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Design of Metal Chelates with Biological Activity. 3.[1] Nickel(II) Complexes of Alkyl and Amino Hydroxamic Acids

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Species distributions and relevant stability constants of species present in aqueous solutions of nickel(II) with acetohydroxamic acid (AHA), propionohydroxamic acid (PHA), glycinehydroxamic acid (GHA), and serinehydroxamic acid (SHA) were obtained by analytical potentiometry. In the solid state, nickel(II) forms normal octahedral complexes, e.g. $Ni(AHA)_2 \cdot 2H_2O$, with the alkyl hydroxamic acids whereas with the amino hydroxamic acids, it forms square-planar complexes involving coordination of the amino nitrogen atom and the NHO^- group. X-ray studies of $Ni(GHA)_2$ show the first case of coordination of the NHO^- group to a transition metal via the *nitrogen* atom. Solution IR studies indicate that this structure persists in solution.

Introduction

This paper is the third part of a series relating the structure of metal chelates to biological activity either of the chelate itself or of the ligand, presumed to be acting biologically via metal complexation. In addition to the trace elements (e.g., Mn, Fe, Co) that are required for many forms of life and the essential elements (e.g., Si, V, Cr, Se, ...), there are other elements such as nickel whose biological role is not yet clear. The nutritional role of nickel has been indicated recently in that nickel deficiency in rats has been associated with retarded growth.[2] Several investigations have shown that albumin is the principal Ni(II)-binding protein in human, bovine, rabbit, and rat serums.[3,4] In the case of metalloenzymes, it has been shown that Jack bean urease contains nickel in stoichiometric amounts, which raises the question as to whether the mechanism of action of this enzyme necessarily involves the nickel atom. Hydroxamic acids are potent and specific inhibitors of urease activity[5] and have been used therapeutically in the treatment of hepatic coma.[6] The inhibition of Jack bean urease by hydroxamic acids has been shown recently[7] to be reversible, and the spectral changes occurring have been interpreted in terms of reversible binding to the active-site nickel ion. In view of these results and the probable involvement of the nickel center, we report in this paper studies of a series of Ni(II) complexes of alkyl and amino hydroxamic acids both in solution and in the solid state.

Experimental Section

Materials. Acetohydroxamic acid (AHA) and propionohydroxamic acid (PHA) were prepared by acylation of hydroxylamine[8] by the appropriate ester in the presence of sodium ethoxide; subsequent treatment with dry HCl liberated the free acid. Glycinehydroxamic acid (GHA) and serinehydroxamic acid (SHA) were prepared by mixing ice-cold aqueous solutions of the amino acid ethyl ester hydrochloride (0.1 mol) and hydroxylamine hydrochloride (0.1 mol), slow addition of 12 N sodium hydroxide (0.33 mol), and subsequent acidification with 12 N hydrochloric acid. When the mixture was cooled, GHA crystallized easily, and washing with a little cold water gave pure crystals of GHA: mp 142 °C dec; yield 40%. SHA required addition of alcohol and subsequent reduction of volume and filtration. Recrystallization from methanol/ethyl acetate gave pure SHA, mp 136 °C.

Preparation of $Ni(AHA)_2 \cdot 2H_2O$ and $Ni(PHA)_2 \cdot 2H_2O$. PHA or AHA (1.0 mol) in ethanol (25 mL) was added to $NiCl_2 \cdot 6H_2O$ (AnalaR) (0.5 mol) in ethanol (25 mL) and the pH adjusted to 6.9 by addition of sodium ethoxide. After filtration of sodium chloride, removal of solvent, and recrystallization from ethanol, washing with hot ethyl acetate gave green crystals of the complex, yield 70%.

$Ni(GHA)_2$ and $Ni(SHA)_2$. GHA or SHA (0.1 mol) in water (50 mL) was added to $NiCl_2 \cdot 6H_2O$ (AnalaR) (0.05 mol) in water (50 mL) and the pH raised to 6.9 or 6.5, respectively. After the solution stood several hours or overnight in the case of $Ni(SHA)_2$, the respective complexes were filtered off; yield 90–95%.

Infrared spectra were recorded on a Perkin-Elmer 283B spectrometer in the solid state as 2% CsBr disks or in solution. UV–visible spectra were recorded on a Perkin-Elmer 552 spectrophotometer and reflectance spectra on a Perkin-Elmer 402 instrument. Magnetic moments were measured on a Newport variable-temperature Gouy balance calibrated with cobalt(II) tetrakis(thiocyanato)mercurate(II). Solution moments were measured by the Evans method[9] using a Perkin-Elmer R12B NMR spectrometer.

Potentiometric Titrations. Distilled and deionized water was used throughout, and all titrations were carried out under an atmosphere of purified argon. The base used for pH measurements was carbonate-free sodium hydroxide (3.0103 M) and was standardized by using over-dried potassium hydroden phthalate.

Stock solutions of $NiCl_2$ in dilute HCl were prepared from AnalaR $NiCl_2 \cdot 6H_2O$ and Volucon (Merck) 0.1 N HCl. The stock nickel(II) solution was 0.094 32 M, as determined by atomic absorption spectroscopy, and was made up in 0.1 M HCl. All other reagents were of analytical grade (Ryvan Chemicals Ltd).

Potentiometric titrations were performed by using a Radiometer (Copenhagen) automatic titration apparatus; pH meter readings were recorded on a Digital PHM64. Small amounts of base were added with the use of an ABU13 autoburet (volume 0.25 mL). Titrations were recorded graphically by using a TTT60 automatic titrator and automatic recorder REC61 servograph. The 50-mL solutions employed were thermostated to 25 ± 0.1 °C by using a water-circulation pump. The electrode pair consisted of a Radiometer G2040C glass electrode and a K4050 KCl electrode.

Methods. (1) Irving–Rossotti Method.[10] The above standard technique was used to determine both proton–ligand and metal–ligand formation constants. In the former case, titration curves were obtained by varying the initial concentration of glycinehydroxamic acid (C_A) from 2.0×10^{-3} to 10.0×10^{-3} M, in steps of 2.0×10^{-3} M in the presence of 0.10 M HCl (15 mL) and 1.50 M NaCl (5 mL). In the determination of metal–ligand constants, the concentration of HCl was kept constant (1.0×10^{-2} M) and two sets of curves were obtained. The first set was at constant ligand concentration ($C_A = 5.0 \times 10^{-3}$ M) with variation of metal concentration (C_M) from 2.00×10^{-4} to 8.00×10^{-4} M in steps of 2.00×10^{-4} M. The second set was at constant metal concentration ($C_M = 4.00 \times 10^{-4}$ M) with variation of ligand concentration (C_A) from 2.50×10^{-3} to 10.00×10^{-3} M in

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Table I. Properties of Ni(II) Complexes of Acetohydroxamic Acid (AHA), Propionohydroxamic Acid (PHA), Glycinehydroxamic Acid (GHA), and Serinehydroxamic Acid (SHA) and Free Ligands

compd	color	mp, °C	anal., % found (% calcd) C	H	N	IR spectra,[c] cm^{-1} ν(NH)	ν(CO)	electronic spectra, nm	β	magnetic moments, μ_{eff}
AHA	white	92.0	31.8 (32.0)	6.95 (6.60)	18.2 (18.6)		1640			
$Ni(AHA)_2 \cdot 2H_2O$	green	300 dec	19.8 (19.8)	4.60 (4.95)	10.8 (11.5)		1600	389, 667, 725 (sh), 1096[a]	0.84	3.07
PHA	white	92.5	40.2 (40.5)	7.90 (7.87)	15.4 (15.7)		1640			
$Ni(PHA)_2 \cdot 2H_2O$	green	250 dec	26.4 (26.6)	5.47 (5.90)	10.4 (10.2)		1600	388, 655, 730 (sh), 1102[a]	0.84	3.08
GHA	white	142	26.4 (26.6)	6.97 (6.65)	31.1 (31.1)	2900 (br)	1604			
$Ni(GHA)_2$	red	218 dec	20.0 (20.3)	4.40 (4.22)	24.0 (23.7)	3290, 3220, 3120	1604	450[b]		diamagnetic
SHA	white	136	30.2 (30.0)	6.42 (6.60)	23.3 (23.3)	2850 (br)	1628			
$Ni(SHA)_2$	orange	196 dec	23.9 (24.2)	5.10 (4.70)	19.1 (18.9)	3290, 3120, 3080	1604	450[b]		diamagnetic

[a] Aqueous solution. [b] Reflectance spectra. [c] CsBr disks.

Table II. Logarithms of the Stability Constants (log β_{pqr}) of Complex Species $M_pH_qA_r$ (M = Ni(II); A = Serine-, Glycine-, Propiono-, and Acetohydroxamic Acids, Respectively) in 0.15 M NaCl at 25 °C

			log β_{pqr}							
			Sarkar-Kruck				Irving-Rossotti			
p	q	r	SHA	GHA	PHA	AHA	SHA	GHA	PHA	AHA
0	1	1			9.560				9.62	
0	2	1	8.953				8.96			
0	1	1	6.795				6.79			
1	0	1	7.430	6.800	5.498	5.369	7.20	6.60	5.42	5.42
1	0	2	14.080	13.495	9.734	9.597	13.63	13.10	9.44	9.48
1	1	2	6.240	6.800						
1	2	2	−2.795							
1	0	3			12.189	12.107			11.71	11.73

steps of 2.50×10^{-3} M. The resulting formation constants are given in Table II.

(2) Sarkar–Kruck Method.[11] A method introduced by Österberg[12] for the measurement of free-ligand concentration during the formation of metal complexes has been extended by Sarkar and Kruck[11] to include the measurement of free-metal concentration. The calculations have been improved by the introduction of computer-based numerical procedures. Full details of the method are given in ref 11. Sample compositions of the various ligands and nickel(II) ions, together with proton liberation and species distribution curves, are available as supplementary material.

Results and Discussion

Complexes of Alkyl Hydroxamic Acids. Since both the solid-state structural results and the solution species distribution are different for Ni(II) complexes of the alkyl hydroxamic acids as compared with those for the amino hydroxamic acids, we shall consider them separately. The properties of both series are given in Table I.

In the case of bis(acetohydroxamato)nickel(II) dihydrate and bis(propionohydroxamato)nickel(II) dihydrate, the infrared spectra (Table I) show shifts of about 40–60 wavenumbers in the broad bands at 1610–1585 cm^{-1} compared to the free-ligand bands, which argues strongly for complexation of the ketonic oxygen atom. Reliable assignment of bands requires a full normal-coordinate analysis such as we have performed previously,[13] but bands in the 1445-cm^{-1} region can be assigned qualitatively to the N–C stretching vibration with contributions from C–O and C–R modes, those at about 1300 cm^{-1} to C–R stretches, and that at about 1100 cm^{-1} to a practically pure N–O stretching mode. The general pattern of the infrared spectra supports normal coordination via the ketonic oxygen atom and the oxygen atom of the deprotonated

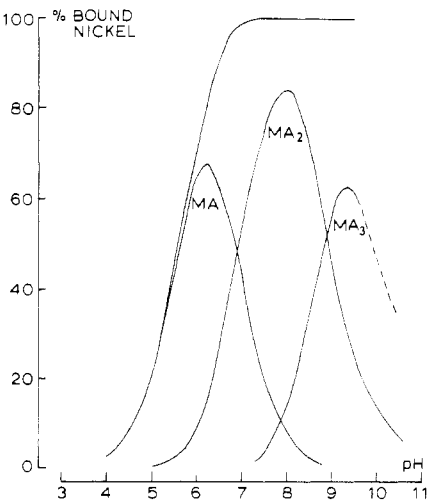

Figure 1. Species distribution in the binary system Ni(II)/AHA as a function of pH. $C_M = 3.0 \times 10^{-3}$ M; $C_A = 3.0 \times 10^{-2}$ M.

NHO^- group, as reported previously for a range of transition-metal complexes of alkyl hydroxamic acids.[13] The solid-state magnetic moments of 3.07 and 3.08 μ_B, measured at 20 °C for $Ni(AHA)_2 \cdot 2H_2O$ and $Ni(PHA)_2 \cdot 2H_2O$, and their relative insensitivity to decrease in temperature provide further support for an octahedral structure.

In solution, the nature of the species present will depend upon the pH. The species distribution for a metal concentration of $C_M = 3.0 \times 10^{-3}$ M and ligand concentration $C_A = 3.0 \times 10^{-2}$ M is given in Figure 1 for the Ni/AHA system (a very similar result is obtained for Ni/PHA—see supplementary material), and the stability constants of the various species are given in Table II.

Below pH 4.0, most of the nickel is present as the hexaaquonickel(II) ion, but as the pH is raised to 6.2, the formation of the MA species is completed, accounting for about 67% of

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the bound metal. The MA_2 species starts forming at pH 5 onward and reaches its maximum of 80% at pH 8.0. The negatively charged anionic species MA_3^- is represented by a dotted line in accordance with its low formation constant, log $K_{MA_2}^{MA_3^-} \sim 2.5$. The larger stability constant for PHA compared to that for AHA presumably reflects the greater inductive effect of the ethyl group compared to methyl. Magnetic moments of aqueous solutions of the Ni/AHA and the Ni/PHA systems are close to 3.0 μ_B and agree with the solid-state values as expected for octahedral systems. The electronic spectra were assigned with octahedral symmetry (see Table I), and the β value was calculated by standard procedures.[14] The β values suggest reasonably ionic M–O bands in these complexes, a conclusion that is supported by the low value of the calculated stability constants (Table II). In conclusion, the nickel(II) complexes of both aceto- and propionohydroxamic acids are octahedral both in the solid state and in solution, with all available evidence supporting coordination from the ketonic oxygen atom and the oxygen of the deprotonated NHO^- group. There is no evidence for the formation of oligomeric species for the simpler alkyl hydroxamic acids (AHA and PHA), in contrast to our previous studies of nickel(II) complexes of *N*-phenyl and *N*-methyl hydroxamic (MAHA) acids, where, for example, the tetrameric $Ni(MAHA)_2$ was found to be quite unreactive to nucleophiles such as pyridine.[13]

Amino Hydroxamic Acids. As mentioned above, hydroxamic acids are very important chelating agents in biological systems and are also potent inhibitors of urease activity. It has also been suggested that amino hydroxamic acids may be particularly active because of a possible surface-active role by an uncoordinated amino group;[15] however, in the case of iron complexes of glycinehydroxamic acid (GHA), we have shown recently that coordination clearly involves not only the deprotonated NHO^- group but also the amino group.[1] In view of the inhibitory action of amino hydroxamic acids of urease activity, we now discuss the interaction of Ni(II) with glycinehydroxamic acid (GHA), $NH_2CH_2CONHOH$, and serinehydroxamic acid (SHA), $HOCH_2CHNH_2CONHOH$, in both solution and the solid state.

In the solid state, it is immediately obvious that the two series of Ni(II) complexes of alkyl and amino hydroxamic acids are quite different. Thus the bis(amino hydroxamato)nickel(II) complexes form as crystalline, red or orange, air-stable solids that are insoluble in most solvents whereas the above bis(alkyl hydroxamato)nickel(II) dihydrates form green powdery solids soluble in polar solvents. Both $Ni(GHA)_2$ and $Ni(SHA)_2$ are diamagnetic, indicating a square-planar arrangement, which is further confirmed by the solid-state electronic spectra (obtained as reflectance when one absorption band appears at 430 nm for both complexes). The infrared spectra of $Ni(GHA)_2$ and $Ni(SHA)_2$ in the 3000–3500-cm^{-1} region are very similar to those of the corresponding amino acid complexes;[16] for example, bis(glycinato)nickel(II) dihydrate shows three bands at 3330, 3250, and 3170 cm^{-1} assigned to N–H stretching modes, and $Ni(GHA)_2$ shows corresponding bands at 3290, 3220, and 3120 cm^{-1} and $Ni(SHA)_2$ bands at 3290, 3120, and 3080 cm^{-1}, all of which shift on deuteration. These results suggest that the amino group is coordinated to the nickel as in the Fe/GHA system.[1] In contrast to the bis(alkyl hydroxamato)nickel(II) dihydrates, the carbonyl frequency in $Ni(GHA)_2$ remains unchanged at 1604 cm^{-1} from that in the free ligand, suggesting that the ketonic oxygen is *not* involved in coordination to the nickel atom; however, in $Ni(SHA)_2$ there is a shift of 24 cm^{-1} in the ketonic frequency, which may be due to intermolecular hydrogen bonding rather than coordination to the nickel atom. The lower frequency region is again consistent with coordination of the deprotonated NHO^- group.[13] It thus appears from the above spectroscopic studies that, in both $Ni(GHA)_2$ and $Ni(SHA)_2$, coordination of the amino hydroxamic acid is via the N atom of the amino group and from the deprotonated NOH^- group, and the square-planar complexes are probably held together by quite strong intermolecular H bonds involving the uncoordinated ketonic oxygen atom. X-ray crystallographic studies of $Ni(GHA)_2$ have been made recently by Pakkanen and co-workers and reported in a joint preliminary communication.[17] The structure is indeed that of a square-planar Ni complex with trans geometry and coordination of the amino nitrogen atom and, surprisingly, of the N atom of the NOH^- group, with intermolecular H bonds between a CO group of one molecule and the uncoordinated oxygen atom of an adjacent NOH^- group. This is the first example of coordination of a hydroxamic acid via the N atom of the hydroxamate function, and it will be very interesting to see whether further examples are found. In view of the X-ray structure and confirmation of intermolecular H bonds, it is rather surprising that no shift in the ketonic CO infrared frequency was observed for $Ni(GHA)_2$. Unfortunately we were not able to obtain crystals of $Ni(SHA)_2$ suitable for X-ray diffraction studies.

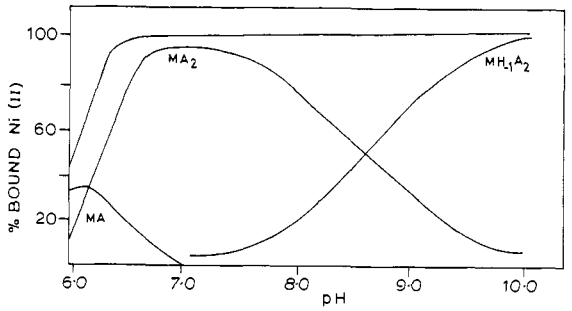

Figure 2. Species distribution in the binary system Ni(II)/GHA as a function of pH. $C_M = 4.0 \times 10^{-4}$ M; $C_A = 5.0 \times 10^{-3}$ M.

Species Distribution. The species distribution for a metal concentration $C_M = 0.4 \times 10^{-3}$ M and ligand concentration $C_A = 5.0 \times 10^{-3}$ M is given in Figure 2 for the Ni(II)/GHA system, and the stability constants of the various species are given in Table II. The data for the Ni(II)/SHA system are similar and are included in the supplementary material. In the acidic region, the MA complex predominates over a narrow pH range from 5.0 to 6.2 for Ni(II)/GHA and from 5.0 to 5.8 for Ni(II)/SHA, accounting for about 35% and 54% of the bound nickel, respectively. At neutral pH the MA_2 species predominates for $Ni(GHA)_2$ between pH 6.2 and 8.6 and for $Ni(SHA)_2$ between pH 5.9 and 7.7. In the alkaline region, there is evidence for possibly hydroxylated species $MH_{-1}A_2$ and $MH_{-2}A_2$ although these may also be due to H-bonded species, as suggested by the solid-state structure of $Ni(GHA)_2$. The molar proton liberation, $\delta[H_1^+]/\delta C_M$ (Figure 3) increases steadily from pH 5.0 to 7.0 for Ni(II)/GHA and from pH 5.0 to 6.3 for Ni(II)/SHA, reaching maxima of 3.6 and 3.7 protons, respectively. These values prove the involvement of the amino group (loss of one proton per NH_3^+ from the ligand A and loss of another proton from the NHOH group per ligand molecule); however, the theoretical value of four protons is not reached since the pK_2 values of GHA and SHA are 7.5 and 6.89 and the MA_2 species is formed in the region pH 6–8.

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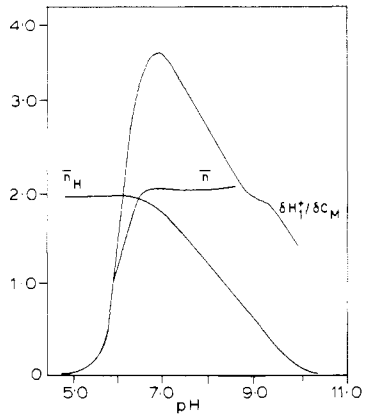

Figure 3. Molar proton liberation, $\delta(H^+)/\delta C_M$, and its relationship to $\bar{n}$ and $\bar{n}_H$ for the Ni(II)/GHA system.

Consequently, some protons will already have dissociated from the ligand before complexation. The above data show that coordination of Ni(II) by these amino hydroxamic acids involves coordination from the amino and NHO^- groups and does *not* involve the carbonyl in agreement with our previous conclusions on the Fe(III)/GHA system in solution.[15] Aqueous solutions of both the Ni(II)/GHA and Ni(II)/SHA systems are diamagnetic over a wide pH range, suggesting a square-planar configuration for the NiA_2 species in solution as well as in the solid state. The solution electronic spectra provide further confirmation of this configuration since both systems show two bands, one in the 370–430-nm region and one in the 490–510-nm region assigned respectively as $^1A_{1g} \rightarrow {}^1A_{2g}$ and $^1A_{1g} \rightarrow {}^1B_{1g}$ transitions in square-planar symmetry by comparison with observed spectra of Ni(II) complexes of confirmed square-planar configuration.[18] Infrared spectra were measured in D_2O in order to investigate further the role of the ketonic oxygen in coordination to nickel in solution. Shifts of about 30 cm^{-1} occur, comparing nickel solutions of pD values corresponding to a predominance of the MA_2 species and the free ligand, respectively; however, these shifts are concentration dependent and so most probably arise from intermolecular H bonding between the carbonyl group of one complex and the hydrogen of an adjacent NHO^- group, similar to that observed in the solid-state structure of $Ni(GHA)_2$,[17] rather than from coordination to the metal. It thus appears from both the species distribution and the solution structural studies that, at least for $Ni(GHA)_2$, the solid-state structure persists in solution.

Registry No. $Ni(AHA)_2 \cdot 2H_2O$, 85749-06-0; $Ni(PHA)_2 \cdot 2H_2O$, 85749-07-1; $Ni(GHA)_2$, 83267-42-9; $Ni(SHA)_2$, 85749-08-2; AHA, 546-88-3; PHA, 2580-63-4; GHA, 5349-80-4; SHA, 31697-35-5.

Supplementary Material Available: Tables of sample compositions and figures showing proton displacement and species distribution (11 pages). Ordering information is given on any current masthead page.

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Synthesis, Characterization, and EPR Spectral Studies of the Multimetal Species $[Fe(MS_4)_2]^{3-}$ (M = Mo, W)

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The complexes $[Et_4N]_3[Fe(MS_4)_2]$ (M = Mo, W) have been prepared by reaction of $[Et_4N]_2MS_4$ with $Fe(S_2CNC_5H_5)_2$ in CH_2Cl_2 and routinely characterized by infrared and visible spectroscopy. Cyclic voltammetry studies show that, for M = Mo, only the 3–/4– couple is reversible but that, for M = W, reversible 1–/2–, 2–/3–, and 3–/4– couples are observed. The complexes exhibit magnetic moments consistent with the presence of three unpaired electrons, prompting low-temperature electron paramagnetic resonance studies. Signals that are consistent with an $S = {}^3/_2$ spin state are observed in a variety of solvents for both M = Mo and M = W, and the absence of high-field resonances is indicative of a relatively high value for the zero-field splitting parameter. Values of $\lambda = E/D$ are estimated for all systems. The potential significance of the similarity of the EPR spectra of these complexes to those of the molybdenum–iron protein of nitrogenase and its cofactor is discussed.

Introduction

The presence of a unique iron–molybdenum–sulfur moiety in the iron–molybdenum protein ([Mo–Fe]) of the enzyme nitrogenase has been implicated by X-ray absorption,[1] EPR,[2] and Mössbauer,[2b,c] spectroscopic studies. Additional studies[4] have now shown that this Fe–Mo–S entity can apparently be extracted intact into *N*-methylformamide after denaturation of the protein. The extracted unit is called the iron–molybdenum cofactor (FeMo-co), and its stability in this organic medium bodes well for attempts to prepare a synthetic analogue for the molybdenum site of nitrogenase. These findings have prompted the recent syntheses of complexes containing these three elements as well as others where tungsten has been substituted for molybdenum. These species are all derived

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