition, $(2-)$ and $(1-)$

477

clusters of type (15) are detectable by cyclic voltammetry, as is the (2−) species of cluster (14). In this regard, the two one-electron oxidations of the cluster (17; R = Bu^t) are particularly well defined by both cyclic voltammetry and differential pulse polarography,[87] but, as yet, no oxidized species have been isolated. There is also electrochemical evidence for the (6−) and (7−) forms of certain clusters (15).[85] The very negative potentials ($\lesssim$ − 2 V) at which these species are produced render their isolation extremely difficult. Consequently, effective experimentation is limited to the reduced members within the series of equilibria (19)—(21). Inasmuch as all transformations of substrates of nitrogenase are reductive, these species are of principal concern in the context of models of the enzyme.
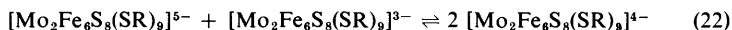
The electron-transfer steps in the series of equilibria (19)—(21) do not meet all electrochemical criteria for reversible transfer of charge, but they are effectively reversible, chemically, in that anodic and cathodic peak currents for each are essentially equal. Chemical reversibility is also demonstrated by the spectroelectrochemical experiment depicted in Figure 6 for a typical cluster of type (15). Here the cycle (3−) → (4−) → (5−) → (4−) → (3−) is traversed over a period of about four hours, with 5—7% loss in intensity of the 394 nm band of the re-oxidized (3−) cluster. Reduction of [Mo$_2$Fe$_6$S$_8$(SPh)$_9$]$^{3-}$ with sodium acenaphthylenide affords [Mo$_2$Fe$_6$S$_8$(SPh)$_9$]$^{5-}$, which, although very
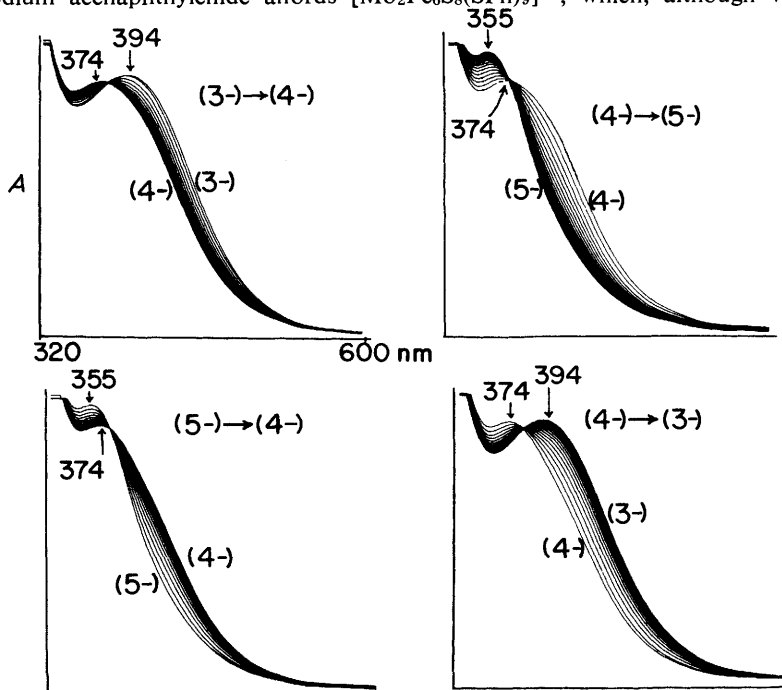
Figure 6 *Absorption spectra of* [Mo$_2$Fe$_6$S$_8$(SEt)$_9$]$^{3-,4-,5-}$, *measured in an optically transparent thin-layer electrochemical (OTTLE) cell in DMF solution*

sensitive to oxidation, has been isolated as its crystalline Et_4N^+ salt. Its structure, shown in Figure 4, reveals that there is retention of the double-cubane arrangement, with a substantial lengthening (by 0.15 Å) of the Mo $\cdots$ Mo distance as compared to $(3-)$ clusters. Oxidized and reduced clusters, in solution, immediately equilibrate by the comproportionation reaction (22), for which $\log K_{com} = (E_1 - E_2)/0.059$. For the redox series based on clusters (14), (15),

$$[Mo_2Fe_6S_8(SR)_9]^{5-} + [Mo_2Fe_6S_8(SR)_9]^{3-} \rightleftharpoons 2\ [Mo_2Fe_6S_8(SR)_9]^{4-} \quad (22)$$

and (17), $(E_1 - E_2) = 200 \pm 20$ mV and $K_{com} \approx 10^4$. The difference of potentials is much greater than the statistical value of 36 mV, indicating that there are interactions between sub-clusters when they are 3.2—3.8 Å apart. In clusters of type (16), where the Mo $\cdots$ Mo separation is 6.9 Å (see Figure 2) prior to reduction, $(E_1 - E_2)$ is much smaller, and usually is not resolved. This behaviour is evident in the cyclic voltammogram of $[Mo_2Fe_7S_8\text{-}(SEt)_{12}]^{3-}$ in Figure 5, where $(E_1 - E_2) < 100$ mV is a reasonable estimate. These separations of potentials provide a simple means of identifying products of synthesis that contain triply bridged double-cubanes, and they are frequently used in the author's laboratory for that purpose. One application has been the demonstration of the retention of bridge structure in the ligand-substitution products [(28)—(31)].[111] Proton n.m.r. spectra, which exhibit isotropically shifted resonances, owing to paramagnetism of the cluster, are also valuable in identifying double-cubane structural types and the positions in which ligands have substituted.[77,84,111]

D. Electron Distribution in Sub-clusters.—In addition to a longer Mo $\cdots$ Mo distance and attendant small increases in bridge Mo–S distances and Mo–S–Mo angles, the structure of $[Mo_2Fe_6S_8(SPh)_9]^{5-}$ (Figure 4) reveals other differences when compared to those of several $[Mo_2Fe_6S_8(SR)_9]^{3-}$ structures.[87] The Mo–S, Mo $\cdots$ Fe, and Fe–S distances in sub-clusters are marginally longer (by 0.01—0.02 Å), resulting in a mean increase in the volume of $MoFe_3S_4$ of 2.6%. The mean terminal Fe–SPh distance is significantly longer (by ~ 0.07 Å). Similar behaviour is found in structural comparisons of the $(2-)$ and $(3-)$ clusters (4). In the pairs $[Fe_4S_4(SPh)_4]^{2-,3-}$ (1.9%, 0.032 Å)[9,38] and $[Fe_4S_4(SCH_2Ph)_4]^{2-,3-}$ (2.6%, 0.046 Å),[68,114] the increases in the volume of the Fe_4S_4 core and of the Fe–SR bond length assume the stated values. Unlike these clusters, in which structural changes of the core are essentially localized in particular bonds, leading to a change from an idealized geometry to another, changes in bond distances in the sub-clusters in $[Mo_2Fe_6S_8(SPh)_9]^{5-}$ are nearly uniformly distributed.

The structural results indicate small alterations of the Fe sites in clusters of type (15) upon reduction of each sub-cluster by one electron. In order to pursue the relationship between these changes and the distribution of electrons in the sub-cluster in different oxidation levels of the cluster, a procedure based on ^{57}Fe isomer shifts (δ), from Mössbauer spectra, has been explored.[75,87] For

[111] J. M. Berg, K. O. Hodgson, and R. H. Holm, *J. Am. Chem. Soc.*, 1979, **101**, 4586.

various Fe–S complexes whose compositions define the (mean) oxidation states s of iron (ranging from $2+$ to $3+$), values of δ are well fitted by the linear equation (23). This equation refers to values of δ for tetrahedral FeS_4 sites that

$$\delta/\text{mm s}^{-1} = 1.44 - 0.43\,s \qquad (23)$$

are measured at 4.2 K and for which the reference is iron metal at this temperature. As examples, the dianionic and trianionic clusters (4) provide cases of $Fe^{2\cdot5+}$ and $Fe^{2\cdot25+}$, respectively. Mössbauer spectra of the trianionic clusters (14)—(17),[75,77,85,87] and of the tetra-anionic (Fe^{II}-bridged) form of (16)[77] have been described. Spectra of sub-clusters have been interpreted in terms of two or three incompletely resolved quadrupole doublets, with very similar values of δ. In none of these clusters (or in any considered below) do isomer shifts correspond to localized iron(II,III) sites; this is a behaviour that has previously been noted for the clusters (4).

Mössbauer spectra of the $[Mo_2Fe_6S_8(SPh)_9]^{3-,4-,5-}$ clusters have been examined in frozen solutions at 4.2—80 K;[87] the $(4-)$ cluster, which has not been isolated, was generated by reaction (22). The spectra were analysed as two quadrupole doublets whose isomer shifts yield, from equation (23), the oxidation-state formulations α and β as indicated in the series of equilibria (20). In these and other formulations the superscripted oxidation state of iron is the mean value of the states of the three iron atoms. Spectra of the $(4-)$ cluster are interpretable by a model involving localized $(\alpha + \beta)$ sub-clusters, indicating that the rate-constant for intramolecular exchange of electrons, k, is $\leqslant 10^7$ s^{-1} at $T \leqslant 80$ K. Similar experiments with the $[Mo_2Fe_6S_9(SEt)_8]^{3-,5-}$ clusters afford the sub-cluster formulations in the series of equilibria (19). The descriptions of the $(4-)$ cluster, whose spectrum was not determined, have been inferred from the results for the $(3-)$ and $(5-)$ clusters. In all cases, the oxidation states of molybdenum were obtained by difference. Reduced members of the series of equilibria (20), based on the clusters (17), and of the series (21) have not been examined by Mössbauer spectroscopy. The descriptions that are shown are based on the indistinguishability of values of δ of the oxidized clusters and of $[Mo_2Fe_6S_8(SR)_9]^{3-}$ and by analogy with the series of equilibria (20) derived from the latter cluster.

The formulations α—δ for the sub-clusters are not intended as literal descriptions but only as the best current estimates of distribution of charge over the metal sites in these electronically complex molecules. The more specific description $Fe^{2\cdot67+}$ is preferred to the value $+2.5$ (±0.1), which has been proposed by other investigators,[85] for clusters of the types (15) and (17). The latter mean oxidation state requires electronically inequivalent sub-clusters in double-cubane trianions of these types; this is a feature for which there is no clear spectroscopic or structural evidence. The presence of localized sub-clusters in $[Mo_2Fe_6S_8(SR)_9]^{4-}$ argues against equilibration of inequivalent electron distributions in sub-clusters by internal transfer of electrons at a rate that is fast when compared to the time-scale of Mössbauer spectra. This may not be the case for the members of the series of equilibria (19), which possibly possess an electronically delocal-

ized Mo–S–Mo bridge. In this event, the description $Mo^{3\cdot5+}$ is more appropriate than ones with integral oxidation states.

The interpretations of charge distribution in the series of equilibria (19)—(21) lead to the proposal that changes in electronic structure that are consequent to electron-transfer reactions of double-cubanes are largely confined to sub-cluster Fe_3 portions and associated sulphur atoms. The oxidation state of the molybdenum atom in a given series is considered to remain nearly constant. Several other observations are supportive of this proposal. The increase in Fe–SPh bond distances upon reduction of a cluster of type (15) to the (5 −) form must be associated with the larger radius of tetrahedral iron(II) *vs.* that of iron(III) (0.14) Å).[98] Redox potentials of the couples $[Mo_2Fe_6S_8(SR)_9]^{3-,4-}$ [series (20)] and $[Fe_4S_4(SR)_4]^{2-,3-}$ vary linearly [according to the relation (24)] as the substituent R is varied, and at parity of R they are very nearly equal.[87] These results indicate that, in the first couple, the orbitals in the sub-cluster that are involved in the redox process are appreciably Fe–S in character. These and other points of evidence concerning changes in electronic structure in the redox series, as well as a fuller description of comparative $[Mo_2Fe_6S_8(SR)_9]^{3-,5-}$ structures, are presented elsewhere.[75,77,87]

$$[E_1(\text{Fe–Mo–S})/\text{V}] = [0.85\, E\, (\text{Fe–S})/\text{V}] - 0.16 \text{ (in DMF, } vs \text{ the s.c.e.)} \quad (24)$$

E. The Reduction of Substrates.—A reduced double-cubane cluster, such as $[Mo_2Fe_6S_8(SR)_9]^{5-}$, presents the uncommon property of being an electron carrier that is capable of delivering two electrons at strongly reducing potentials that are separated by only ~ 200 mV. This property raises the possibility of exploiting reduced clusters in the reduction of substrates that are generally considered to be transformed in one or more 'concerted' two-electron steps, with accompanying protonation. Substrates of nitrogenase, such as those shown in Scheme 1, are of this type. The clusters could act as electron donors to other molecules that contain the site of reaction, or they themselves might function both as carriers and reactants in stoicheiometric or catalytic processes. An example of the first type of behaviour has been found. A water-soluble version of cluster (15) has been shown to be capable of replacing Fd in an illuminated chloroplast/hydrogenase system that evolves dihydrogen.[115] The oxidation level(s) of the cluster under turnover conditions is (are) not known. The presence of hydrogenase is obligatory to activity inasmuch as the cluster does not evolve dihydrogen in the presence of reduced Fd. To examine the second possibility, research on reductive transformations of substrates in systems that contain reduced double-cubanes has been initiated.

Using the isolated salt of $[Mo_2Fe_6S_8(SPh)_9]^{5-}$, and benzenethiol as a protic substrate, the formation of dihydrogen in solutions in *NN*-dimethylacetamide, at 25 °C, has been demonstrated.[116] In the presence of a sufficient excess of thiol (~ 500 equivalents), a quantitative yield of dihydrogen, based on the overall
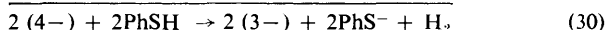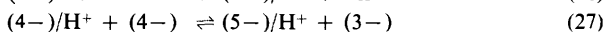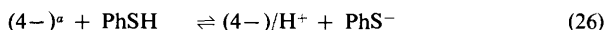
[115] M. W. W. Adams, K. K. Rao, D. O. Hall, G. Christou, and C. D. Garner, *Biochim. Biophys, Acta,* 1980, **589**, 1.
[116] G. Christou, R. V. Hageman, and R. H. Holm, *J. Am. Chem. Soc.,* 1980, **102**, 7600.

reaction (25), is obtained. With smaller excesses of thiol, the yields of dihydrogen

$$[Mo_2Fe_6S_8(SPh)_9]^{5-} + 2PhSH \rightarrow [Mo_2Fe_6S_8(SPh)_9]^{3-} + 2PhS^- + H_2 \qquad (25)$$
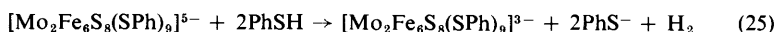
are reduced, and the $(4-)$ cluster accumulates, at least partly as a consequence of reaction (22). Kinetic studies of the evolution of dihydrogen in systems that contain the $(4-)$ cluster [generated by reaction (22)] are in progress.[117] The sequence of reactions (26)—(29), corresponding to the overall reaction (30), is *provisionally* offered as a description of the course of events leading to the formation of dihydrogen. Protonation occurs at a bridging or terminal thiolate sulphur atom. The two-electron-transfer property of the double-cubane occurs in reaction (28), in which a transient hydride, presumably stabilized to an extent by Mo–H⁻ or Fe–H⁻ interactions, is proposed. This species is then protonated in a slow step (29) to afford dihydrogen and the oxidized cluster. The reaction sum $\{(22) + (30)\} = (25)$.

$$(4-)^a + PhSH \rightleftharpoons (4-)/H^+ + PhS^- \qquad (26)$$

$$(4-)/H^+ + (4-) \rightleftharpoons (5-)/H^+ + (3-) \qquad (27)$$

$$(5-)/H^+ \rightarrow (3-)/H^- \qquad (28)$$

$$(3-)/H^- + PhSH \rightarrow (3-) + PhS^- + H_2 \qquad (29)$$

$$\overline{2\,(4-) + 2PhSH \rightarrow 2\,(3-) + 2PhS^- + H_2} \qquad (30)$$

$^a(4-)$ refers to the cluster $[Mo_2Fe_6S_8(SR)_9]^{4-}$; $(3-)$ and $(5-)$ refer to similar clusters.

In similar reaction systems, based on $[Mo_2Fe_6S_8(SPh)_9]^{5-}$ and oxygen acids, or other thiols, the evolution of dihydrogen is found, but yields are less than quantitative when 500 equivalents are used. With only 2 equivalents of Et_3NH^+ as the protic substrate, $\sim 80\%$ of the theoretical amount of dihydrogen (compared to $\sim 30\%$ with 2 equivalents of benzenethiol) is evolved.[117] Kinetic studies on the latter systems are under way. These results are not to be construed as requiring a single two-electron-donor cluster for the formation of dihydrogen. Under the same conditions, a yield of $\sim 30\%$ of dihydrogen, based on reaction (31), is obtained with ~ 500 equivalents of thiol.[116,117] This reaction is the more

$$2[Fe_4S_4(SPh)_4]^{3-} + 2PhSH \rightarrow 2[Fe_4S_4(SPh)_4]^{2-} + 2PhS^- + H_2 \qquad (31)$$
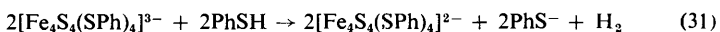
appropriate as a model system for hydrogenase inasmuch as these enzymes[118] contain no molybdenum, and there is some evidence for the presence of Fe_4S_4 clusters.[33,118] A reaction sequence analogous to reactions (26)—(29), in which one reduced cluster acts as a reductant and another as both a reductant and a reaction site, is readily conceived. Incorporation of a reaction site and a capacity to transfer two electrons into the same molecule may provide kinetic and thermodynamic advantages, however. At present we view a reaction system such as reaction (25), together with its final mechanistic description, as being potentially useful in interpreting the formation of dihydrogen in enzymes that contain several clusters that are juxtaposed, so as to promote the efficient internal transfer of electons to the substrate. No substrate-reducing ability has yet been detected for the oxidized clusters $[Mo_2Fe_6S_8(SR)_9]^{3-}$ and $[Fe_4S_4(SR)_4]^{2-}$.

[117] T. Yamamura, G. Christou, and R. H. Holm, unpublished results.
[118] M. W. W. Adams, L. E. Mortenson, and J.-S. Chen, *Biochim. Biophys. Acta*, 1981, **594**, 105.

5 Structural Alterations of the Molybdenum Site in Clusters

At present there is no unequivocal evidence that binding and activation of substrates of nitrogenase takes place at a molybdenum-containing site in the native enzyme or in FeMo-co. The frequently espoused view that such a site is directly involved in enzymatic catalysis derives a measure of support from observations of abiological systems. Stable molybdenum–dinitrogen complexes have been isolated and structurally characterized,[31] patterns of the protonation of dinitrogen that is co-ordinated at electron-rich Mo atom centres have been established,[119-122] and appreciable quantities of ammonia have been detected among reaction products from Mo/N_2 systems that had been treated with protic or hydridic reagents.[119-121,123-128] Detailed consideration of these results, many of which have been reviewed,[31,32] is beyond the purview of this report. While the systems employed are decidedly non-physiological in terms of the ligation of the molybdenum atom and/or its oxidation state, they have provided the stimulating result that dinitrogen can be bound to and activated by molybdenum, and converted into ammonia under ambient conditions. Further, the establishment of modes of protonation of co-ordinated dinitrogen provides a valuable mechanistic baseline for any such systems with more physiologically relevant molybdenum complexes that may be shown to be capable of reducing dinitrogen.

In the present context, it should be remembered that a comparable effort has not been expended by the inorganic chemists to devise dinitrogen-fixing systems that are based on iron complexes. The occurrence of reaction (31) is one indication that Fe–S clusters can effect reductive transformations. Another is reaction (32), which gives $\leqslant 60\%$ yields of ethylene.[129] When a deuterio-acid is present, the predominant stereochemical product is cis-1,2-$C_2H_2D_2$, as is found in the reduction of acetylene by nitrogenase.

$$2[Fe_4S_4(SPh)_4]^{3-} + 2HOAc + C_2H_2 \rightarrow 2[Fe_4S_4(SPh)_4]^{2-} + 2OAc^- + C_2H_4 \quad (32)$$
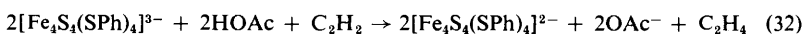
While mindful of these results, we are inclined toward the position that any

[119] J. Chatt, A. J. Pearman, and R. L. Richards, *J. Chem. Soc., Dalton Trans.*, 1977, 1852, 2139; *ibid.*, 1978, 1766.

[120] R. N. F. Thorneley, J. Chatt, R. R. Eady, D. J. Lowe, M. J. O'Donnell, J. R. Postgate, R. L. Richards, and B. E. Smith, in ref. 25, Vol. 1, pp. 171—193.

[121] T. Takahashi, Y. Mizobe, M. Sato, Y. Uchida, and M. Hidai, *J. Am. Chem. Soc.*, 1980, **102**, 7461; *ibid.*, 1979, **101**, 3405.

[122] R. A. Henderson, *J. Organomet. Chem.*, 1981, **208**, C51.

[123] G. E. Bossard, D. C. Busby, M. Chang, T. A. George, and S. D. Iske, Jr., *J. Am. Chem. Soc.*, 1980, **102**, 1001.

[124] J. A. Baumann and T. A. George, *J. Am. Chem. Soc.*, 1980, **102**, 6153.

[125] B. J. Weathers, J. H. Grate, N. A. Strampach, and G. N. Schrauzer, *J. Am. Chem. Soc.*, 1979, **101**, 925.

[126] E. L. Moorehead, P. R. Robinson, T. M. Vickrey, and G. N. Schrauzer, *J. Am. Chem. Soc.*, 1976, **98**, 6555.
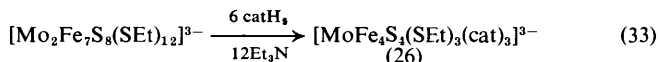
[127] G. N. Schrauzer, P. R. Robinson, E. L. Moorehead, and T. M. Vickrey, *J. Am. Chem. Soc.*, 1976, **98**, 2815.

[128] C. R. Brûlet and E. E. van Tamelen, *J. Am. Chem. Soc.*, 1975, **97**, 911.

[129] R. S. McMillan, J. Renaud, J. G. Reynolds, and R. H. Holm, *J. Inorg. Biochem.*, 1979, **11**, 213.

eventually productive reaction chemistry of MoFe$_3$S$_4$ clusters with dinitrogen is likely to involve the molybdenum site. Although a reaction scheme such as reactions (26)—(29), involving protonation of a ligand followed by bond-breaking and internal transfer of an electron, leading to a transitory metal hydride, can be entertained with protic molecules, clusters of the types (14)—(17) and their reduced forms are likely to prove ineffective in the activation of other substrates of nitrogenase. Co-ordinative saturation of the molybdenum atom is evident. Indeed, the clusters [Mo$_2$Fe$_6$S$_8$(SPh)$_9$]$^{3-,5-}$ {as well as [Fe$_4$S$_4$(SPh)$_4$]$^{2-,3-}$} do not react with dinitrogen at ambient temperature. Current research is directed at the introduction of more labile and chemically manipulable ligand environments external to the MoFe$_3$S$_4$ cluster structure. A related goal is the isolation of single MoFe$_3$S$_4$ clusters in order that their electronic features, unperturbed by a neighbouring cluster [*e.g.* (14), (15), or (17)] or paramagnetic ion [*e.g.* (16)], can be examined.

A. **Bridge-cleavage Reactions and Products.**—Inasmuch as substitution or cleavage of the bridge of clusters (15) and (16) does not result from their treatment with thiols or acetyl chloride under mild conditions, the reactivity of the iron(III)-bridged cluster (16) with other reagents has been explored. Because of the high affinity of catechols for iron(III),[130] reaction (33) was carried out.[105] The unanticipated cluster (26) was isolated as its Et$_4$N$^+$ salt in $\lesssim 50\%$ yield,

$$[Mo_2Fe_7S_8(SEt)_{12}]^{3-} \xrightarrow[\text{12Et}_3\text{N}]{\text{6 catH}_\text{2}} [MoFe_4S_4(SEt)_3(cat)_3]^{3-} \quad (33)$$
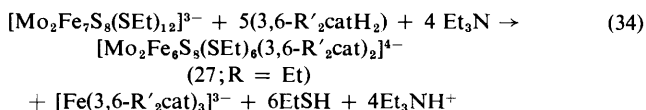$$(26)$$

based on molybdenum. Other molybdenum-containing products have not been purified sufficiently for identification. The structure of (26) (see Figure 4) consists of a single MoFe$_3$S$_4$ cluster with the α electronic formulation, and a [Fe(cat)$_3$]$^{3-}$ subunit is triply bridged to the molybdenum atom.[105] This arrangement leads to a decrease of the trigonal twist angle of the subunit by $\sim 12°$ compared to free [Fe(cat)$_3$]$^{3-}$,[131] and an overall symmetry of the cluster that closely approches C_3. Cluster (26), while of interest in its own right, does not provide a magnetically unperturbed single-cubane species. Means of removing the [Fe(cat)$_3$]$^{3-}$ subunit from the molybdenum site are under investigation. However, this cluster has provided a simple clue leading to entry into a new type of cluster structure. Inspection of molecular scale-models indicates that there are unfavourable steric interactions between ethanethiolate ligands and the 3-substituent on the catecholate ligand when the latter is a methyl or a larger group. This feature suggests that a different reaction product might be obtained from cluster (16) and an appropriate 3,6-disubstituted catechol.

Reaction (34) (where R is C$_3$H$_5$ or Pr^n) proceeds smoothly at room temperature to afford the previously unknown clusters (27), obtained as their Et$_4$N$^+$ salts in
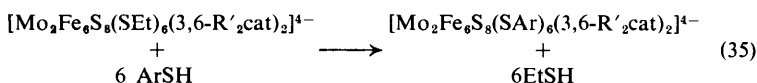
[130] A. Avdeef, S. R. Sofen, T. L. Bregante, and K. N. Raymond, *J. Am. Chem. Soc.*, 1978, **100**, 5362.
[131] K. N. Raymond, S. S. Isied, L. D. Brown, F. R. Fronczek, and J. N. Nibert, *J. Am. Chem. Soc.*, 1976, **98**, 1767.

$$[Mo_2Fe_7S_8(SEt)_{12}]^{3-} + 5(3,6-R'_2catH_2) + 4 Et_3N \rightarrow \quad (34)$$
$$[Mo_2Fe_6S_8(SEt)_6(3,6-R'_2cat)_2]^{4-}$$
$$(27; R = Et)$$
$$+ [Fe(3,6-R'_2cat)_3]^{3-} + 6EtSH + 4Et_3NH^+$$

$\sim 50\%$ yield after purification.[106] Tungsten clusters can be prepared in an analogous manner. The structure of $[Mo_2Fe_6S_8(SEt)_6(3,6-Pr^n_2cat)_2]^{4-}$, shown in Figure 4, is that of a centrosymmetric double-cubane, having sub-clusters of the α electronic formulation.[106] Each molybdenum atom is chelated by a catecholate, and six-co-ordination is completed by thiolate ligation in the form of a $Mo(\mu_2\text{-SEt})Fe$ bridge unit. The bridges, which are of a type that has not previously been encountered, present a particularly interesting point of reactivity. The Fe–S bond is 0.04 Å longer than the average Fe–SEt terminal bonds and the Mo–S bond is ~ 0.12 Å longer than Mo–SR distances in the bridges of clusters of the types (14)—(16). The possible substitutional lability of the bridge that is implied by these results has been investigated.

In contrast to the outcome of reactions (15) and (17), treatment of the clusters (27) with a small excess of arylthiol, as shown in reaction (35), results in the

$$[Mo_2Fe_6S_8(SEt)_6(3,6-R'_2cat)_2]^{4-} \qquad [Mo_2Fe_6S_8(SAr)_6(3,6-R'_2cat)_2]^{4-}$$
$$+ \qquad \longrightarrow \qquad + \qquad (35)$$
$$6\ ArSH \qquad\qquad\qquad 6EtSH$$

substitution of both terminal and bridging ligands. The structure of one reaction product, $[Mo_2Fe_6S_8(SPh)_6\{3,6-(C_3H_5)_2cat\}_2]^{4-}$, as its Et_4N^+ salt, has been determined, and it was found to have the doubly-bridged double-cubane arrangement (27).[106] When dissolved in polar aprotic solvents such as dimethyl sulphoxide (DMSO), at ambient temperatures, the clusters (27) do not appear to retain the bridged structures that are present in the crystalline state. Isotropically shifted ^1H n.m.r. spectra demonstrate the presence of co-ordinated catecholate, and are consistent with a structure having all thiolates bound as terminal Fe–SR ligands. The situation is clearest for clusters with Ar = Ph or *p*-tolyl. Here the integration of the sharp *meta*- and *para*-proton or the methyl resonances *vs* the signals of the catecholate ring and the cation demonstrates that at least $\sim 90\%$ of the signal intensities of the aryl group occur in the form of resonances of terminal ligands. In no cases were signals that possibly originated from thiolate ligands in the bridge observed in spectral scans of ± 400 p.p.m. (from Me_4Si as the reference peak). While the exact structures of the solute species are not known, the presently available results support disruption of the bridge and the attendant formation of single or bridged cubanes, involving the binding of solvent (L) at the molybdenum site. Possible formulations include $[MoFe_3S_4(SR)_3(3,6-R'_2cat)L]^{2-}$ and $[Mo_2Fe_6S_8(SR)_6(\mu_2\text{-L})(3,6-R'_2cat)_2]^{4-}$. Thus the solute clusters present the potentiality of molybdenum sites that have ligands that are sufficiently labile that they can be displaced by substrates of nitrogenase when the clusters are reduced. Further, clusters in which R is aryl are reducible at about the same potential (~ -1.1 V *vs* the s.c.e., in aprotic solvents) as that required for the formation of $[Mo_2Fe_6S_8(SPh)_9]^{5-}$,[87] which

can readily be manipulated under anaerobic conditions. Ongoing experimentation is aimed toward the isolation and determination of the structure of these reduced clusters and the elucidation of their reactivities.

B. **Electronic Features of Bridge-cleavage Products**.—The cluster $[Mo_2Fe_6S_8$-$(SEt)_9]^{3-}$, of type (15), exhibits a magnetic susceptibility behaviour in the solid state, at 4.2—150 K, that is consistent with that of a Curie paramagnet having two sub-clusters, each with $S = \frac{3}{2}$.[87] This is a property that is of considerable interest in view of the presence of clusters in nitrogenase and FeMo-co with a spin system of $S = \frac{3}{2}$ per molybdenum atom. In the solid and solution states, at cryogenic temperatures, this cluster displays rather complicated e.p.r. spectra which may arise from interactions between the sub-clusters and intermolecular interactions that are not well revealed in the magnetic susceptibility behaviour. Certainly these spectra bear little resemblance to an isolated $S = \frac{3}{2}$ spin system of the type that is found in the native enzyme and in FeMo-co.[23,42,52] In this regard, the e.p.r. spectra of solutions obtained from salts of $[Mo_2Fe_6S_8(SAr)_6$-$(3,6-R'_2cat)_2]^{4-}$ are more encouraging.[106] A case in point is the spectrum of a frozen DMSO solution, prepared from $(Et_4N)_4[Mo_2Fe_6S_8(SPh)_6\{3,6-(C_3H_5)_2$-cat$\}_2]$ and presented in Figure 7. This spectrum corresponds to an effectively axial $S = \frac{3}{2}$ species with apparent g values of 4.38 and 1.99, associated with the
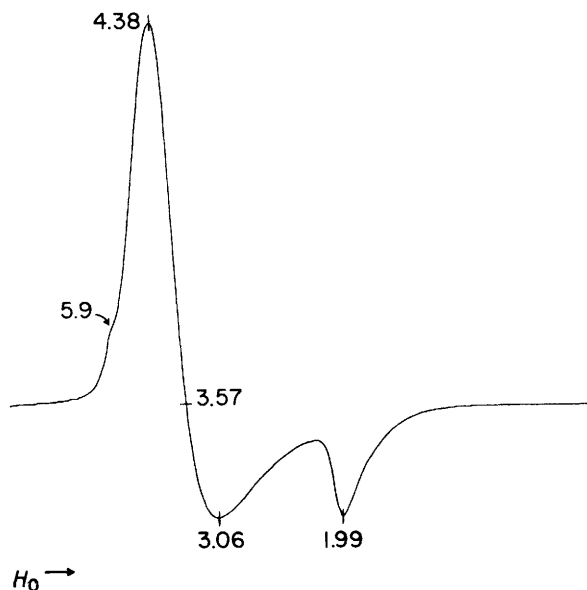
Figure 7 *The X-band e.p.r. spectrum of a ∼10 mmol l⁻¹ solution in DMSO, prepared from* $(Et_4N)_4$ $[Mo_2Fe_6S_8(SPh)_6\{3,6-(C_3H_5)_2cat\}_2]$ *and recorded at 8 K; selected g-values are indicated*

$|\pm\frac{1}{2}>$ Kramers' doublet. Using theoretical expressions for g values,[52] the assignments $g_y \cong 4.38$, $g_x > 3.0$, and $g_z \cong 1.99$ are reasonable for this doublet. If the crossing point on the baseline is taken as $g_x \approx 3.6$, the spectrum corresponds to the spin Hamiltonian parameter ratio, $|E/D|$, of ~ 0.06. This estimate signifies a slightly rhombic g-tensor; purely axial electronic symmetry would afford a spectrum with $g_\perp = 4$ and $g_{\parallel} = 2$. The large linewidth of the g_y feature obscures the small rhombic splitting. In all systems thus far examined, including those prepared from analogous tungsten clusters, the g_x and g_y components have not been resolved. Detailed studies of magnetic susceptibility at low temperatures have not been carried out. Magnetic moments of 4 μ_B per molybdenum atom that were obtained in DMSO solutions at ambient temperature, however, are consistent with a spin-quartet ground state.

Several other features of the e.p.r. spectra that are typified by that in Figure 7 deserve mention. A weak, incompletely resolved, feature at $g \approx 5.9$ is consistently present, and it possibly corresponds to the g_z component of the $|\pm\frac{3}{2}>$ Kramers' doublet. The cluster $[Mo_2Fe_6S_8(SEt)_6(3,6-Pr^n{}_2cat)_2]^{4-}$ has been prepared in two isotopically enriched forms, containing >90 atom percent of ^{95}Mo ($I = \frac{3}{2}$) or ^{57}Fe ($I = \frac{1}{2}$). These species were subjected to the ligand-substitution reaction (35) with benzenethiol and the e.p.r. spectra of the resulting clusters were examined *in situ*. The ^{95}Mo- and ^{57}Fe-enriched clusters and the natural-abundance cluster gave essentially identical spectra. There is no clear evidence for hyperfine broadening of the $g \approx 2$ signal upon ^{57}Fe enrichment. Line broadening of the $g \lesssim 2$ signal has been reported for one enriched FeMo protein.[52]

6 Current Status and Prospects

The information presented here is intended as a conspectus of our approach to a synthetic representation of the Mo–Fe–S cluster catalytic site of nitrogenase. The research is predicated on the viability of MoFe$_3$S$_4$-type clusters as at least preliminary models. The results described are those obtained through August, 1981. For more detailed accounts of the synthesis, structures, and many of the properties of these clusters outlined here, reference is made to the original publications from the author's laboratory.[74–78,87,105,106,111,116] In pursuing the objective, our efforts have benefited from a continually improving state of physicochemical definition of the biological cluster. With due recognition that FeMo-co has not yet been shown to reduce dinitrogen to ammonia[61] and is not the functional equivalent of the native enzyme in the reduction of at least one other substrate,[132] it remains as the most immediate object of synthesis. As a means of assessing the current status of the synthetic approach, the selected properties (1)—(7) of FeMo-co in Table 1 are compared with those of synthetic clusters.

(1) The ratios of Fe, S*, and Mo atoms attained in synthetic species are 3:4.5:1 for (14), 3:4:1 for (15), (17), and (27), 3.5:4:1 for (16), and 4:4:1 for (26). The smaller complexes (18)—(23) have less favourable Fe:Mo ratios. No

[132] C. E. McKenna, J. B. Jones. H. Eran, and C. W. Huang, *Nature (London)*, 1979, **280**. 611.

discrete cluster has yet been prepared that has atom ratios falling within the indicated ranges for cofactor.

(2),(3) All clusters are anionic and all contain organic ligands. Since cysteine is absent in FeMo-co, ligation of other than 'inorganic' sulphur presumably involves one or another of the indicated possible ligands, whose presence is likely to depend on the particular procedure used to prepare the cofactor. Ligation of cysteinate in the native enzymes cannot be discounted. $MoFe_3S_4$ clusters are stable when terminal Fe–SR ligands are replaced by chloride, *e.g.* (29) and (31), and Mo–O interactions are found in clusters (17), (26), and (27). Terminal Fe–O binding appears to be possible, at least with anionic ligands, judging from the isolation of $[Fe_4S_4(OPh)_4]^{2-}$ [109] and $[(PhO)_2FeMoS_4]^{2-}$ [133] and the generation in solution of $[Fe_4S_4(SR)_n-(OAc)_{4-n}]^{2-}$ (n = 0—3).[108] Another conceivable function of oxygen ligands, *viz.* the bridging of metal sites within a cluster by oxide or hydroxide, remains an open question. No such clusters containing also molybdenum and iron and inorganic sulphur have been prepared.

(4) All $MoFe_3S_4$ clusters contain iron atoms that are spin-coupled within sub-clusters; one model for spin-exchange interactions in the form of antiferromagnetic coupling has been proposed.[134] The clusters (27; R = Ph) and (27; R = *p*-tolyl), in solution, give $S = \frac{3}{2}$ e.p.r. spectra; g_x and g_y resonances are not resolved. The representative spectrum in Figure 7 has g values comparable to those of the native enzyme (*e.g.* 4.32, 3.66, and 1.98[52]) and of FeMo-co. Spectral linewidths for the enzyme are narrower than those of FeMo-co, and the extent of rhombicity ($|E/D|$ = 0.055) is very close to that estimated for the synthetic cluster. The sign and value of the zero-field splitting of the Kramers' doublets in the latter species have not been determined. Linewidths for clusters are larger than those of cofactor, whose rhombicity is well resolved and which exceeds that of the native enzyme and those of synthetic clusters. Line broadening was not observed in ^{95}Mo- and ^{57}Fe-enriched clusters.

(5) Dimensions of $MoFe_3S_4$ sub-clusters in (14)—(17), (26), and (27) (Figures 2, 3, and 4) are essentially invariant. Values of Mo–S* and Mo $\cdots$ Fe distances and the presence of three S* and three Fe atoms at these distances are in good accord with conclusions from molybdenum EXAFS analysis of FeMo-co and native enzymes.[18] Clusters (17) and (26) provide possible models of the Mo site in the cofactor, for which recent EXAFS results implicate *O*- or *N*-co-ordination.[53]

(6) Redox activity of synthetic clusters is demonstrated by the existence of the series of electron-transfer equilibria (19)—(21). Sub-clusters with electronic formulation α are those with $S = \frac{3}{2}$. Reduction to the β formulation, as in $[Mo_2Fe_6S_8(SR)_9]^{4-,5-}$, results in species whose magnetic moments at ambient

[133] H. G. Silvis and B. A. Averill, *Inorg. Chim. Acta*, 1981, **54**, L57.
[134] G. Christou, D. Collison, C. D. Garner, F. E. Mabbs, and V. Petrouleas, *Inorg. Nucl. Chem. Lett.*, 1981, **17**, 137.

temperature are consistent with $S = 2$ for their sub-clusters.[87] A change from $S = \frac{3}{2}$ to an even spin (deduced from the lack of an e.p.r. signal) occurs upon reduction of the biological cluster in the native enzyme and in cofactor.

(7) No synthetic cluster has yet been shown to reconstitute the FeMo protein of *Azotobacter vinelandii* UW45, but not all types have been tested. The reported lack of activation of this protein by FeMo-cluster,[60] which appears to be derived from (and to possess a lower ratio of Fe:Mo atoms than) FeMo-co, suggests that reconstitution activity may be the single most stringent criterion of the faithfulness of a synthetic cluster to the cofactor. That certain properties (*e.g.* the MoFe$_{2,3}$S$_{3,4}$ structural arrangement and the $S = \frac{3}{2}$ system) are common to enzyme and cofactor indicates that the basic structure of the cluster core is probably the same in both environments. (Ligands that are external to the core may differ, however.) If these structures are in fact the same, the expressed criterion is appropriate to the assessment of synthetic clusters as models of the Mo–Fe–S cluster of nitrogenase.

It is evident that a model that is consistent with the key properties of FeMo-co has not yet been achieved. Nonetheless we are persuaded by the *collective* properties of MoFe$_3$S$_4$-type clusters that certain elements of these species may be correct. Important individual properties, such as the structural relation of (21) to the site proposal (11), and the e.p.r.[66,94] and magnetic[135] properties of (22), which are indicative of a $S = \frac{3}{2}$ system, are found in other Fe–Mo–S species, as noted earlier. Whatever are their deficits, the clusters described here at least offer the merit of approaching properties (4)—(6) of the biological cluster.

In a structural sense, we regard the MoFe$_3$S$_3$ fragment of a MoFe$_3$S$_4$ cluster to be the most likely element in common with the biological cluster. This fragment, of course, does not carry the full complement of iron (and possibly inorganic sulphur) atoms that are indicated by chemical analysis of FeMo-co. One conception for incorporating this fragment into a larger cluster, consistent with the analytical ranges for the cofactor, has arisen from recent experiments by G. Christou and K. S. Hagen in the author's laboratory. In this work the new cluster [Co$_8$S$_6$(SPh)$_8$]$^{4-}$ has been structurally characterized. It contains the [Co$_8$(μ_4-S)$_6$]$^{4+}$ core, composed of a cube of cobalt atoms with a sulphur atom above each face, forming an octahedron. The structure approaches idealized O_h symmetry. Each cobalt atom occupies a tetrahedral site and has one terminal thiolate ligand. In terms of population of metal- and sulphur-atom sites it is the inverse of [Fe$_6$S$_8$(PEt$_3$)$_6$]$^{2+}$,[136] which has a [Fe$_6$(μ_3-S)$_8$]$^{2+}$ core (of O_h symmetry) and iron(III) atoms in five-co-ordinate sites. From the structure of the cobalt cluster, the species (MoFe$_7$S$_6$(SR)$_7$L$_3$]$^{z-}$, which has structure (32) and is of trigonal symmetry, has been devised by Christou. Iron atoms occupy tetrahedral sites, with terminal thiolate ligands specified for the sake of definiteness. The MoFe$_3$S$_3$ fragment, with a geometry similar to that in MoFe$_3$S$_4$ clusters, is apparent; six-co-ordination at the molybdenum atom is completed by three

[135] A. Simopoulos, V. Papaefthymiou, A. Kostikas, V. Petrouleas, D. Coucouvanis, E. D. Simhon, and P. Stremple, *Chem. Phys. Lett.*, 1981, **81**, 261.
[136] F. Cecconi, C. A. Ghilardi, and S. Midollini, *J. Chem. Soc., Chem. Commun.*, 1981, 640.
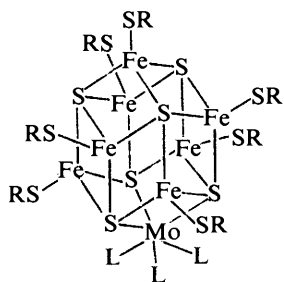
(32)

ligands L. The structure has the appealing feature of being built largely of Fe_2S_2 rhombs such as are present in clusters (3) and (4). In Fe–S cluster chemistry the μ_4-S bridging atoms find precedent in $[Fe_6S_9(SBu^t)_2]^{4-}$.[137] In terms of its topology, the '*n*-type string bag cluster' of Lu^{64} is one-half of the core polyhedron of (32). This structural proposal of the nitrogenase cluster is offered in the spirit of speculative modelling. As with model structures proposed by others,[63–66] the object of the proposal remains to be synthesized.

[137] G. Christou, R. H. Holm, M. Sabat, and J. A. Ibers, *J. Am. Chem. Soc.*, 1981, **103**, 6269.