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Multinuclear NMR studies of some oxomolybdenum(VI) complexes with polyaminocarboxylates

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Abstract

^{95}Mo and ^{13}C NMR spectra of aqueous solutions containing molybdate and either DTPA or TTHA at pH values between 2.0 and 9.5 reflect the presence of two complexes of 1:1 (MoL) and 2:1 (Mo_2L) stoichiometries for both ligands. All complexes give one broad ^{95}Mo signal at 60–70 ppm relative to free molybdate, with linewidth 380–890 Hz which increases when the pH decreases. The ^{95}Mo NMR parameters indicate that the metal center of the complexes consists of the MoO_3 species, which is confirmed by the presence of two ^{17}O NMR signals in the intensity ratio of 1:2 from the corresponding oxo ligands which are *trans* to the nitrogen and oxygen atoms of the ligand IDA-type moities that bind the metal center. The intensities and multiplicities of the ^{13}C signals of the complexes indicate that in the MoL complexes the metal center binds one of the terminal IDA-type moieties of the ligands, whereas in the Mo_2L complexes two metal centers bind the two terminal IDA-type moieties, yielding a very symmetric species. The intensities of the ^{95}Mo and ^{13}C signals give the pH dependence of the concentration of the free ligand, L, free molybdate, Mo, and the MoL and Mo_2L complexes. In general, for both 1:1 and 2:1 solution stoichiometries used, the free ligand and the two complexes are present between pH 2.0 and about 8, Mo only appears above pH 6 and at pH 9 only L and Mo occur. The pH dependence of the ^{13}C complexation shifts reflects the protonation state and sites of the ligand in the complexes. The binding of the MoO_3 center to two nitrogen atoms and one carboxylate oxygen of EDDA leads to two diastereomeric pairs of this complex, which are reflected in the presence of two sets of proton signals. Those spectra were assigned using two-dimensional COSY and *J*-resolved spectra. The vicinal coupling constants obtained were used to define the structures of the species present. Two-dimensional exchange spectra yielded the exchange mechanisms which operate in solution.

Introduction

Studies of the coordination chemistry of molybdenum in the Mo(VI) oxidation state are useful for understanding the mechanistic role of this metal in enzymatic [1–4] and industrial [5] catalysis, as well as in its biological transport processes [6].

Many solution complexation studies of Mo(VI) by aminocarboxylate ligands have been undertaken, including determination of stability constants [7–13], kinetic studies of complex formation [14] using polarography, potentiometry and UV, IR and Raman spectrophotometry. Structural studies in the solid state using X-ray diffraction methods have also been carried out [15, 16]. Various solution NMR studies of Mo(VI)–polyaminocarboxylate complexes have been reported, using ^{1}H [7, 17, 18], ^{13}C [19], ^{17}O [19, 20] and ^{95}Mo [19] observation, while some Mo(V) complexes were also studied [21]. In this paper we complement previous NMR work of Reilley and co-workers [19], further studying the interaction of Mo(VI) with the ligands ethylenediaminediacetic acid (EDDA) and diethylenetriaminepentaacetic acid (DTPA) (Fig. 1) in aqueous solution at various pH values using ^{1}H, ^{13}C, ^{17}O and ^{95}Mo NMR. On the basis of the results obtained we studied for the first time the complexation of Mo(VI) by triethylenetetraaminehexaacetic acid (TTHA), 1,4,7-triazacyclononane ([9]aneN_3) and 1,4,7-triaazacyclononane-*N*,*N'*,*N''*-triacetic acid (NOTA) (Fig. 1) also using ^{1}H, ^{13}C, ^{17}O and ^{95}Mo NMR spectroscopy.

Experimental

The ligands EDDA, DTPA and TTHA were obtained from Aldrich as the free acids. The macrocyclic chelates

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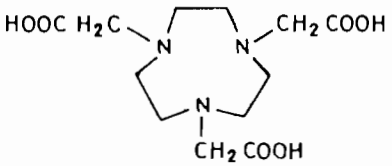
EDDA $HOOCCH_2NHCH_2CH_2NHCH_2COOH$

DTPA $(HOOCCH_2)_2NCH_2CH_2N(CH_2COOH)CH_2CH_2N(CH_2COOH)_2$

TTHA $(HOOCCH_2)_2NCH_2CH_2N(CH_2COOH)CH_2CH_2N(CH_2COOH)CH_2CH_2N(CH_2COOH)_2$

NOTA 

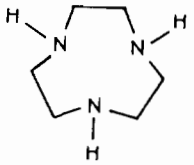
[9]-ane-N_3 

Fig. 1. Chemical structures of the polyaminocarboxylate ligands studied.

[9]aneN_3 and NOTA were synthesized by the method of Richman and Atkins and purified according to published procedures [22, 23]. Sodium molybdate dihydrate, obtained from Merck, was dried under vacuum. The ligands were lyophilized from D_2O solutions.

Solution samples for NMR spectroscopy were prepared by dissolving appropriate amounts of the Mo(VI) salt in aqueous solutions of the ligand. Solutions for ^{13}C, ^{17}O and ^{95}Mo NMR were in H_2O containing 20% D_2O, and solutions for ^1H spectra were in D_2O (99.8% D, from Stohler Isotope Chemicals). The pH was adjusted with dilute solutions of DCl and NaOD (from Stohler Isotope Chemicals). pH values were measured on a Metrohm E520 pH meter, equipped with an Ingold 405-M3A glass electrode, and were uncorrected for the D_2O content of the solvent.

NMR spectra were obtained on a Varian XL-200 (^1H and ^{13}C frequencies of 200.053 and 50.300 MHz, respectively) or on a Varian VXR-400 spectrometer (^1H, ^{13}C, ^{17}O and ^{95}Mo frequencies of 399.952, 100.577, 54.219 and 26.063 MHz, respectively). Some 500 MHz ^1H NMR spectra were obtained on a General Electric GN-500 spectrometer. Proton noise decoupling, with suppression of nuclear Overhauser effects, was used for ^{13}C spectra with a repetition time of 12 s and a 60° pulse. TSS (sodium 3-trimethylsilyl-(2,2,3,3-d_4) propionate) was used as internal reference for ^1H chemical shifts. Carbon chemical shifts were measured relative to internal t-butanol (methyl δ 31.2). The solvent H_2O signal was used as internal reference for ^{17}O shifts, while ^{95}Mo shifts were externally referenced to a 0.5 M aqueous solution of sodium molybdate. The experimental precision of the measured shifts was ± 0.01 ppm for ^1H, ± 0.05 ppm for ^{13}C and between ± 0.1 ppm and ± 1 ppm for ^{17}O and ^{95}Mo, usually 3% of the linewidth value. Signal assignments were carried out on the basis of relative intensities and multiplicities of the signals, spin decoupling experiments as well as by comparison with previous studies of similar systems [18, 19]. All NMR measurements were carried out at 22 ± 1 °C, unless stated otherwise.

The 2D cross relaxation experiment was performed in the phase sensitive mode using a mixing time of 0.8 s and an acquisition delay of 0.8 s. The mixing time was randomized between increments with a maximum variation of 10%. Nitrogen was bubbled through the sample for 15 min prior to the measurement.

Results and discussion

Mo(VI)-EDDA complexes

The ^{95}Mo spectrum of a solution of 0.14 M EDDA and 0.13 M molybdate in D_2O at pH 6 and 25 °C shows, apart from a small sharp signal for free molybdate at 1.1 ppm, a broad signal at +60 ppm ($\Delta\nu_{1/2} = 300$ Hz), which is characteristic of MoO_3 complexes of aminocarboxylates [18–20]. The 400 MHz ^1H NMR spectrum of this sample displays two sets of signals (integral ratio **A**:**B** = 54:46) for MoO_3 complexes and two small singlets for free EDDA. This is in agreement with binding of EDDA by MoO_3 in a tridentate fashion via both N atoms and a carboxylate oxygen of one of the acetate arms, as has been proposed previously by Reilley and co-workers on the basis of ^{13}C and ^{17}O NMR data [19]. Upon binding in this way, the inversion of the N atoms is precluded, which makes them chiral. Therefore, two diastereomeric pairs of this complex are possible (see Fig. 2), which is reflected in the presence of two sets of signals in the ^1H spectrum. The peaks in the ^1H spectrum were assigned with the

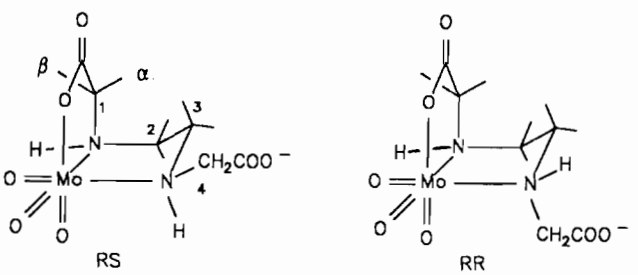


Fig. 2. Two diastereomers of Mo(VI) chelates formed with EDDA.

use of a 2D COSY spectrum using a mixing pulse of flip angle 45° [24]. Since geminal and vicinal HH couplings have opposite signs, the tilt of the cross peaks could be exploited for an unambiguous determination of the connectivities between the ethylene protons. Then, with the use of a 2D *J*-resolved spectrum [25] and spin simulation all HH coupling constants were determined. For each isomer a rather large long range coupling constant was observed between one of the bound acetate protons and one of the ethylene protons (1.4 and 1.0 Hz, respectively), which indicates that the concerning protons are in about a W-configuration. An inspection of molecular models shows that this is the case of $H_{1\beta}$ and $H_{2\beta}$, and now with the use of the connectivities obtained from the COSY spectrum an unambiguous assignment of all signals in the ^1H spectrum is possible. The chemical shifts and coupling constants obtained are compiled in Table 1.

Compound **A** has a large coupling $^3J_{2\beta3\alpha}$ (13.9 Hz), which indicates that it has a rigid conformation. The dihedral angles in the ethylene group were estimated from the set of vicinal coupling constants concerned with the use of the semi-empirical relationship of Altona and co-workers [26]. It was assumed that the angle between geminal protons in the Newman projection is 120°. The electronegativities of the substituents ($\Delta\chi$) are unknown and were, therefore, included in the fitting procedure. Optimal results were obtained for $\Delta\chi = 0.07$, which seems a reasonable value since it may be expected that the electronegativities of α N and β Mo have opposite effects on the magnitudes of the vicinal coupling constants [22]. The proton–proton torsion angles obtained are $H_{2\alpha}H_{3\alpha} = 54.5°$, $H_{2\alpha}H_{3\beta} = 65°$, $H_{2\beta}H_{3\alpha} = 174°$ and $H_{2\beta}H_{3\beta} = 54.5°$. It may be concluded that the five-membered $MoNCH_2CH_2N$ chelate ring adopts an envelope conformation with Mo, the N atoms and C_2 in a plane and C_3 on the flap, which is directed towards the $MoNCH_2COO$ ring (see Fig. 2).

TABLE 1. ^1H chemical shifts and coupling constants of the two diastereomeric pairs of the 1:1 MoO_3–EDDA complex in a solution of 0.14 M EDDA and 0.13 M $Na_2MoO_4 \cdot 2H_2O$ in D_2O at pH 6 and 25 °C

Nucleus	*RS/SR* (**A**)	*RR/SS* (**B**)
Chemical shifts (ppm)		
1_α	3.226	3.372
1_β	3.834	3.975
2_α	2.835	2.611
2_β	3.104	2.935
3_α	2.204	3.314
3_β	3.243	2.583
4_α	2.943	3.769
4_β	3.636	3.292
Coupling constants (Hz)		
$^2J_{1\alpha1\beta}$	−17.6	−17.6
$^4J_{1\beta2\beta}$	1.0	1.4
$^2J_{2\alpha2\beta}$	−13.3	−13.3
$^3J_{2\alpha3\alpha}$	3.9	3.4
$^3J_{2\alpha3\beta}$	2.0	5.3
$^3J_{2\beta3\alpha}$	13.9	13.2
$^3J_{2\beta3\beta}$	3.9	4.0
$^2J_{3\alpha3\beta}$	−13.3	−13.3
$^2J_{4\alpha4\beta}$	−17.1	−17.3

The coupling constants for structure **B** are rather similar to those of **A** and they indicate that the bicyclic ring skeleton adopts about the same conformation as structure **A**. The magnitude of $^3J_{2\beta3\alpha}$ (13.2 Hz), however, is relatively low, whereas that of $^3J_{2\alpha3\beta}$ (5.3 Hz) is rather high. Probably this compound is not conformationally homogeneous and 10–20% of the envelope conformation with the flap directed away from the other ring contributes to the conformational equilibrium. This is an indication that **B** is the *RR/SS* diastereomeric pair, because for this configuration the preferred conformation may be somewhat destabilized by an unfavorable 'syn-diaxial' interaction between the free acetate group and $H_{2\beta}$.

Upon heating the sample severe exchange broadening occurred in the ^1H spectra. The exchange mechanisms could be elucidated with the use of 2D exchange spectra [25] of the sample at 25 °C. Positive cross peaks were observed between the signals of free EDDA and the corresponding signals in the two MoO_3 complexes. In addition, the 2D spectrum reveals large cross peaks for exchange among sites of each diastereomeric pair. Interchange of chemical shifts was observed between the following pairs of nuclei of structure **A**: $H_{1\alpha} \rightleftharpoons H_{4\alpha}$, $H_{1\beta} \rightleftharpoons H_{4\beta}$, $H_{2\alpha} \rightleftharpoons H_{3\alpha}$ and $H_{2\beta} \rightleftharpoons H_{3\beta}$; and for structure **B**: $H_{1\alpha} \rightleftharpoons H_{4\alpha}$, $H_{1\beta} \rightleftharpoons H_{4\beta}$ and $H_{2\beta} \rightleftharpoons H_{3\alpha}$. The ^1H signals of $H_{2\alpha}$ and $H_{3\beta}$ of structure **B** overlap and therefore no exchange cross peak could be observed in the 2D spectrum. Furthermore, smaller negative cross peaks were observed, which can be ascribed to NOE effects and to a combination of exchange and NOE effects. Some typical cross sections through the 2D exchange spectrum are given in Fig. 3. For example, the cross section through the signal of $H_{1\beta}$ of structure **B** shows positive peaks for exchange with the corresponding nucleus of free EDDA and $H_{4\beta}$ and negative peaks at the resonance frequencies of $H_{1\alpha}$ and $H_{4\alpha}$. The former can be ascribed to the NOE between $H_{1\beta}$ and $H_{1\alpha}$ and the latter to a transfer of magnetization from $H_{1\beta}$ to $H_{4\beta}$ via exchange followed by a transfer to $H_{4\alpha}$ via the nuclear Overhauser mechanism, or alternatively first transfer to $H_{1\alpha}$ via NOE and then to $H_{4\alpha}$ via exchange.

These data show that, both in **A** and **B**, an exchange mechanism is operative, in which the N atoms remain coordinated, whereas the bound and free acetate arms exchange (Fig. 4). In the *RS* or the *SR* complex the entering acetate group takes the same position on Mo(VI) as the leaving acetate. The reaction product

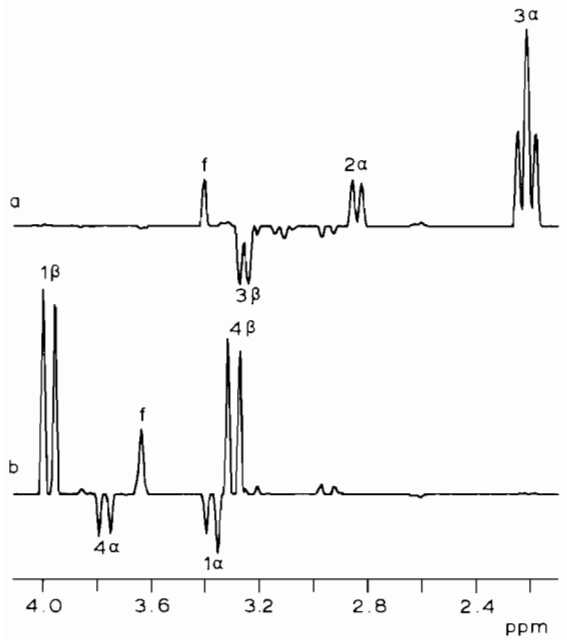


Fig. 3. Cross sections through the 2D exchange spectrum (^{1}H) of MoO_3–EDDA; a, at $\omega_{3\alpha}$ of **A**; b, at $\omega_{1\beta}$ of **B**; f = signal of free ligand.

has an unfavorable conformation of the $MoNCH_2CH_2N$ ring and therefore a ring flip occurs, which is in agreement with the observed exchange between pseudo-axial and pseudo-equatorial protons in the ethylene group in structure **A**. In the *RR/SS* diastereomeric pair the substituting acetate attacks Mo(VI) at the *trans* position with respect to the leaving acetate group and no conformational interconversion of the ethylenediamine chelate ring is required. Since the 2D exchange data show that this is the case for **B**, it can be concluded that **B** is the *RR/SS* pair. It should be noted that in **A** this exchange leads to the interconversion $RS \rightleftharpoons SR$, whereas in **B** the same diastereomer is obtained after rearrangement.

Mo(VI)–DTPA and –TTHA complexes

The 500 MHz ^{1}H spectra of solutions of Mo(VI)–DTPA and Mo(VI)–TTHA, with stoichiometric molar ratios of 1:1 and 2:1 and pH 6.0 (data not shown), are quite broad and not conclusive relative to the nature and structure of the various complexes formed by these ligands.

The lack of useful information provided by the ^{1}H spectra led us to further study the Mo(VI)–DTPA system, previously analysed at pH 6.0 by ^{13}C NMR [19], as well as the Mo(VI)–TTHA system using ^{95}Mo, ^{13}C and ^{17}O NMR as a function of pH and metal-to-ligand stoichiometry.

^{95}Mo NMR spectra were obtained for the Mo(VI)–DTPA and Mo(VI)–TTHA systems at 1:1 and 2:1 metal-to-ligand ratios and pH values between 3 and 9. The characteristics of the ^{95}Mo signals are summarized in Table 2. At high pH (pH ≈ 9) only a sharp signal corresponding to the tetrahedral MoO_4^{2-} species is present. As the pH decreases, a new broad signal, at +64 ppm relative to molybdate, can be observed. Its

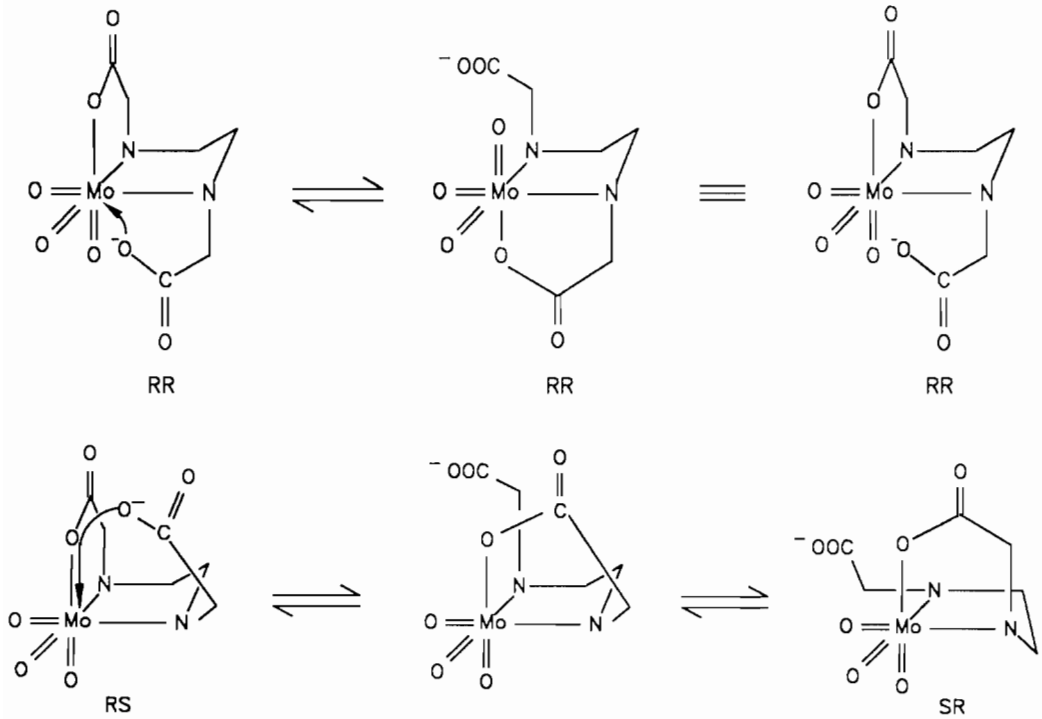


Fig. 4. The mechanism of the exchange of nuclei within the *RS* and the *RR* diastereomers of the MoO_3–EDDA complex.

TABLE 2. Chemical shift (δ), linewidth ($\Delta\nu_{1/2}$) and intensity percentage (p) of the ^{95}Mo NMR signals of Mo(VI)–DTPA and Mo(VI)–TTHA solutions as a function of M/L ratio and pH

System	M/L	pH	Molybdate			Complex		
			δ (ppm)	$\Delta\nu_{1/2}$ (Hz)	p (%)	δ (ppm)	$\Delta\nu_{1/2}$ (Hz)	p (%)
Mo(VI)/DTPA	1:1	9.5	0.0	28	100			0
		8.6	−0.8	40	100			0
		7.6	−0.9	65	18	64	410	82
		6.4	−1.1	66	2	62	483	98
		5.5			0	61	545	100
		4.5			0	60	554	100
		3.5			0	60	558	100
	2:1	9.5	0.0	22	100			0
		8.6	−0.8	51	67	66	369	33
		7.6	−0.9	63	45	64	440	55
		6.4	−1.1	68	6	63	500	94
		5.4			0	60	560	0
		4.3			0	59	571	0
		3.5			0	59	585	0
Mo(VI)/TTHA	1:1	9.0	0.0	30	100			0
		7.8	0.9	73	29	64	380	71
		6.7	−0.8	87	4	62	515	96
		5.1			0	61	642	100
		3.4			0	60	777	100
	2:1	9.0	0.0	30	100			0
		7.6	0.0	70	36	67	620	64
		6.5	−1.1	78	7	64	700	93
		4.3			0	64	890	100

intensity, relative to the MoO_4^{2-} signal, increases steadily and becomes the only species present below pH 6 for 1:1 and 2:1 solutions of both systems. This broad signal, similar to others previously reported for oxomolybdenum(VI) complexes with aminocarboxylate ligands [18–20], is attributed to the possible complex(es) formed between Mo(VI) and DTPA and TTHA. The high frequency shift of the ^{95}Mo NMR signal due to complexation is probably a result of a decreased electron density at the metal center. The large linewidth of the signal of the complex(es) reflects the efficient quadrupolar relaxation of the ^{95}Mo nucleus ($I=5/2$) of the metal center induced by the electric field gradients and an increased rotational correlation time caused by complexation. The broadening of the two signals at pH values where both MoO_4^{2-} and the complex(es) are present in solution results from chemical exchange effects. Even at very low pH, no broad signal at +40 ppm from the $Mo_7O_{24}^{6-}$ species is observed in the presence of the ligands. This is in contrast with what is observed for molybdate solutions in the absence of ligands [19].

^{13}C NMR spectra of Mo(VI)–DTPA and Mo(VI)–TTHA solutions were obtained as a function of pH, between pH 2.0 and 9.0, and for metal-to-ligand ratios of 1:1 and 2:1. These ^{13}C NMR studies were quite informative about the number of complexes formed with these systems at the different solution conditions studied, their respective stoichiometries and binding sites.

The number of signals observed in the ^{13}C NMR spectra indicate the formation of two complexes, C_1 and C_2, for both systems, with metal-to-ligand stoichiometries of 1:1 and 2:1, respectively. For instance, in the case of the C_1 complex of Mo(VI)–TTHA four different ^{13}C signals are observed for the carboxylate carbons or for the respective methylene carbons, with relative intensities of 2:1:1:2 (see Fig. 5). The ^{13}C spectrum of the C_2 complex of the same system is very simple, with only two different types of carboxylate and methylene carbons, as a result of its high symmetry. Taking into account the information available for the complexation of Mo(VI) with the ligands EDTA and DTPA [19], the structures proposed for the complexes C_1 and C_2 of the Mo(VI)–DTPA and Mo(VI)–TTHA systems are shown in Fig. 6 B, C, E and F. In both systems, the 1:1 complexes result from the binding of a MoO_3 metal center to one of the IDA-type terminal moieties of the ligands, whereas in the 2:1 complexes two MoO_3 groups bind their two IDA-type terminal units originating very symmetrical chelates. There is a plane of symmetry through the Mo atoms and, therefore, there is no chirality here (in contrast to the EDDA complexes). This coordination scheme is also in agreement with the ^{13}C complexation shifts defined relative to the shifts of the free ligand at the same pH, observed

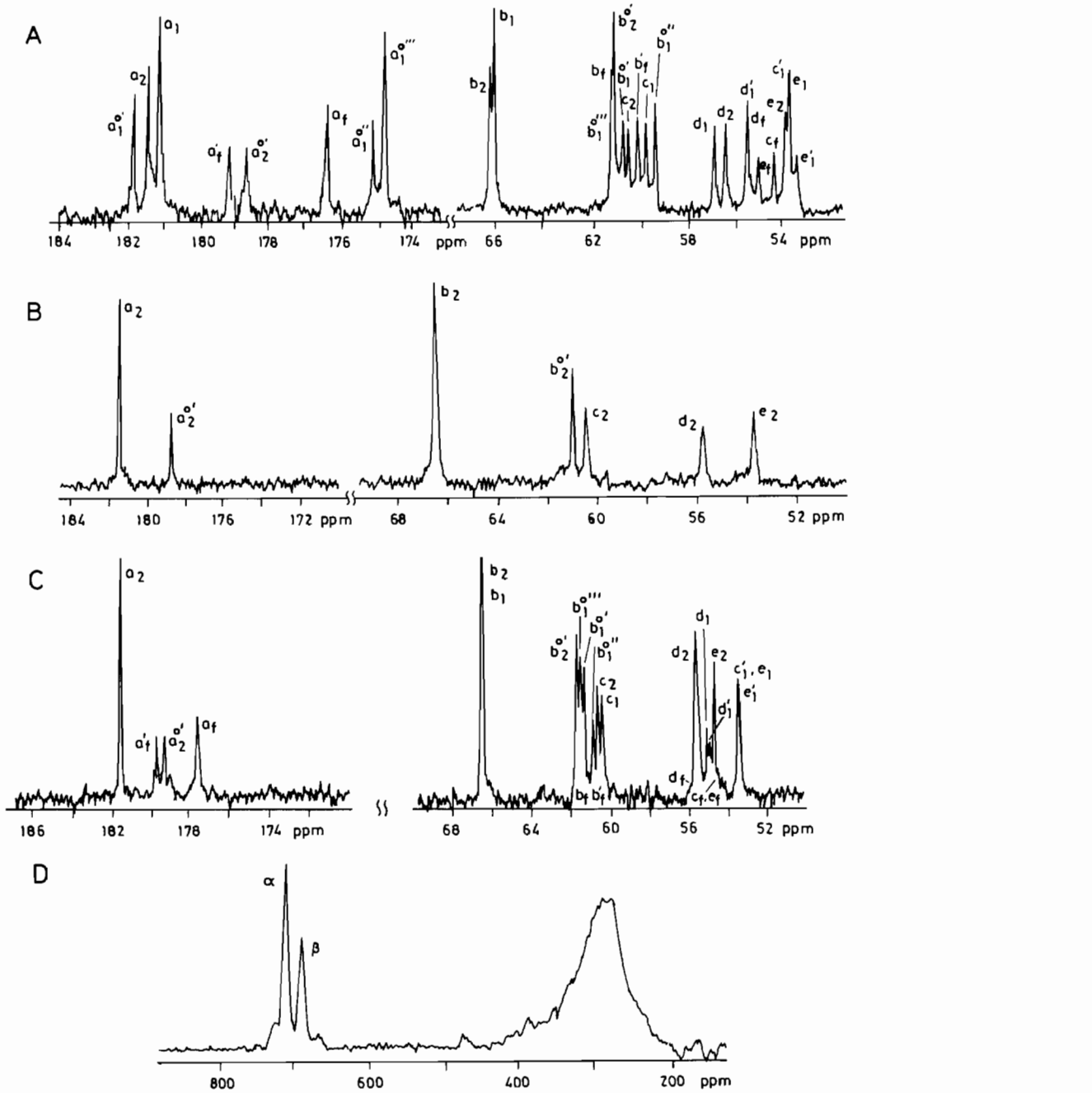


Fig. 5. ^{13}C NMR spectra (100.6 MHz) (A–C) and ^{17}O NMR spectrum (54.2 MHz) (D) of Mo(VI)–TTHA solutions: A, 0.15 M:0.15 M, pH = 5.1; B, 0.30 M:0.15 M, pH = 4.3; C, 0.30 M:0.15 M, pH = 7.6; D, 0.15 M:0.15 M, pH = 5.1.

for the C_1 and C_2 complexes of both systems at pH 6.4 (Table 2). These shifts are similar to the complexation shifts observed in the literature for the corresponding carbon atoms in the Mo(VI)–EDTA and Mo(VI)–DTPA complexes at pH 6.0 [19]. The complexation shifts of the ^{13}C nuclei geminal to the direct binding sites (a,b,c) are all positive and with similar magnitudes as those previously observed [19]. The ^{13}C nuclei belonging to parts of the molecules of the ligands which do not directly bind to the metal center ($a^{o\prime}$, $b^{o\prime}$, $a^{o\prime\prime}$, $b^{o\prime\prime}$, $a^{o\prime\prime\prime}$, $b^{o\prime\prime\prime}$ of the acetate groups and c′, d, d′, e, e′ of the ethylenediamine moieties) have complexation shifts which depend on the coordination and the protonation of the nitrogen atoms of the ligands, which are pH dependent [27–29] (see later).

The ^{13}C NMR assignments were made on the basis of literature data [19, 27, 28] and by comparing the spectra obtained for the 0:1, 1:1 and 2:1 metal-to-ligand ratios, at different pH values, namely the chemical shifts and relative intensities of the fully relaxed

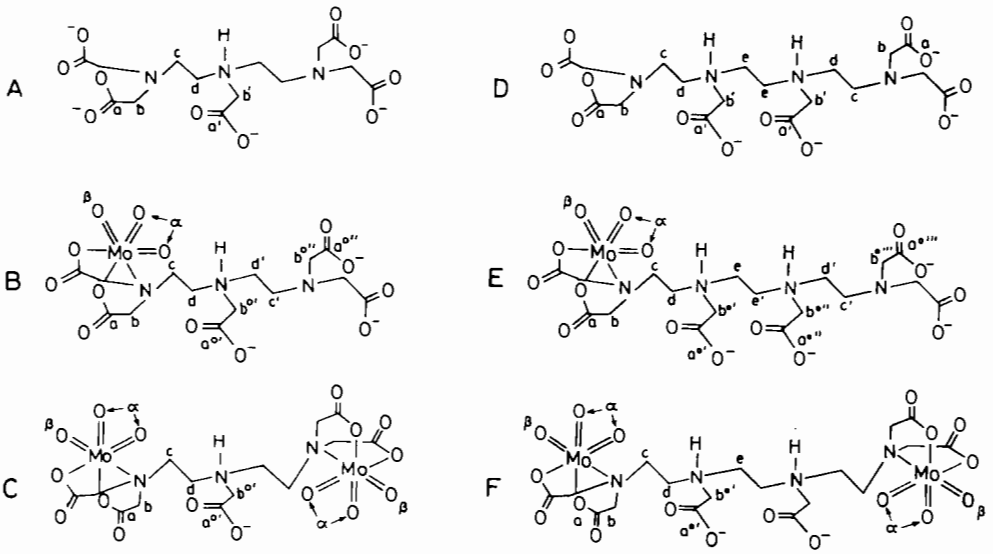


Fig. 6. Structure and carbon atom identifications of the DTPA and TTHA ligands and complexes: A, DTPA (HL form); B, Mo(VI)–DTPA 1:1 complex (C_1, MoHL form); C, Mo(VI)–DTPA 2:1 complex (C_2, Mo_2HL form); D, TTHA (H_2L form); E, Mo(VI)–TTHA 1:1 complex (C_1', MoH_2L form); F, Mo(VI)–TTHA 2:1 complex (C_2', Mo_2H_2L form).

signals of the carboxylate, methylene and ethylene carbons.

^{17}O NMR spectroscopy was also used to directly probe the nature of the Mo(VI) center [18–20]. Figure 5D shows a ^{17}O NMR spectrum of a 1:1 Mo(VI)–TTHA solution at pH = 5.1. Similar spectra were obtained with this system for a metal-to-ligand ratio of 2:1 and for the Mo(VI)–DTPA system (data not shown). Besides a broad signal at +289 ppm relative to the solvent H_2O, attributed to the free and bound carboxylate groups of the ligand, two sharper signals, α and β, are observed at higher frequencies ($\delta = +711$ ppm, $\Delta\nu_{1/2} =$ 407 Hz and $\delta = +692$ ppm, $\Delta\nu_{1/2} = 542$ Hz), with relative intensities of 2:1, respectively. These two signals correspond to the oxo groups of the coordinated MoO_3 metal center [19] and their chemical shifts and linewidths are very similar to the values obtained for the complexes of Mo(VI) with EDTA [19]: signal α results from the oxo ligands in position *trans* relative to the carboxylate groups and signal β is associated with the oxo ligand *trans* to the nitrogen atom (see Fig. 6). The latter signal has a smaller resonance frequency than the former as a result of a larger *trans* effect of the more basic amino nitrogen relative to the carboxylate groups, which causes a smaller π contribution to the Mo=O(β) bond. The corresponding bond lengthening effect is correlated with a smaller ^{17}O shift of the respective oxo ligand [19].

The intensities of the ^{13}C and ^{95}Mo NMR signals of Mo(VI)–DTPA and Mo(VI)–TTHA solutions of 1:1 and 2:1 metal-to-ligand stoichiometries were used to quantitatively analyse the different species present in solution as a function of pH. Considering that both the ^{95}Mo and ^{13}C spectra are in fast exchange for ligand protonation and in slow exchange for ligand complexation, the following equations can be written

$$C_{Mo} = [Mo] + [MoL] + 2[Mo_2L] \quad (1)$$

$$C_L = [L] + [MoL] + [Mo_2L] \quad (2)$$

where C_{Mo} and C_L are the total concentration of molybdate and of the ligand L, respectively, [Mo] is the free molybdate concentration, and [L], [MoL] and [Mo_2L] are the total concentrations of the free ligand, the 1:1 C_1 complex and the 2:1 C_2 complex irrespective of their protonation state. Therefore, [Mo] includes the total concentration of the $[MoO_4H_n]^{(2-n)-}$ species, [MoL] of the $[(MoO_3)LH_n]^{(x-n)-}$ species and [Mo_2L] of the $[(MoO_3)_2LH_n]^{(x-n)-}$ species (protonation state $n = 0.1\ldots$; $x = 5$ for DTPA and 6 for TTHA).

The ^{95}Mo signal intensities and C_{Mo} values yielded values of [Mo] and ([MoL] + 2[Mo_2L]) as a function of pH. Values of [L], [MoL] and [Mo_2L] were obtained from C_L and the normalized intensities of equivalent ^{13}C signals of the three forms (e.g. $c_f/2$, c_1 and $c_2/2$). The carboxylate carbon signal intensities were not used in concentration estimates because of their long spin-lattice relaxation times.

Figure 7 shows the speciations obtained for the two systems at the 1:1 and 2:1 solution stoichiometries. In the 1:1 solutions the free ligand is present at all pH values studied, as well as the two complexes, C_1 and C_2. In the 2:1 solutions, below pH 6.5, the C_2 complex predominates and the free ligand is absent. Above pH 6.5, the free ligand and free molybdate increase and the C_1 and C_2 become comparable. Above pH 9.5, the free ligand and molybdate are the only species present in solution for both systems.

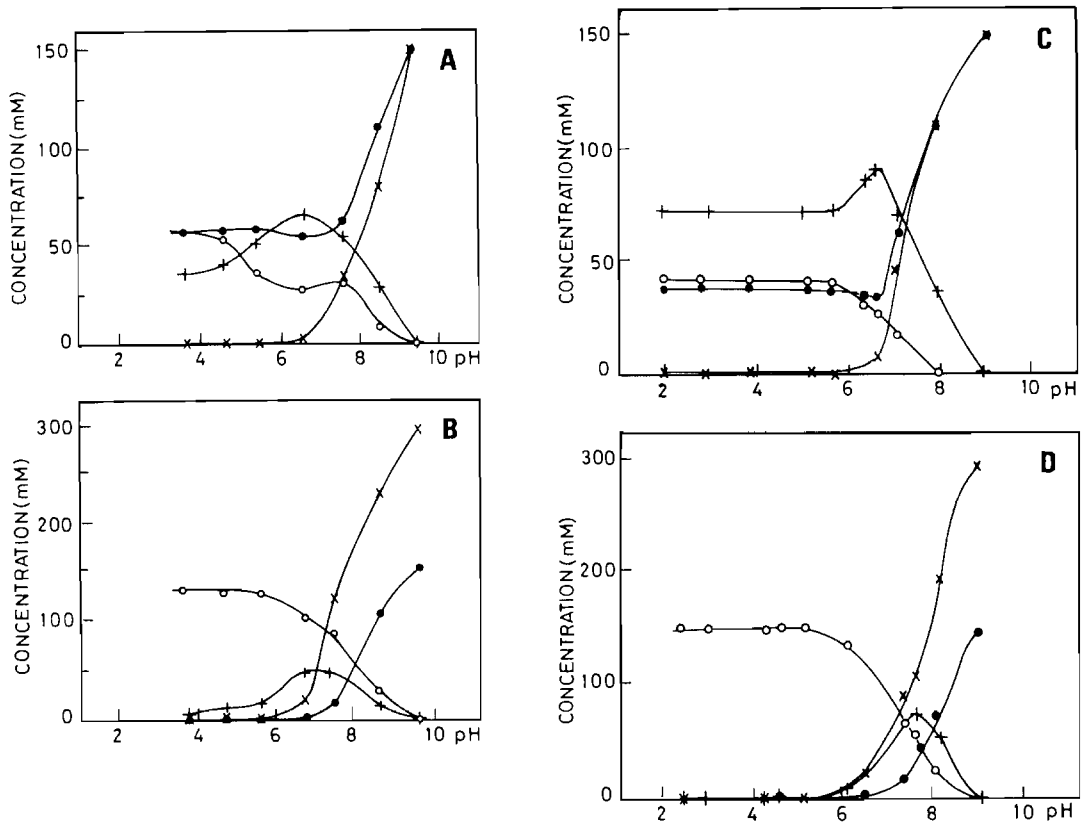


Fig. 7. pH dependence of the concentration of the various species existing in solution, obtained by ^{13}C and ^{95}Mo NMR: A, for Mo(VI)–DTPA, 1:1 (0.15 M:0.15 M); B, for Mo(VI)–DTPA, 2:1 (0.30 M:0.15 M); C, for Mo(VI)–TTHA, 1:1 (0.15 M:0.15 M); D, for Mo(VI)–TTHA, 2:1 (0.30 M:0.15 M). The symbols are: Mo (×), L (●), MoL (+) and Mo_2L (○).

It is useful to compare the pH distribution curves of Fig. 7 obtained for the species L, MoL and Mo_2L in this study at high concentrations ($C_L = 0.15$ M) with speciation curves obtained by potentiometry at much lower concentrations ($C_L = 5 \times 10^{-4}$ M) where the various protonated forms of the C_1 and C_2 complexes present can be distinguished [11]. In the case of DTPA the general shapes of those curves are quite similar, although Mo_2L is present up to higher pH values in the more concentrated solution (pH = 8.5) than in the dilute one (pH = 6.5), and in the 2:1 solution MoL is less favoured relative to Mo_2L in the more concentrated solutions. In the case of TTHA, similar observations are found, including the fact that the MoL species exists up to pH 11 in the dilute solutions and only up to pH 9 in the concentrated ones. The relative preference for the binuclear versus the mononuclear species in the concentrated solutions is to be expected.

Table 3 shows the pH dependence of the complexation shifts of all ^{13}C nuclei of the C_1 and C_2 complexes of Mo(VI) with both DTPA and TTHA. As noticed above, the complexation shifts of the nuclei geminal to the chelating atoms (a,b,c) are large, positive and nearly pH independent. The complexation shifts of nuclei further away are pH dependent, reflecting the effects of protonation of the nitrogens. Therefore, the interpretation of their pH dependence requires a comparison of the preferential protonation of the nitrogens in the free ligand and in the complexes, yielding a rather complex effect of MoO_3 binding upon their basicity [19].

Let us discuss the complexation shifts of Table 3 at the extreme pH values for each ligand: pH 7.5 and 3.6 for Mo(VI)–DTPA and pH 8.0 and 2.1 for Mo(VI)–TTHA. Considering the known pH distribution of protonated forms of the free ligands [27, 29] and of their 1:1 and 2:1 complexes [11], the complexation process of DTPA or of TTHA by Mo(VI) may be described in terms of the predominant protonated forms at each pH value. Therefore, for L = DTPA, at pH 7.5 the reaction of H_2L^{3-} into $[(MoO_3)HL]^{4-}$ predominates, with loss of one proton; at pH 3.6 H_3L^{2-} turns into $[(MoO_3)H_2L]^{3-}$ and/or $[(MoO_4)HL]^{4-}$ with loss of one or two protons. For L = TTHA, at pH 8.0 H_2L^{4-} turns into $[(MoO_3)L]^{6-}$ and/or $[(MoO_3)_2L]^{6-}$, with loss of two protons, whereas at pH 2.1 H_6L is converted into $[(MoO_3)H_2L]^{4-}$ and/or $[(MoO_3)_2H_2L]^{4-}$, with the loss of four protons.

We confine ourselves to a discussion of the carboxylate carbon complexation shifts. In the case of DTPA, at

TABLE 3. pH dependence of the ^{13}C NMR complexation shifts, $\Delta\delta$ (ppm), for the Mo(VI)–DTPA and Mo(VI)–TTHA systems

System	Carbon	pH				
		3.6	4.5	5.4	6.4	7.5
Mo(VI)/DTPA	a_1	+6.9	+7.1	+7.3	+7.3	+6.5
	$a^{o\prime}{}_1$	−3.6	−3.7	−1.7	−0.8	+0.1
	$a^{o\prime\prime}{}_1$	+0.5	+0.7	+1.1	+1.2	+0.8
	a_2	+6.7	+6.8	+6.7	+6.8	+6.8
	$a^{o\prime}{}_2$	−5.5	−7.1	−7.1	−6.5	−5.9
	b_1	+5.1	+5.3	+5.5	+5.7	+5.5
	$b^{o\prime}{}_1$	+1.0	+0.4	+0.2	+0.7	+0.5
	$b^{o\prime\prime}{}_1$	+0.2	+0.3	+1.1	+1.5	+2.1
	b_2	+5.1	+5.3	+5.4	+5.7	+5.5
	$b^{o\prime}{}_2$	+1.5	+0.7	+0.3	+1.4	+2.3
	c_1	+3.2	+3.0	+3.5	+4.1	+4.2
	c'_1	−1.2	−1.0	−0.8	−0.6	−0.4
	d_1	+2.0	+1.6	+1.0	+0.5	+0.3
	d'_1	+1.1	+0.7	−0.2	−0.3	−0.3
	c_2	+2.4	+2.3	+3.0	+3.7	+3.9
	d_2	+1.5	+1.5	+1.2	+0.8	+1.0

	pH						
	2.1	2.9	3.9	5.1	6.1	7.1	8.0
Mo(VI)/TTHA							
a_1	+3.3	+4.3	+5.8	+6.3	+4.8	+3.7	+3.5
$a^{o\prime}{}_1$	−2.1	−2.6	−3.3	−4.2	−3.8	−2.5	+0.2
$a^{o\prime\prime}{}_1$	+4.0	+4.2	+3.6	+3.7	+3.0	+2.0	+2.1
$a^{o\prime\prime\prime}{}_1$	+0.1	0.0	−0.1	0.0	−0.7	−1.2	+0.1
a_2	+6.0	+6.4	+6.6	+6.4	+4.8	+3.6	+3.5
$a^{o\prime}{}_2$	−0.3	−0.3	−0.2	0.0	−0.2	−0.6	−0.7
b_1	+5.4	+5.1	+5.1	+5.0	+5.0	+5.1	+5.2
$b^{o\prime}{}_1$	−0.6	−0.9	−0.5	−0.7	−0.7	0.0	+1.3
$b^{o\prime\prime}{}_1$	+1.5	+1.9	+1.6	+1.2	+0.9	+0.6	+0.7
$b^{o\prime\prime\prime}{}_1$	−0.2	−0.1	+0.1	+0.1	0.0	0.0	+0.4
b_2	+5.4	+5.2	+5.2	+5.2	+5.2	+5.2	+5.3
$b^{o\prime}{}_2$	+0.7	+0.7	+0.6	+0.7	+1.1	+1.1	+1.7
c_1	+4.1	+4.7	+5.1	+5.4	+5.3	+5.5	+6.7
c'_1	0.0	0.0	0.0	0.0	−0.2	−0.4	0.0
d_1	+1.8	+1.8	+1.8	+1.7	+1.7	+1.0	−0.2
d'_1	−0.1	0.0	+0.3	0.0	+0.1	0.0	−0.2
e_1	−2.0	−2.2	−2.2	−1.6	−1.6	−1.4	−1.6
e'_1	−3.3	−3.2	−2.2	−1.9	−1.6	−1.2	0.0
c_2	+5.6	+5.8	+5.9	+5.9	+5.9	+5.7	+5.8
d_2	−0.2	−0.2	0.0	+0.4	+0.7	+0.8	+0.2
e_2	−1.7	−1.7	−1.8	−1.9	−0.8	−0.2	+0.2

pH 7.5, the 1:1 and 2:1 complexes have large positive shifts for the directly bound carboxylate carbons, a_1 and a_2, respectively. This is due to a larger effect of complexation than of protonation of H_2L^{3-}, which predominantly occurs at the terminal N_1 and N_3 atoms. The central carboxylate $a^{o\prime}{}_2$ has a large negative shift indicating that the central nitrogen of the 2:1 complex is protonated. The free carboxylate carbons of the 1:1 complex, $a^{o\prime}{}_1$ and $a^{o\prime\prime}{}_1$, have smaller shifts, indicating that the protonation state of N_2 and N_3 do not change significantly between the H_2L^{3-} and the $[(MoO_3)HL]^{4-}$ species. At pH 3.6, the central free carboxylate $a^{o\prime}{}_2$ carbon of the 2:1 complex maintains a large negative shift, indicating that the central N_2 nitrogen is protonated in the $[(MoO_3)_2HL]^{4-}$ species. In the 1:1 complex, the terminal carboxylate $a^{o\prime\prime}{}_1$ maintains a nearly zero shift whereas the central one, $a^{o\prime}{}_1$, has a large negative shift. This indicates that, when H_3L^{2-} turns into $[(MoO_3)H_2L]^{3-}$, the protonation state of N_3 stays constant but that of N_2 increases.

In the case of TTHA, at pH 8.0, the 2:1 complex has a positive shift for the directly bound carboxylate carbon a_2. Its value, much lower than expected, results from opposing effects of complexation and deprotonation of H_2L^{4-} that predominantly occurs at the terminal N_1 and N_4 nitrogens [25, 26]. The shift of the central carboxylate carbons $a^{o\prime}{}_2$ is close to zero, indicating that complexation does not significantly affect the protonation state of the central nitrogens (N_2 and N_3), which are deprotonated. At pH 2.1, the terminal a_2 shifts become more positive and the central $a^{o\prime}{}_2$ shifts do not change, indicating that in the H_6L form the proton distribution through the four nitrogens is quite uniform, and that there is a concentration of charge in the central nitrogens of $[(MoO_3)_2H_2L]^{4-}$.

The 1:1 complex, less symmetric, has a more complex protonation scheme. At pH 8.0, the terminal a_1 carboxylate directly binding to MoO_3 again has a small, positive shift, resulting from the same factors, described above for the 2:1 complex, applying to transformation of H_2L^{4-} into $[(MoO_3)L]^{6-}$. The shifts of the other carboxylates are close to zero ($a^{o\prime}{}_1$ and $a^{o\prime\prime\prime}{}_1$) or slightly positive ($a^{o\prime\prime}{}_1$), indicating that ligand deprotonation predominantly affects N_3. At pH 2.1, the $a^{o\prime}{}_1$ shift becomes negative, the $a^{o\prime\prime}{}_1$ shift more positive and the $a^{o\prime\prime\prime}{}_1$ shift remains practically zero. These observations show that deprotonation of H_6L, that occurs during complexation to give $[(MoO_3)H_2L]^{4-}$, occurs predominantly at N_3, with a preferential protonation at N_2 and no protonation change at N_4.

Therefore, complexation of N_1 of DTPA or TTHA by Mo(VI) causes an increased basicity of the neighboring nitrogen N_2 [7, 19].

Mo(VI)–[9]aneN$_3$ and –NOTA complexes

The complexes $LMoO_3$ and $L'MoO_3$ (L = 1,4,7-triazacyclononane, [9]aneN$_3$; L′ = *N*,*N*′,*N*″-trimethyl-1,4,7-triazacyclononane, Me$_3$[9]aneN$_3$) have been previously prepared, either from the reaction of $LMo(CO)_3$ (or $L'Mo(CO)_3$ with H_2O_2 in tetrahydrofuran [30] or from the reaction of MoO_3 with L or L′ [31, 32]. The complexes have also been characterized spectroscopically, including ^{95}Mo NMR [33]. It was proposed that the MoO_3 center binds the three nitrogen atoms of the macrocyclic ligand. We found in this work that a 1:1 aqueous solution of [9]aneN$_3$·3HBr and sodium

molybdate (0.05 M:0.05 M) gives two ^{95}Mo NMR signals between pH 6.0 and 10.2, one at +1.5 ppm corresponding to free molybdate and the other at +85 ppm, corresponding to the $LMoO_3$ complex. The signal intensity of free molybdate decreases but does not vanish when the pH increases. Below pH 6.0 the sample becomes turbid. A 2:1 solution of Mo(VI)/L yields very similar ^{95}Mo spectra, except that at pH 6.0 a signal at +34 ppm is also present, corresponding to the molybdate dimer [18–20]. The chemical shift observed for the ^{95}Mo signal of $LMoO_3$ is in good agreement with the value of +86 ppm found in the previous studies [32]. This result and the value of +98 ppm found for $L'MoO_3$ [33] show that these Mo(VI) centers are more deshielded than those of a variety of anionic complexes, which resonate at $+66\pm4$ ppm [20, 34].

The 1:1 solution of Mo(VI)/L at pH 6.0 gives a ^{13}C NMR spectrum with a single resonance with a complexation shift of +4.0 ppm relative to the free ligand. The equivalence of all the carbon nuclei of the ethylenediamino bridges of the ligand in the complex shows that the three chelate rings formed due to binding of the MoO_3 center to the three macrocyclic ligand nitrogens are equivalent and undergo a δ/λ isomerization process which is fast on the NMR time scale. Accordingly, the proton spectrum of this solution (see Fig. 8) is of the AA'XX' type, also indicating the flexibility of the ligand rings. A spectral simulation yielded geminal proton couplings $J_{AA'}=J_{XX'}=-13.40$ Hz, and vicinal couplings $J_{AX}=J_{A'X'}=+6.00$ Hz and $J_{AX'}=J_{A'X}=+7.59$ Hz.

A 1:1 (0.05 M:0.05 M) solution of Mo(VI)/NOTA at pH values between 5.5 and 10.9 was also investigated by multinuclear NMR. The ^{95}Mo spectra showed a very similar behavior to that of molybdate, with a signal close to zero ppm (1.3 ppm, 63 Hz linewidth at pH 5.5; −1.1 ppm, 33 Hz linewidth at pH 10.9) due to free molybdate, whereas a signal at +33 ppm (386 Hz linewidth), attributed to the molybdate dimer was present at pH 5.5. The ^{13}C spectrum of the 1:1 Mo(VI)/NOTA solution at pH 5.5 was also found to be similar to that of free NOTA, and the ^{1}H spectrum showed the two signals from the ethylenediamine and the acetate protons unshifted but slightly broadened relative to the signals of free NOTA [23]. These data indicate that the interaction of the MoO_3 center with the ligand NOTA is very weak, possibly involving carboxylate groups only.

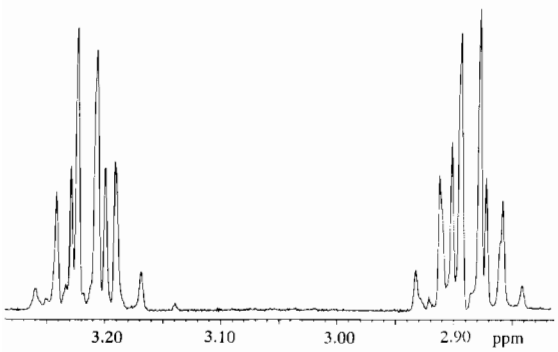


Fig. 8. ^{1}H spectrum (400 MHz) of Mo(VI)–[9]aneN$_3$, 1:1 (0.05 M:0.05 M), pH = 6.0.

Conclusions

Multinuclear NMR spectroscopy is quite a powerful technique to characterize the complexation of oxomolybdenum(VI) by polyaminocarboxylates in aqueous solution: we used ^{13}C complexation shifts for the ligand and ^{95}Mo and ^{17}O shifts for the metal centers. As opposed to the ^{17}O shifts of the oxo groups, which are sensitive to *trans* group effects, ^{95}Mo shifts are quite independent of the type of coordinating groups present [18–20]. ^{13}C complexation shifts depend on metal binding and the protonation state of the nitrogen and acetate oxygen atoms of the ligand [19]. Direct binding to Mo(VI) causes large, positive shifts on the neighboring carbon resonances, but the carbons located further away from the complexation site(s) have smaller, positive or negative shifts, which reflect the change of percent protonation of the free nitrogens in the free ligand relative to the coordinated ligand.

The interaction of molybdate with the polyaminocarboxylate ligands studied yields octahedral complexes with trioxomolybdenum(VI) (MoO_3) metal centers [18–20]. These centers bind preferentially IDA-type ligand moieties (one nitrogen and two directly attached acetate oxygens) in a tridentate facial mode. In the case of DTPA and TTHA, like for EDTA [19], binding of MoO_3 centers to their two terminal IDA-type moities leads to formation of both 1:1 and 2:1 complexes. Speciation of Mo(VI)/ligand solutions can be determined from the ^{95}Mo and ^{13}C signal intensities. The mononuclear and binuclear complexes coexist in solution in a variety of metal-to-ligand ratios and pH values, as a consequence of their relatively similar stability constants [11].

The tridentate facial mode of binding of MoO_3 to IDA-type moieties is kinetically relatively labile, as indicated by acetate pendant arm intramolecular scrambling in the NTA 1:1 complex [18, 19]. It is also not very stable, as geometrical (e.g. dipicolinate requires meridional coordination) or steric (e.g. CDTA or NOTA) ligand constraints may destabilize this binding mode [19].

When the above coordination mode is not possible, the MoO_3 center also binds EDDA-type ligand moities (two ethylenediamine nitrogens, each with one directly attached acetate oxygen) in a tridentate mode, yielding,

in the case of EDDA, two diastereomeric pairs, as shown by ^{13}C [19] and the present ^{1}H NMR studies. In these structures there is an intramolecular exchange of the free and bound acetate arms, in which the nitrogen atoms remain coordinated. The IDA-type of MoO_3 coordination does not result in structural isomers.

The preference of the MoO_3 center for IDA-type rather than EDDA-type ligand moieties contrasts with the observed preference of the VO_2^+ center of V(V) for the latter relative to the former moiety in linear polyaminocarboxylates [35, 36]. However, in the case of triazamacrocyclic ligands both centers preferentially bind the three ring nitrogens [30–33, 37, 38]. The above differences of preferential binding modes to linear polyaminocarboxylates are, at least in part, a consequence of the different number of available binding sites in the octahedral coordination polyhedron of the MoO_3 and VO_2^+ species, which contain a different number of oxo ligands. These differences could be of interest in the modelling of metal centers in molybdenum or vanadium containing enzymes.

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