The Structure and Mode of Action of the Cofactor of the Oxomolybdoenzymes

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1 Introduction

The concept that a small molybdenum-containing unit might act as a cofactor for the molybdoenzymes was first suggested by Pateman *et al.* 30 years ago; as a result of work on a series of mutant cells lacking both nitrate reductase and xanthine oxidase activity, it was proposed that the two enzymes share a common cofactor.[1] Support for this idea came from work by Nason *et al.*[2] who showed that a molybdenum-deficient nitrate reductase from a mutant strain of *Neurospora crassa*, Nit-1, could be reactivated by an acid-denatured extract from 'any molybdoenzyme.' Subsequent studies, principally by Brill *et al.*, achieved the isolation[3] of a cofactor from Component I of nitrogenase, containing iron, molybdenum and acid-labile sulfide. However, although this cofactor, *FeMoco*, was capable of reconstituting molybdenum-deficient nitrogenase Component I from the *Azotobacter vinelandii* mutant strain, UW45, it did *not* reconstitute the nitrate reductase mutant, Nit-1. On the other hand, a cofactor isolated from xanthine oxidase *did* reactivate the Nit-1 strain – but not the nitrogenase mutant, UW45 – this cofactor is now termed *Moco*, and is the subject of this review.[3—7] Subsequently, activity has been returned to Nit-1 nitrate reductase using cofactor produced from other oxomolybdoenzymes: aldehyde oxidase, sulfite oxidase, and nitrate reductase.[5] Moco, or structural variants thereof, is also the cofactor for xanthine dehydrogenase, pyridoxal oxidase, nicotinate oxidase, carbon monoxide oxidase, formate dehydrogenase, tetrathionate reductase, chlorate reductase, biotin sulfoxide reductase, purine hydroxylase, and dimethyl sulfoxide (DMSO) reductase, all enzymes involved in redox–oxygen-transfer processes. In man, hepatic sulfite oxidase is an essential enzyme serving a detoxification purpose, converting sulfite into sulfate; lack of the enzyme leads, in most cases, to death soon after birth.[6] The majority of these enzymes are large and complex, containing haem, Fe-S, and/or flavin centres in addition to Moco.[7]

2 Moco: Structural Characterisation

Moco is unstable when released from its associated protein and has only been characterised by conversion into derivatives, all of which lack the molybdenum. Therefore, ideas for the exact location of the metal have been developed using a combination of the evidence for the structure of the organic moiety, termed *molybdopterin*, spectroscopic information as to the oxidation state of the metal and its primary coordination sphere in the enzyme (Section 2.2), and indications from the structures of pteridine-containing model compounds (Section 5).

2.1 The Organic Ligand

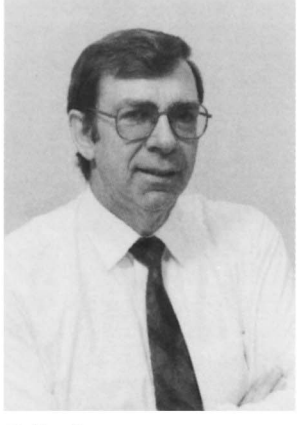
From experiments on denatured enzyme extracts, Rajagopalan *et al.*[8] isolated two pteridines,[9] 'Form A', **1** (after exposure to $H^+/I_2/KI$) and 'Form B', **2a** ($K_3[Fe(CN)_6]/HO^-$). They noted that the fluorescence of these compounds was not present in the whole enzyme and concluded that molybdopterin in Moco does not contain a fully aromatic pteridine and that **1** and **2a**, therefore, must be formed by oxidative degradations. The phosphate in Form B could be removed by treatment with *phosphatase*, the resulting material, **2b**, being shown to contain a 1,2-diol by its positive reaction with periodic acid. The absolute configuration of the alcohol-bearing side-chain carbon in **1** was later established by comparison of the CD spectrum of the corresponding diol with that of synthetic material (the synthesis is summarised in Section 4).[10]

Dr. Joule graduated (University of Manchester) in 1958 and after a PhD (Manchester, with G. F. Smith), and post-doctoral work (Princeton, R. K. Hill, Stanford, C. Djerassi), returned to Manchester as a lecturer, with subsequent sabbatical periods at the University of Ibadan, Nigeria, Johns Hopkins Hospital, Baltimore, and the University of Maryland (UMBC), USA. He is now a Reader. Dr Joule's research concentrates on nitrogen heterocyclic chemistry and synthesis of heterocyclic natural products

Professor Garner graduated (University of Nottingham) in 1963 and, after a PhD (Nottingham, with Professor C. C. Addison, Drs. D. Sutton and S. C. Wallwork), and post-doctoral research (Caltech, H. B. Gray; Nottingham, ICI Research Fellow) was appointed as a lecturer at the University of Manchester in 1968. He has been visiting Professor at the Universities of Lausanne, Strasbourg, Melbourne and Florence, and was awarded the Tilden Medal of the Royal Society of Chemistry in 1985/6. He is now Professor of Inorganic Chemistry and Head of Department. His research is concentrated upon coordination chemistry relevant to the biological chemistry of the d-transition metals.

Dr. Collison graduated (University of Manchester) in 1976 and after a PhD (Manchester, with C. D. Garner and I. H. Hillier), and post-doctoral work (Manchester, F E. Mabbs; Manchester, C. D. Garner; UMIST, N. J. Blackburn), became an SRC Fellow (Manchester) and subsequently a Royal Society University Research Fellow (Manchester) and was appointed to his current position of Senior Lecturer at the University of Manchester in 1994. His research concentrates on the electronic structure of d-transition metal compounds.

J. A. Joule



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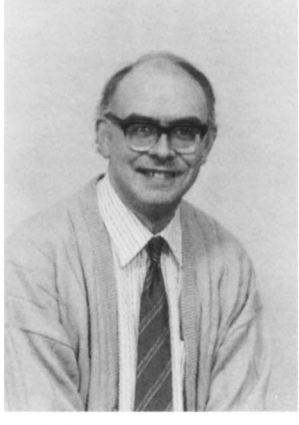
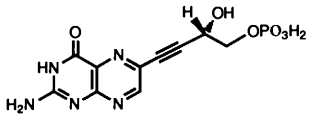


D. Collison

The stereochemistry shown for **2** is based on the assumption that it is the same as in **1**.



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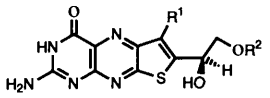
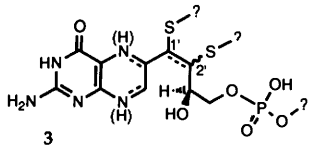


	R^1	R^2
2 a	H	PO_3H_2
b	H	H
c	SMe	H

Urothione **2c** is present in normal urine, and is believed to be a metabolite of Moco;[11] as such, it gives a clue to the locations of two sulfur atoms in molybdopterin. Rajagopalan and Johnson subjected urothione to Raney nickel hydrogenolysis; four compounds were produced, one of which, presumed to have a dihydrothiophene ring, was dehydrogenated with selenium dioxide producing material identical with 'dephospho Form B', **2b**.

Key information[12] on the structure of molybdopterin was gained when Rajagopalan and coworkers, working with chicken liver sulfite oxidase and cows' milk xanthine oxidase, produced a compound which they believed to represent a trapped molybdopterin. These and other data led to a formulation for Moco as **3** with uncertainties as to: (*i*) the oxidation level of the pyrazine ring; (*ii*) whether the sulfur atoms are attached *only* (*cf.* urothione and Form B) at C-1′ and C-2′ of the side-chain; (*iii*) the oxidation levels of the side-chain carbons carrying the sulfur atoms; (*iv*) the tautomeric form of the (reduced) pteridine moiety, and (*v*) the substituent on the phosphate.



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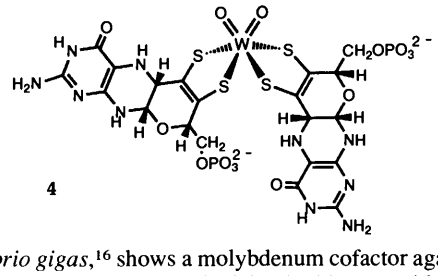
Other studies have demonstrated that in carbon monoxide dehydrogenase the pterin unit is linked *via* the side-chain phosphate to a guanosine-5′-phosphate and, in DMSO reductase, to a cytosine-5′-phosphate, in each case *via* a pyrophosphate link; latterly, hypoxanthine and adenine have been shown to be associated with the molybdopterin in the same way.[13]

Very recently, two X-ray crystal structure determinations have shed new light on molybdopterin-cofactors. A hyperthermophilic tungstopterin enzyme, ferredoxin aldehyde oxidoreductase from *Pyrococcus furiosus*, contains[14] a cofactor which is fascinatingly similar yet subtly different from that proposed by Rajagopalan and other previous workers. The first difference is that in the tungsten cofactor, **4**, the metal has *two* pterin ene-dithiolate ligands. The second significant difference is the presence of a dihydropyran ring formed by the formal cyclisation of a side-chain hydroxy oxygen onto the pteridine 7-position of a 5,6-dihydropteridine. Representation **4** includes the two oxo-groups indicated by a tungsten edge EXAFS study;[15] the phosphates link to the same Mg^{2+} ion.[14] The crystal structure determination, of the aldehyde oxidase from



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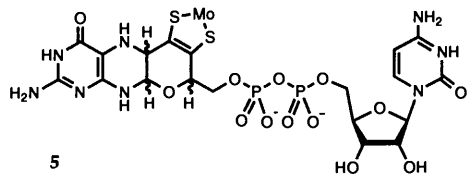
Desulfovibrio gigas,[16] shows a molybdenum cofactor again in a tricyclic form, comparable to that in **4**, but in this case with a cytosine linked at the terminal phosphate and with *only one ene-dithiolate* ligating the metal centre, **5**.

Considering the quantities of material available for the earlier chemical degradative and spectroscopic work on Moco, the closeness of the deductions to the structures now determined is



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commendable. A *caveat* must be included regarding the accuracy with which the protein crystal structure determinations can delineate the state of oxidation at the pyrazine ring carbon atoms, or at the side-chain sulfur-bearing carbon atoms.

2.2 The Metal Centre

2.2.1 Introduction

Protein crystallographic studies on oxomolybdoenzymes and tungsten-containing enzymes (*tunzymes*) have only recently become available (see above). However, these studies do not unambiguously define the coordination at the metal. The clearest evidence concerning the coordination at the metal centre has been derived from a variety of spectroscopic studies. The complementary use of X-ray absorption spectroscopy (XAS), notably the extended X-ray absorption fine structure (EXAFS) of the molybdenum *K*-edge, and electron paramagnetic resonance (EPR) spectroscopy have dominated these investigations, because both are able to probe the metal's environment selectively. Interpretations of the data obtained from the enzyme studies have been significantly strengthened by recording corresponding XAS and EPR information for fully characterised chemical analogues.

The prefix *oxo* for this group of enzymes is appropriate; not only does each enzyme catalyse a conversion, the net result of which can be represented as oxygen atom transfer, but also XAS studies have indicated the presence of at least one terminal oxo ligand (Mo=O) in (virtually) every system and state examined. Six-coordination and an octahedral geometry dominate the chemistry of molybdenum(VI), molybdenum(V) and molybdenum(IV).[17] For molybdenum(VI) the *cis*-dioxo moiety $\{MoO_2\}^{2+}$ achieves the pseudo-octahedral geometry by binding four donor atoms. Each bond *trans* to an Mo=O group is generally longer than an equivalent bond *cis*; neutral ligands are often found in the former positions and anionic ligands in the latter. The *cis*-dioxo geometry maximises the $Mo(d_\pi)-O(p_\pi)$ overlaps.

Binuclear complexes of molybdenum(VI) exist, but the tendency to dimerisation *via* μ_2-OH linkages becomes dominant on reduction to molybdenum(V). This aspect of the chemistry means that the synthesis of most monomeric analogue complexes and studies of their spectroscopy and reactivity are performed in non-aqueous media. One terminal oxo (or sulfido) group is generally found for monomeric molybdenum(V) complexes and hence there is a single *trans*-site at which easy substitution chemistry can take place. Thus, both five- and six-coordinate geometries are common.

Both mono- and di-oxo complexes of molybdenum(IV) are found, the latter possessing a mutually *trans*-dioxo geometry, which places the d^2 electrons in the same metal (d_π) orbital leaving the two remaining d_π orbitals for $Mo(d_\pi)-O(p_\pi)$ overlaps.

2.2.2 Spectroscopic characterisations

2.2.2.1 X-Ray absorption spectroscopy

XAS has played a vital role in defining the chemical nature of molybdenum centres in enzymes and how they respond to changes in the oxidation level of Moco and/or to the presence of substrates, substrate analogues, or inhibitors of enzymic activity.[18] The molybdenum K-edge EXAFS results achieved[19] for chicken liver sulfite oxidase are the clearest such data and the interpretation achieved represents a prototype for other oxomolybdoenzymes. The molybdenum site has been investigated in each of its three accessible oxidation levels [(VI), (V) and (IV)] as a function of pH and chloride concentration. The molybdenum(VI) is coordinated by two oxo-groups, at *ca.* 1.70 Å, one oxygen (or nitrogen) and three

sulfur-donor ligands at *ca.* 2.06 and 2.42 Å, respectively; two of these sulfur atoms presumably derived from the molybdopterin.[8] The molybdenum(VI) centre is not affected by changing the pH from 6 to 9 or by a variation in the chloride concentration.

The molybdenum-(V) and -(IV) centres each possess a single oxo-ligand, at *ca.* 1.69 Å, one oxygen (or nitrogen) and three sulfur-donor ligands at *ca.* 2.00 and 2.37 Å, respectively. Both of these centres appear to bind chloride at pH 6 in 0.3 mol l^{-1} KCl. EPR spectroscopy showed that the centre can exist in two different forms, which are in a pH- and anion-dependent equilibrium. George *et al.*[19] concluded that the molybdenum K-edge EXAFS data were consistent with one chloride ligand binding to the low pH form and that the number of oxo-groups remains the same upon transition from the high-pH to the low-pH molybdenum(V) form. Thus, reduction of molybdenum(VI) results in the loss of one oxo-group, presumably due to protonation, and the generation of an anion binding site. This behaviour is consistent with the chemistry of molybdenum in its higher oxidation states since a *cis*-$\{Mo^{VI}O_2\}^{2+}$ centre is generally converted into a $\{Mo^{V}O\}^{3+}$ or $\{Mo^{IV}O\}^{2+}$ centre upon reduction.

Xanthine oxidase is the most accessible of the oxomolybdoenzymes and is readily extracted from cows' milk. This enzyme exists in two forms: an active and an inactive form caused by loss of a sulfur atom (desulfo). Molybdenum K-edge EXAFS studies[20] have shown that the environments of molybdenum(VI) and molybdenum(IV) in desulfo xanthine oxidase closely resemble that[19] of the corresponding oxidation state for chicken liver sulfite oxidase. The principal difference between the centre of the oxidized active form, as compared to the oxidized desulfo form, is the presence of one sulfido-group (at *ca.* 2.18 Å) plus one oxo-group, rather than two oxo-groups.[21] The molybdenum centre of xanthine oxidase is very reactive and both molybdenum K-edge EXAFS and EPR data indicate that the centre of this reactivity is the Mo=S bond. The terminal sulfido-group is lost upon reduction, presumably being protonated to form an Mo−SH moiety. Arsenite is a potent inhibitor of xanthine oxidase, clear evidence for an Mo−S−As interaction, and an interbond angle of *ca.* 80° has been obtained from combined Mo and As K-edge EXAFS studies.

2.2.2.2 Electron paramagnetic resonance spectroscopy

EPR spectroscopy selectively probes the molybdenum(V), d^1, centres of the oxomolybdoenzymes. The EPR parameters (*g*- and *A*-values) of the centre are extremely sensitive to the nature of the coordination sphere. The role of the molybdenum(V) state in the enzymatic reactions has been questioned, but nonetheless this state is important since it can be generated within biological samples and it is the only state (other than possibly Mo^{III}) which can be detected by EPR spectroscopy. The work of Bray *et al.*,[22] using EPR spectroscopy has profoundly influenced views as to the nature of the active sites of the oxomolybdoenzymes. Indeed the initial experiments of Bray *et al.*[23] in 1959 and Meriwether *et al.*[24] in 1966 were indicative of sulfur-donor ligands bound to molybdenum.

The presence of nuclei such as ^1H, ^{13}C, ^{14}N, ^{17}O, ^{31}P or ^{33}S in or near to the coordination sphere of molybdenum(V) can be revealed by EPR spectroscopy as super-hyperfine splittings of resonances. More recently, it has become technically feasible to use ^1H or ^{31}P electron nuclear double resonance (ENDOR) on enzyme samples containing molybdenum(V).[25]

2.2.2.3 Magnetic circular dichroism spectroscopy (MCD)

DMSO-reductase from *Rhodobacter capsulatus*[26] and *Rhodobacter sphaeroides*[27] is a soluble periplasmic enzyme (M_r = *ca.* 82000) which contains only Moco as a prosthetic group. This simplicity greatly facilitates spectroscopic studies of the molybdenum centre and this has been exploited in an MCD spectroscopic study of the molybdenum(V) state of these enzymes.[28] The spectrum shows six oppositely signed bands ranging in wavelength from 701 to 358 nm which are assigned as dithiolene S to Mo^V charge-transfer transitions.

2.2.2.4 Resonance Raman spectroscopy

Resonance Raman spectroscopy has been used to probe the metal coordination in a variety of metalloproteins.[29] However, for most pterin-containing molybdenum enzymes, other strongly absorbing prosthetic groups dominate the electronic and resonance Raman spectra and, to date, only DMSO reductase has been studied by this technique. The oxidized and reduced forms of DMSO reductase show vibrations in the 335—385 cm^{-1} region that shift upon enrichment of the enzyme with ^{34}S and therefore have been assigned to Mo−S vibrations. The most prominent feature is the band at 350 cm^{-1} in oxidized DMSO-reductase which shifts to 343 cm^{-1} upon ^{34}S enrichment and has been assigned to a Mo−S (dithiolene) vibration by comparison with the bands at 351 and 348 cm^{-1} in $[C_5H_5Mo^{IV}\{S_2C_2[C(O)Me]\text{-quinoxalino}\}]$ and $[(C_5H_5)_2Mo^{IV}\{S_2C_2[C(O)Me]\text{-pterin}\}]$, respectively, which shift by the same amount.[30]

3 Synthesis and Properties of Compounds which model the Bioactivities of Moco-containing Enzymes

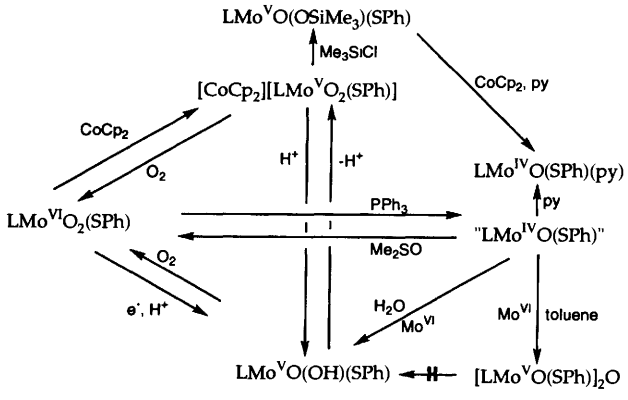
3.1 Oxygen Atom Transfer

'Molybdenum . . . lies at the epicentre of oxo transfer chemistry. More oxo compounds have been prepared and characterized, more oxo transfer reactions are known, and more catalytic systems based on these reactions have been devised than for any other element',[31b] and these have been comprehensively reviewed.[31] Molybdenum enzymes catalyse the overall reaction shown by equation 1, where X is the enzyme substrate. The electrons and protons produced by the oxidation of the substrate (or consumed in the reduction of the substrate) may be involved with the molybdenum as the (formal) $\{Mo^{VI}O_2\}^{2+}/\{Mo^{IV}O(OH_2)\}^{2+}$ couple. Alternatively, the molybdenum centre may be involved in direct oxygen atom transfer (equation 2.).

$$X + H_2O \rightleftarrows XO + 2H^+ + 2e \quad (1)$$

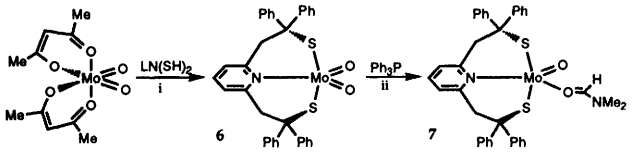
$$\{Mo^{VI}O_2\}^{2+} + X \rightleftarrows \{Mo^{IV}O\}^{2+} + XO \quad (2)$$

Both routes can be employed, depending on the enzyme and the operating conditions. Recent work by Holm *et al.* has demonstrated that DMSO-reductase from *R. sphaeroides* is an oxotransferase. Thus, the overall transfer of an oxygen atom (^{18}O) from DMSO to 1,3,5-triaza-7-phosphatricyclo[3.3.1.$1^{3,7}$]decane (PTA) is catalysed by the enzyme and the labelling of the substrate demonstrated that the oxygen atom transferred did not arise from the solvent.[32] Mechanistic versatility for oxomolybdenum complexes has been demonstrated by the reaction sequence summarised in Scheme 1.[33] This system, based on the tris(pyrazolyl)borate ligand ($L-N_3$) models some aspects of the reaction chemistry of sulfite oxidase. $[(L-N_3)Mo^{VI}O_2(SPh)]$ reacts with PPh_3 to produce $[(L-N_3)Mo^{IV}O(SPh)]$ and is capable of catalysing oxygen atom transfer from Me_2SO to PPh_3. In the presence of H_2O a one-electron reduction takes place to yield $[(L-N_3)Mo^{V}O(OH)(SPh)]$ which can be oxidised by O_2 to regenerate the starting material. Oxygen isotope labelling reveals that H_2O is the source of the oxo ligand, not O_2 and the oxidation state of the molybdenum is suggested to control the level of protonation of the water-derived ligand.



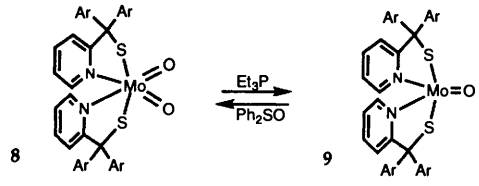
Scheme 1

Holm *et al.* have developed some elegant ligands[34] capable of sustaining molybdenum as a monomeric centre during oxygen atom transfer. The compound **6**, synthesised by the reaction of $[MoO_2(acac)_2]$ with the dithiol pro-ligand oxidises phosphines and the resultant molybdenum(IV) form, **7**, reduces *N*- and *S*-oxides.



Scheme 2 *Reagents*: i, MeOH, CH_2Cl_2, room temp. (92%); ii, DMF, room temp. (65%).

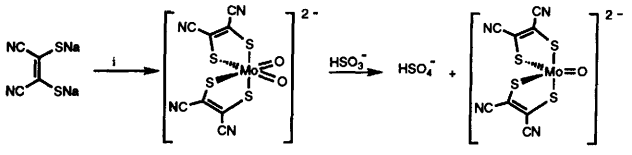
The redox pair **8** and **9** oxidise/reduce a variety of substrates such as *P*-, *Se*-, and *N*-oxides, some of which are substrates for oxomolybdoenzymes.



Ar = 4-Bu^t C_6H_4

3.2 Dithiolene Complexes

The coordination of molybdenum by sulfur demonstrated by spectroscopic studies of the oxomolybdoenzymes, together with the constitution of Moco, has usually been interpreted to indicate dithiolene (or ene-1,2-dithiolate) ligation (see **5**). The results of the crystallographic studies of the aldehyde oxidoreductase from *D. gigas*[16] and *P. furiosus*[14] are consistent with the coordination of one dithiolene to molybdenum and two dithiolenes to tungsten, respectively.



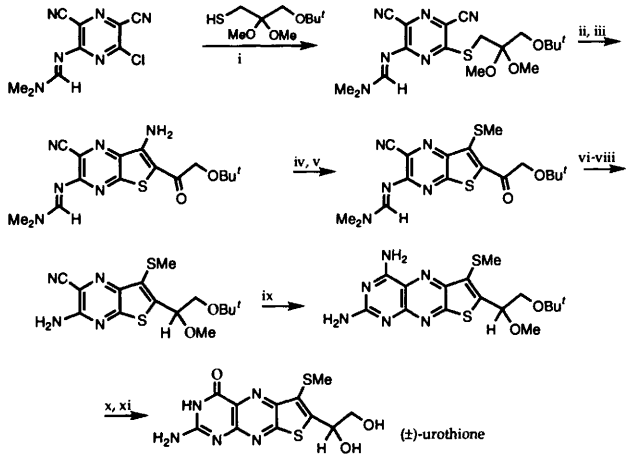
Scheme 3 *Reagents*: i, $Na_2MoO_4 \cdot 2H_2O$, H_2O, pH 6, room temp. (61%).

Dithiolene complexes generally display reversible redox properties. This behaviour is vital for any chemical analogue of the molybdenum centre of the oxomolybdoenzymes. The maleonitrile dithiolate (mnt) ligand has been shown[35] to afford $[MoO_2(mnt)_2]^{2-}$ and $[MoO(mnt)_2]^{2-}$ (Scheme 3) as well as $[MoOCl(mnt)_2]^{2-}$; a set of complexes which further reinforce the comparison between these systems and the molybdenum centres of the oxomolybdoenzymes. However, the redox potentials reported for oxomolybdoenzymes: two couples, corresponding to Mo^{VI}/Mo^{V} and Mo^{V}/Mo^{IV} processes separated by only *ca.* 200 mV, contrast with the observation of two one-electron processes for $[Mo(dithiolene)_3]^{n-}$ and $[MoO(dithiolene)_2]^{n-}$ (n = 0, 1, 2) complexes which are separated by $\geqslant$ 500 mV.[35,36]

An intriguing aspect of the properties of Moco is the extent to which the molybdenum and the pterin jointly participate in the redox changes of the centre. Chemical support for this postulate has been demonstrated by studies of $[Mo(qdt)_3]^{2-}$ (qdt = quinoxaline-2,3-dithiolate) and $[(\eta^5\text{-}C_5H_5)Co\{S_2C_2H(quinoxalin\text{-}2\text{-}yl)\}]$ systems.[37] Thus, not only may the extent and nature of this cooperation vary from enzyme to enzyme but also it may be meaningless to attempt to separate the metal and ligand redox contributions. Also, such behaviour provides an attractive mechanism for modulating the redox potential of Moco by a protein *via* a control of the state and extent of protonation of the pyrazine ring *and* the stabilisation of particular tautomers of the partially reduced forms of the pteridine.

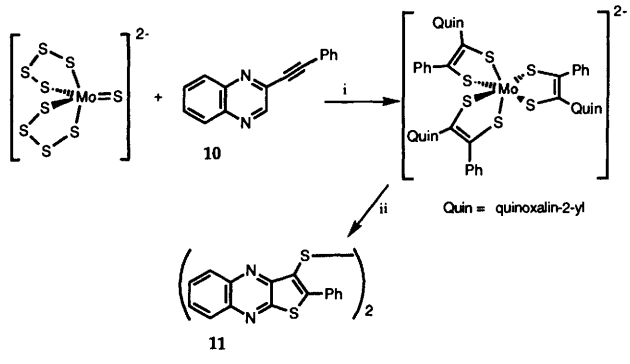
4 Synthesis of Degradation Products of Moco

Following syntheses[38] of deoxyurothione, the total synthesis[39] of (±)-urothione is a triumph for Taylor's strategy for the regioselective synthesis of 6-substituted pteridines; Scheme 4 summarises the key steps.



Scheme 4 *Reagents*: i, Et_3N, EtOH, room temp. (92%); ii, $LiBF_4$, aq. MeCN, room temp. (98%); iii, NaOAc, Bu^{t}OH, reflux (99%); iv, Bu^{t}ONO/$CuBr_2$, MeCN, reflux (56%); v, NaSMe, THF, room temp. (93%); vi, $NaBH_4$, EtOH, THF, room temp. (90%); vii, $HC(OMe)_3$, *p*-$MeC_6H_4SO_3H$, room temp. (88%); viii, *p*-$MeC_6H_4SO_3H$, MeOH, reflux (86%); ix, guanidine hydrochloride, NaOMe, reflux; x, CF_3CO_2H, room temp.; xi, 3 mol l^{-1} H_2SO_4, reflux (79%, two steps).

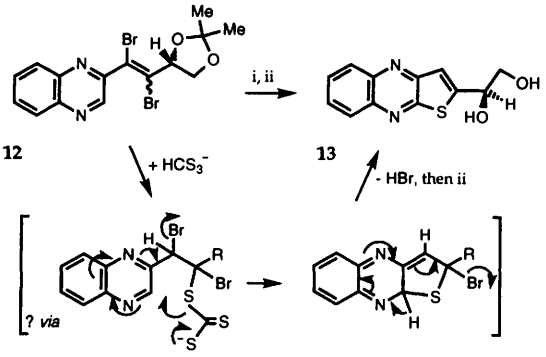
If, in molybdopterin, the sulfur at C-2′ is *not* also linked to the pteridine C-7, then both the formation of urothione as a metabolite and the production of Form B during degradation must involve a cyclisation to produce the thiophene ring: this has been inadvertently modelled.[40,41] Reaction of the phenyl quinoxalin-2-yl alkyne **10** with $[Mo(S_4)_2S]^{2-}$ (see Section 5 below for fuller discussion of such additions) gave a *tris*(dithiolene) complex of molybdenum. Oxidation of this complex produced, as well as higher oxidation states of the molybdenum complex, a small amount of a metal-free substance shown to be **11** (Scheme 5).



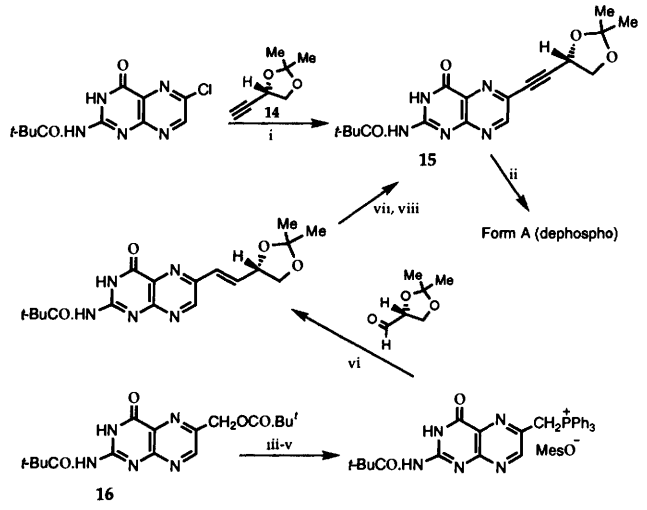
Scheme 5 *Reagents*: i, MeCN, reflux (68%); ii, *e.g.* I_2, −50 °C (7%).

Reaction of the quinoxaline-dibromoalkene **12** with dipotassium trithiocarbonate, produced, as the main product, a tricyclic thiophene, then hydrolysed to give **13**. It was suggested that the mechanism proposed (Scheme 6) to rationalise this unexpected product may well have a bearing on the formation of urothione, and of Form B.[8]

Taylor was the first to prove unequivocally the structure of 'Form A' (dephospho) by total synthesis (Scheme 7), in racemic[42] and then later in homochiral form.[43] In the key step, a homochiral alkyne **14**, obtained from D-mannitol, was coupled with a pivaloyl-protected 6-chloropteridine using palladium(0) methodology. Taylor's use of an *N*-pivaloyl group, as a lipophilic protecting group for pteridines, considerably facilitates their handling – they are otherwise often very insoluble.



Scheme 6 *Reagents*: i, aq. K_2CS_3, MeOH, room temp. (72%); ii, 48% HBr, MeOH, CH_2Cl_2, room temp. (70%).



Scheme 7 *Reagents*: i, $Pd(OAc)_2$, (o-Tol)$_3$P, CuI, Et_3N, MeCN, 100 °C (20%); ii, 0.5 mol l^{-1} HCl, reflux; iii, K_2CO_3, MeOH (42%); iv, Et_3N/$MeSO_2Cl$, CH_2Cl_2, 0 °C (72%); v, Ph_3P, MeCN, 90 °C (76%); vi, Bu^{n}Li, THF, −78 °C, then room temp. in solution (equilibration to all *E*-alkene) (72%); vii, Br_2, CH_2Cl_2, 0 °C (67%); viii, DBU, dioxane, 100 °C (40%).

An alternative route to alkyne-acetal **15**, starting from ester-amide **16**, itself available[44] either *via* degradation of folic acid or from synthetic 6-hydroxymethylpterin, is also shown in Scheme 7.[45]

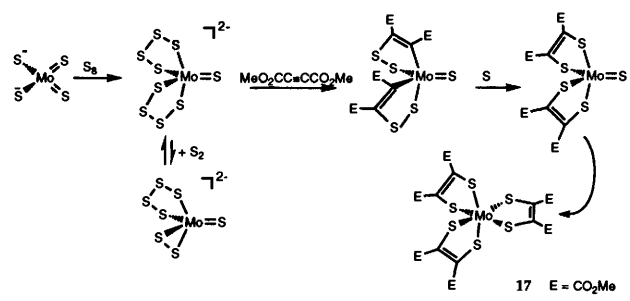
5 Towards a Total Synthesis of Moco

Any strategy aimed at a total synthesis of Moco must have several components; one of these is a means for the generation of a molybdenum-complexed 1,2-dithiolate (probably an ene-1,2-dithiolate – a 'dithiolene'). Several methods have been described for the production of such units, around molybdenum and other metals. We summarise below those routes which we believe to be of greatest relevance for such synthetic endeavours.

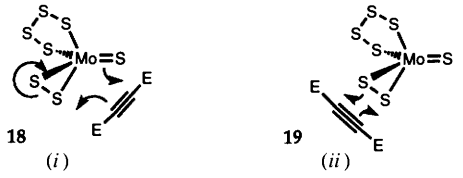
Coucouvanis' studies[46] of the reactions of the polythiomolybdenum anions, generated by reaction of $[MoS_4]^{2-}$ or $[MoS_xO_{4-x}]^{2-}$ (x = 4, 3 or 2) with sulfur, may be relevant to the biosynthesis of the dithiolene unit in Moco and could be of value in a laboratory synthesis of molybdenum-dithiolene complexes (see also below). Mechanistic sequences were suggested for the reaction of such anions with dimethyl acetylenedicarboxylate; illustrated in Scheme 8 is the reaction of $[(S_4)_2Mo{=}S]^{2-}$ under anaerobic conditions giving **17**.

Shown below are two possible interpretations for the key C–S bonding step: the process is (*i*) initiated by nucleophilic attack by a terminal sulfur on the acetylene, with the electron reorganisation shown by the curly arrows on **18**, or (*ii*) viewed as a [2 + 2] cycloaddition **19**.

Taylor and Stiefel elegantly utilised similar additions in their syntheses of pteridinyl- and quinoxalinyl-dithiolenes **20** and **21**.[47] The



Scheme 8



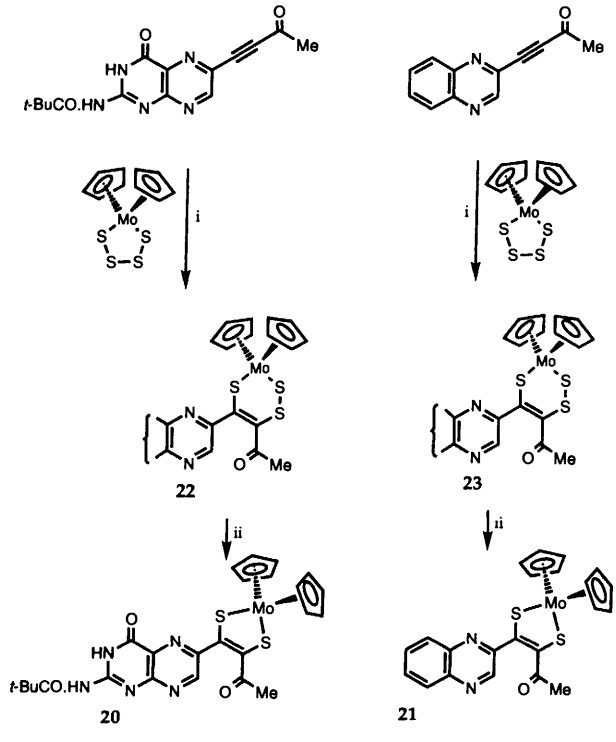
initial complexes **22** and **23** could be transformed into the ene-dithiolate systems believed to exist in molybdopterin, by treatment with a phosphine (Scheme 9).

Hartzler had demonstrated[48] that alkynes carrying at least one electron-withdrawing substituent (ester) react with the betaine ($Ph_3P^+-CS_2^-$), produced from tributylphosphine and carbon disulfide, generating an ylide which can be trapped in a Wittig process. Taylor capitalized on this idea, showing that addition to the pteridinyl ketone, **25** occurred in the same fashion (Scheme 10).[49]

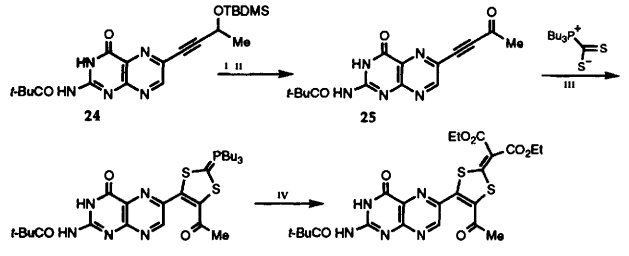
It is important to note that an attempt to utilize (protected) alcohol **24** yielded 'only a minute yield' of addition product – it seems that the electron-withdrawal provided by the heterocycle does not activate the alkyne sufficiently for addition to occur.

de Mayo showed that diphenyldithiolene-thione **26** reacts with $Mo(CO)_6$, in the presence of light, generating complex **27**, though in low yield; a somewhat better yield was obtained in a thermal process, using the same metal complex but starting from diphenylacetylene and sulfur (Scheme 11).[50]

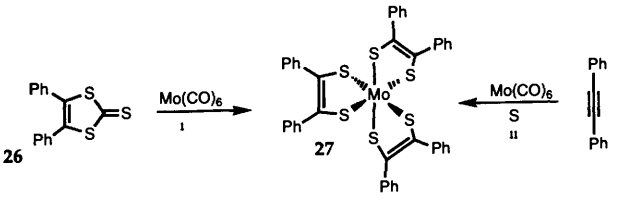
An alternative for the further processing of tri- or di-thiocarbonates could rest on Rauchfuss' work on tetrathiapentalenedione **28**.



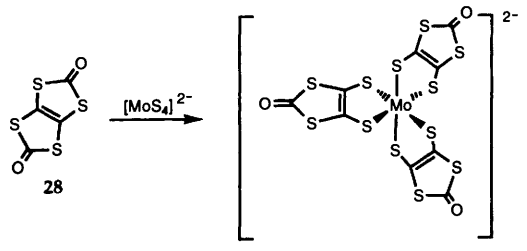
Scheme 9 *Reagents*: i, DMF, 70 °C (62% **22**, 36% **23**); ii, Ph_3P, DMF, 70 °C (95%).



Scheme 10 *Reagents* i, Bu_4NF, THF, room temp (93%), ii, CrO_3, aq H_2SO_4, butan-2-one, room temp, iii, THF, −40 °C, iv, $(EtO_2C)_2C{=}O$, −50 °C (69% two steps)

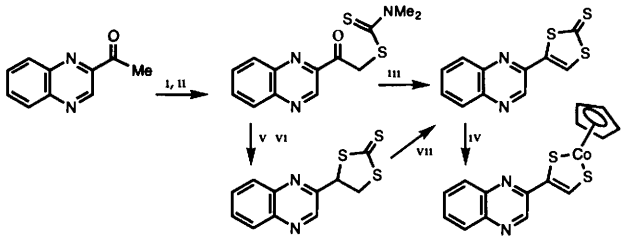


Scheme 11 *Reagents* i, *hν*, $CHCl_3$ (9%), ii, PhMe, 120 °C (25%)



Scheme 12

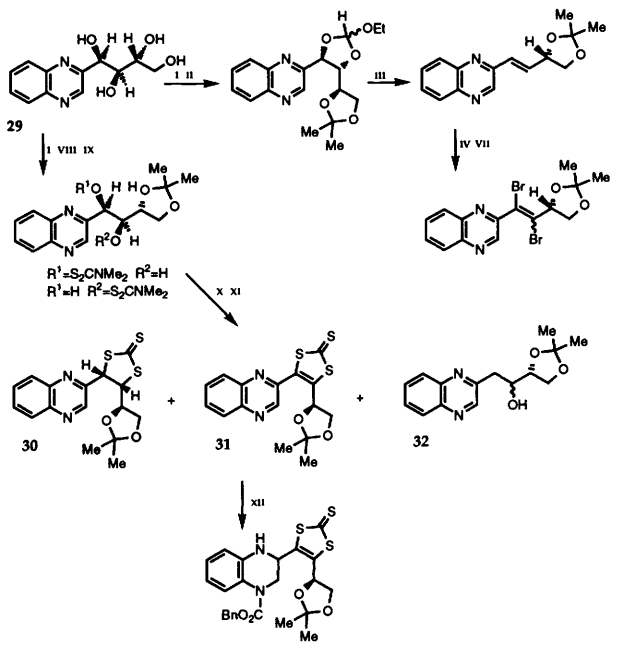
This compound served as a precursor to 1,2-dithiolene complexes of molybdenum by reacting with tetrathiomolybdate (Scheme 12) [51] Early studies in Manchester established a route to unsymmetrically substituted trithiolenes, hydrolysis of the immediate precursors for the trithiolenes generated ene-dithiolates in solution which could be trapped by reaction with, for example, $[MoO_2(acac)_2]$ [52] In the light of remaining uncertainties regarding the oxidation level of sulfur-bearing carbons in molybdopterin, the option of producing 1,2-dithiolane precursors was addressed in a model system, as shown in Scheme 13 [53] It was possible to convert the model dithiolane into the dithiolene *via* peracid oxidation



Scheme 13 *Reagents* i, Br_2, AcOH, 90 °C (85%), ii, NaS_2CNMe_2, EtOH, reflux (67%), iii, conc H_2SO_4, room temp (58%) then NaHS, aq AcOH, room temp (64%), iv, $[Co(C_5H_5)(cyclooctadiene)]$, xylene, reflux (51%), v, $NaBH_4$, MeOH, 0 °C (87%), vi, $MeSO_2Cl$, pyridine, 0 °C (67%), vii, *m*-$ClC_6H_4CO_3H$, $CHCl_3$, 0 °C then $(CF_3CO)_2O$, to room temp (84%)

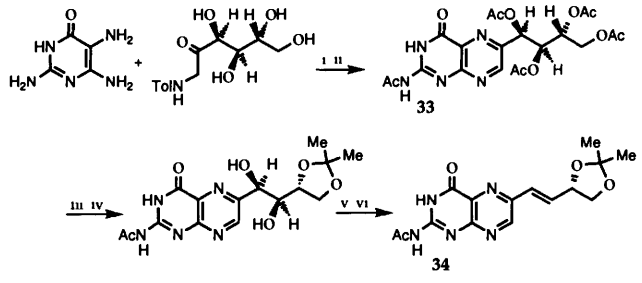
It has been known for more than 100 years that *ortho*-phenylenediamine reacts with glucose giving 2-(D-*arabino*-tetrahydroxybutyl)quinoxaline, **29** Manchester studies[41 54] have utilised this readily available substance as the starting point for studies aimed at modelling methods for the elaboration of the C_4-side-chain of molybdopterin (Scheme 14)

An extrapolation of the strategy illustrated above into pteridine chemistry required the development of a practical route to a 6-tetrahydroxybutylpterin Although the condensation of



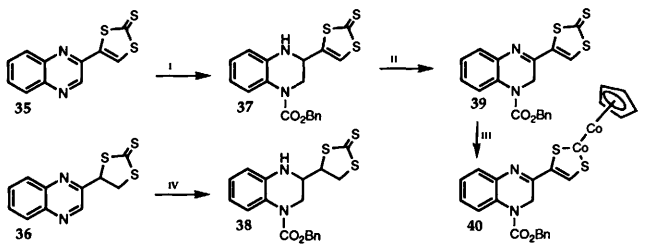
Scheme 14 *Reagents* i, Me_2CO, conc H_2SO_4, room temp (51%), ii, $HC(OEt)_3$, *p*-$MeC_6H_4SO_3H$, CH_2Cl_2, room temp (93%), iii, Ac_2O, reflux (76%), iv, Br_2, CH_2Cl_2, 0 °C (67%), v, NaS_2CNMe_2, MeOH, reflux (40%), vi, Br_2, CH_2Cl_2, 0 °C (86%), vii, NaOMe, MeOH, room temp (34%), viii, $MeSO_2Cl$, pyridine, room temp (53%, plus 30% dimesylate), ix, NaS_2CNMe_2, EtOH, reflux (73%), x, $(CF_3CO)_2O$, pyridine, reflux, xi, H_2S, room temp (**30** 9%, **31** 25%, **32** *ca* 50%) xii, BnO_2CCl, $NaB(CN)H_3$, MeOH, room temp (65%)

5,6-diaminopyrimidines with 1,2-dicarbonyl compounds leading to pteridines is a well-known approach to pteridines – the Isay synthesis – it usually produces mixtures of 6- and 7-substituted pterins – early reports of the regioselective reactions of sugars with 4,5-diaminopyrimidines had been shown to be in error However, a very careful examination of reaction conditions produced a recipe for the synthesis of (D-*arabino*)-tetrahydroxybutylpterin, as its tetraacetate-acetamide **33**, in acceptable yields on a multigram scale, as shown in Scheme 15 [55] The pterin **33** has been processed, following the quinoxaline model sequence, to afford alkene **34**



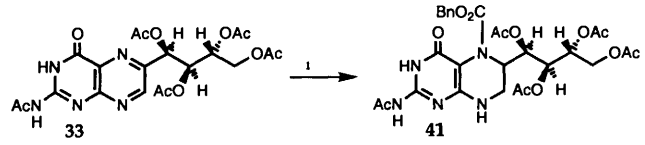
Scheme 15 *Reagents* i, N_2H_4 H_2O, AcOH, 100 °C (38%), ii, Ac_2O, pyridine, 100 °C, then recrystallise (70%), iii, K_2CO_3, MeOH, room temp (55%), iv, Me_2CO, *p*-$MeC_6H_4SO_3H$, room temp, v, $HC(OEt)_3$, *p* $MeC_6H_4SO_3H$, pyridine, CH_2Cl_2, room temp (44%, two steps), vi, 160 °C (67%)

The oxidation level and the precise tautomeric form adopted by the pteridine in Moco are still not certain because of this, any synthetic strategy must allow variation in the oxidation level of the pyrazine ring [56] To this end it was shown that reductions of quinoxalinyltrithiolene **35** and quinoxaline-trithiolane **36** produced tetrahydro-*N*-protected derivatives **37** and **38**,[57] in each of which, importantly, the sulfur-containing units were untouched It has subsequently been shown that **37** can be selectively oxidized to the mono-protected-*dihydro*quinoxaline **39** which can then be transferred to a metal centre to give a dithiolene complex **40** (Scheme 16) [58]



Scheme 16 *Reagents* i, BnO_2CCl, $NaB(CN)H_3$ (97%), ii, MnO_2, CH_2Cl_2, room temp (72%), iii, [Co(C_5H_5)cyclooctadiene)] (13%), iv, BnO_2CCl, $NaB(CN)H_3$ (42%)

A comparable reduction of 6-substituted-pteridines, of which the most relevant to molybdopterin synthesis is **33**, produced, interestingly, tetrahydro-derivatives (**40**) but with the protecting group on N-5, adjacent to C-6-substituents (Scheme 17) [59]



Scheme 17 *Reagents* i, BnO_2CCl, $NaB(CN)H_3$ (65%)

6 Conclusion

This is an exciting time in the development of the understanding of the nature and mode of action of the molybdenum cofactors of the oxomolybdoenzymes and their tungsten counterparts Thus, protein cystallography is now providing vital information which complements earlier spectroscopic and chemical studies, the total synthesis of Moco is in prospect and with this, and the preparation of variants and close analogues, studies of the natural systems and the mode of action will be considerably augmented

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