Inorganica Chimica Acta, 172 (1990) 25–33

Molybdenum(VI) Complexation by Amino Acids
Part II*. Species in Aqueous Solutions of (*R*)-Cysteine and Sodium Molybdate or Tungstate

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(Received September 14, 1989)

Abstract

Complex formation between Mo(VI) and (*R*)-cysteine, abbreviated H_2cys, was studied in aqueous solutions ($C_{Mo} \leqslant 0.1$ mol dm^{-3}), using circular dichroism (CD) spectrophotometry and NMR spectrometry. Only one Mo(VI) complex is present in neutral or basic solutions, $[MoO_3(cys)]^{2-}$ (I), in which cysteine is bonded to the metal as a terdentate ligand. Its formation constant was found to be $\log K \doteq 18.8 \pm 0.2$, at $T = 18 \pm 1$ °C and $I = 1$ mol dm^{-3}. In solutions with pH values between 4.5 and 6.5–7.0 at least one other complex species **II** is formed, in addition to complex **I**. Complex **II** is believed to have the same stoichiometric ratio L/M = 1 and to be formed by reaction of complex I with acid. All solutions were unstable, the time necessary for disappearance of the Mo(VI) complexes varying with concentration, C_L/C_{Mo} and pH.

A single complex $[WO_3(cys)]^{2-}$ is formed in aqueous solutions of sodium tungstate and (*R*)-cysteine at pH > 6.7, and its formation constant was determined by CD spectrophotometry. The influence of the method used in the preparation of the solutions on the reactions of W(VI) with (*R*)-cysteine is reported.

Circular dichroism, ^1H and ^{13}C NMR spectra of the Mo(V) complex $[Mo_2O_4(cys)_2]^{2-}$ and its aquation products are also presented and discussed.

Introduction

The search for possible models for biological systems has prompted a number of authors to study the reactions of molybdenum(VI) and (*R*)-cysteine, in aqueous solutions [2–6]. An early report [2], based on spectrophotometric studies, suggested the formation, in aqueous solutions with pH values in the range 4.6–6.5, of complexes with ligand to metal ratio, L/M, between 3 and 1. Subsequently, conflicting claims about the nature of the species formed and the pH region adequate for the study have been published [3–6]. The presence of only one complex, with L/M = 2, in acidic solutions has been proposed by some authors [3, 4]. ^1H NMR results, obtained from basic solutions, were interpreted in terms of two complexes, with stoichiometric ratio, L/M, equal to one and three, respectively [5]. No evidence was found for complex formation in solutions with a phosphate buffer at pH 7.50 [6].

Tungsten(VI) has been found to have, in certain cases, a chemical behaviour similar to molybdenum(VI) [7]. In particular, complexation may lead to the formation of similar species with analogous values of formation constants [5, 8, 9]. The reactions of W(VI) and cysteine have been studied less than the corresponding Mo(VI) ones. An early report refers to the absence of complexation [10]. ^1H NMR studies in solutions of high pH were interpreted in terms of two complexes, with L/M = 1 and 3 [5].

The reduction of molybdenum(VI) by (*R*)-cysteine has been noted by different authors [2–5] and its kinetics studied at pH 7.50 [6]. Reaction of sodium molybdate and (*R*)-cysteine hydrochloride, in aqueous solution, is known [3] to yield the well studied [11, 12] dimeric complex $[Mo_2O_4(cys)_2]^{2-}$ (cys = (*R*)-$C_3H_5NO_2S^{2-}$) and cystine. Depending on the conditions used, other molybdenum(V) complexes [6, 13] and molybdenum 'blues' [2] have been found as products.

A programme including the study of the complexation of Mo(VI) and W(VI) with some naturally

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occurring ligands, in aqueous solution, is being carried out in our laboratories [1, 14, 15]. In a preliminary note [16], we have shown that the method of continuous variations used by Spence and Chang [2] should not be used to determine the presence of complex species with an L/M ratio higher than one, in solutions of molybdate and cysteine. Solutions with excess cysteine show higher absorbance than would correspond to the Mo(VI) complexes: this is due to the presence of previously undetected, highly absorbing, Mo(V) species.

In this paper, the study of dilute aqueous solutions of molybdate (or tungstate) and (*R*)-cysteine using circular dichroism spectrophotometry, complemented by a number of ^1H NMR spectrometry experiments, is described. Particular attention was given to the conditions needed to study complexation between Mo(VI) or W(VI) and the ligand without redox or other possible reactions taking place. Characterization of the Mo(V) complexes by CD, ^1H and ^{13}C NMR spectroscopy is presented. Only complexes of Mo(VI) and W(VI) with stoichiometric ratio L/M = 1 were detected in the solutions studied.

Experimental

Materials and Methods

All reagents were of analytical grade, except (*R*)-cysteine and (2*R*,2'*R*)-cystine, which were Merck biochemical reagents, and were used without further purification. $Na_2MoO_4 \cdot 2H_2O$ was dehydrated in an oven at 110 °C for preparation of the solutions for NMR studies. Deuterated reagents from Sigma were used. $Na_2[Mo_2O_4(cys)_2] \cdot 5H_2O$ was prepared by literature procedures [3].

Circular dichroism measurements were carried out using either a Jobin-Yvon Dichrographe III or a Jobin-Yvon Dichrographe IV. Perkin-Elmer 202 and Perkin-Elmer 683 spectrophotometers were used to obtain UV and IR spectra, respectively. ^1H NMR spectra were recorded at 60, 300 and 360 MHz using Varian EM-360, Brucker CXP-300S and Brucker WM-360 instruments, respectively. ^{13}C NMR spectra were obtained with a Varian XL-200 spectrometer. A Metrohm E520 and a WTW 521 pH-meter, fitted with combined glass electrodes, were used to determine pH and pH*. The pH* values of D_2O solutions are the direct readings obtained with the pH-meter calibrated with commercial aqueous buffers.

Solutions for NMR studies

Solutions for ^1H NMR spectra of Mo(VI) complexes were prepared mixing appropriate D_2O solutions of (*R*)-cysteine and Na_2MoO_4 so that the desired pH* value would result and the spectra were run immediately. The ligand solutions were acidified with DCl and the Mo(VI) ones made basic with NaOD. Preliminary experiments were needed to determine the appropriate conditions. The pH* measurement was done after recording the spectra, as it was found that the pH did not vary significantly in the time needed to perform the experiment.

Solutions of $Na_2[Mo_2O_4(cys)_2] \cdot 5H_2O$ for ^1H and ^{13}C spectroscopy were prepared by dissolving the solid in D_2O and 20% D_2O/H_2O mixtures, respectively. The spectra were run at once.

Tert-butanol and dioxan were used as internal references for ^1H and ^{13}C NMR spectra, respectively.

Solutions for CD studies

Acid solutions of (*R*)-cysteine were prepared by dissolving the ligand in HCl solutions of appropriate concentration. An amount of base (NaOH) necessary to neutralize the acid was added to the molybdate solution prior to the mixing. This was done to prevent a temporary decrease of the pH that would favour the reduction of Mo(VI). The necessary pH was obtained by addition of some drops of HCl or NaOH solutions and the spectra run immediately after preparation.

Solutions of W(VI) and (*R*)-cysteine were prepared mixing deaerated (flushed with N_2) basic solutions of both metal and ligand, with predetermined concentrations and pH to obtain the desired final solution. Each spectrum was run immediately after preparation of the solution, followed by the pH determination.

Ionic strength was adjusted by adding the appropriate amount of NaCl to all reagent solutions and using a 1 mol dm^{-3} NaCl solution as solvent.

No special technique was used to prepare solutions of $Na_2[Mo_2O_4(cys)_2] \cdot 5H_2O$, but the spectra were run immediately after the preparation of the solutions.

Determination of the Stoichiometry and Stability Constants of the Complexes

The process of complexation is represented by eqn. (1), with a formation constant (M = Mo, W) $K = [MO_3(cys)^{2-}]/([MO_4^{2-}][cys^{2-}][H^+]^2)$.

For a fixed pH value, a conditional formation constant $K' = [MO_3(cys)^{2-}]/([MO_4^{2-}][cys])$ can be defined, so that $K' = K\alpha_3[H^+]^2$ and $\alpha_3 = [cys^{2-}]/[cys]$. The value of α_3 is given by $K_1K_2K_3/([H^+]^3 + K_1[H^+]^2 + K_1K_2[H^+] + K_1K_2K_3)$, where K_1, K_2 and K_3 are the ionization constants of H_3cys^+.

$$MO_4^{2-} + cys^{2-} + 2H^+ \rightleftarrows [MO_3(cys)]^{2-} + H_2O \quad (1)$$

Equations (2), (3) and (4) were used to determine K' as described previously [15]. In these equations, H is the intensity of the CD per unit path length and $\Delta\epsilon$ is the molar CD per unit path length of the considered complex, at a certain wavelength. Other symbols have been defined elsewhere [15].

$$1/H = (1/C_L)\cdot 1/(K'\Delta\epsilon C_M) + 1/(\Delta\epsilon C_M) \qquad (2)$$

$$1/\nu = C_M \Delta\epsilon (C_L{}^0/V) K'(1/H) - K'(C_L{}^0/V) \qquad (3)$$

$$H/C_L = K'\Delta\epsilon C_M - K'H \qquad (4)$$

The relationship between eqn. (3) and Asmus' equation [17], deduced for UV–Vis spectrophotometry data has been described previously [15]. Equation (3) is directly deduced from eqn. (2). These equations are valid for complexes with L/M = 1 and were also used to determine the stoichiometry of the complexes [15].

$$K = (10^{2\mathrm{pH}} \times K')/\alpha_3 \qquad (5)$$

The expression (5) was used to determine the formation constant K. This is, strictly speaking, a mixed formation constant [18]. To calculate α_3 the values $pK_2 = 8.07$ and $pK_3 = 9.95$ were used. These values correspond to the mixed constants at I = 1 mol dm^{-3} and 25 °C and were determined as recommended in ref. 18. Only these two ionization constants need to be considered for the pH range studied.

Determination of the Number of Species in Solution

The matrix rank analysis [19] of the CD results was performed as previously described [15].

NMR Spectral Analysis and Ligand Conformation

When possible, ABX spectral analysis was carried out by standard methods [20]. To calculate the rotamer populations the recommended methods were used [21, 22].

Results and Discussion

Reactions of Molybdate with (R)-Cysteine in Neutral and Alkaline Aqueous Solutions

Circular dichroism studies

The CD spectra of freshly prepared neutral and alkaline solutions of molybdate and (*R*)-cysteine ($C_{Mo} \leqslant 0.1$ mol dm^{-3}) show two bands (one, negative, centered at 277 and another, positive, at 325 nm). Changes in the ratio C_L/C_{Mo} (C_L and C_{Mo} being the analytical concentrations of ligand and molybdenum, respectively) have no influence on the number and position of the bands observed, provided that a large excess of ligand is avoided. Solutions with ionic strength equal to 1 mol dm^{-3} (adjusted with NaCl) have also the same type of spectra (Fig. 1).

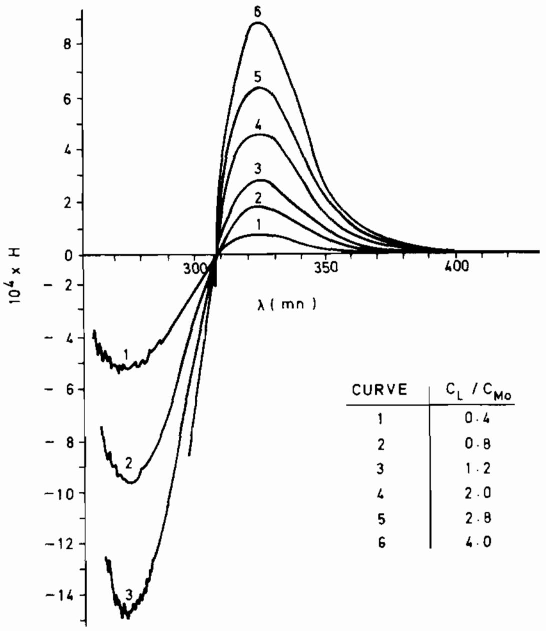


Fig. 1. Circular dichroism spectra of aqueous solutions of sodium molybdate and (*R*)-cysteine (C_{Mo} = 1.00 × 10^{-2} mol dm^{-3}, pH = 6.65, I = 1 mol dm^{-3}).

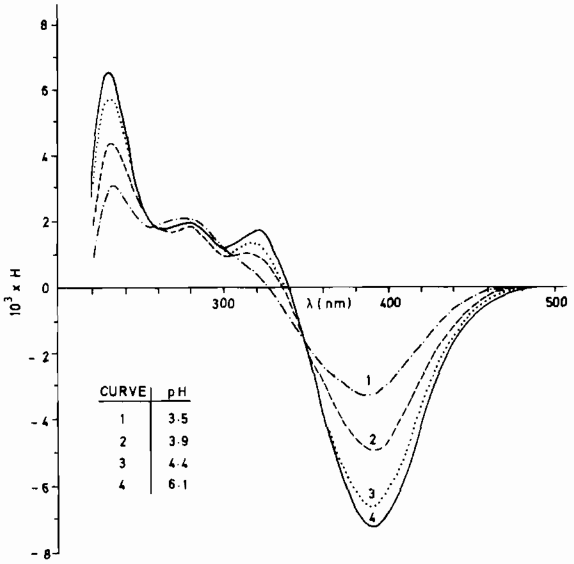


Fig. 2. Circular dichroism spectra of aqueous solutions of $Na_2[Mo_2O_4(cys)_2]\cdot 5H_2O$ (6.4–7.0 × 10^{-4} mol dm^{-3}).

These spectra provide evidence for the complexation between Mo(VI) and (*R*)-cysteine, as no other likely species than a Mo(VI) complex can be held responsible for them. The CD spectra of aqueous (*R*)-cysteine have only one positive peak: this occurs below 214 nm [23, 24], with its wavelength depending on pH. The Mo(V) complexes with cysteine give rise to spectra (Fig. 2) that are easily distinguished from those represented in Fig. 1. Cystine, in strongly alkaline solutions, originates a relatively weak negative CD band centered at 270 nm, in addition to others at lower wavelengths [24]. This compound is virtually insoluble in neutral solutions. No complexation between molybdate and cystine could be detected.

The CD spectra of solutions containing molybdate and (*R*)-cysteine vary slowly with time. The negative band, centered at around 380 nm, characteristic of the Mo(V) complexes, can then be observed in the spectra of all but the more basic solutions. The time necessary for that band to be visible in the spectra varies with C_{Mo}, C_L/C_{Mo} and pH. For a large excess of ligand, the reduction can be detected immediately after the preparation of the solutions. Formation of very small amounts of the Mo(V) complex can be detected as the intensity of its CD is much larger than that corresponding to the Mo(VI) complexes. The colour of the solutions changes from pale to deep yellow and precipitation of a white solid, identified by IR spectroscopy as cystine, may occur.

Air oxidation of cysteine, in the presence of molybdate, occurred in the colourless solutions with pH higher than about nine. The bands associated with the Mo(VI) complex disappear as the spectra change with time. After 24 h only the CD of cystine is observed.

To determine the number of Mo(VI) complex species formed, solutions with $C_{Mo} \leqslant 0.01$ mol dm^{-3}, pH > 6.5 and C_L/C_{Mo} less than five were considered: no reduction of Mo(VI) occurred in the 20–30 min immediately after the preparation of such solutions. The application of the matrix rank analysis method [19] shows that only one complex is formed in these solutions. A stoichiometric ratio L/M = 1 was found for it, using the equations given in 'Experimental' (Fig. 3).

When only one complex gives rise to an absorption spectrum the absorbances at any two wavelengths plotted against each other give a straight line [19, 25]. The same principle can be applied to the CD spectra. Figure 4 represents such a plot for the present case. For values of C_L/C_{Mo} higher than those indicated in Fig. 4, a non-linear plot was seen. This fact, together with the observation of the negative band at about 380 nm, was used to detect the occurrence of redox reactions. Formation of cystine could only be detected by that method.

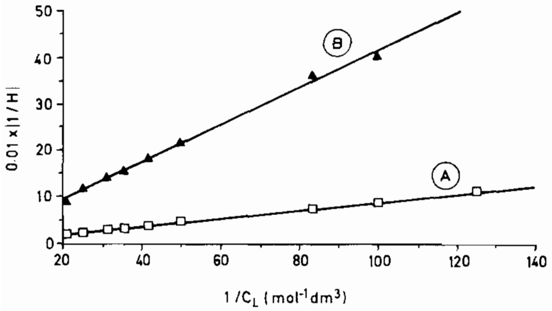


Fig. 3. Determination of the stoichiometry and the conditional formation constant of the complex $[MoO_3(cys)]^{2-}$ by application of eqn. (2) (C_{Mo} = 1.00 × 10^{-2} mol dm^{-3}, pH = 6.65, C_L/C_{Mo} = 0.8–4.8, I = 1 mol dm^{-3}, T = 18 °C): (A) λ = 277 nm, (B) λ = 325 nm.

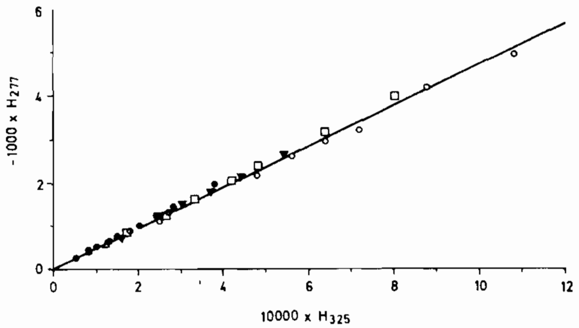


Fig. 4. Verification of the relation H_{277} = constant × H_{325} for solutions of sodium molybdate and (*R*)-cysteine (I = 1 mol dm^{-3}): ○, C_{Mo} = 1.00 × 10^{-2} mol dm^{-3}, pH = 6.65, C_L/C_{Mo} < 6; ▼, C_{Mo} = 1.00 × 10^{-2} mol dm^{-3}, pH = 7.85, C_L/C_{Mo} < 4; ●, C_{Mo} = 5.00 × 10^{-3} mol dm^{-3}, pH = 6.75, C_L/C_{Mo} < 8; □, C_{Mo} = 1.00 × 10^{-2} mol dm^{-3}, pH = 7.28; C_L/C_{Mo} < 4.8.

Proton NMR results

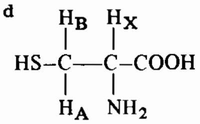
The ^{1}H NMR spectra of solutions of (*R*)-cysteine show several peaks corresponding to an ABX or to a deceptively simple A_2X spectra, depending on the pH of the solution and on the frequency of the spectrometer used [22, 26, 27]. Some ^{1}H NMR data and results from (*R*)-cysteine spectra are collected in Table 1. The ^{1}H NMR spectra of neutral and basic solutions of molybdate and (*R*)-cysteine do not show distinguishable signals for the free and coordinated ligand. Spectra obtained at room temperature, in 60 or 100 MHz spectrometers, are usually well defined, indicating that complex and free ligand undergo rapid exchange in comparison with the chemical shifts. Depending on conditions, spectra run on a higher frequency instrument may be broader and have a smaller number of peaks. The presence of small quantities of products of redox reactions was obvious in 300 MHz spectra in all conditions studied (C_L/C_{Mo} between 0.5 and 2, $C_{Mo} + C_L$ = 0.2 or 0.5 mol dm^{-3}, pH* between 6.5 and 9.8).

In most cases, it is possible to obtain the sum of the coupling constants $J_{AX} + J_{BX}$ of the averaged spectra, even if the complete analysis is not feasible. This value is consistently smaller than that for the free ligand, in the entire pH-range studied. This fact is interpreted as an indication that the population of rotamer **III** of (*R*)-cysteine (Fig. 5) is higher in the solutions with the Mo(VI) complex than in solutions of the ligand alone [21, 22]. The increase of the population of this conformer is accounted for by the formation of a complex in which the ligand is preferentially coordinated terdentate.

TABLE 1. ^{1}H NMR results and data from spectra of (*R*)-cysteine and its molybdenum complexes

	pH*	Coupling constant (Hz)				Chemical shift[a] (ppm)				Reference
		J_{AX}	J_{BX}	$J_{AX}+J_{BX}$	J_{AB}	H_A		H_B	H_X	
(*R*)-Cysteine [d]	0.4	4.1	5.6		−15.00	1.89		1.94	3.15	26
	6.0						1.87		2.76	27
	6.5			10.0						b
	7.3			10.3			1.85		2.73	b
	8.5			11.2			1.78		2.57	b
	9.5	3.8	8.1	11.9	−13.7	1.77		1.62	2.42	b
	10.5	3.7	8.9	12.6	−13.6	1.73		1.48	2.24	b
	>11	3.3	9.5	12.8	−12.8					22
Mo(V) complex **III**	~6			<8–8.5			~1.7		2.66	c
Mo(V) complex **IV**	~6	3.49		~12.5	−12.6	2.34		1.78	3.22	c
Λ-[$MoO_2(Mecys)_2$]	dmso	4.4	11.2		−12.3					28
(2*R*,2′*R*)-Cystine	<0	4.3	7.7		−15.2					29
	10.0	4.1	8.0		−13.8	1.89		1.70	2.39	b
	12.7	4.1	8.2		−13.4	1.87		1.65	2.33	b

[a] t-BuOH was used as reference. [b] This work, 60 MHz spectra. [c] This work, 360 MHz spectra. [d]



$HS{-}C(H_B)(H_A){-}C(H_X)(NH_2){-}COOH$

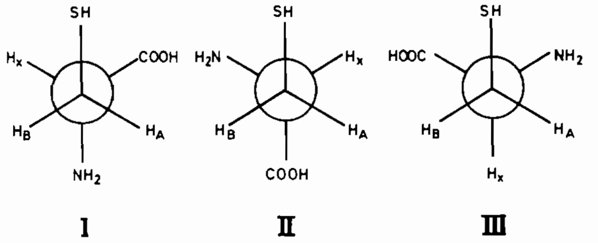


Fig. 5. Conformers of (*R*)-cysteine.

The presence of a Mo(VI) complex with (*R*)-cysteine coordinated as a terdentate ligand and a stoichiometric ratio L/M equal to one, in solutions with pD > 8.5, was proposed by others [5]. This work shows that the same complex is present in solutions with lower pH values (pH* ⩾ 6.5).

Determination of the formation constant of the complex [$MoO_3(cys)]^{2-}$

Equation (2) was used to determine the formation constant, K', of the title complex at specified values of pH. To apply this method it was necessary to ensure that, apart from the acid–base reaction of the ligand, only reaction (1) occurs. This implies conditions of no redox reaction or formation of isopolymolybdates. The former was checked using the graphical method [25] already mentioned. The absence of condensations involving molybdate in the range of concentration and pH used had been demonstrated previously [1].

A plot for one typical experiment is shown in Fig. 3. The conditional stability constant, K', is obtained from this plot and used to calculate the formation constant. The value $\log K = 18.8 \pm 0.2$ was obtained for solutions with $I = 1$ mol dm^{-3} and $T = 18 \pm 1$ °C. Table 2 summarizes the results.

This value agrees with that previously reported, $\log K = 19.88$ [5], determined using ^{1}H NMR spectroscopy. The apparent difference (our value being lower) could be eliminated if the same macroscopic ionization constants for cysteine had been used.

Reactions of Tungstate with (R)-Cysteine in Neutral and Alkaline Aqueous Solutions

The complexations of W(VI) and Mo(VI) with (*R*)-cysteine were found to be analogous. Only one complex is formed in solutions of tungstate and the ligand with pH in the 6.7–8.0 range. The CD spectrum corresponding to this complex (Fig. 6) has one negative and one positive band, centered at 244 and 280 nm, respectively. Confirmation of the presence of only one complex in solution and the determination of its stoichiometric ratio (found to be L/M = 1) were performed using the same methods described above for the Mo(VI) species.

The similarity of the CD spectra for the W(VI) and Mo(VI) solutions was considered to be due to analogous complexes and the formula [$WO_3(cys)]^{2-}$ was attributed to the tungsten complex. The determination of the formation constant was carried out using eqns. (3) and (4); a value of $\log K = 18.8 \pm 0.2$

TABLE 2. Determination of the formation constant[a] of the complex $[MoO_3(cys)]^{2-}$ in solutions with $I = 1$ mol dm^{-3} (NaCl), $T = 18 \pm 1$ °C

Exp	C_{Mo} [b]	pH	C_L/C_{Mo}	K'	K	log K	$-\Delta\epsilon$ [c]	$\Delta\epsilon$ [d]
1	1.00×10^{-2}	6.65	1.0–4.8	2.92	5.98×10^{18}	18.78	4.44	0.75
2	1.00×10^{-2}	7.28	1.0–4.0	3.04	6.79×10^{18}	18.83	5.22	0.55
3	1.00×10^{-2}	7.85	1.0–3.6	1.80	5.18×10^{18}	18.71	5.52	0.73
4	5.00×10^{-3}	6.75	1.0–4.8	4.08	8.41×10^{18}	18.92	3.65	0.58
5[e]	1.00×10^{-2}	7.68	1.0–3.3	2.20	5.69×10^{18}	18.76		1.00
				$K = 6.4(1.3) \times 10^{18}$ $\log K = 18.8 \pm 0.2$			4.71	0.72

[a] Average values, obtained from CD determinations at 277 and 325 nm. [b] mol dm^{-3}. [c] mol^{-1} dm^3 cm^{-1}, $\lambda = 277$ nm. [d] $\lambda = 325$ nm. [e] Only CD results at 325 nm considered.

TABLE 3. Determination of the formation constant of the complex $[WO_3(cys)]^{2-}$ in solutions with $I = 1$ mol dm^{-3} (NaCl), $T = 18 \pm 1$ °C[a]

Exp.	pH [b]	C_W [c]	C_L/C_W	K' [d]	K	log K	K' [e]	K	log K
1	7.45(0.04)	1.25×10^{-2}	8.0–16.0	2.23	5.23×10^{18}	18.72	2.27	5.32×10^{18}	18.73
2	7.86(0.08)	1.25×10^{-2}	3.2–11.2	3.24	9.38×10^{18}	18.97	2.77	8.00×10^{18}	18.90
3	7.99(0.06)	1.25×10^{-2}	4.8–16.0	1.97	6.32×10^{18}	18.80	1.91	6.12×10^{18}	18.7
					$K = 6.7(1.6) \times 10^{18}$ $\log K = 18.8 \pm 0.2$				

[a] CD determinations at 290 nm. [b] Average values and standard deviation. [c] mol dm^{-3}. [d] Application of eqn. (3). [e] Application of eqn. (4).

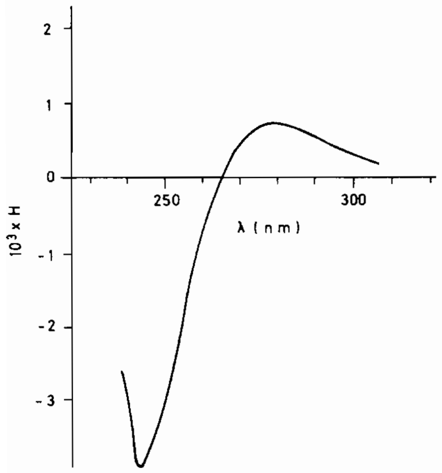


Fig. 6. Circular dichroism spectrum of an aqueous solution of sodium tungstate and (*R*)-cysteine ($C_W = 1.00 \times 10^{-2}$ mol dm^{-3}, pH = 6.78, $C_L/C_W = 8.2$, $I = 1$ mol dm^{-3}).

was obtained for $I = 1$ mol dm^{-3}. Results are summarized in Table 3.

The process used in the preparation of the solutions studied was found to influence the results obtained. The solutions were prepared by mixing neutral or slightly basic deaerated reagent solutions. In these conditions, the CD was constant during, at least, the 15–20 min interval necessary to run the spectra. If strongly basic solutions of (*R*)-cysteine were added to W(VI) in acid media, a blue colour developed instantaneously (reoxidation and fading of the colour was obtained in contact with atmosphere, or by bubbling O_2 through the solution). Colourless, unstable solutions resulted from the addition of acid cysteine to tungstate, even when the final pH was in the range 6.7–8.0.

No evidence was found for the formation of a W(V) complex with (*R*)-cysteine, in our conditions. CD spectra of the blue solutions showed the presence only of W(VI) species. Addition of such reduction agents as zinc or sodium dithionite to slightly acidic solutions of W(VI) and the ligand again gave blue solutions. Similar behaviour has been observed with other ligands like aminopolycarboxylates and the methyl ester of (*R*)-cysteine [5]. The formation of a W(V) complex with (*R*)-cysteine, by reduction of a sodium tungstate solution (in the presence of the ligand) with excess sodium borohydride, has been mentioned previously [10].

Reactions of Molybdate with (R)-Cysteine in Acidic Solutions (pH > 4.5)

The CD bands due to complex **I** are still present in the spectra of acidic solutions of molybdate and (*R*)-cysteine with pH > 4.5. The extent of its formation

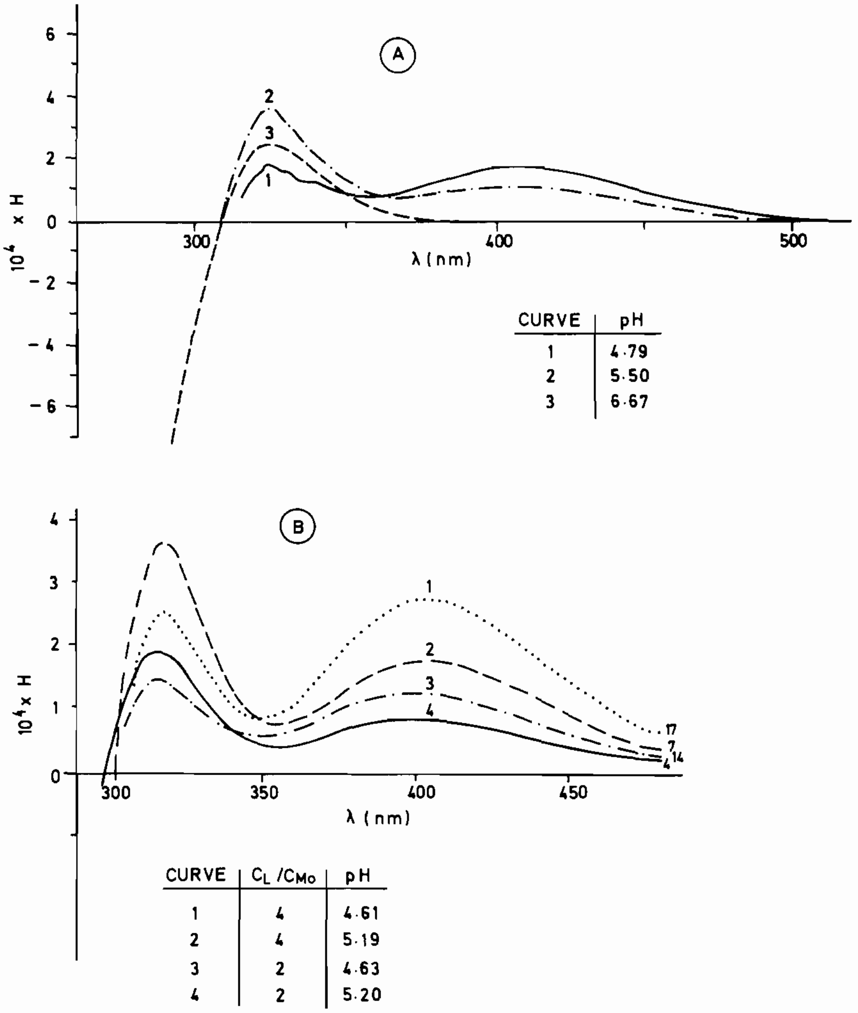


Fig. 7. Circular dichroism spectra of aqueous solutions of sodium molybdate and (*R*)-cysteine (I = 1 mol dm^{-3}): (A) C_{Mo} = 1.00 × 10^{-2} mol dm^{-3}, C_L/C_{Mo} = 1; (B) C_{Mo} = 5.00 × 10^{-3} mol dm^{-3}.

is greatest at pH values around 5.5 (when C_{Mo} = 2 × 10^{-3}–1 × 10^{-2} mol dm^{-3}). Yet, those CD spectra have another band, at 412 nm, whose intensity increases as pH decreases (Fig. 7). Spectra of solutions with a slight excess of cysteine ($C_L/C_{Mo} >$ 3–4) give evidence for fast reduction of the Mo(VI) complex and formation of Mo(V) complexes.

As can be seen in Fig. 7, the relative intensities of the bands in the CD spectra of solutions with the same pH value are fairly independent of the ratio C_L/C_{Mo}. This suggests that the complex which gives rise to the band at 412 nm (species **II**) has the same stoichiometric ratio (L/M = 1) as complex **I**. Several possibilities are open for the structure of complex **II**. It may be a protonated derivative of species **I** or any of the dimeric or polymeric species frequently found in Mo(VI) chemistry [12].

No quantitative study and characterization of species **II** was attempted. In the pH range considered, the extent of formation of isopolymolybdates is quite large and polymerization is competing with complexation. The reduction of Mo(VI) was observed to interfere more severely than at higher pH values. However no other complex of Mo(VI) with (*R*)-cysteine seemed to be present in solution, in the conditions studied ($C_L/C_{Mo} < 8$), and no evidence for species with L/M > 1 could be found.

Aquation of the Anion [$Mo_2O_4(cys)_2$]$^{2-}$

The structure of the complex [$Mo_2O_4(cys)_2$]$^{2-}$ (**III**) has been determined by X-ray crystallography of its sodium salt [30]. The complex contains the doubly oxygen bridged core $Mo_2O_4^{2+}$ and the ligand is coordinated by the S, N and O atoms. The oxygen atom of the carboxylate group is *trans* to the terminal Mo=O bonds.

The dissociation of the coordinated carboxylate groups in acid solutions with pH < 6 (due to protonation) and their replacement by water in the coordination sphere of the metal has been proposed previously

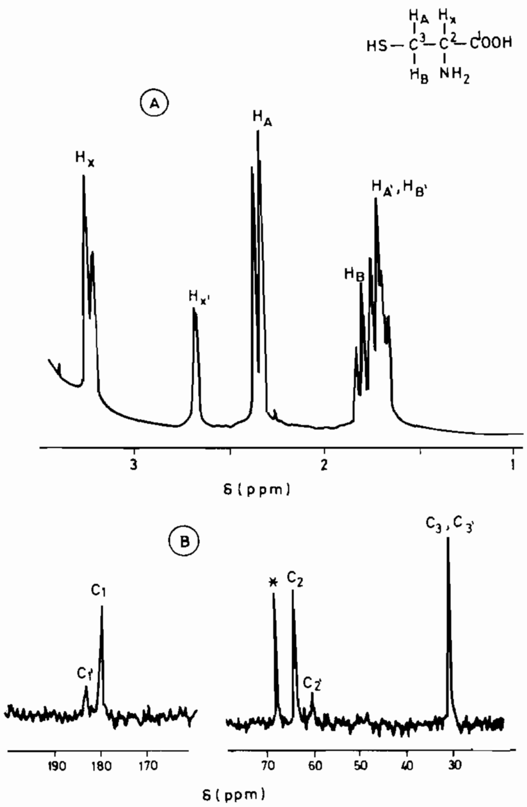


Fig. 8. NMR spectra of solutions of $Na_2[Mo_2O_4(cys)_2]\cdot 5H_2O$, 0.2 mol dm^{-3}: (A) 360 MHz ^1H spectrum; (B) 50.3 MHz ^{13}C spectrum (* dioxan, considered to resonate at 67.6 ppm against TMS).

[13]. Complex III is slowly oxidized in solution by oxygen, at a rate depending on the conditions [13, 31].

The ^1H and ^{13}C NMR spectra of a 0.2 mol dm^{-3} solution of $Na_2[Mo_2O_4(cys)_2]\cdot 5H_2O$, in D_2O (Fig. 8), provide evidence for the existence of two complex species and no free ligand in solution. The analysis of the 360 MHz ^1H NMR spectra indicates that different ligand conformations, corresponding to different coordination modes of (*R*)-cysteine, are present in these complexes.

Complex **IV** corresponds to an AMX spectra, with coupling constants similar to those of (*R*)-cysteine in basic media. These values indicate the predominance of rotamer **II** (Fig. 5) [21, 22] in solution and thus the conformation of the ligand is adequate for bidentate coordination via the N and S atoms. Analogous values for the coupling constants have been found for other N,S-cysteinates [28]. We therefore consider that species **IV** is the complex resulting from the substitution, in the coordination sphere, of the carboxylate group of (*R*)-cysteine by water.

TABLE 4. ^{13}C NMR results and data of (*R*)-cysteine[a] and its complexes with Mo(V)

Species[b]		Chemical shift[c] (ppm)			Reference
		C^1	C^2	C^3	
(*R*)-Cysteine	pH 6.5–7.0	173.9	57.0	25.8	32
Complex **III**	pH* 6	183.0	59.9	29.9	d
Complex **IV**	pH* 6	179.8	63.3	29.9	d

[a] $HS–H_2C^3–HC^2(NH_2)–C^1OOH$. [b] Complex **III** = $[Mo_2O_4(cys)_2]^{2-}$, complex **IV** = $[Mo_2O_4(cys)_2(H_2O)_2]^{2-}$ or protonated derivatives. [c] Referred to TMS (dioxane was used as internal reference and δ (dioxane) = 67.6 ppm. [d] This work.

The spectra of the second species are less well resolved, but the value of $J_{AX}+J_{BX}$ agrees with terdentate coordination of the ligand. It was, therefore, taken to correspond to species **III**. The concentration of species **IV** is about three times larger than that of species **III**, in our concentration. ^{13}C NMR spectra confirm the presence of those species in solution. The NMR results are collected in Tables 1 and 4.

The CD spectra of aqueous solutions of $Na_2[Mo_2O_4(cys)_2]\cdot 5H_2O$ have been reported previously and used to conclude that no isomerization or ligand dissociation took place [33]. No mention was made of the concentration range used.

Freshly prepared solutions of $Na_2[Mo_2O_4(cys)_2]\cdot 5H_2O$ (concentration between 6×10^{-5} and 7×10^{-4} mol dm^{-3} and pH around 6) give CD spectra that have four bands in the range 220–500 nm (Fig. 2). These agree reasonably well with the previously reported spectra. The intensity of the circular dichroism at 385 nm (corresponding to the center of the negative band) varies linearly with the concentration of the solutions. The intensity of the CD decreases slowly with time, reflecting slow oxidation.

Circular dichroism spectra of solutions of $Na_2[Mo_2O_4(cys)_2]$ with different pH values are also shown in Fig. 2. The relative intensities of the bands change with the pH of the solutions and the negative band shifts slightly to lower wavelengths. We assume that this is due to the aquation of species **III**. The CD signals of the bands at higher wavelength are considered to be related to the asymmetric distortion of the $Mo_2O_4^{2+}$ core around the Mo–Mo axis [33]. The substitution of the carboxylate group by water in the coordination sphere of the metal seems not to influence that distortion.

The different conditions used to study the solutions of $[Mo_2O_4(cys)_2]^{2-}$ by NMR and CD spectroscopy do not permit comparison of the results obtained so far. Further study is in progress to assess the extent of formation and of protonation of species **IV**.

Acknowledgements

We wish to thank I.N.I.C. for financial support to carry out this work and NATO for a travel grant (no. 1752).

We are grateful to Professor A. V. Xavier and his NMR laboratory in the Centro de Química Estrutural, IST, Lisbon for the 300 MHz spectra.

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