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NMR Study of uronic acids and their complexation with molybdenum(VI) and tungsten(VI) oxoions

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Abstract

A multinuclear 1D and 2D NMR study of D-galacturonic and D-glucuronic acids in aqueous solution and their complexation with tungstate and molybdate ions for variable concentration and pH conditions has been undertaken. The acids exist mainly in the pyranose forms, but complexes were detected involving the less stable α- and β-furanose anomers as well as the α-pyranose form. Thus, NMR evidence was gathered for the formation of two 1:2 (metal–ligand) complexes of W(VI) with the furanose forms. These are stronger with D-galacturonic acid and when the β forms are involved. The same was found with Mo(VI), but, in addition, 2:1 complexes also form. In the case of D-galacturonic acid, three such complexes were detected, two involving the α-pyranose form, in an approximately $^4C_1$ and a $^1C_4$ conformation, respectively, and the other presumably involving the β-furanose isomer. With D-glucuronic acid, only one such complex could be characterized, involving the α-pyranose isomer in a distorted $^1C_4$ conformation. More detailed information on the structure of the various complexes was obtained from ^{1}H, ^{13}C, ^{17}O, ^{95}Mo, and ^{183}W NMR data. The 2:1 complexes with the α-pyranose forms, insofar as they involve metal binding to the ring oxygen atom, are considered to play an important role in the oxidation of the acids especially by Mo(VI). © 1996 Elsevier Science Ltd.

Keywords: Tungsten; Molybdenum; Complexes; Uronic acids

1. Introduction

Studies of the interaction of metal ions with sugar molecules, particularly with uronic acids, are of interest in pharmacology and health sciences [1–3], and especially in soil science in view of its biological role in plant nutrition [4]. D-Galacturonic acid, which is

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the main sugar derivative in plant pectins, is associated with extracellular organic chelating agents that can remove nutrient cations from poorly soluble salts and make its storage in the walls of plant cells [5,6]. It may also play an important role in heavy metal detoxification processes [7].

Microorganisms like *Pseudomonas aeruginosa* (a rhizosphere bacterium) are known to produce large quantities of extracellular polysaccharides capable of binding molybdenum and thereby reducing its availability as a micronutrient to plants [8,9]. It was suggested that the uronic acid residues in the extracellular polysaccharides are active in this binding process [9] and, as a result, the interaction of molybdate with uronic acids in aqueous solution was investigated [10]. The complexation of sugars and derivatives was recently reviewed [11].

In the present work we report on the structure of D-galacturonic acid and D-glucuronic acids in aqueous solution and on their complexation with tungstate and molybdate, using ^{1}H, ^{13}C, ^{17}O, ^{183}W, and ^{95}Mo NMR spectroscopy. In our comparative study of complexation of sugar acids with Mo(VI) and W(VI) [12–15], multinuclear NMR spectroscopy as a single technique proved to have clear advantages over other methods for studying these systems. This is mainly due to relatively slow exchange phenomena that enable distinct spectra to be obtained for different species.

The ^{1}H and ^{13}C signals due to free and bound ligand and their changes with molar metal–ligand ratio and pH, together with the number and intensity of the ^{95}Mo and ^{183}W signals, enabled the determination of the number of complexes. From ^{1}H and ^{13}C shifts it was possible to establish the ligand coordination sites, and ^{17}O, ^{95}Mo, and ^{183}W shifts provided information about the metal centre. Information about the stoichiometry of the species was obtained by comparing ^{13}C and ^{1}H with ^{95}Mo, ^{183}W, and/or ^{17}O NMR spectra of the same solutions. The proton vicinal coupling constants were used to establish the approximate conformations of the bound ligands.

2. Experimental

Analytical grade sodium tungstate and sodium molybdate and commercially available D-galacturonic and D-glucuronic acids were used.

The pH was adjusted (cautiously, to reduce the possibility of drastic local disturbances of equilibria that may be slow to disappear) by addition of DCl and NaOD; the pH* values quoted are the direct pH-meter readings (room temperature) after standardization with aqueous (H_2O) buffers.

The ^{1}H and ^{13}C spectra were obtained on a Varian Unity-500 NMR spectrometer (at 499.843 and 125.695 MHz, respectively). The ^{13}C spectra were recorded using proton-decoupling techniques (Waltz-16) with suppression of the nuclear Overhauser effect. The methyl signal of *tert*-butyl alcohol was used as internal reference for ^{1}H (δ 1.3) and ^{13}C (δ 31.2) shifts. The ^{95}Mo and ^{183}W spectra were obtained on a Varian Unity-500 NMR spectrometer (32.576 and 20.825 MHz, respectively), using D_2O solutions of Na_2MoO_4 and Na_2WO_4 at pH* 9.0 and pH* 9.5, respectively, ($\delta = 0$) as external reference. The ^{17}O spectra were obtained on a Varian Unity-500 NMR spectrometer (67.760 MHz), using D_2O ($\delta = 0$) as external reference. Detailed conditions can be

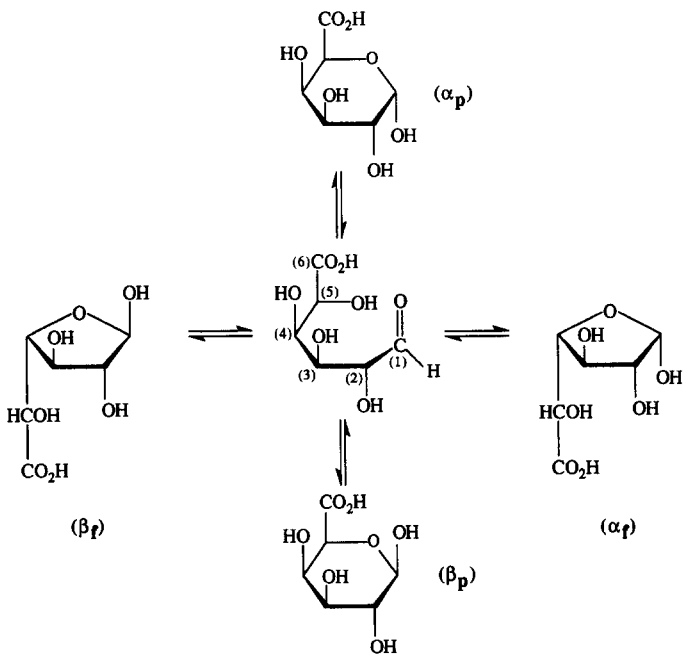
found in previous papers [13,15]. Whenever intensity considerations had to be drawn from the ^{183}W spectra, longer delay times (10 s) were used.

The methyl (methyl α,β-D-galactofuranosid)uronates were obtained by mixing D-galacturonic acid (1.5 g, 6.0 mmol), MeOH (200 mL), and Dowex 50X8-100 (H^+ form) resin (2.0 g) and boiling the mixture in a Soxhlet extraction apparatus, whose porous thimble was filled with zeolite KA. After 4 h, 100% conversion was achieved. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was characterized by ^1H and ^{13}C NMR data; much weaker signals, probably due to pyranosides, also formed.

The 1D TOCSY [16] and the 2D NMR spectra, COSY [17], DQFCOSY [18], HETCOR [19], and COLOC [20] experiments, were performed on a Varian Unity-500 NMR spectrometer.

3. Results and discussion

Structure of D-*galacturonic acid in aqueous solution.*—In aqueous solution, D-galacturonic acid can exist as the equilibrium shown in Scheme 1. Only the pyranose forms, α_p and β_p, have so far been detected by ^1H and ^{13}C NMR spectroscopy [10,21]. Our results at 500 MHz, assisted by homonuclear and heteronuclear correlation experiments (COSY and HETCOR, respectively), confirmed the assignments of the previous work



Scheme 1.

Table 1
^{1}H NMR parameters [a] for D-galacturonic acid, W(VI) + D-galacturonic acid, and Mo(VI) + D-galacturonic acid

	H-5	H-4	H-3	H-2	H-1	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
D-Galacturonic acid [b]									
pH 2.5*									
pyranosidic forms									
α_p	4.75	4.36	3.96	3.83	5.34	3.84	10.50	3.42	1.27
β_p	4.43	4.29	3.74	3.52	4.63	7.90	9.93	3.53	1.28
furanosidic forms									
α_f	4.39	4.17	4.32	4.12	5.28	4.91	6.35	7.69	2.35
β_f	4.45	4.41	4.22	4.03	5.23	3.63	5.50	6.84	2.30
pH 4.0*									
pyranosidic forms									
α_p	4.57	4.36	4.00	3.89	5.38	3.85	10.47	3.42	1.27
β_p	4.22	4.29	3.78	3.57	4.67	7.91	9.94	3.53	1.28
furanosidic forms									
α_f	4.41	4.16	4.36	4.15	5.31	4.92	7.50	7.30	3.30
β_f	4.40	4.42	4.23	4.09	5.28	3.42	5.34	7.00	2.35
W(VI) + D-Galacturonic acid (pH 4.0, 294 K)									
complex **a** [c]									
δ	5.04	4.44	4.35	4.01	5.28	4.00	6.00	6.00	2.00
$\Delta\delta$ (β_f)	0.64	0.02	0.12	−0.08	0.00				
complex **a'** [c]									
δ	5.00	4.17	4.41	4.15	5.28	4.50	8.00	8.00	2.50
$\Delta\delta$ (α_f)	0.59	0.01	0.05	0.00	−0.03				
Mo(VI) + D-Galacturonic acid (pH 4,0, 294 K)									
complex **a** [d]									
δ	4.89	4.47	4.31	4.01	5.27	3.66	5.13	5.86	2.19
$\Delta\delta$ (β_f)	0.49	0.05	0.08	−0.08	−0.01				
complex **a'** [d]									
δ	4.88	4.18	4.38	4.12	5.29	5.12	7.32	7.32	2.93
$\Delta\delta$ (α_f)	0.47	0.02	0.02	−0.03	−0.02				
complex **b** [e]									
δ	5.23	5.01	4.38	4.63	5.22	0	0	3.66	5.86
$\Delta\delta$ (α_p)	0.66	0.65	0.38	0.74	−0.16				
complex **c** [e]									
δ	5.10	4.78	4.99	5.11	5.52	0	0	0	4.43
$\Delta\delta$ (α_p)	0.53	0.42	0.99	1.22	0.14				
complex **d** [e]									
δ	4.92	4.30	3.89	4.96	5.52	0	2.2	10.5	0
$\Delta\delta$ (β_f)	0.52	−0.12	−0.34	0.87	−0.13				

[a] δ Values, in ppm, relative to Me_4Si, using *tert*-butyl alcohol (δ_H 1.3) as internal reference; J values in Hz.
[b] 0.50 M D-Galacturonic acid solution.
[c] 0.25 M:0.50 M W(VI)–D-galacturonic acid solution.
[d] 0.25 M:0.50 M Mo(VI)–D-galacturonic acid solution.
[e] 0.20 M:0.10 M Mo(VI)–D-galacturonic acid solution.

(Tables 1 and 2). But, in addition, new signals of small intensity were detected. A detailed comparison of the ^{13}C signals with the corresponding spectrum of the furanose forms of D-galactose [22] suggests that those additional signals are due to the two

Table 2
^{13}C NMR chemical shifts [a] for D-galacturonic acid, W(VI) + D-galacturonic acid, and Mo(VI) + D-galacturonic acid

	C-6	C-5	C-4	C-3	C-2	C-1
D-Galacturonic acid [b]						
pH ≈ *2.5*						
pyranosidic forms						
α_p	174.45	71.22	71.76	70.22	69.39	93.94
β_p	173.52	75.55	71.69	73.87	72.83	97.77
furanosidic forms						
α_f	176.86	70.95	83.16	74.78	77.54	96.47
β_f	176.79	70.60	84.20	76.46	82.57	102.62
pH ≈ *4.0*						
pyranosidic forms						
α_p	176.84	72.63	72.17	70.67	69.46	93.76
β_p	176.12	76.74	71.72	74.25	73.00	97.50
furanosidic forms						
α_f	179.08	72.01	83.64	75.00	77.48	96.23
β_f	178.93	71.80	84.80	76.87	82.63	102.48
W(VI) + D-Galacturonic acid (pH 4.0, 294 K)						
complex **a** [c]						
δ	183.75	83.15	85.28	75.67	81.52	101.66
$\Delta\delta$ (β_f)	4.82	11.35	0.48	−1.20	−1.11	−0.82
complex **a'** [c]						
δ	183.78	82.68	83.15	73.88	75.90	95.78
$\Delta\delta$ (α_f)	4.70	10.67	−0.49	−1.12	−1.58	−0.45
Mo(VI) + D-Galacturonic acid (pH 4.0, 294 K)						
complex **a** [d]						
δ	183.80	84.12	85.99	76.25	81.87	102.00
$\Delta\delta$ (β_f)	4.87	12.32	1.19	−0.62	−0.76	−0.39
complex **a'** [d]						
δ	183.80	83.67	83.67	74.41	75.75	96.15
$\Delta\delta$ (α_f)	4.72	11.66	0.03	−0.59	−1.73	−0.08
complex **b** [e]						
δ	187.81	81.87	87.07	82.78	80.77	99.33
$\Delta\delta$ (α_p)	10.97	9.24	8.90	12.11	11.31	5.62
complex **c** [e]						
δ	186.34	82.87	86.47	88.25	86.00	104.97
$\Delta\delta$ (α_p)	9.50	10.24	14.30	17.58	16.54	11.21
complex **d** [e]						
δ	183.60	86.70	80.15	82.48	87.50	102.21
$\Delta\delta$ (β_f)	4.67	14.90	−4.65	5.61	4.87	−0.27

[a] δ Values relative to Me_4Si, using *tert*-butyl alcohol (δ_C 31.2) as internal reference.
[b] 0.50 M D-Galacturonic acid solution.
[c] 0.25 M:0.50 M W(VI)–D-galacturonic acid solution.
[d] 0.25 M:0.50 M Mo(VI)–D-galacturonic acid solution.
[e] 0.20 M:0.10 M Mo(VI)–D-galacturonic acid solution.

Table 3
^1H and ^{13}C NMR parameters [a] for methyl (methyl α- and β-D-galactofuranosid)uronates

	H-5	H-4	H-3	H-2	H-1	OCH_3	$COOCH_3$	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
α	4.47	4.19	4.32	4.17	4.92	3.45	3.86	4.91	7.52	8.00	4.05
β	4.57	4.35	4.23	4.10	4.96	3.40	3.87	2.31	4.04	6.36	2.89
	C-6	C-5	C-4	C-3	C-2	C-1	OCH_3	$COOCH_3$			
α	174.53	71.18	82.62	74.12	76.84	109.92	53.82	56.42			
β	174.57	70.28	84.45	76.69	81.43	103.51	53.84	56.01			

[a] δ Values, in ppm, relative to Me_4Si, using *tert*-butyl alcohol (δ_H 1.3, δ_C 31.2) as internal reference; *J* values in Hz.

anomeric furanose forms of the acid. Further confirmation was gained by running the proton and the carbon spectra of the methyl ester methyl glycosides (Table 3). These were found, in comparison with D-galactose, to involve predominantly the furanose forms. In this way, the full assignment of the proton and carbon signals of the furanose forms of D-galacturonic acid was possible as is shown in Tables 1 and 2.

At room temperature, the ratio between the pyranose and the furanose isomers is about 9 and the β anomers are in both cases the most stable ones. The acyclic form, however, was not detected in the pH range (2–8) and temperature range (20–80 °C) covered. In this temperature range, the relative concentrations of the various ring forms change as shown in Fig. 1. Based on these data, a rough estimate of 17 kJ mol^{-1} is made for the energy difference between the pyranose isomers taken as a whole and the furanose forms.

Structure of D-*glucuronic acid in aqueous solution.*—In aqueous solution, D-glucuronic acid can exist as the equilibrium shown in Scheme 2. In parallel with the relative stability of the pyranose and the furanose forms of glucose in comparison with galactose [23,24], the furanose anomers of D-glucuronic acid are even less abundant than with D-galacturonic acid, as found by NMR at 500 MHz. While our 2D correlation experiments confirm the assignments of ref. [10], being therefore at variance with the ^1H data of ref. [21], only a few proton and carbon-13 assignments were possible for the furanose forms (Tables 4 and 5). This was due not only to the very low concentrations

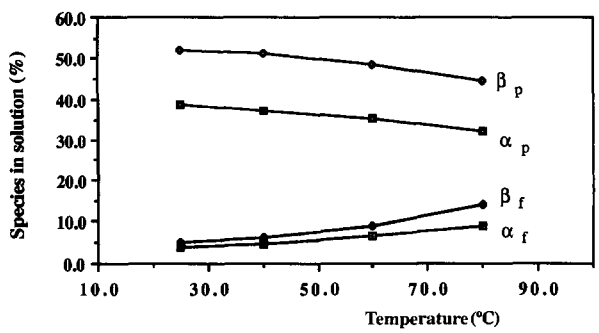
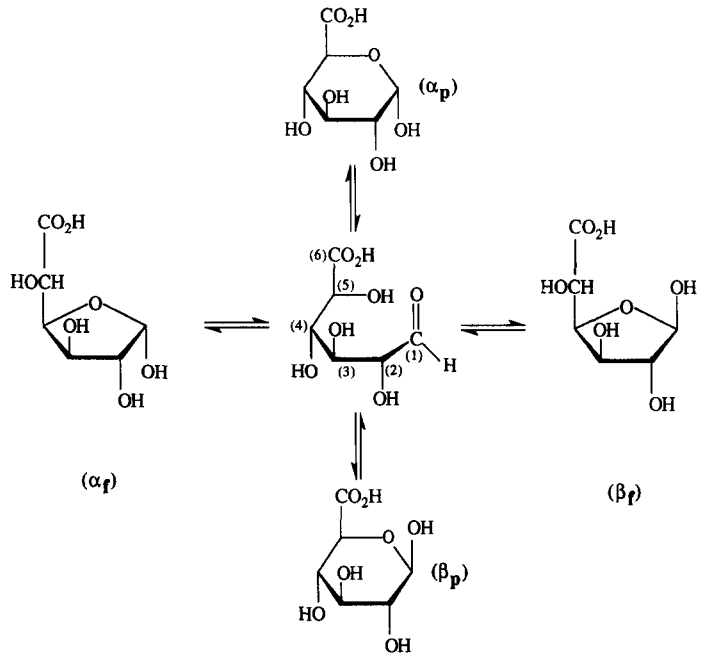


Fig. 1. Concentration of species as a function of temperature, obtained by ^1H NMR for a 0.10 M D_2O solution of D-galacturonic acid at pH * 2.5.



Scheme 2.

of these species, but also to superposition with the pyranose signals and to the presence of α,β-D-glucofuranurono-6,3-lactone [22] (Scheme 3).

The relative concentration of the furanose forms could not in this case be increased by heating because of degradation of the acid at temperatures approaching 60 °C.

Complexes W(VI)–D-galacturonic acid.—In the presence of sodium tungstate, the proton and carbon-13 spectra of aqueous solutions of D-galacturonic acid show new signals which are due to complexation, while the signals of the free ligand become weaker (Fig. 2). The existence of separate signals for bound and free ligand means that ligand exchange is slow in the NMR time-scale as has been found for related systems already studied [12–15].

A systematic recording of spectra was performed for metal–ligand molar ratios ranging from 2 to 0.25, the total concentration of species ranging from 1.25 M to 0.10 M, and pH values ranging from 3 to 8. Besides some weak signals due to minor species formed, the spectra show two intense sets of signals due to bound ligand, one set having an intensity which is about double that of the other, at room temperature.

The ^{1}H and ^{13}C assignments were done with the aid of homonuclear and heteronuclear correlation experiments (COSY and HETCOR, respectively), starting with the H-1 signal related to the well-separated signal due to the C-1 anomeric carbon atom. Tables 2 and 3 show the carbon and proton chemical shifts, respectively. Comparison with the values for the free ligand (namely for positions 2, 3, and 4 in the rings) shows that the bound ligand molecules are the α- and β-furanose forms, the β anomer being responsible for the signals of larger intensity. Therefore, complexation involves almost

Table 4
^{1}H NMR parameters [a] for D-glucuronic acid and Mo(VI) + D-glucuronic acid

	H-5	H-4	H-3	H-2	H-1	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
D-Glucuronic acid [b] *(pH* ≈ *4.0)*									
pyranosidic forms									
α_p	4.16	3.56	3.78	3.63	5.30	3.80	10.30	10.30	10.08
β_p	3.81	3.56	3.56	3.34	4.71	8.06	8.06	– [c]	9.62
furanosidic forms									
α_f	– [d]	– [d]	– [d]	– [d]	5.26	1.84	– [d]	– [d]	– [d]
β_f	– [d]	4.59	4.41	– [d]	5.46	4.27	– [d]	– [d]	– [d]
Mo(VI) + D-Glucuronic acid (pH 4.0, 294 K)									
complex **a** [e]									
δ	– [f]	– [f]	– [f]	– [f]	5.43				
$\Delta\delta$ (β_f)					−0.03				
complex **a'** [e]									
δ	– [f]	– [f]	– [f]	– [f]	5.20				
$\Delta\delta$ (α_f)					−0.06				
complex **b** [g]									
δ	5.51	4.92	4.66	4.05	5.11	4.1	1.8	0	0
$\Delta\delta$ (α_p)	1.35	1.36	0.88	0.42	−0.19				

[a] δ Values, in ppm, relative to Me_4Si, using *tert*-butyl alcohol (δ_H 1.3) as internal reference; J values in Hz.
[b] 0.50 M D-Glucuronic acid solution.
[c] Not obtained due to superposition with other signals.
[d] Not obtained due to experimental difficulties related with its small concentration.
[e] 0.25 M:0.50 M Mo(VI)–D-glucuronic acid solution.
[f] Very broad signals.
[g] 0.20 M:0.10 M Mo(VI)–D-glucuronic acid solution.

exclusively the furanose forms although these are the less stable ones for the free ligand in aqueous solution. The data in Tables 1 and 2, namely the pronounced high frequency shifts for the nuclei of positions 6 and 5 (carbon shifts of ca. 5 ppm for the carboxylic group and ca. 11 ppm for the adjacent carbinol group, and shifts of ca. 0.6 ppm for H-5), are evidence for complexation involving the carboxylic group and the adjacent carbinol group [12–15,25–30].

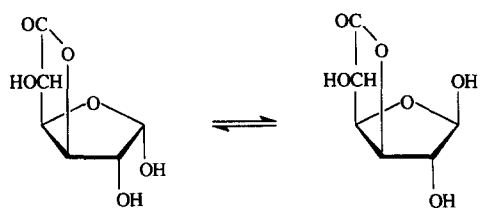
The relative intensities of the signals due to bound β_f and α_f ligands change from 2.2 at room temperature to 1.7 at 80 °C, in accordance with the hypothesis of a more stable complex with the β_f form. Two close tungsten-183 signals at δ 35.4 and 37.4, relative to external Na_2WO_4 (aq) (pH ≈ 9.5), are detected, the more intense one (complex with the β_f form) lying at lower frequency. These values fall in the range of those found for the systems W(VI)–galactaric acid and W(VI)–D-mannaric acid, and are typical of WO_2^{2+} centres [15]. In agreement with this finding is the observation of a ^{17}O resonance at δ 642, relative to an external D_2O reference, a value which is characteristic of a W=O group [15,31,32]. No signal characteristic of a W–O–W bridge was detected.

The fact that the acid acts as a bidentate ligand, associated with the presence of WO_2^{2+} centres, suggests a 1:2 stoichiometry, as found for simpler systems [26,28,29]. Verchère and Chapelle have obtained similar results by using spectrophotometric methods [33]. This is further supported by the effect of the metal–ligand molar ratio on

Table 5
^{13}C NMR chemical shifts [a] for D-glucuronic acid, W(VI) + D-glucuronic acid, and Mo(VI) + D-glucuronic acid

	C-6	C-5	C-4	C-3	C-2	C-1
D-Glucuronic acid [b] (pH ~ 4.0)						
pyranosidic forms						
α_p	177.70	72.75	73.48	74.05	73.08	93.69
β_p	176.86	77.02	73.26	77.44	75.45	97.42
furanosidic forms						
α_f	178.77	– [c]	– [c]	– [c]	– [c]	104.48
β_f	178.61	71.80	– [c]	– [c]	– [c]	96.99
W(VI) + D-Glucuronic acid (pH 4.0, 294 K)						
complex **a** [d]						
δ	184.45/184.75	– [e]	– [e]	– [e]	– [e]	96.79/96.23
$\Delta\delta$ (β_f)						
complex **a'** [d]						
δ	183.06/183.19	– [e]	– [e]	– [e]	– [e]	102.55/103.22
$\Delta\delta$ (α_f)						
Mo(VI) + D-Glucuronic acid (pH 4.0, 294 K)						
complex **a** [f]						
δ	183.30	– [g]	– [g]	– [g]	– [g]	96.17
$\Delta\delta$ (β_f)	4.69					−0.82
complex **a'** [f]						
δ	183.30	– [g]	– [g]	– [g]	– [g]	102.42
$\Delta\delta$ (α_f)	4.53					−2.06
complex **b** [h]						
δ	183.94	90.46	87.14	77.49	– [i]	101.96
$\Delta\delta$ (α_p)	6.24					8.27

[a] δ Values relative to Me_4Si, using *tert*-butyl alcohol (δ_C 31.2) as internal reference.
[b] 0.50 M D-Glucuronic acid solution.
[c] Not obtained due to experimental difficulties related with its small concentration.
[d] 0.25 M:0.50 M W(VI)–D-glucuronic acid solution.
[e] Not obtained due to experimental difficulties related with the signal's small intensity and large line-width.
[f] 0.25 M:0.50 M Mo(VI)–D-glucuronic acid solution.
[g] Broad signals and in some cases superposed with other signals.
[h] 0.20 M:0.10 M Mo(VI)–D-glucuronic acid solution.
[i] Not obtained due to superposition with other signals.



(α_f) (β_f)

Scheme 3.

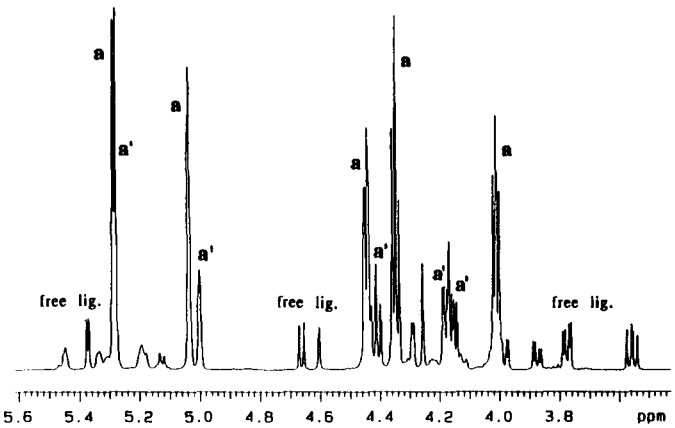
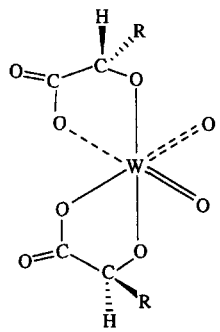


Fig. 2. 500-MHz ^{1}H NMR spectrum of a 0.20 M:0.30 M D_2O solution of sodium tungstate(VI) and D-galacturonic acid at pH * 3.4, and 298 K.

the concentration of the various species measured by the intensity of the proton signals. A rough estimation of the formation constant for the two complexes (**a** and **a'**), based on $M + 2\ L = ML_2$, leads to conditional pK values of -5.4 and -5.5, at pH * 3.0.

Molecular models suggest that the carboxylate groups must be *cis* to each other in these complexes, so that steric hindrance is minimized. Structure **1** is proposed for the 1:2 complex involving the β_f form (complex **a**).



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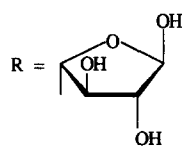


The values of the vicinal H–H coupling constants for the complexes are not significantly different from those of the free ligand forms. This shows that there are no major changes of conformation of the ligand on complexation.

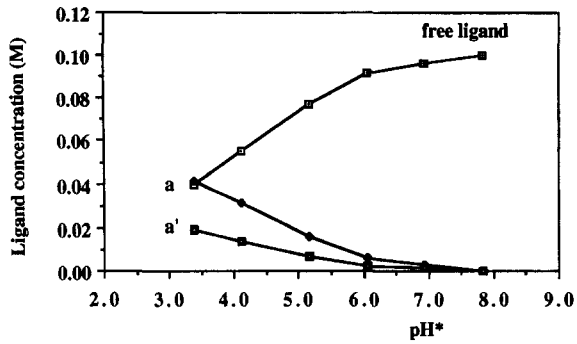


Fig. 3. Concentration of species as a function of pH*, obtained by ^{1}H NMR for a 0.05 M:0.10 M D_2O solution (2 M $NaNO_3$) of sodium tungstate(VI) and D-galacturonic acid at 294 K.

Fig. 3 shows the effect of pH on the concentrations of total bound ligand and of total free ligand for a typical situation.

Complexes Mo(VI)–D-galacturonic acid.—This system was studied in a manner similar to that for tungsten–D-galacturonic acid, except that concentrated solutions were precluded because of rapid redox phenomena.

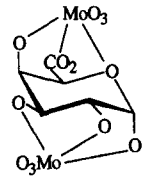
Again, strong evidence was found for the formation of two 1:2 complexes (**a** and **a'**) homologous to the complexes formed with W(VI). The corresponding ^{1}H and ^{13}C NMR parameters are shown in Tables 1 and 2, respectively. The ^{95}Mo spectrum is a broad signal that can be analyzed as two close signals (δ 94, $\Delta\nu_{1/2} = 214$ Hz; and δ 102, $\Delta\nu_{1/2} = 446$ Hz; shifts relative to an external solution of sodium molybdate at pH* 9) as expected for the two complexes. The Mo chemical shifts are consistent with the presence of MoO_2^{2+} groups [12–14,34]. The ^{17}O NMR spectrum shows two signals (δ 842 and δ 833 relative to an external reference of D_2O) which are characteristic of Mo=O groups; no signal corresponding to Mo–O–Mo bridges was found [32,35–37].

In this system, however, new complexes are detected for metal–ligand molar ratios larger than 1: complexes **b, c, d**. Such complexes were not found (or were of very small concentration) in the tungsten system, presumably because of the competing polymerization equilibria of tungstate.

The total assignment of complexes **b**, **c**, and **d** is very difficult, mainly due to the observation of several single signals in the ^{1}H NMR spectrum. In addition, all the possibilities point to the involvement of all (with a possible exception, see below) OH groups and CO_2H groups in complexation. This fact is consistent with a 2:1 composition for these complexes. The ^{95}Mo spectrum is a complex pattern which has been analyzed as four signals with the following chemical shifts: δ 32, 42, 59, and 72. These values are consistent with the presence of MoO_3 or $Mo_2O_5^{2+}$ centres [12,13,38,39].

In the case of complex **b**, COLOC (correlation through long-range couplings) C–H second-order correlation experiments were performed to eliminate some of the possibilities. By considering the vicinal H–H coupling constants and the approximate conformations they suggest for the bound ligand, models were built. In this way, we concluded that the most likely structure for the complex is a 2:1 species (**2**) involving the α-pyranose form in an approximate $^{4}C_1$ conformation. The shift undergone by C-5 and

H-5 can be explained by involving the oxygen ring atom in complexation, besides the CO_2H and the various OH groups. Thus, we propose that one MoO_3 centre is bonded to the carboxylate group, the deprotonated OH-4 group, and the ring oxygen atom, whereas the other MoO_3 group is bonded to positions OH-3, OH-2, and OH-1.



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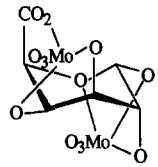
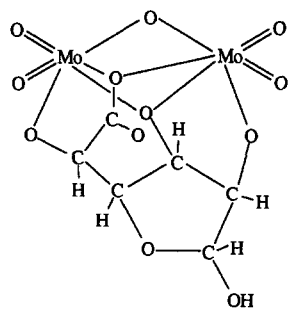


3

According to a molecular model, a similar complex (**3**) involving the $^1C_4$ conformation of α_p is possible, and having the HCCH dihedral angles close to 90° for the positions 1 and 2, 2 and 3, and 3 and 4. This is taken as complex **c**, showing proton coupling constants $J_{1,2} = J_{2,3} = J_{3,4} = 0$ Hz. Now, one metal centre is bonded to the carboxylate group and to the deprotonated OH-4 and OH-3 groups, and the other metal centre is bonded to the ring oxygen atom and to the OH-2 and OH-1 positions.

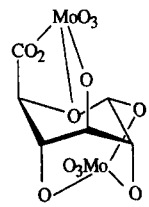
For complex **d**, again presumably a 2:1 species, the proton and carbon chemical shifts for position 4 are the less affected by complexation. This suggests that the ligand is a furanose form. By making use of molecular models and considering the proton coupling constant $J_{1,2} = 0$ Hz, the β_f isomer is selected. The proposed structure (**4**) for this complex has a dinuclear core bridged by three oxygen atoms, resembling the structure of Mo(VI) with lyxose [40].



4

Complexation of W(VI) and Mo(VI) with D-*glucuronic acid.*—No reference to complexation of this acid with tungstate has been found in the literature. In fact, the additional signals observed in the carbon and proton spectra on adding tungstate to the acid are very weak and broad. The only assignment possible is the anomeric region, the corresponding carbon and proton shifts being very similar to those of the furanose forms. By analogy with D-galacturonic acid as ligand, two weak 1:2 complexes (**a** and **a'**) seem to be formed.

The system Mo(VI)–D-glucuronic acid has been studied by Stojkovski et al. [10] who suggested the presence of a dominant complex with a bidentate pyranose form, involving the carboxylate and OH-4 groups. They based their proposal on 'dramatic' upfield shifts for the C-4 position and the disappearance of the carboxylic signal after one week. We were not able to reproduce this result; we attribute those shifts to extensive oxidation of the acid. Instead, in spite of the rapid redox phenomena (which, for example, precluded 2D correlation experiments) we have gathered evidence for 1:2 complexes (**a** and **a'**) involving the furanose forms as before, and for a 2:1 complex (**b**) involving the α_p isomer (Tables 4 and 5). The carbon chemical shifts observed on complexation suggest that all the donor groups are involved in the complexation, including the ring oxygen atom. By making use of molecular models and the values of the proton vicinal coupling constants, the ligand seems to be adopting a distorted $^1C_4$ conformation (**5**). One of the metal centres is bonded to the carboxylate group, to the deprotonated OH-3 group, and to the ring oxygen atom; the other is bound to the deprotonated OH-4, OH-2, and OH-1 groups. Two non-equivalent MoO_3 centres are therefore expected which is confirmed by the ^{95}Mo chemical shifts of two broad signals (δ 56, $\Delta\nu_{1/2} = 652$ Hz; and δ 25, $\Delta\nu_{1/2} = 720$ Hz). In general accordance with these observations, all the proton signals undergo a downfield shift except H-1 which can be affected by magnetic anisotropy and electric effects due to the close Mo=O groups.



5

4. Conclusion

Although Mo(VI) forms complexes with the pyranose forms of D-galacturonic and D-glucuronic acids (which are the most stable isomers of the acids) in solutions of higher metal–ligand molar ratio (2:1 complexes), it also stabilizes the furanose isomers specially by leading to two 1:2 complexes with both the α and β anomers. This

stabilization is much more pronounced in the case of W(VI), which only complexes significantly the α- and β-furanose forms, leading to 1:2 species.

It was also found that Mo(VI) is easily reduced [to Mo(V)] by the uronic acids, faster with D-glucuronic acid, in solutions of higher metal–ligand molar ratios in which 2:1 complexes involving the α-pyranose form are dominant. As has been suggested for the reduction of Cr(VI) to Cr(III) by D-galacturonic acid [41], the involvement of the ring oxygen atom in such a complexation is expected to play a role in the redox phenomenon. In this way, ring opening is favoured, leading to the acyclic form having a readily oxidizable aldehyde group.

Acknowledgements

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