THE JOURNAL OF THE ARGENTINE CHEMICAL SOCIETY - VOL. 90 - Nº 1/3 - 1/19 (2002)

# EPR SPECTROSCOPIC CHARACTERIZATION OF $CR^{V}$ - SACCHARIDE COMPLEXES

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*Received: March 28, 2002. Accepted: April 23, 2002*

## CONTENTS



**ABSTRACT**

*This review describes the characterization of $Cr^{V}$ complexes formed with O-donor chelate ligands employing solution EPR spectroscopy. The discussion is particularly focused on $Cr^{V}$ complexes formed with neutral and acid saccharides, although $Cr^{V}$ complexes of 2-hydroxyacids and 1,2-diols are also described. The review emphasizes the pH dependence of the $Cr^{V}$ speciation and the intramolecular competition of different functional groups and different orientations within the same functional group of saccharides for binding $Cr^{V}$.*

## INTRODUCTION

$Cr^{VI}$ is a potential hazard both in a biological and an ecological context [1,2]. The observation of $Cr^{V}$ intermediates in the selective oxidation of organic substrates by $Cr^{VI}$ and their implication in the mechanism of Cr-induced cancers [3-5] has generated a considerable amount of interest in the chemistry and biochemistry of this Cr oxidation state [6-9]. The reduction of $Cr^{VI}$ occurs with a wide variety of biological reductants [10-15]. The major coordination sites involved in Cr binding are hydroxo, alcoholato, carboxylato, and thiolato donors groups [2,14]. Five-membered, *O*-donor, chelate ligands,

such as 1,2-diols and 2-hydroxy acids, are effective as non-enzymatic reductants and chelates for high Cr oxidation states [1,14,16]. The $Cr^{V}$ speciation is pH dependent and the dependence of the formation of $Cr^{V}$ complexes with different ligands types is likely to be important with respect to their biological activities. In particular, the knowledge of the $Cr^{V}$ speciation at low pH values has relevance with respect to phagocytosis of insoluble chromates, where the pH of the vacuole becomes more acidic (pH = 4-5), and to the transport of $Cr^{V}$ in soils [1-3]. The interaction with soil components facilitates the transport of chromium from chemical dumps to the environment through the formation of soluble and stable hypervalent chromium species contributing to the environmental pollution [3]. Carbohydrates constitute 5-20% (average 10%) of soil organic matter and they constitute more than 50% of dry matter in plants [17]. Because of their potential biological relevance, it is important to know the ability of saccharides to reduce or stabilize hypervalent Cr.

This review is centered mainly in the studies conducted in our research group and our results are discussed in the context of our own research and those of other laboratories. The review describes the $Cr^{V}$ species formed by reaction of $Cr^{VI}$ with diols, 2-hydroxyacids, alditols (ald-ol), aldoses (ald), deoxyaldoses (dald), glycosides (gly), disaccharides, aldonic (alda) and uronic acids, at room temperature, as well as $Cr^{V}$ species generated (a) in the reaction of $Cr^{VI}$ with cysteine (cys) or glutathione (GSH) stabilized by the presence of excess of saccharide, and (b) in the ligand-exchange reaction between the saccharide and $[Cr^{V}O(ehba)_2]^{-}$.

## 2. CHARACTERIZATION OF $CR^{V}$ BOUND TO *O*-DONORS CHELATE LIGANDS

### 2.1 EPR spectroscopy of $Cr^{V}$

The most common mean of characterizing $Cr^{V}$ complexes in solution is EPR spectroscopy, where strong isotropic signals are observed at room temperature in X-band spectra. Typical $Cr^{V}$ EPR spectra exhibit a single narrow signal (1-5 G) centered at $g_{iso} \sim 1.98$ with four weak $^{53}Cr$ (9.55% abundance, I = 3/2) hyperfine peaks. In the case of $Cr^{V}$-alcohol species, the central signal can be further split by superhyperfine (shf) coupling with the carbinolic hydrogen atoms ($^{1}H$ $a_{iso} \sim 0.4 - 1.0 \times 10^{-4}$ $cm^{-1}$). An empirical relationship between the nature and number of donor groups and the EPR spectral parameters of $Cr^{V}$ complexes has been established [18]. Five-co-ordinate $Cr^{V}$ species show higher $g_{iso}$ and lower $^{53}Cr$ $A_{iso}$ values than the corresponding in six-co-ordinate species [18-20]. The assignment of the structures of new oxo-$Cr^{V}$ species in solution can then be made according to the isotropic EPR parameters ($g_{iso}$ and $A_{iso}$ values) and the shf pattern of the signal [21-22]. The criteria for assigning the structures of the species described below rely on a number of independent observations. These include: a.- the [substrate] and $[H^{+}]$ dependences of the EPR signals resulting from the ligand-exchange reactions of $Na[Cr^{V}O(ehba)_2]$ or from chelation of $Cr^{V}$ generated in the reaction of $Cr^{VI}$ with GSH or cys; b.- the $[H^{+}]$, [ligand] and $[Cr^{VI}]$ dependences of the EPR signal intensities for the $Cr^{V}$ complexes formed during the $Cr^{VI}$ + substrate reaction and c.- empirical correlations between the known structure of $Cr^{V}$ complexes and their EPR parameters. Together they provide evidence for the structure of the $Cr^{V}$ complexes corresponding to the EPR signal.

### 2.2 Alditols

Polyols usually form weak complexes with metal cations. However, in order to compete with water molecules for the occupancy of the first co-ordination sphere of a metal cation, these acyclic ligands must posses some pre-organization and contain particular spatial arrangements of the ligating groups [23]. The *threo* arrangement of two vicinal hydroxy groups is particularly suitable for the complexation of hypervalent metal cations, which reinforces the $CrO^{3+}$ preference to form five-membered $Cr^{V}$ chelate

rings [24-26]. The redox and complexation chemistry of $Cr^{VI}$/alditol systems has been recently examined [27]. When an excess of alditol over $Cr^{VI}$ is used, the secondary OH groups of the substrate are inert to oxidation and alditols are selectively oxidized by $Cr^{VI}$ at the primary OH group to yield the aldonic acid as the only oxidation product. The reaction involves a $Cr^{VI} \rightarrow Cr^{V} \rightarrow Cr^{III}$ reduction path and the relative rate of the $Cr^{VI}/Cr^{V}$ and $Cr^{V}/Cr^{III}$ steps depends of the $[H^{+}]$. At $[H^{+}] > 0.1$M the $Cr^{VI}/Cr^{V}$ step is rate determining, while at pH > 1 the rate of the $Cr^{V}/Cr^{III}$ reduction is the slow step.

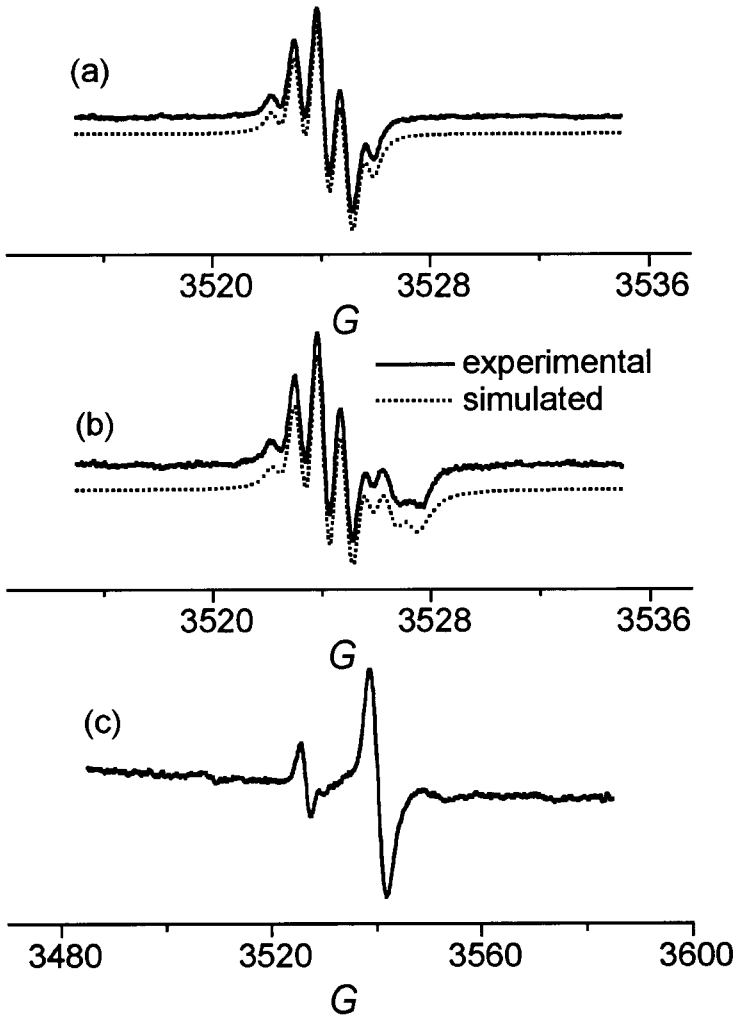



**FIGURE 1.** X-band EPR signal of solutions of glc-ol and $Cr^{VI}$. (a) glc-ol:$Cr^{VI}$ = 10:1, pH = 5, mod. ampl. = 0.4 G; (b) glc-ol:$Cr^{VI}$ = 20:1, pH = 2, mod. ampl. = 0.4 G; (c) glc-ol:$Cr^{VI}$ = 10:1, $[H^{+}]$ = 0.1 M, mod. ampl. 1.01 G. $T$ = 25°C, frequency = 9.7815 GHz. $[Cr^{VI}] = 2.5 \times 10^{-3}$ M.

$Cr^{V}$ generated by redox reaction of $Cr^{VI}$ with the alditol, is stabilized by the excess substrate or by the oxidation product (alda) and an estimation of the $Cr^{V}$ species formed in the reaction can be made at different pH, $[Cr^{VI}]$ and [ald-ol], according to the shf pattern and the isotropic EPR parameters [27]. The $g_{iso}$ and $A_{iso}$ values reveal that five- and six-co-ordinate oxo-$Cr^{V}$ complexes are formed depending on the $[H^{+}]$. At pH > 2, only five-co-ordinate $Cr^{V}$ species form ($g_{iso} \approx 1.9799$, $A_{iso} \approx 16.5 \times 10^{-4}$ $cm^{-1}$),

intramolecular redox reactions are very slow and the $Cr^{V}$ complexes remain in solution several days to weeks (Figure 1-a).

It is known that in $Cr^{V}$-diolato complexes formed with linear diols all the protons are equivalent in isotropic EPR spectra [28-29]. The shf structure of the EPR signal at $g_{iso}$ 1.9799 (quintet) indicates that the five-coordinate oxo-$Cr^{V}$ complexes formed with excess ald-ol involve metal binding to four secondary alcoholate donors, belonging to the *vic*-diolate donor sites of two bidentate ald-ol bound to $Cr^{V}$ ($[CrO(O,O\text{-ald-ol})_2]^-$, type A complex, Figure 2). It is interesting to note that the more stable oxo-$Cr^{V}$ complexes do not involve $Cr^{V}$ coordinated to the primary hydroxyl group. Besides $[CrO(O,O\text{-ald-ol})_2]^-$, a second type of oxo-$Cr^{V}$ bis-chelate ($g_{iso}$ 1.9787) appears at pH 1–2 (Figure 1-b). The spectroscopic EPR parameters correspond to oxo-$Cr^{V}$ species formed with the oxidation product (alda), with co-ordination occurring *via* the 1-carboxylate-2-hydroxy donor groups, $[CrO(O^1,O^2\text{-alda})_2]^-$ (type B complex, Fig. 2)). At $[H^+] \geq 0.1$ M, positively charged six-co-ordinate $Cr^{V}$ monochelates ($g_{iso} \approx 1.9719$, $A_{iso} \approx 20.0$, type C complex, Figure 2) form and are the dominant $Cr^{V}$ species in solution (Figure 1-c).

### 2.3. Cyclic diols

The EPR studies of cyclic diol-$Cr^{V}$ complexes reveal that the multiplicity of the EPR signal of $Cr^{V}$-diolate complexes is dependent upon whether the ligand is cyclically strained or not. The EPR shf pattern of $Cr^{V}$-cyclic diols systems was examined for the $Cr^{V}$-diolato$_2$ complexes formed by reduction

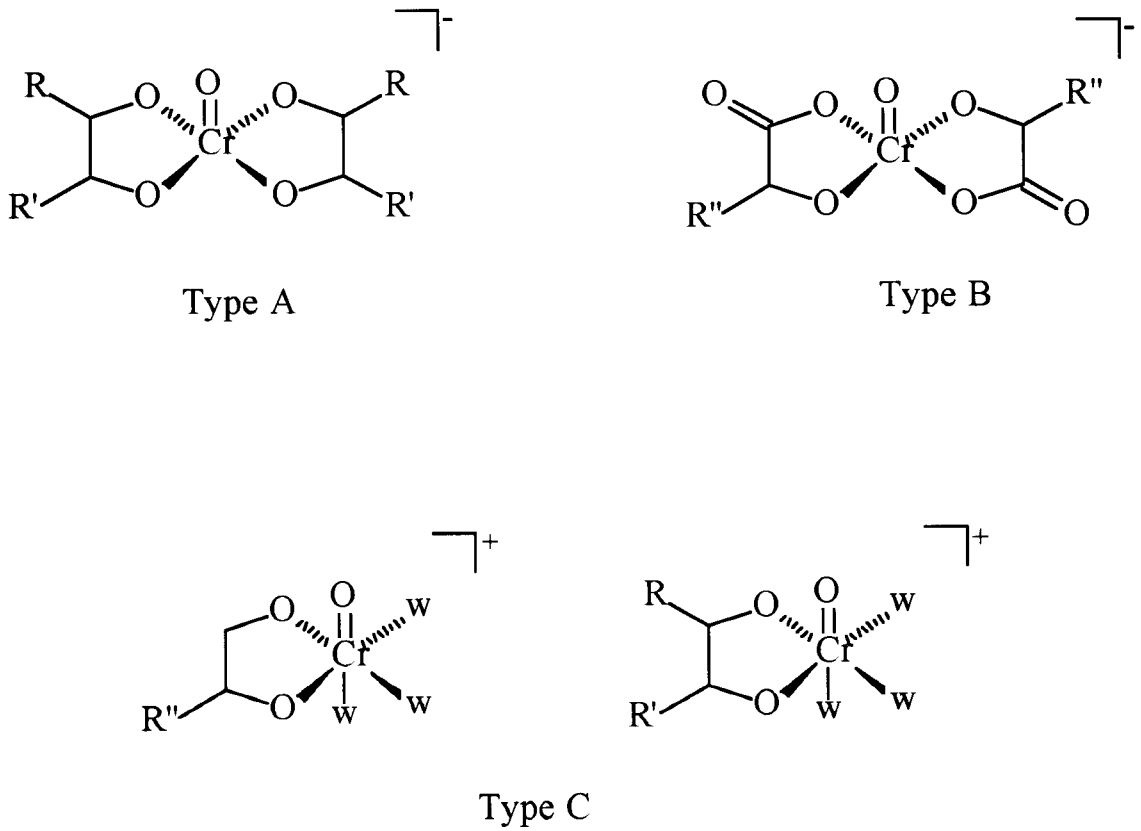



**FIGURE 2.** $Cr^{V}$ species formed in the reaction between $Cr^{VI}$ and alditols.

of $Cr^{VI}$ by GSH in the presence of *cis*- or *trans*-1,2-cyclohexanediol [2,30]. It was observed that the strain of the six-member ring imparts inequality to the magnetic environment of the protons in the second co-ordination sphere. *Trans*-1,2-cyclohexanediol affords a singlet corresponding to [CrO(*trans*-1,2-cyclohexanediolato)$_2$]$^-$ ($g_{iso}$ 1.9800), while the *cis* isomer affords two triplets corresponding to the two geometric isomers of [$Cr^{V}$(O)(*cis*-1,2-cyclohexaneidolato)$_2$]$^-$, each with two equivalent $^1H_{eq}$ coupled to the $Cr^{V}$ electronic spin ($g_{iso}$ 1.9799, $^1H$ $a_{iso}$ 0.97x10$^{-4}$ cm$^{-1}$; $g_{iso}$ 1.9796, $^1H$ $a_{iso}$ 0.89x10$^{-4}$ cm$^{-1}$) (Figure 3) [30]. The different EPR shf pattern is explained considering that when protons lay in the $Cr^{V}$-ligand plane there is a maximal overlap between the proton orbital and the $Cr^{V}$ orbital containing the unpaired electron density [21]. In the EPR spectral simulations, values for $^1H$ $a_{iso}$ are included only where the $^1H$ $a_{iso}$ value is greater than the LW (line width) of the $Cr^{V}$ species, since the signal is not significantly affected where the $^1H$ $a_{iso}$ value is ≤ LW. Consequently, the singlet observed for the *trans*-isomer means that the two carbinolic protons are very weakly coupled to the $Cr^{V}$ electronic spin resulting in a $^1H$ $a_{iso}$ < LW.

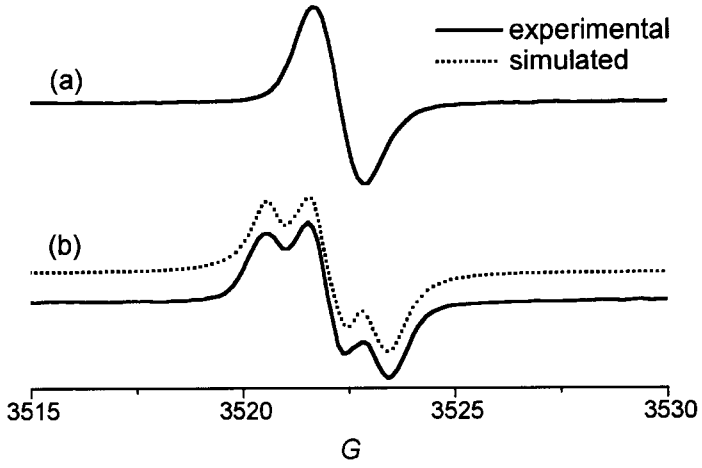



**FIGURE 3.** X-band EPR spectra of mixtures of (a) GSH:$Cr^{VI}$:*trans*-1,2-cyclohexanediol = 1:1:10000, frequency = 9.7743 GHz., mod. ampl. = 0.4 G; (b) GSH:$Cr^{VI}$:*cis*-1,2-cyclohexanediol = 1:1:10000, frequency = 9.7739 GHz., mod. ampl. = 0.2 G. [$Cr^{VI}$] = 0.25 mM, pH 7.5.

The study of $Cr^{V}$-diolato complexes formed by reduction of $Cr^{VI}$ by GSH in the presence of *cis*- or *trans*-1,2-cyclopentanediol extends the analysis of the EPR shf pattern to $Cr^{V}$-diolate species formed between $Cr^{V}$ and five-membered ring 1,2-diols [22]. Unlike $Cr^{V}$ complexes formed with *cis*- and *trans*-1,2-cyclohexanediol, 1,2-cyclopentanediols afford [CrO(*cis*-1,2-cyclopentanediolate)$_2$]$^-$ and [CrO(*trans*-1,2-cyclopentanediolate)$_2$]$^-$ with indistinguishable EPR spectral patterns (Figure 4) corresponding to two $Cr^{V}$ components split by non equivalent carbinolic protons coupled to the $Cr^{V}$ electronic spin: one $Cr^{V}$ component ($g_{iso}$ 1.9803/4, $A_{iso}$ 15.9x10$^{-4}$ cm$^{-1}$) with two "pseudo-equatorial" protons ($^1H$ $a_{iso}$ 0.84, 0.81x10$^{-4}$ cm$^{-1}$ (*cis*-diolate) and 0.84, 0.76x10$^{-4}$ cm$^{-1}$ (*trans*-diolate)) and two protons disposed at angles in between the "pseudo-axial/equatorial" orientations, and a second $Cr^{V}$ species ($g_{iso}$ 1.9798/99, $A_{iso}$ 15.9x10$^{-4}$ cm$^{-1}$) with two "pseudo-axial" protons ($^1H$ $a_{iso}$ 0.41, < LW (*cis*-diolate) and 0.47,< LW (*trans*-diolate)), and two protons protruding at intermediate angles. Low modulation amplitude and very large (5000 times) excess of diol over $Cr^{V}$ are required for the shf pattern of the EPR signal of the $Cr^{V}$-1,2-pentanediol species resolves [22].

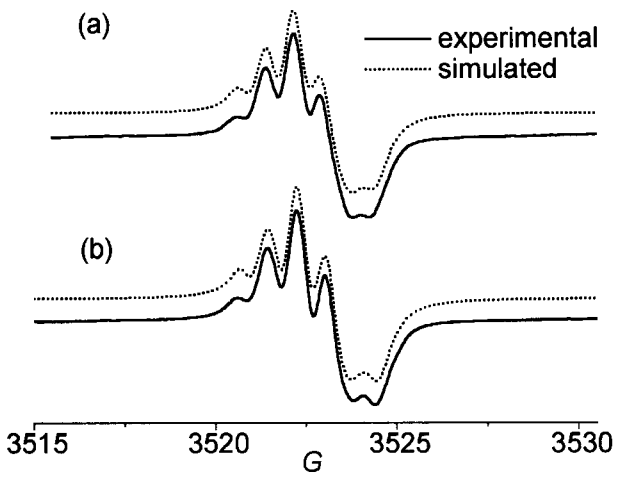



**FIGURE 4.** X-band EPR spectra of mixtures of (a) GSH:$Cr^{VI}$:*trans*-1,2-cyclopentanediol= 1:1:5000, [$Cr^{VI}$] = 1.0 mM, frequency = 9.7620 GHz., mod. ampl. = 0.4 G; (b) GSH:$Cr^{VI}$:*cis*-1,2-cyclopentanediol = 1:1:5000, [$Cr^{VI}$] = 0.5 mM, frequency = 9.7634 GHz., mod. ampl. = 0.4 G.

It results evident that the $[Cr^{V}(O)(1,2\text{-cyclohexanediolate})_2]^-$ system differentiates the axially and equatorially disposed carbinolic protons, and consequently, the resulting shf coupling corresponds to the maximal and minimal values expected for the $^1H$ coupled to the $Cr^{V}$ electronic spin; while the strained bi-cycle arising from the two fused five-membered rings in $[Cr^{V}(O)(1,2\text{-cyclopentanediolato})_2]^-$ yields isotropic EPR spectra corresponding to the average conformations of two geometric isomers in which all the carbinolic protons are non equivalent.
For either the 1,2-cyclopentanediol/$Cr^{V}$ or 1,2-cyclohexanediol/$Cr^{V}$ systems, it was found that $CrO^{3+}$ shows a marked preference for binding to the *cis*- rather than the *trans*-diol groups of the cyclic diol [22,30].

## 2.4. Methyl glycosides

The preference of $CrO^{3+}$ for binding *cis*- rather than *trans*-diol groups was confirmed by experiments where $Cr^{V}$ was generated by reaction of $Cr^{VI}$ with cys in the presence of excess of methyl glycoside and in the ligand-exchange reaction of the glycosides with $[Cr^{V}O(ehba)_2]^-$ [31-32]. It was observed that at pH 5.5 and 7.5 an EPR signal appears when excess methyl D-galactopyranoside (gal1Me) or methyl D-mannopyranoside (man1Me) is added to a cys:$Cr^{VI}$ equimolar mixture but not upon addition of excess methyl D-glucopyranoside (glc1Me) or methyl D-xilopyranoside (xil1Me). The potential binding modes in these cyclic glycosides to afford five-membered $Cr^{V}$ species are the 2,3-*cis*- and 3,4-*trans*-diolato moieties in man1Me, the 2,3-*trans* and 3,4-*cis*-diolate moieties in gal1Me, the 2,3-*trans* and 3,4-*trans*-diolato moieties in glc1Me and the 2,3-*trans*-diolate moieties in xil1Me. The observation of the EPR signal only for man1Me and gal1Me evidences the higher ability of the *cis vs* the *trans*-diolate for binding $Cr^{V}$. This result was taken as the base for the interpretation of the EPR spectral features of man1Me and gal1Me/$Cr^{V}$ mixtures in terms of $Cr^{V}$-diolate species involving mainly binding through $O^2,O^3$-man1Me and $O^3,O^4$-gal1Me. The contribution to the EPR signals from other species, involving $O^3,O^4$-man1Me and $O^2,O^3$-gal1Me, was considered to be small or negligible.

A low intensity EPR singlet at $g_{iso}$ 1.9792 is observed at the early stages of the ligand-exchange reactions of $[Cr^{V}O(ehba)_2]^-$ with xil1Me and glc1Me in HEPES buffer (pH 7.5) with the $g_{iso}$ value and

multiplicity expected for $[Cr^{V}O(ehba)(trans\text{-}O,O\text{-sugar})]^{-}$ ($g_{calc}$ 1.9791) [32]. With time, this singlet is replaced by another weak singlet at $g_{iso}$ 1.9799 due to $[Cr^{V}O(trans\text{-}O,O\text{-gly1Me})_2]^{-}$ ($g_{calc}$ 1.9800). The relatively poor ability of the *trans*-diolato groups to bind $Cr^{V}$ means the disproportionation of $[Cr^{V}O(ehba)_2]^{-}$ (which is fast at pH 7.5) [9] occurs much faster than the ligand-exchange reaction with the result that the $[Cr^{V}O(ehba)_2]^{-}$ EPR signal quickly disappears and only a very weak $[Cr^{V}O(O,O\text{-gly1Me})_2]^{-}$ signal is observed in the final spectrum. Similarly, the $[Cr^{V}O(O,O\text{-gly1Me})_2]^{-}$ EPR signal is not observed for the $Cr^{VI}$/cys reaction in the presence of excess xil1Me or glc1Me because the cys/$Cr^{V}$ redox reaction [33] is much faster than the $Cr^{V}$-cys ligand-exchange reactions with these glycosides. From these results it was concluded that kinetic control favors the formation of $Cr^{V}$-diolato species of *cis*- over *trans*-diolato moieties of six-membered sugar rings [32].

The $Cr^{VI}$/cys reaction in the presence of excess α-man1Me or α(β)-gal1Me, at pH 5.5 or 7.5, affords two EPR triplets (shown in Figure 5-a) at $g_{iso1}$ 1.9802 and $g_{iso2}$ 1.9800/1 ($A_{iso}$ 16.5(3)×$10^{-4}$ $cm^{-1}$, ~50:50 $g_{iso1}/g_{iso2}$ ratio). The $g_{iso}$ and $A_{iso}$ values and the shf splitting –the triplet means that two (one from each chelate ring) carbinolic protons are coupled to the $Cr^{V}$ electronic spin- are consistent with the presence of the two geometrical isomers of $[Cr(O)(cis\text{-}O,O\text{-gly1Me})_2]^{-}$ (**I-II** with man1Me and **III-IV** with gal1Me, Figure 6).[32] The formation of $[Cr(O)(cis\text{-}O,O\text{-gly1Me})_2]^{-}$ was found to be faster at pH 5.5 than at pH 7.5, and it was interpreted as the consequence of the faster generation of $Cr^{V}$ from the redox reaction between cys and $Cr^{VI}$ at the lower pH value [33].

Figure 5-b,c show the EPR spectra taken at the beginning of the ligand-exchange reaction of the methyl glycoside. At pH 7.5, the rapid ligand-exchange reaction of $[CrO(ehba)_2]^{-}$ with excess α-man1Me or α(β)-gal1Me affords the two geometric isomers of $[Cr^{V}O(cis\text{-}O,O\text{-gly1Me})_2]^{-}$ as the final $Cr^{V}$ species observed in the EPR spectra in a ~50:50 $g_{iso1}/g_{iso2}$ ratio. At pH 5.5, ligand-exchange reactions of $[CrO(ehba)_2]^{-}$ with excess α-man1Me or α(β)-gal1Me are not complete and after 2 h >50% of the total $Cr^{V}$ is $[CrO(ehba)_2]^{-}$, while oxo-$Cr^{V}$-diolate$_2$ species represent ~5% of total $Cr^{V}$. A fact attributed, in part, to the lower reactivity of $[CrO(ehba)_2]^{-}$ toward disproportionation and ligand exchange at the lower pH values.

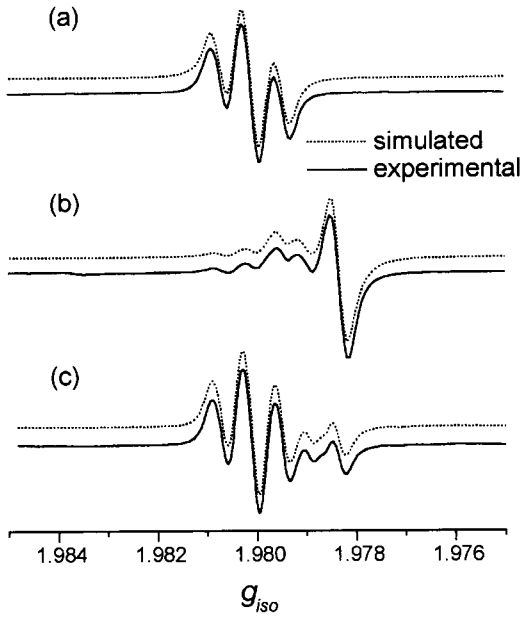



**FIGURE 5.** X-band EPR spectra of mixtures of (a) cys:$Cr^{VI}$: αman1Me = 1:1:100, pH 5.5; $t$ = 120 min; (b) $[Cr^{V}O(ehba)_2]^{-}$:αman1Me = 1:100, pH 5.5, $t$ = 3 min; (c) $[Cr^{V}O(ehba)_2]^{-}$:αman1Me = 1:100, pH 7.5, $t$ = 3 min. $T$ = 25°C, frequency ≈ 9.67 GHz, mod. ampl. = 0.2 G.

Under more acidic conditions (pH < 1), gly1Me reduces both $Cr^{VI}$ and $Cr^{V}$. Complexation of $Cr^{V}$ takes place prior to the redox paths, but the distribution of $Cr^{V}$ species is rather different than at pH ≥ 5.5. In the reaction of α-man1Me with $Cr^{VI}$, at pH ≤ 1 and a 10:1 α-man1Me:$Cr^{VI}$ ratio, the EPR spectrum is composed of a triplet at $g_{iso1}$ 1.9800, a doublet at $g_{iso2}$ 1.9798, and three signals with no shf structure at $g_{iso3}$ 1.9788, $g_{iso4}$ 1.9746 and $g_{iso5}$ 1.9716 [31]. The intensity of signals at $g_{iso4}$ and $g_{iso5}$, relative to those of the $g_{iso1}$, $g_{iso2}$ and $g_{iso3}$ signals, increases with higher $[H^+]$ and at $[H^+] > 0.3$ M the signal at $g_{iso5}$ is the dominant one and is attributed to six-coordinate $[CrO(O^4,O^6\text{-man1Me})(OH_2)_3]^+$ monochelates ($g_{calc}$ = 1.9724). Among the six-co-ordinate complexes, **V** (Figure 6) is proposed to be the precursor of the redox steps. The results show that the formation of positively charged $Cr^{V}$-monochelates are favored in acid medium and low glycoside:$Cr^{VI}$ ratios, while the formation of anionic $Cr^{V}$-bis-chelates are favored at the higher pH and larger glycoside:$Cr^{VI}$ ratios.

## 2.5. Hydroxy acids

The relatively stable $Cr^{V}$-2-hydroxycarboxylato complexes ($Na[Cr^{V}O(ehba)_2]$ (ehba = 2-ethyl-2-hydroxybutanoate(2-)) and $K[Cr^{V}O(hmba)_2]$ (hmba = 2-hydroxy-2-methylbutanoate(2-)) were the first studied as model compounds of $Cr^{V}$ biological activity [34-35]. $K[CrO(hmba)_2]$ and $Na[CrO(ehba)_2]$ have been isolated and their structure determined by X-ray diffraction [36-38]. These complexes have distorted trigonal bipyramidal coordination geometry, with carboxylate and alcoholate donors occupying the axial and equatorial sites, and the remaining equatorial site occupied by an oxo ligand. The stability of these complexes in aqueous solution ranges from minutes to days depending of the pH but they are very stable in aprotic solvents. EPR and $^1H$ ENDOR studies revealed the presence of more than one geometrical isomer of these complexes in solution ($[CrO(O^1,O^2\text{-hmba})_2]^-$: $g_{iso}$ 1.9785, $A_{iso}$ 17.4x10$^{-4}$ cm$^{-1}$; $g_{iso}$ 1.9785, $A_{iso}$ 16.3x10$^{-4}$ cm$^{-1}$. $[CrO(O^1,O^2\text{-ehba})_2]^-$: $g_{iso}$ 1.9784, $A_{iso}$ 17.2x10$^{-4}$ cm$^{-1}$; $g_{iso}$ 1.9784, $A_{iso}$ 16.1x10$^{-4}$ cm$^{-1}$).[19,39] $Cr^{V}$-$hmba_2$ intermediate complexes are also formed in the $Cr^{VI}$ + hmba reaction in 0.25 – 1.0 M acid solutions, where they rapidly decay to the redox products [40].

$K[CrO(qa)_2]\bullet H_2O$ (qa = 1R,3R,4R,5R-1,3,4,5-tetrahydroxycyclohexane carboxylic acid) has been isolated and the analysis of the XAFS data shows a coordination geometry analogous to that of $K[CrO(hmba)_2]$ and $Na[CrO(ehba)_2]$ [41]. In aqueous solutions of pH < 4.0, $K[CrO(qa)_2]\bullet H_2O$ gives two EPR signals ($g_{iso}$ 1.9787, $A_{iso}$ 17.2x10$^{-4}$ cm$^{-1}$; $g_{iso}$ 1.9791, $A_{iso}$ 16.4x10$^{-4}$ cm$^{-1}$) which are consistent with those found for $K[CrO(hmba)_2]$ and $Na[CrO(ehba)_2]$ and are assigned to the two geometrical isomers of the $[CrO(O^1,O^7\text{-qa})_2]^-$ linkage isomer [21]. At pH 5.0-7.5, solution EPR spectroscopic studies show that quinic acid is able to coordinate $Cr^{V}$ by either the 2-hydroxyacid and/or the diolate moieties. In this pH range, three additional signals appear ($g_{iso}$ 1.9791, 1.9794 and 1.9799) which have EPR spectral data consistent with the presence of $[CrO(O^1,O^7\text{-qa})(O^3,O^4\text{-qa})]^-$ and $[CrO(O^1,O^7\text{-qa})(O^4,O^5\text{-qa})]^-$, with $[CrO(O^1,O^7\text{-qa})(O^3,O^4\text{-qa})]^-$ being the major species in solution in this pH range. At pH values > 7.5, the $Cr^{V}$-qa EPR spectra show two triplets ($g_{iso}$ 1.9800 and $g_{iso}$ 1.9802) which are ascribed to the geometric isomers of the bis-diol $Cr^{V}$-qa complex, $[CrO(O^3,O^4\text{-qa})_2]^-$. This assignment was supported by the similarity of the EPR spectral data with those formed in the reduction of $Cr^{VI}$ by GSH in the presence of shikimic acid (sa, 3R,4R,5R-3,4,5-trihydroxycyclohexenecarboxylic acid) which does not possess a 2-hydroxyacid moiety.[21] At pH > 5.5, the EPR spectra reveal that two $Cr^{V}$-sa species forms with $g_{iso}$ 1.9800 and 1.9801, ascribed to the two geometric isomers of $[CrO(O^3,O^4\text{-sa})_2]^-$, with no alternative linkage isomers present in significant concentrations.

The redox reaction of lactic acid (la) with $Cr^{VI}$ involves $Cr^{V}$ as a redox intermediate [42]. At pH 4.5, the EPR spectra (Figure 7) show two triplets ($g_{iso}$ 1.9787, $^1H$ $a_{iso}$ 0.52x10$^{-4}$ cm$^{-1}$; $g_{iso}$ 1.9783, $^1H$ $a_{iso}$ 0.60x10$^{-4}$ cm$^{-1}$) with spectral parameters consistent with those expected for the two geometrical isomers of $[CrO(O^1,O^2\text{-la})_2]^-$[43].

Naturally occurring acid saccharides are relevant ligands well suited for stabilization of $Cr^{V}$ since

they posses the possibility of 2-hydroxycarboxylato and *vic*-diolato potential chelate sites for $Cr^{V}$. It was found that five coordinated oxo-$Cr^{V}$ complexes are formed in the oxidation of galactonic acid (gala) by $Cr^{VI}$ [44]. The redox reaction occurs through $Cr^{VI}\rightarrow Cr^{III}$ and $Cr^{VI}\rightarrow Cr^{V}\rightarrow Cr^{III}$ paths. The rate of reduction of both $Cr^{VI}$ and $Cr^{V}$ is first order on [gala] and is catalyzed by protons. $Cr^{V}$ is formed in a rapid step by reaction of $Cr^{VI}$ with the $CO_2^{\bullet-}$ radical generated in the reaction. The EPR spectra show that several intermediate $[Cr(O)(gala)_2]^-$ linkage isomers are formed in rapid pre-equilibria before the redox steps. In the 2-6 pH range, the EPR signals are composed of one triplet ($g_{iso}$ 1.9781) and one double triplet ($g_{iso}$ 1.9788), which have EPR spectral data consistent with the presence of $[Cr(O)(O^1,O^2\text{-gala})_2]^-$ (**VI**, Figure 8) and $[Cr(O)(O^{2(4)},O^{3(5)}\text{-gala})(O^1,O^2\text{-gala})]^-$ (**VII**). The relative proportion of the signals depends on the pH, but is independent of the reaction time. At pH 6-8, $[Cr(O)(O^{2(4)},O^{3(5)}\text{-gala})(O^1,O^2\text{-gala})]^-$ is still present, but the major species corresponds to $[Cr(O)(O^{2(4)},O^{3(5)}\text{-gala})_2]^-$ (**VIII**, $g_{iso}$ 1.9795, quintet). At pH 7.4, the last complex represents the 92% of the total $Cr^{V}$ in the reaction mixture. These results show that at the higher pH, the $Cr^{V}$-diolate binding mode is preferred over the $Cr^{V}$-2-hydroxyacid coordination mode, such as observed for the $Cr^{V}$/qa system.

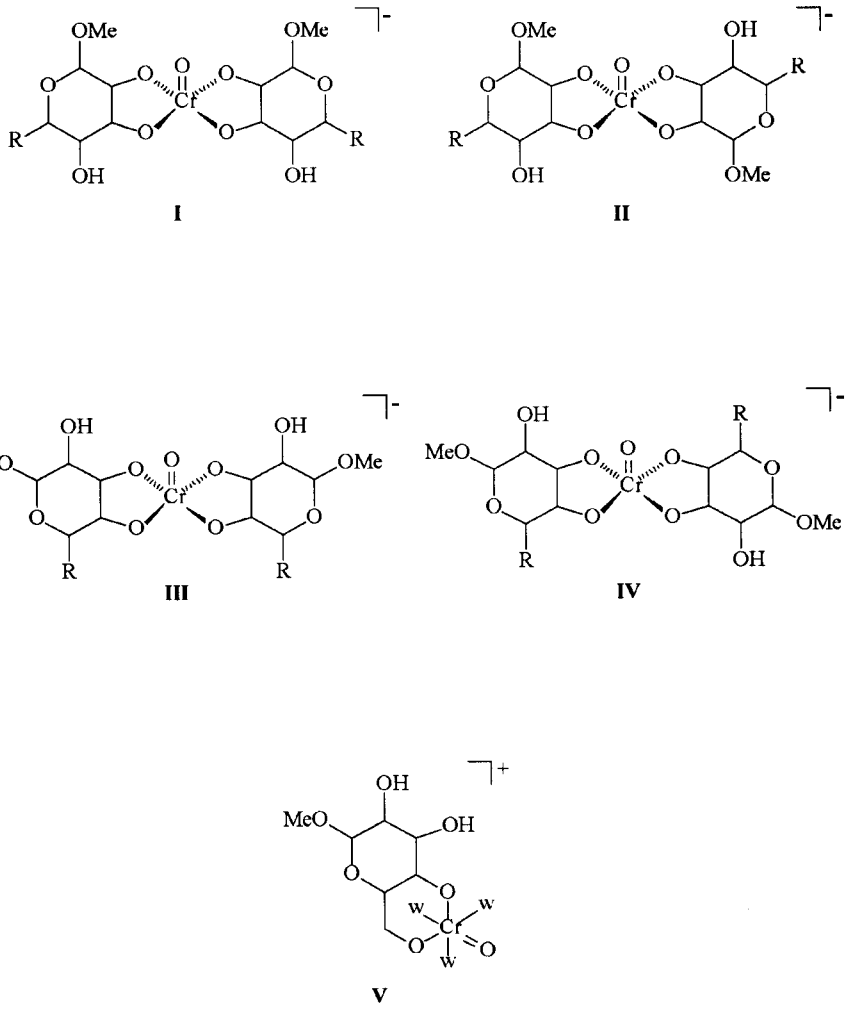



**FIGURE 6.** Oxo-$Cr^{V}$ chelates formed with gly1Me.

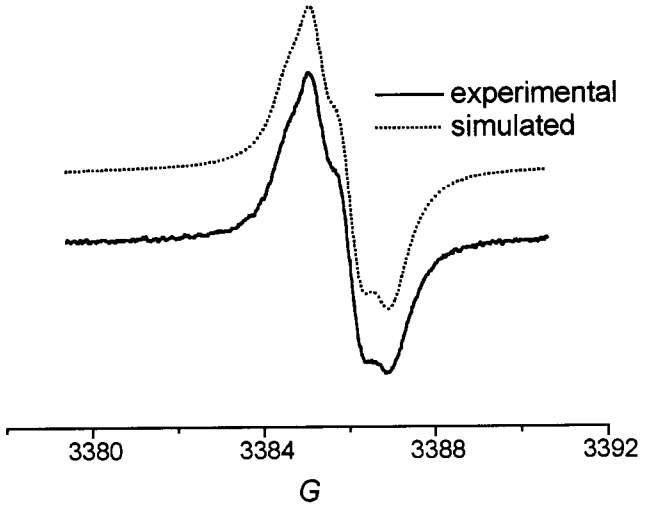
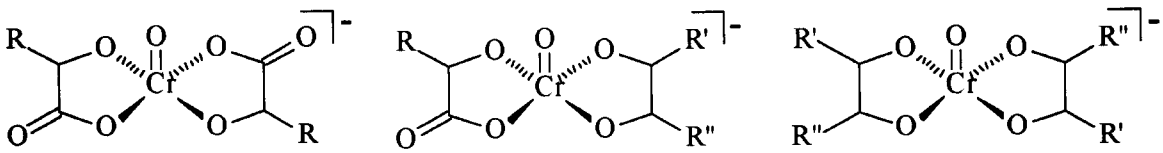



**FIGURE 7.** X-band EPR spectra of a mixture of Ia and $Cr^{VI}$. $T = 25°C$, pH = 4.5, mod. ampl. = 0.4 G, frequency = 9.3759 GHz. $[Cr^{VI}] = 4.8 \times 10^{-4}$ M. Ia:$Cr^{VI}$ = 7000:1.

$Cr^{V}$ was observed in the reaction between gluconic acid (glca) and $Cr^{VI}$ [44,45]. In aqueous solutions of pH 2 – 6, the $Cr^{VI}$ oxidation of glca affords only one $Cr^{V}$ species with EPR spectral parameters attributable to $[Cr(O)(O^{2(3)},O^{3(4)}\text{-glca})(O^{1},O^{2}\text{-glca})]^{-}$ [44].



VI VII VIII

**FIGURE 8.** Five-coordinated oxo-$Cr^{V}$ bis-chelates formed in the reaction between $Cr^{VI}$ and gala. R = $C_4H_9O_4$. R' = $CO_2$, R'' = $C_3H_7O_3$ or R' = $C_3O_4H_4$, R'' = $CH_2OH$, for the $Cr^{V}$-$O^{2},O^{3}$-gala and $Cr^{V}$-$O^{4},O^{5}$-gala coordination modes.

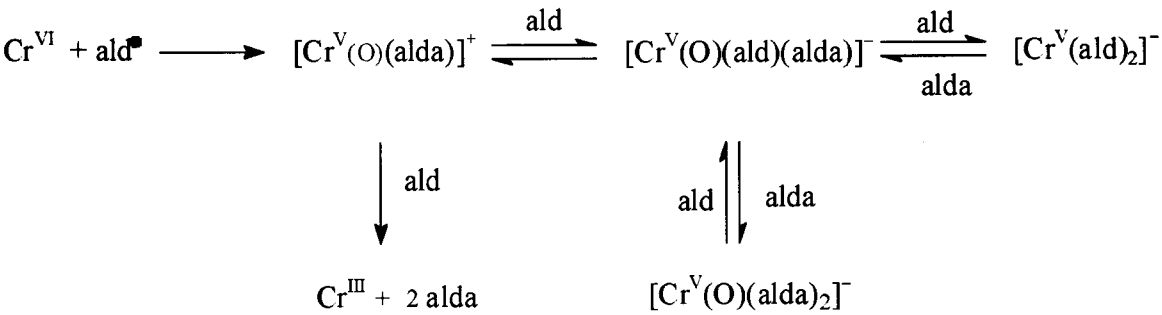
EPR spectroscopy was used to monitor the oxidation of D-ribonic acid (riba) by $Cr^{VI}$ at $[H^{+}] > 0.1M$.[46] The EPR spectra consist of a single detectable sharp signal at $g_{iso}$ 1.978, attributable to $[Cr^{V}(O)(O^{1},O^{2}\text{-riba})_2]^{-}$. At this $[H^{+}]$, the $Cr^{V}$ signal grows and decays rapidly with time to yield the broad $Cr^{III}$ signal ($g_{iso}$ 1.981) as the ultimate fate of chromium in this reaction.

In the reaction of 4-hydroxybutyric acid with $Cr^{VI}$, $Cr^{V}$ is not observed [47], a result which reinforces the fact that the 2-hydroxy-1-carboxylato moiety is the best suited to stabilize $Cr^{V}$ by forming five-membered chelates.

## 2.6. Aldohexoses

In acid medium, aldohexoses are selectively oxidized by $Cr^{VI}$ at the C1-OH hemiacetalic function yielding the aldonic acid (and/or the aldonolactone) and $Cr^{3+}$ as final products when an excess of sugar over $Cr^{VI}$ is used [48-52]. The redox reaction occurs through $Cr^{VI}\rightarrow Cr^{IV}\rightarrow Cr^{III}$ and $Cr^{VI}\rightarrow Cr^{V}\rightarrow Cr^{III}$ paths [49-51]. In 0.75 M $[H^+]$, D-glucose (glc), D-allose (allo), D-mannose (man) and D-galactose (gal) are oxidized at comparable rate by $Cr^{VI}$ and $Cr^{V}$, specially at high [ald] [49]. The observed rate constants for the reduction of $Cr^{VI}$ and $Cr^{V}$ by the aldoses ($k_6$ and $k_5$) depend on $[H^+]$. When the excess of sugar over $Cr^{VI}$ is large, $k_6 < k_5$ at high $[H^+]$, but the relative values of the kinetic parameters reverse ($k_5 < k_6$) at high pH. At pH > 2, the $Cr^{V}$ reduction rate becomes extremely slow and $Cr^{V}$ species generated in the $Cr^{VI}$ + ald reaction remain in solution for long time [18, 49, 52]. The EPR spectra show that a mixture of five-co-ordinate oxo-$Cr^{V}$ bis-chelates ($g_{iso} \approx 1.979$, $A_{iso}$ 16.4(3)x$10^{-4}$ $cm^{-1}$) are formed, and the relative proportion of each species in the reaction mixture depends on the reaction extent for each particular sugar [49]. For the glc + $Cr^{VI}$ reaction, the EPR spectra show one triplet at $g_{iso}$ 1.9789 and one quartet at $g_{iso}$ 1.9793, with spectral parameters consistent with those expected for [CrO($O^1$,$O^2$-glca)(*cis*-$O^1$,$O^2$-glc)]$^-$ and [CrO(*cis*-$O^1$,$O^2$-glc)($O^{2(3)}$,$O^{3(4)}$-glca]$^-$. The man + $Cr^{VI}$ reaction affords one triplet at $g_{iso}$ 1.9791 and one quartet at $g_{iso}$ 1.9795, which can be assigned to [CrO(*cis*-$O^{1(2)}$,$O^{2(3)}$-man)($O^1$,$O^2$-mana)]$^-$ and [CrO(*cis*-$O^{1(2)}$,$O^{2(3)}$-man)($O^3$,$O^4$-mana]$^-$ (mana = D-mannonic acid). Allo yields two triplets at 1.9794 ([CrO(*cis*-$O^{1(2,3)}$,$O^{2(3,4)}$-allo)$_2$]$^-$) and 1.9785 ([CrO($O^1$,$O^2$-alloa)$_2$]$^-$; alloa = D-allonic acid). In the gal +$Cr^{VI}$ system, the two main species present in solution are [CrO(*cis*-$O^3$,$O^4$-gal)($O^{2(4)}$,$O^{3(5)}$-gala)]$^-$ (quartet at $g_{iso}$ 1.9789) and [CrO($O^{2(4)}$,$O^{3(5)}$-gala)$_2$]$^-$ (quintet at $g_{iso}$ 1.9796). The observation of mixed $Cr^{V}$-ald-alda and/or $Cr^{V}$-alda$_2$ species with the open-chain alda bound to $Cr^{V}$ *via* the *vic*-diolate for glc, gal and man, but not for allo (the only observed binding mode for alloa is *via* the 2-hydroxy-1-carboxylato moiety- was taken as a point of evidence for the preferential $Cr^{V}$ chelation of *threo- vs erythro*-diolate moiety.

At $[H^+] \geq 0.1$ M, the reaction of $Cr^{VI}$ with excess aldose affords two or three new EPR signals ($g_{iso}$ 1.974, 1.971 and 1.966) besides the signal at g 1.979 [49,51]. The $g_{iso}$ values of the new species are consistent with those expected for six-coordinated oxo-$Cr^{V}$ complexes. The relative intensity of the $Cr^{V}$ EPR signals depends on the $[H^+]$, but is independent of the ald:$Cr^{VI}$ ratio employed, and, at $[H^+]$ > 0.75 M, the six-coordinated species are the major ones. The six- and five-coordinated oxo-$Cr^{V}$ species decay at essentially the same rate, which means that these complexes are in rapid equilibrium and react at the same rate to yield the redox reactions. Scheme 1 summarizes the observed $Cr^{V}$-sugar redox and complexation chemistry. The proposed first step in the scheme is the formation of a six-coordinated oxo-$Cr^{V}$ which can yield either the redox products or the $Cr^{V}$ bis-chelates depending of the $[H^+]$. When the $[H^+]$ is high, the redox reaction –which is acid catalyzed- is faster than complexation by a second molecule of the aldose; but as the $[H^+]$ decreases, complexation competes favorably with the redox reaction and five-coordinated $Cr^{V}$ bis-chelates form and stabilize.




**Scheme 1.** Redox and complexation chemistry of $Cr^{V}$ in the $Cr^{VI}$/aldose reaction

Five- and six-coordinated oxo-$Cr^{V}$ species are also formed in 50 – 100 % acetic acid solutions of L-rhamnose and $Cr^{VI}$ (10:1 ratio), with $g_{iso}$ 1.978 and 1.973, respectively [53]. In aqueous solutions, the five-coordinated oxo-$Cr^{V}$ species are observed at $g_{iso} \approx 1.977$ ($A_{iso}$ $16.4x10^{-4}$ $cm^{-1}$) and prevail at pH 3-7 [51]. The six-coordinated oxo-$Cr^{V}$ species have $g_{iso} \approx 1.970$ and dominate the EPR spectra at pH < 1.

The reduction of $Cr^{VI}$ by α-D-glucose and β-D-glucose was studied in dimethylsulfoxide in the presence of pyridinium p-toluensulphonate, a medium where mutarotation is slower than the redox reaction [48]. The two anomers reduce $Cr^{VI}$ and $Cr^{V}$ by formation of an intermediate $Cr^{VI}$ (and $Cr^{V}$) ester precursor of the slow redox step. The equilibrium constant for the formation of the intermediate chromic ester and the rate of the redox steps are different for each anomer. α-D-glucose forms the $Cr^{VI}$-glc ester with a higher equilibrium constant than β-D-glucose, but the electron-transfer within this complex is slower than for the β-anomer. The difference is attributed to the better chelating ability of the 1,2-cis-diolate moiety of the α-anomer. The EPR spectra show that the α-anomer forms several five-co-ordinate $Cr^{V}$ bis-chelates with $g_{iso}$ 1.9820 ($A_{iso}$ $15.9x10^{-4}$ $cm^{-1}$), 1.9785 and 1.9756, while β-D-glucose affords a mixture of six-co-ordinate $Cr^{V}$ monochelate ($g_{iso}$ 1.9700) and five-coordinated $Cr^{V}$ bis-chelates ($g_{iso}$ 1.9820, $A_{iso}$ $15.8x10^{-4}$ $cm^{-1}$, 1.9789 and 1.9767). The fact that the six-coordinated oxo-$Cr^{V}$ monochelates are observed only when the β-anomer is used was interpreted as evidence for the binding of the *trans*-1,2-diolate moiety of the β-anomer *vs* the *cis*-1,2-diolate moiety of the α-anomer. The last affords a bis-chelate easier than the former because of the enhanced ability of the *cis*-1,2-diolate to bind $Cr^{V}$.

Complex EPR signals have been obtained from the ligand-exchange reaction between $[CrO(ehba)_2]^-$ and glc [18]. The resulting $Cr^{V}$-glc solutions have been analyzed by using Q-, X- and S-band EPR spectroscopies and partially or fully deuterated glc. The Q-band EPR spectra of $Cr^{V}$/$^{2}H_5$-glc-C solutions were shown to contain at least six $Cr^{V}$ species [54].

## 2.7. Disaccharides

The observation of long lived lactose/$Cr^{V}$ species generated by direct reaction of $Cr^{VI}$ with lactose in milk contaminated with chromates pointed out the capability of these compounds to stabilize $Cr^{V}$ [55]. Further work revealed that disaccharides form stable hypervalent chromium chelates at pH > 2, while at higher $[H^+]$ their oxidation by $Cr^{VI}$/$Cr^{V}$ is favoured [56]. When excess disaccharide over $Cr^{VI}$ is used, the oxidation occurs selectively at the hemiacetalyc $C^1OH$ group, with first-order kinetics on both $[Cr^{VI}]$ and [disaccharide] [56]. The redox and complexation chemistry observed for the disaccharide-$Cr^{VI}$ systems parallels that of aldoses. However, the glycoside moiety can retard the redox rate of the aglycone when additional binding to $Cr^{VI}$ by the glycoside moiety is possible. In the case of maltose (mal), this effect reduce the observed rate constant to one half of the value obtained for glc [56]. The relative ability of four disaccharides to reduce $Cr^{VI}$, melibiose (mel) > lactose (lac) > cellobiose (cel) > mal, was attributed to the increasing stability of the intermediate disaccharide-$Cr^{VI}$ chelates formed with mel through mal. EPR spectroscopy was used to determine the $Cr^{V}$ species formed in the reaction of $Cr^{VI}$ with mel, lac, cel and mal or in the reaction of $Cr^{VI}$ with GSH in the presence of excess disaccharide, in the 5-7 pH range [56]. The EPR spectral features of disaccharide/$Cr^{V}$ mixtures were interpreted in terms of $Cr^{V}$-diolato species involving mainly $Cr^{V}$ binding through the *cis*-$O^1$,$O^2$-diolato of mal and cel and the *cis*-$O^1$,$O^2$-diolato and *cis*-$O^{3'}$,$O^{4'}$-diolato of lac and mel.

In 0.2 M $HClO_4$ the EPR spectra of the reaction between lac and $Cr^{VI}$ show three signals at $g_{iso}$ 1.9797, 1.9743 and 1.9715 (Figure 9-a). The signal at $g_{iso}$ 1.9715 is attributed to the positively charged six-co-ordinate oxo-$Cr^{V}$ monochelate $[Cr(O)(cis\text{-}O,O\text{-lac})(H_2O)_3]^+$ monochelate (**IX**, Figure 10) and that at $g_{iso}$ 1.9743 to the $[Cr(O)(O^1,O^2\text{-lactobionato})(H_2O)_3]^+$ complex (**X**). The positive charge of these species is consistent with their appearance at high $[H^+]$, and coordination to the oxidized ligand agrees

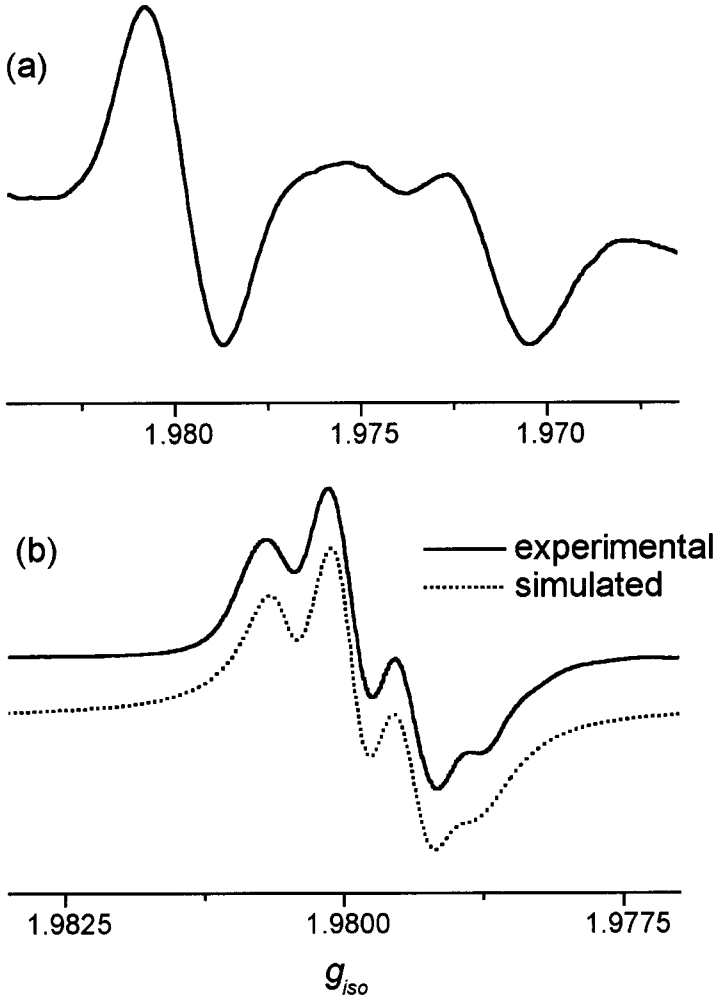



**FIGURE 9.** X-band EPR spectra of mixtures of (a) $Cr^{VI}$:lac = 1:33, $[H^+]$ = 0.20 M; $t$ = 6 min, mod. ampl. = 4 G; (b) $Cr^{VI}$:lac = 1:19, pH = 5.0, $t$ = 24 h, mod. ampl. = 0.4 G, $I$ = 1.0 M, $T$ = 20 °C, frequency ≈ 9.7 GHz.

with the fact that $Cr^{VI}$ is a stronger oxidant in more acidic medium. Only the signal at $g_{iso}$ 1.9797 persists at higher pH and could be deconvoluted into two triplets at $g_{iso1}$ 1.9800 and $g_{iso2}$ 1.9794 ($A_{iso}$ (16.5(3)×$10^{-4}$ $cm^{-1}$, ~50:50 $g_{iso1}/g_{iso2}$ ratio) (Figure 10-b). These EPR spectral parameters are consistent with those expected for any of the three linkage isomers of the $[Cr(O)(cis\text{-}O,O\text{-lac})_2]^-$ (**XI - XIII**). In the reaction of $Cr^{VI}$ with mal, the EPR spectra consist of two triplets at $g_{iso}$ 1.9795 and 1.9794, which are attributed to the two geometric isomers of the $[Cr(O)(O^1,O^2\text{-mal})_2]^-$ (**XII**), with mal bound to $Cr^{V}$ *via* the 1,2-*cis* diolate moiety. The EPR spectra of Cel/$Cr^{VI}$ reaction mixtures consist of one triplet at $g_{iso}$ 1.9793, which corresponds to the $[Cr(O)(O^1,O^2\text{-Cel})_2]^-$ chelate (**XII**). In the reaction of $Cr^{VI}$ with mel, the EPR spectra show two triplets at $g_{iso1}$ 1.9802 and $g_{iso2}$ 1.9798 (~50:50 $g_{iso1}/g_{iso2}$ ratio), corresponding to different linkage isomers of the five-coordinated oxo-$Cr^{V}$ complex $[Cr(O)(O,O\text{-mel})_2]^-$ (**XI – XIII**).

Species **XI – XIII** are also formed when $Cr^{V}$ is generated by reaction of $Cr^{VI}$ with GSH (1:1 ratio) at pH = 5.0 and stabilized by the presence of 10-times excess disaccharide over $Cr^{VI}$ [56]. This result

evidences that $Cr^{V}$ species observed at this pH effectively correspond to $Cr^{V}$ chelates formed with the disaccharide and not with the oxidized product (aldobionic acid).

The fact that only $Cr^{V}$-disaccharide species are formed in the $Cr^{VI}$/disaccharide reaction at pH 3-7, indicates that the disaccharides are better chelating agents for $Cr^{V}$ than aldoses. The latter are poorer complexation agents than the corresponding aldonic acids so that $Cr^{V}$-ald-alda and $Cr^{V}alda_2$ bis-chelates are the major species observed in the EPR spectra of the $Cr^{VI}$/aldose reaction mixtures.

## 2.8. Aldopentoses

The biological relevance of the interaction of $Cr^{V}$ with diolate groups of furanoses was first pointed out in a work where it was shown that $Cr^{V}$ complexation occurred with single ribonucleotide units but not with the analogous deoxyribonucleotides [57]. This suggested that the *cis*-diolate group of the ribose unit was involved in the $Cr^{V}$ coordination. $Cr^{V}$ species formed with ribonucleotides and with D-ribose 5'-monophosphate have been characterized and yield EPR spectroscopic parameters ($g_{iso} \approx 1.979$ and $A_{iso} \approx 16.3x10^{-4}$ $cm^{-1}$) indicative of $Cr^{V}$-diolato binding [58]. The redox chemistry of the D-ribose (rib)/$Cr^{VI}$ system has been studied [59]. It was found that the reduction of $Cr^{VI}$ by rib is six-times higher than that of allo -the aldohexose with the same configuration of C2 and C3-. The fact that the furanose ring favors oxidation *vs* complexation processes was interpreted as the consequence of the influence of the strain-induced instability of the chromate ester on the redox rate. This effect is enhanced in the reaction between $Cr^{V}$ and the furanose, and can be evidenced when the observed rate constants for the reduction of $Cr^{V}$ ($k_5$) and $Cr^{VI}$ ($k_6$) by the aldose are compared for the pyranose and furanose forms. In the chromic oxidation of aldohexoses (pyranose form) in 0.75 M $HClO_4$, $k_5$ are no more than 10-times higher than $k_6$ [49]; whereas for rib, $k_5 > 100\ k_6$ [59]. The higher $k_{ox}/K_{complexation}$ ratio for the rib/$Cr^{VI}$ system, means that intramolecular electron transfer paths are faster within the less stable hypervalent chromium intermediate complexes.

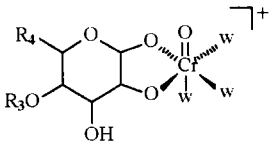
The reaction of $Cr^{VI}$ with an excess of rib, in the 4-6 pH range, yields an EPR spectrum dominated by a single detectable signal ($g_{iso} = 1.9787$, $A_{iso}$ $15.7x10^{-4}$ $cm^{-1}$), corresponding to five-coordinated oxo-$Cr^{V}$ complexes. In this pH range, $Cr^{V}$ remains in solution several days. At low modulation amplitude, the shf pattern partially resolves, and the $Cr^{V}$ EPR spectrum results to be a composite of several $Cr^{V}$ species. Based on the EPR shf spectral pattern derived for the cyclopentanediol/$Cr^{V}$ system the spectra can be deconvoluted into three signals with spectroscopic EPR parameters consistent with the presence of the [Cr(O)($O^{1(2)}$,$O^{2(3)}$-rib)($O^{1}$,$O^{2}$-riba)]$^{-}$ ($g_{iso}$ 1.9791 and 1.9792) and [Cr(O)($O^{1}$,$O^{2}$-riba)$_2$]$^{-}$ ($g_{iso}$ 1.9785) [59].

In [$HClO_4$] ≥ 0.1 M, the EPR spectra of the reaction of $Cr^{VI}$ with excess rib show that six-coordinated oxo-$Cr^{V}$ monochelates ($g_{iso}$ 1.9711) form besides the five-coordinated oxo-$Cr^{V}$ species at $g_{iso}$ 1.9787 (Figure 11) [59], and they are the major $Cr^{V}$ species in 0.15-1.0 M $HClO_4$. Unlike that observed for pyranoses, the two $Cr^{V}$ signals decay at different rates, and these rates increase with decreasing pH. Probably, the two types of complexes -five- and six-coordinated observed in the rib/$Cr^{VI}$ reaction are formed independently and yield the final $Cr^{III}$ by two parallel paths.

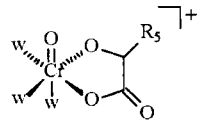
## 2.9. 2-Deoxyaldoses

The study of the reaction of 2-deoxyaldoses with $Cr^{VI}$ show that the lack of the $C_2$-OH group favors the redox reactions and reduce the capability of the aldose for binding hypervalent Cr [60,61].

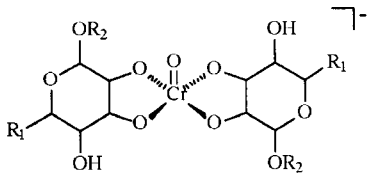
The reaction of 2-deoxy-D-glucose (2dglc) and $Cr^{VI}$ results in the formation of two $Cr^{V}$ species with $g_{iso}$ 1.9781 and 1.9754 in a 2.6 ratio independently of the pH and [2dglc] [61]. The two species disappear at comparable rates, meaning that these $Cr^{V}$ intermediates are in rapid equilibrium compared to the time-scale of their subsequent reduction to $Cr^{III}$. The spectroscopic EPR parameters indi-



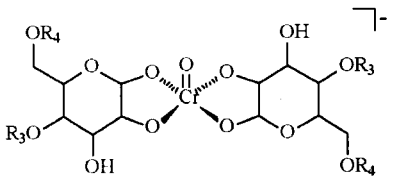
IX



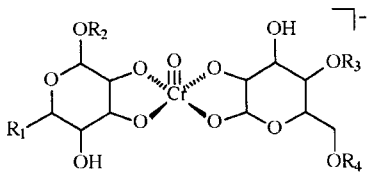
X



XI



XII



XIII

**FIGURE 10.** Oxo-$Cr^{V}$ mono- and bis-chelates formed in the reaction between $Cr^{VI}$ and disaccharides. $R_1$ = $CH_2OH$. $Cr^{V}$-$Lac_2$: $R_2$ = $O^4$-glc; $Cr^{V}$-$Mel_2$: $R_2$ = $O^6$-glc. $Cr^{V}$-$Lac_2$ ($Cr^{V}$-$Mal_2$, $Cr^{V}$-$Cel_2$): $R_3$ = glycoside, $R_4$ = H; $Cr^{V}$-$Mel_2$: $R_3$ = H, $R_4$ = glycoside. w = water. $R_5$ = $C_{10}O_9H_{19}$.

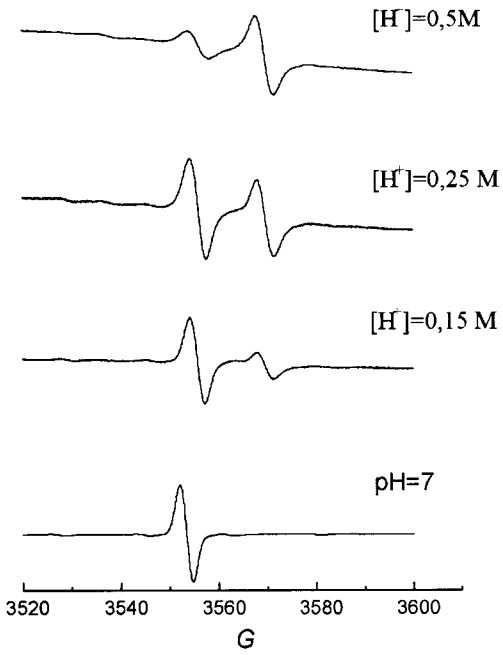



**FIGURE 11.** X-band EPR spectra of a mixture of rib and $Cr^{VI}$ (10:1 mole ratio) at different $[H^+]$. $T$ = 20°C, mod. ampl. = 4 G, frequency ≈ 9.85 GHz.

cate the signals can be assigned to $Cr^{V}$ bis- and mono-chelates formed with the substrate: $[Cr(O)(O^3,O^4\text{-}2dglc)(O^1\text{-}2dglc)(H_2O)]^-$ ($g_{calc}$ 1.9783) and $[Cr(O)(O^1\text{-}2dglc)(H_2O)_3]^+$ ($g_{calc}$ 1.9756).

2-acetamido-2-deoxy-D-glucose is oxidized by $Cr^{VI}$ to the aldonic acid and the reaction is proposed to occur by a reaction path involving $Cr^{V}$. However, $Cr^{V}$ intermediates could not be detected by EPR in the 0.15 – 1.0 M $[H^+]$ used in the kinetic measurements [62].

The reaction of $Cr^{VI}$ with an excess of 2-deoxy-D-ribose (2drib), in the 4-7 pH range, yields only one $Cr^{V}$ intermediate species, probably $[Cr(O)(2drib)(2driba)]^-$, with $g_{iso}$ 1.9791 and $A_{iso}$ $16.6x10^{-4}$ $cm^{-1}$.[59] At pH 2, a minor signal with $g_{iso}$ 1.9735 appears, and when the $[H^+] > 0.1$ M a third signal at $g_{iso}$ 1.9707 is observed (Figure 12) [59]. The $g_{iso}$ values of the two signals at higher field correspond to six-coordinated oxo-$Cr^{V}$ complexes. The relative proportions of the three species depend essentially on the acidity. The two (or three) signals decay at comparable rates, and these rates increase with decreasing pH, such as observed for 2dglc.

### 2.10. Uronic acids

The redox reaction between $Cr^{VI}$ and galacturonic acid has been studied and relatively long-lived $Cr^{V}$ intermediates were found by EPR spectroscopy [26,43]. Galacturonic acid is oxidized by $Cr^{VI}$ to mucic acid, when an excess of substrate over $Cr^{VI}$ is used. The reaction is catalyzed by acid and proceeds through a $Cr^{VI} \rightarrow Cr^{V} \rightarrow Cr^{III}$ path [43]. When a large excess of galacturonic acid over $Cr^{VI}$ is used (2000-7000 : 1), in the 1-6 pH range, the EPR signal is a composite of at least three five-coordinated oxo-$Cr^{V}$ species, with $g_{iso}$ 1.9786, 1.9785 and 1.9784 and $A_{iso} \approx 17.2x10^{-4}$ $cm^{-1}$ (Figure 13). The EPR spectral parameters are indicative of the presence of three different isomers of $[Cr(O)(O^5,O^6\text{-}galacturonate)_2]^-$, with $Cr^{V}$ bound to the carboxylato and alcoholate donor groups of the ligand [43].

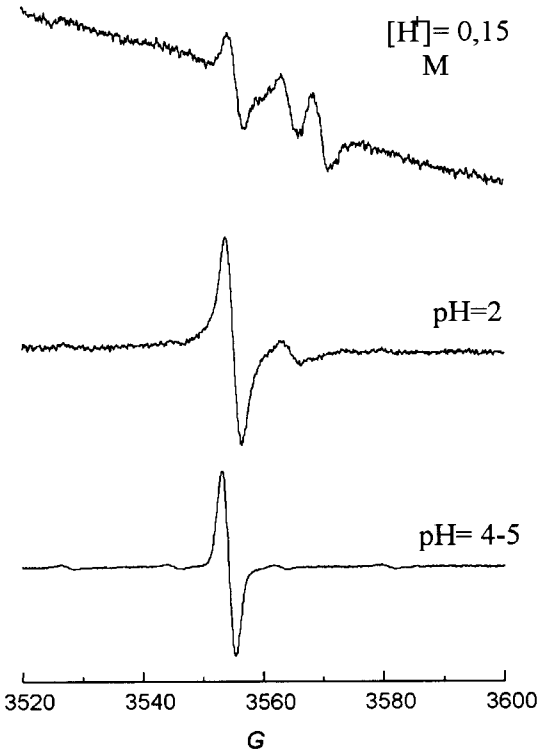



**FIGURE 12.** X-band EPR spectra of a mixture of 2drib and $Cr^{VI}$ (10:1 mole ratio) at different $[H^+]$. $T$ = 20°C, mod. ampl. = 8 G, frequency ≈ 9.84 GHz.

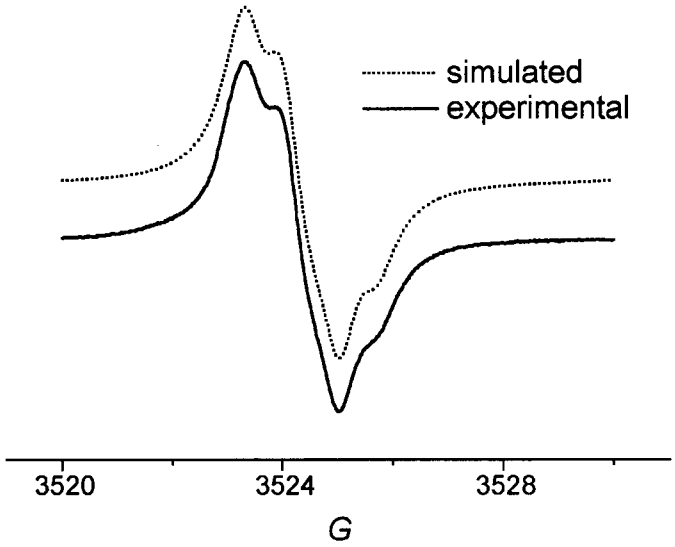



**FIGURE 13.** X-band EPR spectra of a mixture of $Cr^{VI}$:galacturonic acid = 1:7000, pH 1.0, mod. ampl. = 0.3 G, $I$ = 1.0 M, $T$ = 20 °C, frequency = 9.7746 GHz.

## 3. Conclusions

The above results show that saccharides are effective complexation agents for $Cr^{V}$, affording very stable $Cr^{V}$-bis-chelates over a large range of pH values. Strong acid conditions ($[H^{+}]$ >0.1M) are required to favor the redox reaction between the saccharide and $Cr^{V}$ over the complexation by a second molecule of the substrate. The EPR spectroscopic studies of $Cr^{V}$-cyclic saccharides systems show that, when *cis*- and *trans*-diol groups are present in the saccharide, $Cr^{V}$ prefers to bind the *cis*-diolato moieties, with very small to negligible proportion of species with $Cr^{V}$ bound to the *trans*-diol groups. The 2-hydroxyacid donor groups are favored with respect to 1,2-diols for stabilizing $Cr^{V}$ at low pH values ($\approx$ 4), but the opposite is the case at pH $\approx$ 7. At neutral pH, the $Cr^{V}$-2-hydroxycarboxylato complexes rapidly decompose by disproportionation, while $Cr^{V}$ species formed *via* the *vic*-diolate moiety of saccharides are very stable and stay in solution for long time. Both the 2-hydroxycarboxylato and the *vic*-diolate moieties of saccharides compete effectively for complexation of $Cr^{V}$ compared to GSH or cys at all the pH values studied.

## ACKNOWLEDGEMENTS

We wish to thank all the collaborators who participated in the research and the National Research Council of Argentina (CONICET), the Third World Academy of Sciences (TWAS), the National University of Rosario (UNR), the International Foundation for Sciences (IFS) and Antorchas Foundation for the financial support.

## REFERENCES

1. Klein, C. B. in *Toxicology of Metals*, Chang, L. W., ed., CRC-Lewis Publishers, New York, 1996, pp. 205-220.

2. Codd, R.; Dillon, C. T.; Levina, A.; Lay, P. A. *Coord. Chem. Rev.* **2001**, *216-217*, 533-577.
3. Katz, S. A.; Salem, H. *The Biological and Environmental Chemistry of Chromium*, VCH Publishers. Inc., New York, 1994, pp. 65-119.
4. Shi, X.; Chiu, A.; Chen, C. T.; Halliwell, B.; Castranova, V.; Vallyathan, V. *J. Toxicol. Environm. Health, Part B*, **1999**, 87-104.
5. Costa, M. *Crit. Rev. Toxicol.* **1997**, *27*, 431-442.
6. Gould, E. S. *Coord. Chem. Rev.* **1994**, *135/136*, 651-684.
7. Zhang, L.; Lay, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 12624-12637.
8. Geiger, D. K. *Coord. Chem. Rev.* **1997**, *164*, 261-288.
9. Levina, A.; Lay, P. A.; Dixon, N. E. *Inorg. Chem.* **2000**, *39*, 385-395.
10. Stearns, D. H.; Wetterhahn, K. E. *Chem. Res. Toxicol.* **1994**, *7*, 219-230.
11. Wetterhahn Jannette, K. E. *J. Am. Chem. Soc.* **1982**, *104*, 874-875.
12. O'Brien, P.; Barrett, J.; Swanson, F. *Inorg. Chim. Acta* **1985**, *108*, L19-L20.
13. Lay, P. A.; Levina, A. *J. Am. Chem. Soc.* **1998**, *120*, 6704-6714.
14. Ciéslak-Golonka, M. *Polyhedron* **1996**, *15*, 3667-3689.
15. Zhang, L.; Lay, P. A. *Aust. J. Chem.* **2000**, *53*, 7-13.
16. S. Signorella, C. Palopoli, M. Santoro, S. García, V. Daier, J. C. González, V. Roldán, M. I. Frascaroli, M. Rizzotto, L. F. Sala; *Research Trends*, in press
17. Mehta, N.; Dubach, P.; Deuel, H. *Adv. Carbohyd. Chem. Biochem.*, Vol. 16, **1958**, 335-355.
18. Barr-David, G.; Charara, M.; Codd, R.; Farrell, R. P.; Irwin, J. A.; Lay, P. A.; Bramley, R.; Brumby, S.; Ji, J.-Y.; Hanson, G. R. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 1207-1216.
19. Bramley, R.; Ji, J.-Y.; Judd, R. J.; Lay, P. A. *Inorg. Chem.* **1990**, *29*, 3089-3094.
20. Farrell, R. P.; Judd, R. J.; Lay, P. A.; Bramley, R.; Ji, J.-Y. *Inorg. Chem.* **1989**, *28*, 3401-3403.
21. Codd, R.; Lay, P. A. *J. Am. Chem. Soc.* **1999**, *121*, 7864-7876
22. Signorella, S.; Daier, V.; Santoro, M.; García, S.; Palopoli, C.; González, J. C.; Korecz, L.; Rockenbauer, A.; Sala, L. F. *Eur. J. Inorg. Chem.* **2001**, 1829-1833.
23. Israeli, Y.; Detellier, C. *Carbohyd. Res.* **1997**, *297*, 201-207
24. Mitewa, M.; Bontchev, P. *Coord. Chem. Rev.*, **1985**, *61*, 241-272.
25. Kaiwar, S. P.; Raghavan, M. S. S.; Rao,C. P. *Carbohyd. Res.*, **1994**, *256*, 29-40.
26. Branca,M.; Micera, G.; Dessi, A. *Inorg. Chim. Acta*, **1988**, *153*, 61-65.
27. Roldán, V.; Daier, V.; Goodman, B.; Santoro, M.; González, J. C.; Calisto, N.; Signorella, S.; Sala, L. *Helv. Chim. Acta*, **2000**, *83*, 3211-3228.
28. Bramley, R.; Ji, J-Y.; Lay, P. A. *Inorg. Chem.* **1991**, 30, 1557-1564.
29. Branca, M.; Micera, G.; Segre, U.; Dessi, A. *Inorg. Chem.* **1992**, *31*, 2404-2408.
30. González, J. C.; Signorella, S.; Sala, L. F., unpublished results.
31. Signorella, S.; Frascaroli, M. I.; García, S.; Santoro, M.; González, J. C.; Palopoli, C.; Casado, N.; Sala, L. F. *J. Chem. Soc., Dalton Trans.* **2000**, 1617-1623.
32. Rizzotto, M.; Levina, A.; Santoro, M.; García, S.; Frascaroli, M. I.; Signorella, S.; Sala, L. F.; Lay, P. A., unpublished results.
33. Lay, P. A.; Levina, A. *Inorg. Chem.* **1996**, *35*, 7709-7717.
34. Sugden, K. D.; Wetterhahn, K. E. *Chem. Res. Toxicol.* **1997**, *10*, 1397-1405.
35. Levina, A.; Barr-David, G.; Codd, R.; Lay, P. A.; Dixon, N. E.; Hammershøi, A.; Hendry, P. *Chem. Res. Toxicol.* **1999**, *12*, 371-381.
36. Krumpolc, M.; DeBoer, B. G.; RoËek, J. *J. Am. Chem. Soc.* **1978**, *100*, 145-153.
37. Krumpolc, M.; RoËek, J. *J. Am. Chem. Soc.* **1979**, *101*, 3206-3209.
38. Judd, R. J.; Hambley, T. W.; Lay, P. A. *J. Chem. Soc., Dalton Trans.* **1989**, 2205-2210.
39. Branca, M.; Dessi, A.; Micera, G.; Sanna, D. *Inorg. Chem.* **1993**, *32*, 578-581.
40. Signorella, S.; García, S.; Sala, L. F. Polyhedron **1992**, *11*, 1391-1396.
41. Codd, R.; Levina, A.; Zhang, L.; Hambley, T. W.; Lay, P. A. *Inorg. Chem.* **2000**, *39*, 990-997.
42. Haight, G.; Jwisich, G.; Kelso, M.; Merril, P. *Inorg. Chem.*, **1985**, *24*, 2740-2746.
43. González, J. C.; Signorella, S.; Goodman, B.; Daier, V., unpublished results.
44. Signorella, S.; Santoro, M.; Palopoli, C.; Brondino, C.; Salas-Peregrin, J. M.; Quirós M.; Sala, L. F. *Polyhedron*, **1998**, *17*, 2739-2749.
45. Signorella, S.; Santoro, M. I.; Mulero, M.; Sala, L. F. *Can. J. Chem.* **1994**, *72*, 398-402.
46. Signorella, S.; García, S.; Sala, L. F. *Polyhedron* **1997**, *16*, 701-706.
47. Palopoli, C.; Signorella, S.; Sala, L. F. *New J. Chem.* **1997**, *21*, 343-348.
48. Signorella, S.; Lafarga, R.; Daier, V.; Sala, L. F. *Carbohyd. Res.* **2000**, *324*, 127-135.

49. Signorella, S.; Daier, V.; García, S.; Cargnello, R.; González, J. C.; Rizzotto, M.; Sala, L. F. *Carbohyd. Res.* **1999**, *316*, 14-25.
50. Signorella, S.; García, S.; Sala, L. F. *J. Chem. Ed.* **1999**, *76*, 405-408.
51. Sala, L. F.; Signorella, S.; Rizzotto, M.; Frascaroli, M. I.; Gandolfo, F. *Can. J. Chem.* **1992**, *70*, 2046-2052.
52. Branca, M.; Dessi, A.; Kozlowski, H.; Micera, G.; Swiatek, J. *J. Inorg. Biochem.* **1990**, *39*, 217-226.
53. Rizzotto, M.; Frascaroli, M. I.; Signorella, S.; Sala, L. F. *Polyhedron* **1996**, *15*, 1517-1523.
54. Lay, P. A., personal communication.
55. Goodgame, D. M. L.; Joy, A. M. *Inorg. Chim. Acta* **1987**, *135*, L5-L7.
56. Roldán, V.; González, J. C.; Santoro, M.; García, S.; Casado, N.; Olivera, S.; Salas-Peregrin, J. M.; Signorella, S.; Sala, L. F., unpublished results.
57. Goodgame, D. M. L.; Hayman, P. B.; Hathway, D. E. *Polyhedron* **1982**, *1*, 497-499.
58. Rizzotto, M.; Moreno, V.; Signorella, S.; Daier, V.; Sala, L. F. *Polyhedron* **2000**, *19*, 417-423.
59. Daier, V.; Signorella, S.; Rizzotto, M.; Frascaroli, M. I.; Palopoli, C.; Brondino, C.; Salas-Peregrin, J. M.; Sala, L. F. *Can. J. Chem.* **1999**, *77*, 57-64.
60. Rizzotto, M.; Signorella, S.; Frascaroli, M. I.; Daier, V.; Sala, L. F. *J. Carbohyd. Chem.* **1995**, *14*, 45-51.
61. Signorella, S.; Rizzotto, M.; Daier, V.; Frascaroli, M. I.; Palopoli, C.; Martino, D.; Bousseksou, A.; Sala, L. F. *J. Chem. Soc., Dalton Trans.* **1996**, 1607-1611.
62. Sala, L. F.; Palopoli, C.; Signorella, S. *Polyhedron* **1995**, *14*, 1725-1730.