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Chemistry

Fluorine-19 chemical shifts as structural probes of metal-sulfur clusters and the cofactor of nitrogenase

(¹⁹F NMR/iron-molybdenum-sulfur clusters)

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ABSTRACT **Several properties of the FeMo-cofactor (co) of nitrogenase in *N*-methylformamide solution at ambient temperature have been investigated by means of ¹⁹F NMR spectroscopy. With $C_6H_5CF_3$ reference signals the magnetic moment per Mo atom was found to be ≈3.9 BM, consistent with $S = 3/2$ ground state identified by other spectroscopic methods at low temperature. Reaction of FeMo-co with 1.0 eq of R_FS^- ($R_F = p$-$C_6H_4CF_3$, p-C_6H_4F) afforded isotropically shifted signals indicative of binding to a paramagnetic cluster. By comparison with the spectra of Fe-S and Fe-Mo-S species derivatized with R_FS^-, including the cubane-type $MoFe_3S_4$ clusters with $S = 3/2$ ground states, it was concluded that the essential FeMo-co cluster structure remains intact and a Fe atom is the probable thiolate binding site. An interaction of FeMo-co with $C_6H_5S^-$ had been detected earlier by low temperature EPR spectroscopy. The binding site assignment is based on large observed isotropic shifts (*ca.* −12 ppm) compared to the much smaller values found for Mo-SR_F ligands in $MoFe_3S_4$ clusters and anticipated in FeMo-co on the basis of recent spectroscopic results. Isotropic ¹⁹F shifts have proven extremely sensitive to electronic and structural features of Fe-S and Fe-Mo-S clusters. The inclusion of a ¹⁹F NMR label in FeMo-co should prove of utility in further investigation of cofactor properties and reactions.**

The FeMo-cofactor (co) of nitrogenase, first obtained in 1977 (1) by *N*-methylformamide (NMF) extraction of acid-denatured FeMo protein, is of considerable current interest because of the possibility that it may function as the catalytic site for reduction of dinitrogen and other substrates. Present analytical data indicate the atom ratios to be 7–8 Fe/4–6 S/Mo (1–3). Spectroscopic results have provided partial elucidation of certain properties of FeMo-co in NMF solution and in the native FeMo proteins. Among these are the findings that six Fe atoms and the Mo atom form a spin-coupled cluster having $S = 3/2$ ground state with an EPR spectrum unique in biology (4–7). Analysis of Mo atom extended x-ray absorption fine structure (EXAFS) substantiates a cluster structure (8), the most recent results indicating three Fe atoms at 2.68 Å, three S atoms at 2.36 Å, and three O,N atoms at 2.09 Å from the Mo atom (K. O. Hodgson, personal communication). These and other properties of FeMo-co have been reviewed (9).

Our synthetic analogue approach to the investigation of clusters in metallobiomolecules (10, 11) has resulted in the preparation of complexes **1–11** shown in Fig. 1. Terminal ligands are thiolates (RS^-) or fluorinated variants R_FS^- (see below). Of potential relevance to FeMo-co, although not conforming to its composition, are clusters **8–10** (12–15). These present $S = 3/2$ ground states and Mo atom coordination units similar to what has been deduced for FeMo-co. The clusters exhibit well-resolved, isotropically shifted NMR spectra that have proven indispensable in establishing solution structures. NMR spectroscopy has not been applied to FeMo-co because of the lack of any endogenous organic component. However, the observation (5, 16) that the EPR spectrum of FeMo-co in frozen NMF solution is sharpened in the presence of one PhSH or PhS^-/Mo atom suggests thiolate binding. Thiolate/FeMo-co interactions have been further examined with the ligands R_FS^- ($R_F = p$-$C_6H_4CF_3$, p-C_6H_4F) and ¹⁹F NMR spectroscopy in conjunction with the spectra of **1–11**. This technique obviates the lack of commercially available deuterated NMF and the expense in preparing sufficient quantities for FeMo-co extraction.

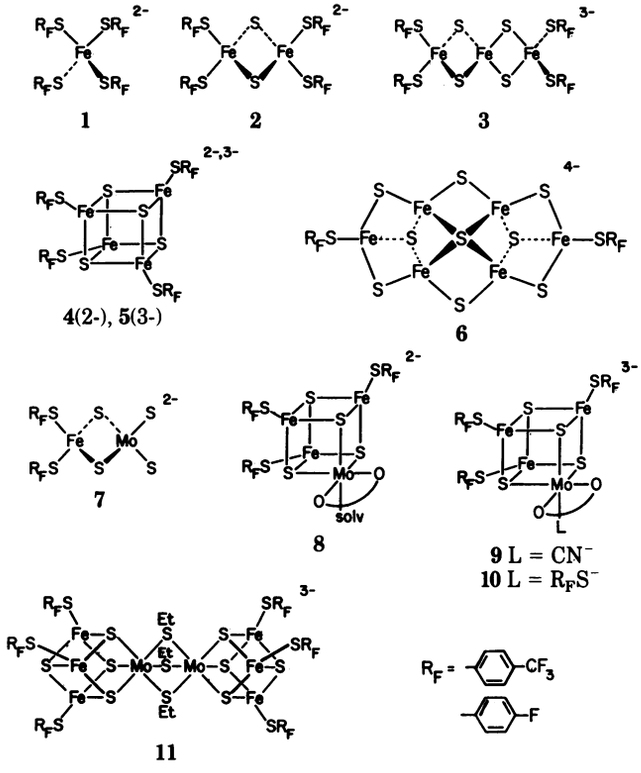

FIG. 1. Schematic structures of Fe-S (**1–6**) and Fe-Mo-S (**7–11**) complexes; O⁻O, 3,6-diallylcatecholate $(al_2cat)^{2-}$ ligand.

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Abbreviations: co, cofactor; NMF, *N*-methylformamide; EXAFS, extended x-ray absorption fine structure; al_2cat, 3,6-diallylcatecholate; DMF, *N*,*N*-dimethylformamide; Me_2SO, dimethyl sulfoxide; ENDOR, electron nuclear double resonance.

MATERIALS AND METHODS

Preparation of Compounds. Compounds R_FSH and $(Et_4N)_2[Fe_nS_n(SR_F)_4]$ (R_F = *p*-$C_6H_4CF_3$; n = 2,4) were prepared as described (17); *p*-fluorobenzenethiol (Aldrich) was distilled prior to use. Et_4N^+ thiolate salts were obtained by a known procedure (18). Preparations were conducted under strictly anaerobic conditions.

Fe-S compounds. $(Me_4N)_2[Fe(S\text{-}p\text{-}C_6H_4F)_4]$ (**1**). Reaction of 10 mmol of $FeCl_2{\cdot}2H_2O$ with 52 mmol of $Na(S\text{-}p\text{-}C_6H_4F)$ in 220 ml of ethanol gave a pale greenish black solution, from which a pale green solid separated upon addition of a solution of 22 mmol of Me_4NBr in 50 ml of ethanol. The solid was extracted with 150 ml of warm (45°C) acetonitrile. The extract was concentrated to ≈50 ml and cooled to −20°C, affording the product (82%) as greenish white crystals after washing with cold ethanol and drying *in vacuo*. *Anal.* Calculated for $C_{32}H_{40}F_4FeN_2S_4$: C, 53.92; H, 5.65; Fe, 7.83; N, 3.93; S, 18.00. Found: C, 53.48; H, 5.56; Fe, 7.31; N, 4.09; S, 17.93

$(Me_4N)_2[Fe_2S_2(S\text{-}p\text{-}C_6H_4F)_4]$ (**2**). Reaction of compound **1** and sulfur in acetonitrile, in a manner analogous to the preparation of $[Fe_2S_2(SPh)_4]^{2-}$ (19), gave the product (80%) as a dark purple crystalline solid. *Anal.* Calculated for $C_{32}H_{40}F_4Fe_2N_2S_6$: C, 46.15; H, 4.84, Fe, 13.41; N, 3.36; S, 23.10. Found: C, 46.41; H, 4.95; Fe, 13.38; N, 3.58; S, 23.31.

$(Et_4N)_2[Fe_4S_4(S\text{-}p\text{-}C_6H_4F)_4]$ (**4**). A standard procedure (20) involving reaction of $FeCl_3$, sulfur, and the sodium thiolate gave the product (80%) as a black crystalline solid. *Anal.* Calculated for $C_{40}H_{56}F_4Fe_4N_2S_8$: C, 42.86; H, 5.04; Fe, 19.93; N, 2.50; S, 22.88. Found: C, 42.94; H, 4.85; Fe, 19.84; N, 2.44; S, 22.70. The *n*-Pr_4N^+ salt was obtained similarly.

$(n\text{-}Pr_4N)_3[Fe_4S_4(S\text{-}p\text{-}C_6H_4F)_4]$ (**5**). Reduction of $(n\text{-}Pr_4N)_2[Fe_4S_4(S\text{-}p\text{-}C_6H_4F)_4]$ by a standard method (21) afforded the product (55%) as a black crystalline solid after recrystallization from acetonitrile/tetrahydrofuran 1:2 (vol/vol). *Anal.* Calculated for $C_{60}H_{100}F_4Fe_4N_3S_8$: C, 50.77; H, 7.10; Fe, 15.74; N, 2.96; S, 18.07. Found: C, 50.77; H, 6.96; Fe, 15.44; N, 2.88; S, 18.00.

Mo-Fe-S compounds. $(Et_4N)_4[Mo_2Fe_6S_8(S\text{-}p\text{-}C_6H_4CF_3)_6(al_2cat)_2]$. By use of a thiolate ligand substitution reaction (18) the product (45%) was obtained as a black crystalline solid. *Anal.* Calculated for $C_{98}H_{128}F_{18}Fe_6Mo_2N_4O_4S_{14}$: C, 42.89; H, 4.70; Fe, 12.21; N, 2.04; S, 16.36. Found: C, 43.23; H, 4.62; Fe, 12.56; N, 1.98; S, 16.21. $(Et_4N)_4[Mo_2Fe_6S_8(S\text{-}p\text{-}C_6H_4F)_6(al_2cat)_2]$ was prepared similarly; it was found to be pure by the NMR criterion for similar compounds (14). Both compounds form the solvated clusters **8** in solution.

Ligand Substitution Reactions. The following species were generated in NMF or acetonitrile solution from the indicated reactants: **1**, $[Fe(SEt)_4]^{2-}$ (22) + R_FSH; **3**, $[Fe_3S_4(SEt)_4]^{3-}$ (22) + R_FSH; **6**, $[Fe_6S_9(SEt)_2]^{4-}$ (unpublished data) + R_FSH; **7**, $[FeMoS_4Cl_2]^{2-}$ (23) + $(Et_4N)(SR_F)$; **9**, **8** + $(Et_4N)CN$; **10**, **8** + $(Et_4N)(SR_F)$; **11**, $[Mo_2Fe_6S_8(SEt)_9]^{3-}$ (24) + R_FSH. Reactions were monitored by NMR to ensure their completion. NMF (Aldrich) was stirred over anhydrous Na_2CO_3 for 48 hr, filtered, and distilled *in vacuo*; the central fraction was used. The set of species isolated or generated is given in Table 1.

FeMo-co was isolated from 2 g of purified *Azotobacter vinelandii* FeMo protein (specific activity, 2,850 nmol H_2/min per mg of protein) by the large-scale HCl/NaOH modification (25) of the original isolation method (1). NMF extracts of FeMo-co were concentrated (2) to give two solutions (mM Mo, mM Fe, activity): (*i*) 0.944 ± 0.097, 6.69 ± 0.63, 259 ± 11; and (*ii*) 1.47 ± 0.07, 10.51 ± 0.76, 239 ± 16. Activities (nmol C_2H_4 formed/min per nmol of Mo) were determined by reconstitution of crude extracts from *A. vinelandii* UW45 (1).

^1H (300 MHz) and ^{19}F (282.4 MHz) NMR spectra were obtained with a Brüker WM-300 spectrometer. Chemical shifts at fields above and below the internal references (Me_4Si, $PhCF_3$) are designated as positive and negative, respectively. Magnetic susceptibilities were determined by the NMR method (26) with 5-mm coaxial tubes, the above internal references, and the solvent susceptibilities of Gerger *et al.* (27). All measurements were made under anaerobic conditions.

Table 1. ^{19}F NMR shifts* of Fe-S and Fe-Mo-S clusters at ≈297 K

	Species	R_F =	δ, ppm vs. $PhCF_3$	
			p-$C_6H_4CF_3$	*p*-C_6H_4F
	R_FSH		−0.34	55.6
	$(Et_4N)(SR_F)$		−1.51	64.2
1	$[Fe(SR_F)_4]^{2-}$		−65.1	−9.00
2	$[Fe_2S_2(SR_F)_4]^{2-}$		−6.66	50.5
3	$[Fe_3S_4(SR_F)_4]^{3-}$		−80.3	†
4	$[Fe_4S_4(SR_F)_4]^{2-}$		−3.31	54.6
5	$[Fe_4S_4(SR_F)_4]^{3-}$		†	46.8
6	$[Fe_6S_9(SR_F)_2]^{4-}$		−4.48	55.8
7	$[(R_FS)_2FeS_2MoS_2]^{2-}$		−80.4	†
8	$[MoFe_3S_4(SR_F)_3(al_2cat)(NMF)]^{2-}$		−18.7	37.1
9	$[MoFe_3S_4(Sr_F)_3(al_2cat)CN]^{3-}$		−13.0‡,	24.6§,
	(C^2H_3CN)		−33.5§	47.0‡
10	$[MoFe_3S_4(SR_F)_4(al_2cat)]^{3-}$		−3.08¶,	23.3§,
	(C^2H_3CN)		−12.5‡,	48.1‡,
			−34.7§	60.8¶
11	$[Mo_2Fe_6S_8(SEt)_3(SR_F)_6]^{3-}$		−19.0	35.6
	FeMo-co + 1.0 $(Et_4N)(SR_F)$		−13.7	44.1

* In NMF solution unless noted otherwise.
† Not prepared.
‡ m′ ligand.
§ m ligand.
¶ Mo-bound ligand.

RESULTS AND DISCUSSION

Magnetic Susceptibilities. The molar magnetic susceptibility of a solute is given by Eq. 1

$$\chi_M = -\frac{3}{4\pi}\frac{\Delta\nu}{\nu}\frac{1000}{c} + M\chi_0 - \chi_{dia} \qquad [1]$$

in which $\Delta\nu$ (Hz) is the shift difference of sample and reference solution signals, ν is the spectrometer frequency, c is the molar concentration, M is the molecular weight, χ_0 is the solvent mass susceptibility, and χ_{dia} is the molar diamagnetic correction for solute. (A numerically small term involving the density difference between sample and reference solutions is omitted.) Because the molecular weight of FeMo-co is not known with certainty a procedure based on the molar concentration of molybdenum was used. Both FeMo-co and $[MoFe_3S_4(S\text{-}p\text{-}C_6H_4Cl)_4(al_2cat)]^{3-}$ afforded resolvable $PhCF_3$ signal separations at molybdenum concentrations of 1 mM (Fig. 2). The latter species is representative of a number of clusters **8**–**10** whose $S = 3/2$ spin system has been demonstrated by measurement of solution susceptibilities (12–14) and solid state magnetization at low temperatures (unpublished data). For the cluster the following results were obtained for 1.00 mM solutions at 300 K [$\Delta\nu$, first term in Eq. 1, χ_M, $\mu = 2.829\,(\chi_M T)^{1/2}$]: acetonitrile ($Me_4Si$ reference), −8.10 Hz, 6.42×10^{-3} cm^3/mol, 6.31×10^{-3} cm^3/mol, 3.89 BM; acetonitrile ($PhCF_3$ reference), −7.93 Hz, 6.68×10^{-3} cm^3/mol, 6.57×10^{-3} cm^3/mol, 3.97 BM; NMF/DMF-d_7, 68:32 ($PhCF_3$ reference), −8.17 Hz, 6.89×10^{-3} cm^3/mol, 6.96×10^{-3} cm^3/mol, 4.08 BM. The good agreement between results of measurements using Me_4Si and $PhCF_3$ establishes the validity of the latter as a susceptibility reference. Whereas Me_4Si is a frequently used reference for

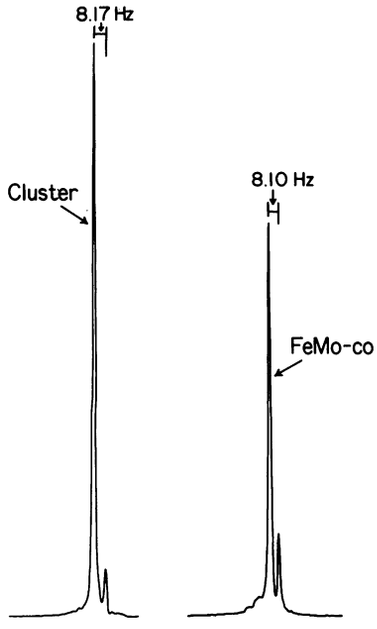

FIG. 2. Spectra illustrating ^{19}F shift differences of $PhCF_3$ in susceptibility determinations of $(Et_4N)_3[MoFe_3S_4(S\text{-}p\text{-}C_6H_4Cl)_4(al_2cat)]$ (*Left*) and FeMo-co (*Right*) in NMF/DMF-d_7, 68:32 (vol/vol) at 300 K. Both sample solutions were 1.00 mM in molybdenum and were in the outer tube; in this medium the cluster exists in the solvate form **8**. Reference and sample solutions contained 1 μl of $PhCF_3$; DMF-d_7 was used as a spectrometer lock. Signal amplitudes differed because of the use of concentric tubes of unequal volumes in the two experiments.

susceptibility determinations by ^1H NMR, we are unaware of a previous use of ^{19}F NMR for this purpose.

Duplicate measurements of FeMo-co solutions in NMF/DMF-d_7, 68:32, containing 1.00 mM molybdenum gave these average results at 300 K: $\Delta\nu = -7.71$ Hz; first term, 6.52×10^{-3} cm^3/mol of Mo. If, as is the case for $[MoFe_3S_4(S\text{-}p\text{-}C_6H_4Cl)_4(al_2cat)]^{3-}$, the second and third terms of Eq. 1 nearly cancel, the first term yields $\mu_{Mo} \cong 3.96$ BM. If the second and third terms are approximated by use of 1,500 as a possible maximum molecular weight (3) and 8 Fe/6 S/Mo as a minimal content, $\chi_{Mo} \cong 5.95 \times 10^{-3}$ cm^3/mol of Mo and $\mu_{Mo} = 3.78$ BM result. The set of χ_{Mo}, μ_{Mo} values for FeMo-co agree satisfactorily with the more accurately calculated molar quantities for the synthetic cluster. The magnetic moments of both species are close to the spin-only moment of 3.87 BM for $S = 3/2$ ground state. Thus, the magnetic moment of FeMo-co at ambient temperature is consistent with its EPR spectrum at $\leq$15 K and the small ($\approx$6 cm^{-1}) zero-field splitting of the $|\pm 1/2>$, $|\pm 3/2>$ Kramers' doublets of the $S = 3/2$ system (4–6).

Reactions of FeMo-co and R_FS^-. The EPR spectrum at 8 K of a NMF solution of FeMo-co and 1.0 eq of (Et_4N) $(S\text{-}p\text{-}C_6H_4CF_3)$ per Mo, frozen after 10–15 min of reaction, exhibited the same line sharpening and small g-value shifts noted previously in the presence of PhSH and PhS^- (5, 16). Reactions in fluid solutions were monitored by ^{19}F NMR; chemical shifts are given in Table 1. Addition of 1.0 eq of the thiolate salt per Mo to 0.94 mM FeMo-co in NMF afforded the spectrum in Fig. 3. The nonstoichiometric nature of the reaction is indicated by the 1.6/1 intensity ratio of the -13.7 and -1.34 ppm signals; the latter arises from a thiolate/thiol mixture in fast exchange. Triplicate runs gave the same result. Several analogous experiments with a 1.47 mM FeMo-co solution and (Et_4N) $(S\text{-}p\text{-}C_6H_4F)$ gave solutions with a signal at 44.1 ppm and no free ligand resonance. Addition of 2–6 eq of R_FS^- markedly decreased (-13.7 ppm) and increased (-1.3 ppm) signals ($R_F = p\text{-}C_6H_4CF_3$), a behavior suggestive of cluster decomposition‡ or caused cloudy solutions that were not further examined ($R_F = p\text{-}C_6H_4F$). The large shift differences between the -13.7 and 44.1 ppm features and the positions of R_FS^- or R_FSH resonances at parity of R_F can only arise from isotropic paramagnetic interactions of a contact or dipolar nature, defined by

$$(\Delta H/H_0)_{iso} = (\Delta H/H_0)_{obs} - (\Delta H/H_0)_{dia} = (\Delta H/H_0)_{con} + (\Delta H/H_0)_{dip}. \quad [2]$$

It has been shown that ^{19}F shifts readily distinguish between clusters **2** and **4** with $R_F = p\text{-}C_6H_4CF_3$ (17). The structure sensitivity of ^{19}F shifts has been further assessed by synthesis or *in situ* generation of the species **1–6** with one or both R_F substituents. This set encompasses all known structural types of synthetic or biological Fe-S clusters stabilized by monodentate thiolates except protein 3-Fe clusters (28, 29), synthetic analogues of which have not yet been achieved. It does not include the one-electron oxidized forms of **1** and **4** and the reduced form of **2**, which correspond to biological oxidation levels but are unstable. All species are paramagnetic and exhibit isotropically shifted resonances; chemical shifts in NMF are given in Table 1. The integrity of these complexes and the Fe-Mo-S clusters **7–11** (see below) in freshly prepared NMF solutions was established by similarities of absorption and ^{19}F spectra with those in acetonitrile solutions. None of the resonances of the species **1–6** occurs in the spectra of FeMo-co/R_FS^- systems. A more appropriate comparison involving species with one R_FS^- ligand and other binding sites occupied by solvent or inorganic ligands is difficult to achieve. Such species cannot be directly isolated, and appropriate ligand substitution precursors for the desired variants of all members of the set **1–6** are unavailable. With this caveat, the present observations are compatible with the earlier findings that excess PhSH does not disrupt FeMo-co (5) and excess $p\text{-}CF_3C_6H_4SH$ does not give $[Fe_nS_n(SR_F)_4]^{2-}$ ($n = 2,4$) from FeMo-co (30). Similarly, thiolate reactions do not produce **7**, derived from MoS_4^{2-} (23), which is reported to be released by acid/base treatment of a FeMo protein (31). In this case the complexes $[FeMoS_4Cl_{2-n}(S\text{-}p\text{-}C_6H_4CF_3)_n]^{2-}$ with $n = 1$ (-81.7 ppm) and $n = 2$ (-82.6 ppm) were observable in ligand substitution reactions in acetonitrile.

Thiolate Binding Site in FeMo-co. Under the C_s symmetry of clusters **8–10** the Fe atoms and their ligands divide into the sites m, on, and two m', related by the mirror plane bisecting the cluster and perpendicular to the catecholate chelate ring. The clusters **8** undergo rapid exchange of bound and bulk solvent molecules with attendant degenerate reorientation of the catecholate ring, averaging sites m and m' (13–15). This effective trigonal symmetry is like that of the very weakly coupled $S = 3/2$ subclusters of **11** (32) and, as in that case, affords a single ^{19}F resonance, illustrated for one NMF solvate in Fig. 3. Chemical shifts of Fe-Mo-S clusters are listed in Table 1. In acetonitrile solution, addition of 1.0 eq of $L = CN^-$ (**9**), RS^- or R_FS^- (**10**), and PEt_3 results in stoichiometric binding at the Mo atom with solvent displacement and the appearance of two resonances. For $R_F = p\text{-}C_6H_4CF_3$ (Fig. 3) the m and m' resonances occur at about -34 and -13 ppm, respectively; in this and other clusters, m ligands experience the larger isotropic shift. Assignment of the remaining ^{19}F signals in the spectra of **10**, at -3.08 ($R_F = p\text{-}C_6H_4CF_3$) and 60.8 ppm ($R_F = p\text{-}C_6H_4F$), to Mo-bound ligands follows from their absence in the spectra of **9** and the phosphine adducts. In NMF, *N*,*N*-dimethylformamide (DMF), and dimethyl sulfoxide (Me_2SO) solutions con-

‡ This behavior was not apparent in EPR spectra of rapidly frozen solutions containing 2–8 eq of thiolate per Mo; FeMo-co signal intensities did not diminish and new resonances did not appear.

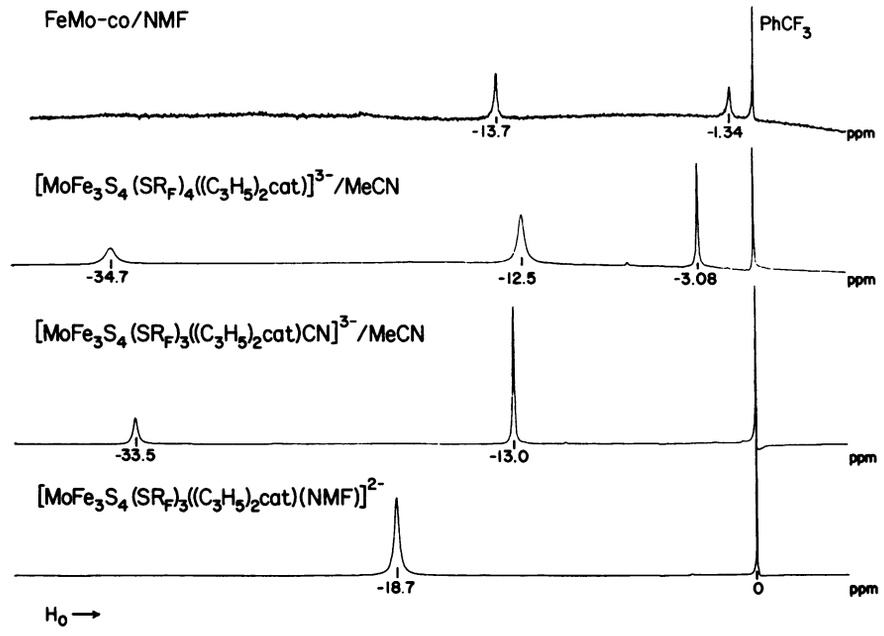

FIG. 3. ^{19}F NMF spectra of $S = 3/2$ clusters and FeMo-co in NMF or acetonitrile solutions at 297 K. Top spectrum is for NMF solution 0.94 mM in molybdenum to which 1.0 eq of $(Et_4N)(SR_F)$ was added per Mo. It was recorded after 15 min of reaction and was unchanged after 24 hr. In all spectra, $R_F = p\text{-}C_6H_4CF_3$.

taining 1.0 eq of thiolate the spectra of **8** and the free ligand appear, demonstrating the lack of binding at the Mo site.

The ^{19}F shifts of Fe-S-p-$C_6H_4CF_3$ ligands in **8**–**10** and the PEt_3 adduct are remarkably sensitive to the extent of thiolate substitution at the Fe sites. Titration of $[MoFe_3S_4Cl_3(al_2cat)(MeCN)]^{2-}$, which has the structure **8** with chloride in place of thiolate (unpublished data), with 1–3 eq of p-$CF_3C_6H_4S^-$ in acetonitrile affords $[MoFe_3S_4Cl_{3-n}(SR_F)_n(al_2cat)(MeCN)]^{2-}$. These species have signals at -3.06 ($n = 1$), -12.9 ($n = 2$), and -19.7 ppm ($n = 3$). With 2–3 eq, the $n = 2,3$ species with Mo-bound thiolate also appear; the fully substituted cluster **10** is produced with ≥4 eq. These results have been confirmed by generation of $[MoFe_3S_4Cl_3(al_2cat)(PEt_3)]^{2-}$ followed by titration with p-$CF_3C_6H_4S^-$ in acetonitrile. The stable Mo-PEt_3 ligation affords five species in the **n** = 1–3 set, all of which have been detected. Chemical shifts of Fe-bound ligands in the thiolate and phosphine adducts occur in the -40 to $+5$ ppm range. In contrast, shifts of the Mo-S-p-$C_6H_4CF_3$ ligand in partially and fully substituted clusters in acetonitrile fall in the narrow interval of -2.9 to -3.1 ppm. Because the shifts of **8** and **11** with

Table 2. Isotropic ^1H and ^{19}F shifts of Fe-Mo-S clusters at ≈297 K

Cluster (R, R_F)	^1H (C^2H_3CN), ppm*			^{19}F, ppm*	
	o	*m*	*p*	C^2H_3CN	NMF
8 (p-$C_6H_4CH_3$)	+9.64	−6.05	−9.81	—	—
8 (p-$C_6H_4CF_3$)	+9.94	−6.07	—	−19.3	−18.5
8 (p-C_6H_4F)	+9.69	−5.70	—	−18.0	−18.5
10 (p-$C_6H_4CH_3$)					
m	+16.3	−10.3	−17.0	—	—
m'	†	−3.21	−5.44	—	—
Mo‡	†	+0.13	−0.94	—	—
10 (p-$C_6H_4CF_3$)					
m	+16.6	−9.85	—	−34.5	§
m'	†	−3.25	—	−12.3	
Mo	−0.60	≈0	—	−2.85	
				−0.85¶	
10 (p-C_6H_4F)					
m	+17.3	−10.1	—	−32.5	§
m'	†	−2.91	—	−7.69	
Mo	−0.13	+0.43	—	+4.99	
				−7.67¶	
FeMo-co + 1.0 p-$CF_3C_6H_4S^-$	—	—	—	—	−13.4, −12.2¶
+ 1.0 p-$FC_6H_4S^-$	—	—	—	—	−11.5, −20.1¶

* Referenced to thiol unless otherwise noted.
† Not resolved.
‡ For R = Ph: *o*-H, −0.30; *m*-H, +0.10; *p*-H, +1.16 ppm.
§ Solvated cluster formed.
¶ Referenced to R_FS^-.

either R_F substituent differ by ≤1 ppm in NMF and acetonitrile, shifts in the latter solvent are considered good estimates for those of the clusters **10** were they observable in NMF. Isotropic shifts of selected clusters and FeMo-co-SR_F (Table 2), calculated by using thiols as diamagnetic references in Eq. **2**, are much smaller for Mo-SR_F ligands than for Fe-bound thiolates in FeMo-co. The clusters contain the $[MoFe_3S_4]^{3+}$ core unit which is electronically delocalized with coupled spins. An analysis of ^{57}Fe isomer shifts has led to the preferred (mean) oxidation state description of +2.67 for the Fe atoms and, by difference, +3 for the Mo atom (32). Further indication that this unit produces intrinsically small isotropic interactions of Mo-bound ligands is found in the ^1H shift data presented in Table 2. Also, ^1H isotropic shifts of bridging ligands in $[Mo_2Fe_6S_8(SR)_9]^{3-}$ (R = Ph, p-$C_6H_4CH_3$) are relatively small (≤2 ppm), being ≤20% of those of terminal ligands (32, 33).

Recent ^{95}Mo electron nuclear double resonance (ENDOR) results for a FeMo protein have been interpreted in terms of an even-electron diamagnetic Mo atom structurally integrated in a cluster containing six Fe atoms (7). Such an arrangement appears likely to produce small contact shifts of a Mo-thiolate ligand, and small dipolar shifts are probable at a *para* substituent owing to the r^{-3} dependence on metal–nucleus mean distance (34). Any differences in the electronic structures of synthetic and native S = ½ clusters notwithstanding, small isotropic shifts of Mo-thiolate ligands are an experimental property of the former and a reasonable proposition (based on ENDOR results) for the latter. For these reasons and in the absence of superior S = ½ structural models of the Mo atom site in FeMo-co, it is concluded that the thiolate binding site in FeMo-co·SR_F is a Fe atom. This conclusion is in agreement with that from an analysis of the Mo EXAFS of FeMo-co·SPh (K. O. Hodgson, personal communication).

The signs and, to a lesser extent, the magnitudes of the ^1H and ^{19}F isotropic shifts of Fe-thiolate ligands in Table 2 and ^1H shifts of other clusters **8–11** (14, 15, 32, 33) are consistent with dominant hyperfine contact interactions (34–36). The signs of all shifts conform to a ligand→metal antiparallel spin delocalization mechanism such as is found with clusters **4** and **5** (37). Both FeMo-co·SR_F species exhibit negative ^{19}F isotropic shifts, as do **8–11**, suggesting that, at the *para* positions, contact shifts prevail and the same type of delocalization mechanism is operative. Using the Mo-thiolate ligands in the clusters **10** as the only examples available, the signs of the p-H, p-CH_3, and p-CF_3 (but not necessarily of the o-H and m-H) isotropic shifts are also suggestive of dominant contact shifts but their magnitudes are usually small compared to Fe-thiolate shifts in the same cluster. Because of the variability of the latter shifts with extent of substitution, the assignment of a Fe binding site in FeMo-co is better founded on demonstrated or anticipated small shifts of Mo-thiolate ligands in synthetic or native S = ½ clusters. Lastly, the structure of FeMo-co in NMF must be such as to allow binding of at least one thiolate ligand at ambient temperature. The species FeMo-co·SR_F are chemical derivatives of the cofactor, and the ^{19}F NMR label provides an opportunity to monitor directly certain reactions, including oxidation-reduction.

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