Complexes of cobalt(III) with D-fructose and phenanthroline

Exequiel Moraga [a], Sergio Bunel [a], Carmen Ibarra [a], Andrei Blaskó [b], Clifford A. Bunton [b,*]

[a] *Departamento de Quimica Inorganica y Analitica, Facultad de Ciencias Quimicas y Farmaceuticas, Universidad de Chile, Santiago, Casilla 233, Chile*

[b] *Department of Chemistry, University of California, Santa Barbara, CA 93106, USA*

Abstract

D-Fructose and 1,10-phenanthroline form complexes with Co(III). Configurations about Co(III) are assigned from the CD and ORD spectra of the separated Λ and Δ diastereomers, and Λ strongly predominates in the mixture. Most of the ^{1}H NMR signals of the complexes are shifted strongly upfield, relative to those of D-fructose, due to shielding by aromatic residues, and the effect is especially strong for hydrogens at positions 1 and 6. Signals of the fructose residue are assigned for the Λ diastereomer and coupling constants estimated, but only some of the signals can be assigned for the Δ diastereomer. The marked changes in chemical shifts on formation of the complexes from fructose are rationalized in terms of predicted conformations based on molecular-mechanics calculations with MM2 parameters, which predict the higher stability of the Λ over the Δ diastereomer, and also complexation at positions 2 and 3.

Keywords: Cobalt(III); D-Fructose; Phenanthroline

1. Introduction

There is extensive evidence for complexation of sugars and their derivatives with metal ions [1–4]. The sugar may be a bi- or tri-dentate ligand and stabilities of the complexes depend on the conformations of the sugars. Several mixed complexes of sugars and bidentate amines with transition metal ions have been identified, generally with amine:sugar = 2:1 [3,4].

We had examined complexes of α- and β-glucosamine (2-amino-2-deoxy-α- and β-D-glucose) and Co(III), with the other ligands being ethylenediamine (en), 1,10-phenanthroline (phen), or ammonia [4]. The absolute configuration at the metal ion can be determined by circular dichroism (CD) or optical rotatory dispersion (ORD) spectroscopy [5,6]. Cobalt(III) is diamagnetic, and so the NMR spectra can be examined [7].

Solutions of equimolar fructose and Co(III)$(phen)_2$ at pH 8 develop a well defined CD spectrum in the visible region, demonstrating formation of a complex which appears to be stable in solution for several weeks. There is eventual decomposition, probably because the equilibrium mixture contains some Co(III)$(phen)_2$ which disproportionates to Co(III)$(phen)_3$ and a Co(III) aquo species which gives Co(II) [8].

Based on these preliminary results, we synthesized and characterized the complex from its absorbance and CD spectra which give information on the stereochemistry at Co(III) [5,6], and its NMR spectra which are powerful tools for elucidation of sugar structures [7–11]. The NMR spectra of the isomers of fructose have been studied very thoroughly, signals have been assigned and coupling constants determined [10,11]. The ^1H signals of phenanthroline do not interfere with those of the sugar residue, and this avoids possible problems of signal overlap present in mixed complexes of sugars and aliphatic diamines [7].

2. Results and discussion

Preparation of the complex.—D-Fructose reacts with Co(III)$(phen)_2$ at pH 8 without any change in the pH of the solution (see Experimental section). The cobalt phenanthroline complex was added as *cis*-$(Co(III)phen_2Cl_2)^+$ which, at pH 8, rapidly gives $(Co(III)(phen)_2(OH)(OH_2)^{2+}$ based on the pK_a of diaquo cobalt(III) complexes [12].

$$(phen_2Co(III)Cl_2)^+ \xrightarrow{+H_2O} (phen_2Co(III)(H_2O)_2)^{3+}$$

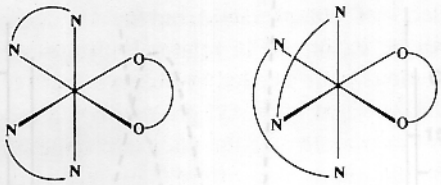
$$\rightleftharpoons (phen_2Co(III)(H_2O)(OH))^{2+}$$

$$\xrightarrow[pH8]{Fructose} (phen_2Co(III)(fructose))^{2+}$$

Therefore, the complex formed with fructose must be dicationic. Anomeric OH groups are relatively acidic [13], and it was assumed that complexation involves alkoxide oxygen at position 2 [2,4]. The appearance of a visible CD signal demonstrates the formation of mixed complexes of Co(III) with phenanthroline and fructose. These complexes could involve either fructopyranose or furanose, because in water at ca. 25°C the equilibrium mixture of fructose contains ca. 70% β-pyranose, ca. 25% β-furanose, and a small amount of α-furanose [10,11]. The configuration at Co(III) is given by the form of the CD spectrum [5,6].

The mixture of complexes can be separated chromatographically on Sephadex SP-C-25 and the predominant isomer is Λ (Scheme 1).

The CD and absorbance spectra of a solution of the Λ complex are very similar to those of a mixture of the complexes obtained from fructose and $(Co(III)(phen)_2Cl_2)^+$



Λ Δ

Scheme 1.

without isolation or separation of the complexes, and, based on the visible CD spectrum, equilibrium strongly favors the Λ complex.

Absorbance and CD spectra of the complexes.—The CD spectrum of the Λ complex has a positive signal at 510 nm and a negative signal at 400 nm, and for the Δ complex the corresponding extrema are at 510 nm (negative) and 410 nm (positive) (Fig. 1 and Table 1). The ORD spectra agree with the CD in the slopes of plots of molar rotation against wavelength and the positions of zero rotation [5,6] (data not shown). The assignment of configuration at metallic centers, based on CD or ORD spectra, has been discussed in detail [5,6], and there are a number of examples of the application of this method to complexes with ligands of biological interest, e.g., amino and hydroxy acids and sugar derivatives [4,7,14–16].

The CD spectrum of the Λ complex in the UV region is shown in Fig. 2. It has strong excitonic bands at 278 nm (positive) and 263 nm (negative) and a weak negative signal at 320 nm. There is a strong negative signal at 238 nm and a positive signal at lower wavelength, but this maximum could not be observed quantitatively because of the strong absorbance of the complex at low wavelengths.

The strong absorbance, and relatively weak CD signal of the Δ complex, meant that a good CD spectrum could not be obtained in the UV region, but there is a negative signal at 295 nm and a positive signal at 270 nm, consistent with the configuration at Co(III). The CD spectrum of the mixed complexes is very similar to that of the isolated Λ complex (Fig. 2), and $\Lambda/\Delta \approx 7$.

The absorbance and CD spectra of these fructose complexes are very similar to those of the corresponding complexes of D-glucosamine with Co(III) bis(phenanthroline) [4], apart from a slight red shift because of differences in the numbers of nitrogen and oxygen atoms coordinated to Co(III). Assignments of the absorbance bands and CD signals are essentially the same as those given for Co(III)–glucosamine complexes [4], and for other metal complexes with ligands of biological interest [14–18].

The factors that control the relative stabilities of Λ and Δ diastereomer polyol complexes have been discussed in terms of intramolecular forces, e.g., steric, hydrophobic, and hydrogen-bonding interactions as well as interactions with solvent [17]. Molecular modeling, based on MM2 parameters for intramolecular interactions, predicts the preference for the Λ diastereomer.

The magnitude of $\Delta\epsilon$ (Table 1) is very similar to that seen with other mixed complexes of Co(III) with ammonia or amines and sugars [4,18]. In these complexes

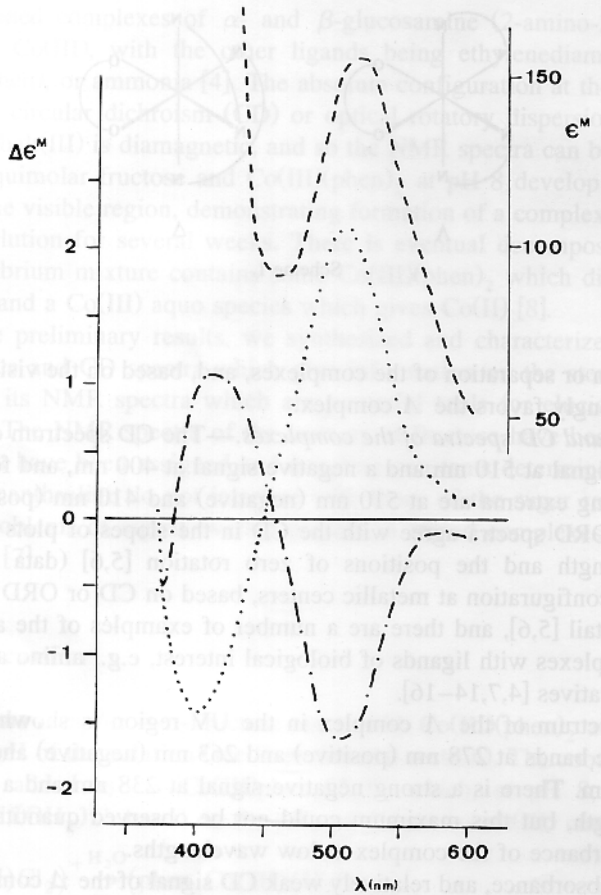


Fig. 1. Absorbance and CD spectra in the visible region. Absorbance (—), CD (· · ·) and (-·-·-·) of the *Λ*- and *Δ*-complexes of Co(III)(phen)$_2$ with D-fructopyranose, respectively.

Table 1
Electronic absorbance and circular dichroism spectra of the complexes

Complex	λ_{max} (nm)	log ϵ	λ (nm)	$\Delta\epsilon$
Λ	220(s)	4.20	220	63.6
			240	−31.6
			265	−40.7
	275	4.03	275	46.9
			320	−6.7
	515	2.19	510	2.2
			400	−1.4
Δ	515	2.19	510	−1.6
			410	1.1

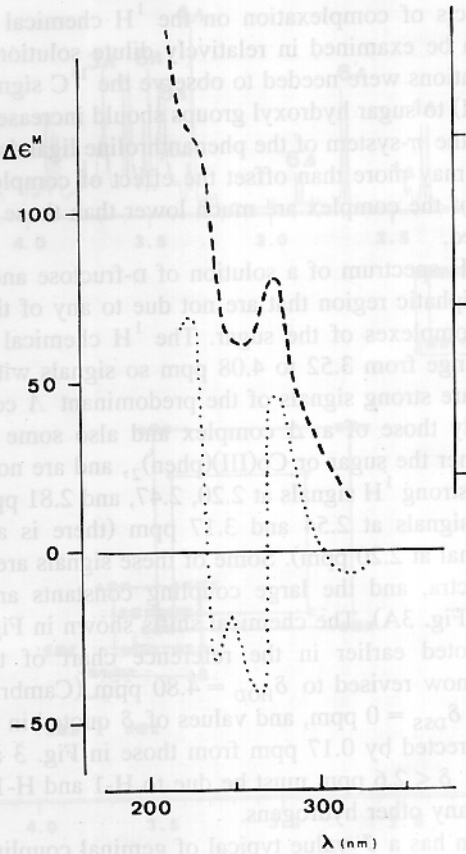


Fig. 2. Absorbance and CD spectra of the *Λ*-complex in the UV region. Key as in Fig. 1.

there is evidence for binding of Co(III) to the anomeric OH or O^- residue and to vicinal amino or hydroxyl groups. Therefore, the CD spectra of the Co(III)(phen)$_2$ complex (Figs. 1 and 2, and Table 1) are consistent with bonding of Co(III) to *cis*-alkoxide and hydroxyl residues at positions 2 and 3 or possibly 2 and 1, respectively. There are strong preferences for chelation by *cis* (*ax*, *eq*) groups [1,2].

NMR spectroscopy.—We examined the ^{1}H NMR spectrum of a mixture of the complexes formed by reactions in situ of D-fructose and Co(III)(phen)$_2$ in D_2O, because of problems with decomposition of solid Co(III) complexes [7] and spectral evidence that they are stable in solution. The ^{1}H and ^{13}C NMR spectra of *β*-D-fructopyranose and related sugars have been analyzed [10,11]. Our NMR spectra of D-fructose in D_2O at 25°C and chemical shifts relative to DSS agree with those published earlier [10,11]. There is interference by signals of uncomplexed fructose, because in dilute solution the complex does not form quantitatively. The downfield signals of the phenanthroline ligands do not interfere with those of the sugar and were not examined.

We first consider effects of complexation on the ^1H chemical shifts of the sugar residue, because they can be examined in relatively dilute solution (see Experimental section). Higher concentrations were needed to observe the ^{13}C signals.

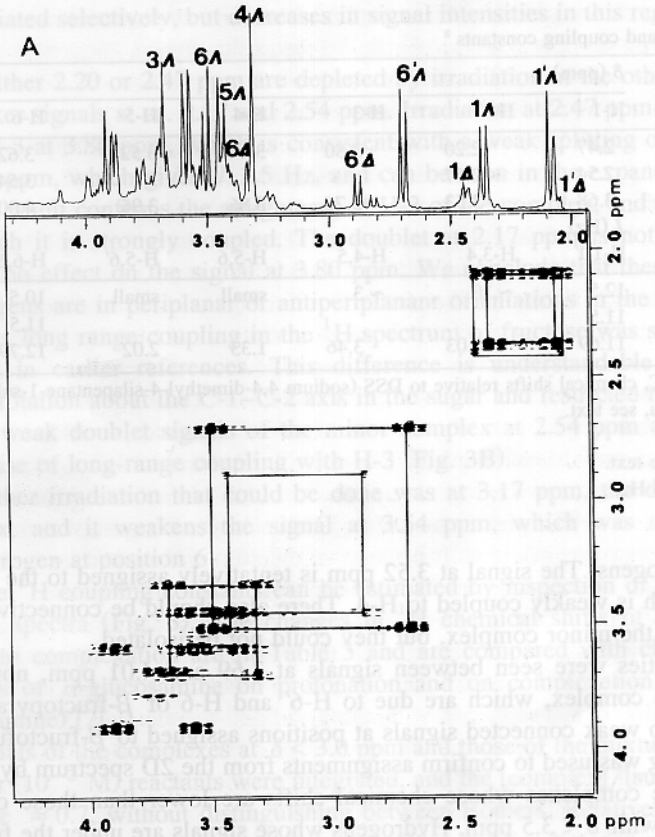
Complexation of Co(III) to sugar hydroxyl groups should increase the chemical shifts of the adjacent C ^1H, but the π-system of the phenanthroline ligands can strongly shield nearby hydrogens, which may more than offset the effect of complexation. As a result some ^1H chemical shifts of the complex are much lower than those of the parent sugar and can be easily identified.

The one-dimensional ^1H spectrum of a solution of D-fructose and Co(III)$(phen)_2$ in D_2O has signals in the aliphatic region that are not due to any of the fructose isomers [10,11], and are due to complexes of the sugar. The ^1H chemical shifts of the main tautomers of D-fructose range from 3.52 to 4.08 ppm so signals with $\delta < 3.5$ ppm are due to complexes. There are strong signals of the predominant Λ complex and weaker signals which are probably those of a Δ complex and also some very weak signals which are not those of either the sugar or Co(III)$(phen)_2$, and are not considered.

Coupling constants for strong ^1H signals at 2.20, 2.47, and 2.81 ppm are very similar to those for the weaker signals at 2.54 and 3.17 ppm (there is also a weak signal overlapping the strong signal at 2.20 ppm). Some of these signals are readily assignable from the ^1H–^1H 2D spectra, and the large coupling constants are characteristic of geminal coupling [19,20] (Fig. 3A). The chemical shifts shown in Fig. 3 are referred to $\delta_{HOD} = 4.63$ ppm, as quoted earlier in the reference chart of the GE-500 NMR instrument. This value is now revised to $\delta_{HOD} = 4.80$ ppm (Cambridge Isotope Labs, Andover, MA), which fits $\delta_{DSS} = 0$ ppm, and values of δ quoted in the text, and given in Tables 2 and 3, are corrected by 0.17 ppm from those in Fig. 3 and are referred to DSS. The strong signals at $\delta < 2.6$ ppm must be due to H-1 and H-1′ because they are not strongly coupled with any other hydrogens.

The doublet at 2.81 ppm has a J value typical of geminal coupling and is probably that of a pseudoaxial methylene hydrogen at position 6′ which is strongly coupled with H-6 whose signal at 3.62 ppm is not well separated from fructose signals in the one-dimensional ^1H spectrum of the equilibrium mixture (Fig. 3). There are also connectivities involving weaker signals of the minor component, e.g., between signals at 2.17 and 2.54 ppm. The doublet at 3.17 ppm, which is probably that of H-6′ of the minor (Δ) complex, is connected with a signal which is under the fructose signals in the one-dimensional spectrum, and it is assigned to H-6 at 3.54 ppm. There is a strong signal at 3.44 ppm, not of fructose, which is not connected to hydrogens at positions 1 or 6, but is connected to a signal at 3.80 ppm, and signals at 3.80 and 3.44 ppm are tentatively assigned to H-3 and H-4, respectively, because they are pseudoaxial in a pyranose ring. There is an additional connectivity between the signal at 3.44 ppm and a signal at 3.52 ppm which is almost lost in the noise due to connectivities involving

Fig. 3. (A) DQF-COSY ^1H spectrum of the complex mixture. The numbering indicates signals of the complexes. Chemical shifts of the uncomplexed sugar are given in Table 2 and [10] and [11]. (B) Expanded ^1H spectrum of the complex showing long-range couplings. Chemical shifts in these figures are referred to $\delta_{HDO} = 4.63$ ppm rather than to $\delta_{DSS} = 0$, see text.


A
4A
3A
6A
5A
6A
6'A
1A
1'A
6'Δ
1Δ
1'Δ
4.0
3.5
3.0
2.5
2.0 ppm
2.0
2.5
3.0
3.5
4.0

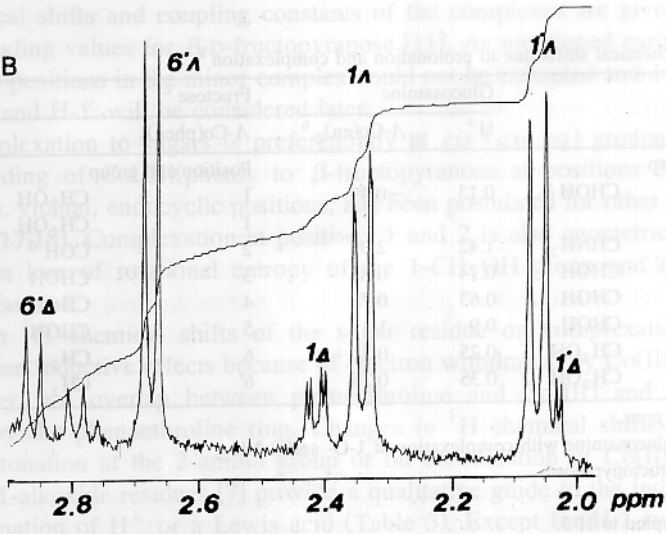

B
6'A
1A
1'A
6'Δ
1Δ
1'Δ
2.8
2.6
2.4
2.2
2.0 ppm

Table 2
Chemical shifts and coupling constants [a]

	δ (ppm)						
	H-1	H-1′	H-3	H-4	H-5	H-6	H-6′
Λ-Complex [b]	2.47	2.20	3.80	3.44	3.52	3.62	2.81
Δ-Complex [b]	2.54	~2.17				3.54	3.17
β-Pyranose [c]	3.68	3.53	3.76	3.86	3.96	4.00	3.68
	J (Hz)						
	H-1,1′	H-3,4	H-4,5	H-5,6	H-5,6′	H-6,6′	
Λ-Complex [d]	12.5	~10	~3	small	small	10.5	
Δ-Complex [e]	11.5					11.5	
β-Pyranose [c]	11.69	10.03	3.46	1.33	2.02	12.79	

[a] In D_2O at 25°C, chemical shifts relative to DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate).
[b] Prepared in situ, see text.
[c] Refs. [10,11].
[d] $J_{1,3}$ 1.5 Hz, see text.
[e] $J_{1',3}$ or $J_{1,3}$ 2.5 Hz.

fructose hydrogens. The signal at 3.52 ppm is tentatively assigned to the pseudoequatorial H-5 which is weakly coupled to H-4. There also should be connectivities involving hydrogens of the minor complex, but they could not be isolated.

Connectivities were seen between signals at 3.69 and 4.01 ppm, not connected to signals of the complex, which are due to H-6′ and H-6 of β-fructopyranose [10c,11]. There are also weak connected signals at positions assigned to β-fructofuranose [11].

Decoupling was used to confirm assignments from the 2D spectrum by irradiating ^1H signals of the complexes whose chemical shifts are lower than those of the fructose isomers, i.e., with $\delta < 3.5$ ppm. Hydrogens whose signals are under the fructose signals

Table 3
Changes in ^1H chemical shifts due to protonation and complexation [a]

		Glucosamine		Fructose [c]		
Coordination to:		H^+	Λ-Co(en)$_2$ [b]	Λ-Co(phen)$_2$		
Position and group				Position and group		
1	CHOH [d]	0.13	−0.49	1	CH_2OH	−1.21 [e]
				1′	CH_2OH	−1.33
2	$CHNH_2$	1.42	2.05	2	COH [d]	
3	CHOH	0.74	0.83	3	CHOH	0.04
4	CHOH	0.63	0.73	4	CHOH	−0.42
5	CHOH	0.9	1.1	5	CHOH	−0.46
6	CH_2OH	0.25	0.31	6	CH_2	−0.38
6′	CH_2OH	0.35	0.25	6′	CH_2	−0.87

[a] Values of Δδ, ppm.
[b] Relative to α-glucosamine with complexation at 1-O- and 2-NH_2.
[c] Relative to β-fructopyranose.
[d] Anomeric centers.
[e] Long-range coupled to H-3.

cannot be irradiated selectively, but decreases in signal intensities in this region could be seen.

Signals at either 2.20 or 2.47 ppm are depleted by irradiation at the other frequency, as are the weaker signals at ca. 2.17 and 2.54 ppm. Irradiation at 2.47 ppm also depletes the signal of H-3 at 3.80 ppm, which is consistent with a weak splitting of the doublet signals at 2.47 ppm, which gives J 1.5 Hz, and can be seen in the expanded spectrum (Fig. 3B). This result confirms the assignment of H-3 of the complex, and therefore that of H-4 to which it is strongly coupled. The doublet at 2.17 ppm is not split and its irradiation has no effect on the signal at 3.80 ppm. We conclude that these long-range coupled hydrogens are in periplanar or antiperiplananr orientations in the complex. No evidence of this long range coupling in the ^{1}H spectrum of fructose was seen, and it is not mentioned in earlier references. This difference is understandable in terms of relatively free rotation about the C-1–C-2 axis in the sugar and restricted rotation in the complex. The weak doublet signals of the minor complex at 2.54 ppm are also split, probably because of long-range coupling with H-3 (Fig. 3B).

The only other irradiation that could be done was at 3.17 ppm, the doublet of the minor complex, and it weakens the signal at 3.54 ppm, which was assigned to a methylene hydrogen at position 6.

Some of the ^{1}H coupling constants can be estimated by inspection of the expanded 1D or the 2D spectra (Fig. 3). The changes in ^{1}H chemical shifts of the β-fructopyranose due to complexation are in Table 3 and are compared with changes in the chemical shifts of α-glucosamine on protonation and on complexation with Co(III) bis(ethylenediamine) [7].

The ^{1}H signals of the complexes at $\delta < 3.0$ ppm and those of the mixture at higher δ with equimolar (10^{-2} M) reactants were integrated, and the [complex]/[sugar] ratio was estimated to be ≈ 0.7, without distinguishing between isomers. Relatively more complex was obtained by increasing reactant concentrations but line broadening due to high electrolyte concentration then became a major problem.

The chemical shifts and coupling constants of the complexes are given in Table 2 with corresponding values for β-D-fructopyranose [11]. As mentioned earlier, values of δ or J at some positions in the minor complex could not be extracted and assignments at positions H-1 and H-1′ will be considered later.

Metal complexation to sugars is preferentially at *cis* (*ax*, *eq*) groups [1,2], which points to bonding of Co(III)(phen)$_2$ to β-fructopyranose at positions 2 and 3, and bonding at *cis*, vicinal, endocyclic positions, has been postulated for other Co(III) sugar complexes [4,17,18]. Complexation at positions 1 and 2 is also geometrically feasible, but it involves loss of rotational entropy of the 1-CH_2OH group and to this extent should be disfavored.

Changes in ^{1}H chemical shifts of the sugar residue on complexation involve a balance between inductive effects because of electron withdrawal by Co(III), which will be affected by π-d overlap between phenanthroline and Co(III) and shielding, or deshielding, by the phenanthroline ring. Changes in ^{1}H chemical shifts of α-glucosamine on protonation at the 2-amino group or on coordination of Co(III)(en)$_2$ to the 2-amino and 1-alkoxide residues [7] provide a qualitative guide to the inductive effects due to coordination of H^+ or a Lewis acid (Table 3). Except for H-1 (which is at an

alkoxide center in the glucosamine complex), chemical shifts increase especially at H-2, because of build up of a positive charge on the amino group.

An approximate estimation of the effect of deprotonation of β-fructose on its ^1H chemical shifts was made by examining the spectrum in D_2O, pD 13.4, where the anomeric OH group should be partially deprotonated [13]. Signals are broad, but upfield shifts are not large, and we estimate that complete conversion to the monoanion would reduce δ by ca. 0.1 ppm at positions 1, 3, and 6, and less at the other positions.

The downfield shifts due to complexation of α-glucosamine with $Co(III)(en)_2$ [7] are in marked contrast to the upfield shifts at most positions on complexation of β-fructose and $Co(III)(phen)_2$ (Table 3). The phenanthroline ligands should decrease δ by interactions of hydrogens with faces of the aromatic residues and also by π-d conjugation with Co(III). These electron donating effects overcome those due to coordination of O^- and OH centers to Co(III) for all hydrogens except at position 3, and the effects are especially large (ca. 1–2 ppm) for H-1 and H-1′ in both complexes.

The marked upfield shifts of H-1 and H-1′ on complexation with $Co(III)(phen)_2$ (Table 3) show that these hydrogens must be close to the face of a phenanthroline ligand [14,19,20], and if $Co(III)(phen)_2$ bonds at position 1, shielding must be strong enough to more than offset a downfield shift because of electron withdrawal by Co(III). Complexing at position 3 merely requires an approximate compensation of effects.

The upfield shifts of signals of H-4, H-5, H-6, and especially H-6′ (Table 3) indicate that these hydrogens are interacting with the π-system of the phenanthroline ligands. These upfield shifts depend upon through-space distances. Shifts due to the inductive effect of Co(III) should decrease with through-bond distances as it is relayed through sigma bonds, but the effects seem to be small, based on partial deprotonation of fructose.

The ^1H chemical shift of H-1′ in β-fructopyranose is lower than that of H1 [10c,11], and so H-1′ is probably *syn* to the endocyclic oxygen, based on a Newman projection with 1-OH and 2-OH *anti*. In the complexes, chemical shifts of these hydrogens will be controlled largely by shielding by the phenanthroline rings. It was noted earlier that the hydrogen with the higher chemical shift has long-range coupling with H-3 (J 1.5 Hz) in both the Λ and the minor Δ complex (Fig. 3B), consistent with a periplanar orientation with H-3. If the orientation were antiperiplanar, the coupled hydrogen at position 1 would be the closer to the phenanthroline groups and shielding would give it the lower chemical shift.

We were unable to examine the $^1H-^{13}C$ spectrum of the complex prepared in situ. High concentrations of D-fructose and $\{(phen)_2Co(III)Cl_2\}Cl$ (ca. 0.1 M) were needed in order to obtain the spectrum in a reasonable time, and the high electrolyte concentration broadened signals and increased the noise. However, the ^{13}C spectrum of the complex, prepared in situ, had signals at 59.5 and 111.8 ppm (relative to external Me_4Si) that were respectively upfield and downfield of signals of all the fructose isomers [10b,d]. There were also signals at > 120 ppm due to the phenanthroline residues which were not assigned. Based on the attached proton test (APT) [21], and consistent with the chemical shifts, the signal at 59.5 ppm was that of C-1 and that at 111.8 ppm was of C-2 which was linked to Co(III) via alkoxide oxygen.

Some ^{13}C signals had the chemical shifts assigned to free β-fructopyranose and β-fructofuranose [10b,d]. Some very weak signals could have been due to α-fructo-

furanose, or to small amounts of the Δ-complex. Considering the large ^{13}C signals between 61 and 99 ppm, which encompass C-1, C-3, C-4, C-5, and C-6 of β-fructopyranose, signals of the sugar carbons were seen at 63.9, 67.5, 69.6, 68.9, and 62.7 ppm, respectively, based on accepted values [10b,d]. The signal of C-4 of β-fructopyranose at 69.6 ppm is overlapped with a signal of a complex giving a prominent peak. There were also weaker signals in the regions 61–63, 74–76, and 80–82 ppm which were probably those of free or complexed β-fructofuranose which were not assigned. There were also signals at 66.8, 69.6, 69.1, and 61.8 ppm which are those of C-3, C-4, C-5, and C-6, respectively, of the Λ-fructopyranose complex, if the sequence of chemical shifts is the same as that of the sugar [10b,d]. There were also weaker signals of minor complexes, e.g., of the Δ-complex and/or furanose forms. No attempt to assign these signals was made.

These ^{13}C chemical shifts can be interpreted in terms of binding of Co(III) to C-2-O^- and C-3-OH which would place C-1 close to a phenanthroline ring and decrease its ^{13}C chemical shift, just as the chemical shifts of H-1 and H-1′ are decreased (Table 3). However, complexation of C-1–OH to Co(III) cannot be excluded, although in that event shielding by a phenanthroline ligand has to more than offset the downfield shift due to the electron withdrawal by Co(III).

Stick models of Λ-complexes were examined with coordination at positions 1–2 or 2–3 and Co–O distances of 1.9 Å, consistent with X-ray data on Co(III) complexes[22] and other distances as used in Dreiding models, and a molecular modeling program based on MM2 parameters was also used (Fig. 4). Molecular modeling predicts that 2–3 complexation gives lower energy complexes than 1–2 complexation (with an enthalpy difference of 4.5 kcal/mol) and that, regardless of sites of coordination, Λ-complexes should be more stable than Δ. These calculations are only qualitatively useful because they neglect solute–water interactions, which are very large in sugar derivatives.

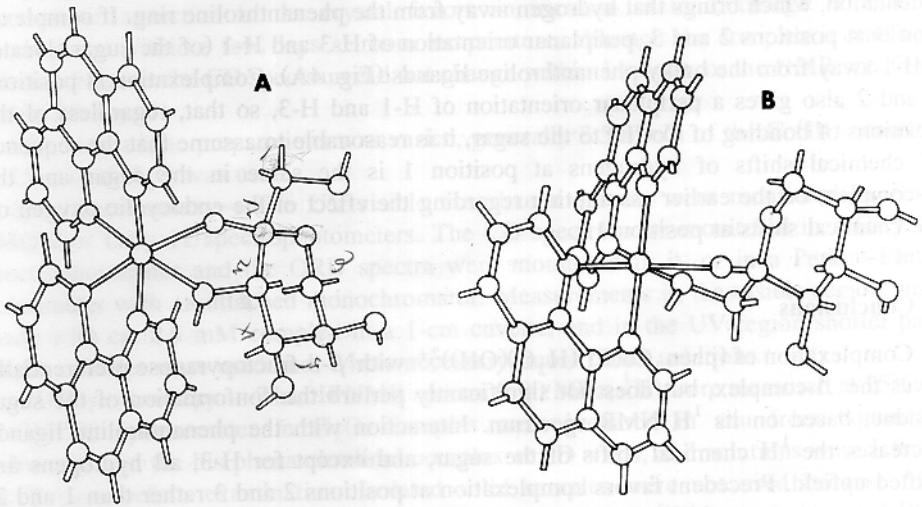


Fig. 4. Simulated structures of the Λ-complexes of Co(III)(phen)$_2$ with D-fructopyranose with coordination at (A) oxygens at positions 2 and 3 and (B) oxygens at positions 1 and 2. Hydrogens are indicated by sticks.

Some qualitative conclusions may be drawn from inspection of models. Complexation at positions 2 and 3 or 1 and 2 brings H-1 and H-1′ close to the face of a phenanthroline ligand. The hydrogen at 2.47 ppm, which is furthest from the phenanthroline and has a long-range coupling with H-3, is periplanar to H-3. The quasiaxial hydrogen at position 6′ should also be close to the face of a phenanthroline ligand and is therefore strongly shielded, and in any model of the complex the quasiaxial H-3 is directed away from the phenanthrolines.

The coupling constants of the predominant *Λ*-complex are similar to those of *β*-fructopyranose [10c,11]. Only the geminal coupling constants and $J_{3,4}$ 10 and $J_{4,5}$ 3 Hz can be estimated, but $J_{5,6}$ and $J_{5,6'}$ are small, based on the 2D spectra. It appears that complexation does not markedly change the conformation of the sugar residues. The NMR data do not fit coordination to fructofuranose and MM2 calculations predict that this structure is disfavored.

It is highly probable that there is binding between Co(III) and an alkoxide residue at position 2 and precedent indicates that the second bond will be at OH at position 3 [1,2]. Differences in ^{1}H chemical shifts of *β*-D-fructopyranose and the complex (Table 3) are consistent with this structure on the assumption of opposing effects of shielding by the phenanthroline ligands and inductive effects of Co(III). If the second bond is at position 1 it must be assumed that, despite electron withdrawal to Co(III), shielding by the phenanthroline ligands can decrease δ by ca. 1–2 ppm, relative to *β*-fructopyranose (Table 2).

There is little evidence on the conformation of the sugar residue in the minor *Δ*-complex, although MM2 calculations predict that complexation at positions 2 and 3 will be preferred, as in the *Λ* complex, and, regardless of the structure of the complex hydrogens at positions 1, and probably 6′, are close to phenanthroline ligands.

Observation of long range coupling between H-3 and the hydrogen at position 1 that has the higher chemical shift (Table 2) is understandable only in terms of a periplanar orientation, which brings that hydrogen away from the phenanthroline ring. If complexation is at positions 2 and 3, periplanar orientation of H-3 and H-1 (of the sugar) locates OH-1 away from the bulky phenanthroline ligands (Fig. 4A). Complexation at positions 1 and 2 also gives a periplanar orientation of H-1 and H-3, so that, regardless of the positions of bonding of Co(III) to the sugar, it is reasonable to assume that the sequence of chemical shifts of hydrogens at position 1 is the same in the sugar and the *Λ*-complex, on the earlier assumption regarding the effect of the endocyclic oxygen on the chemical shifts at position 1.

3. Conclusions

Complexation of $(phen_2Co(III)(H_2O)(OH))^{2+}$ with *β*-D-fructopyranose preferentially gives the *Λ*-complex, but does not significantly perturb the conformation of the sugar residue, based on its ^{1}H NMR spectrum. Interaction with the phenanthroline ligands decreases the ^{1}H chemical shifts of the sugar, and except for H-3, all hydrogens are shifted upfield. Precedent favors complexation at positions 2 and 3 rather than 1 and 2, [1,2] in agreement with molecular modeling based on MM2 parameters, which also predicts the higher stability of the *Λ*- over the *Δ*-complex. Electron withdrawal by

Co(III) should give downfield ^{1}H and ^{13}C chemical shifts, and this shift is observed at C-2, but for most positions, including C-1, and H-1,1′, H-4, H-5, and H-6,6′, shielding by the phenanthroline rings more than offsets this effect.

The long-range coupling between H-1 and H-3 in the *Λ*- and *Δ*-complexes is consistent with a periplanar orientation of these hydrogens, which is required by complexation at positions 1 and 2, or with complexation at positions 2 and 3 with interference between the 1-OH and phenanthroline groups. Inspection of models indicates that complexation at either positions 1 and 2 or 2 and 3 should not materially perturb the pyranoid conformation and should allow shielding by the phenanthroline ligands, in agreement with NMR data (Fig. 4).

4. Experimental

Complex formation.—The mixed complexes of phen and fructose with Co(III) were prepared by treating *cis*-$[Co(phen)_2Cl_2]Cl \cdot 3H_2O$ [23] with equimolar D-fructose in water at pH 8, adjusted with NaOH. The solution was kept at room temperature for 3–5 days, with no change in pH and *Λ* and *Δ* diastereomers were separated on a Sephadex SP-C-25 column by elution with a K_2SO_4 gradient. The *Λ*-complex eluted first and a small amount of $Co(III)(phen)_2$ remained on the column. Spectral measurements were made on these eluents.

A solid sample of mixed *Λ*- and *Δ*-complexes was prepared from the sulfate form of diaquo $Co(III)(phen)_2$ and fructose. The chloride counterion of *cis*-$[Co(phen)_2Cl_2]Cl$ was first replaced by SO_4^{2-} on a Merck Lewatit column in the sulfate form, and fructose was added at pH 8. The solution was concentrated in vacuo and a red solid separated. Calcd for $[Co(phen)_2fructose]_2(SO_4)_3 \cdot H_2O$: C, 47.00; H, 3.90; N, 7.30; Co, 7.70; S, 6.25%. Found: C, 48.00; H, 4.50; N, 7.30; Co, 7.73; S, 6.20%. The charge of the complex is different in the solid and in the aqueous solution at pH 8, probably because electrostatic attraction in the crystal favors an increase in ionic charge. The solid decomposed within a few days at room temperature, although the complex is stable in solution because the CD spectrum did not change within 1 month, or the NMR spectrum within 10 days.

The scale of the preparations was varied, generally 3.5 mmol of the Co(III) complex in 25 mL of H_2O was used.

Spectrophotometry.—The UV-visible absorbance spectra were monitored in Zeiss PMQ-2 or Cary 11 spectrophotometers. The CD spectra were monitored in a Cary 60 spectrophotometer and the ORD spectra were monitored in it, or in a Perkin–Elmer polarimeter with an attached monochromator. Measurements in the visible region were made with ca. 2.5 mM complex in a 1-cm cuvette, and in the UV region shorter path length cuvettes were used and, where necessary, more dilute solutions.

NMR spectroscopy.—The ^{1}H NMR spectra (500 MHz, ^{1}H) were measured at 25°C in a GN-500 spectrometer in D_2O. Samples were prepared in situ from equimolar D-fructose and the bis(phenanthroline) complex at pD ≈ 8.4, and solutions were left for ca. 2 days before examination. Isolated solid samples were not used because they decompose readily. Concentrations of fructose and the Co(III) complex were in the range of 0.01–0.2 M. Dilute solutions were used to avoid line broadening due to

electrolyte, because there is considerable overlap of some of the signals of fructose with those of the complex. However, in these dilute solutions, the reaction does not go to completion and fructose and $Co(III)(phen)_2(OH_2)_2^{3+}$ are present, and so conditions were used that provided a reasonable compromise between signal strength and line width. The signals of the phenanthroline ligands are in the aromatic region and were not examined in detail.

The ^1H NMR spectrum did not change as the pD was gradually increased over a period of 10 days. There was decomposition at pD 11. The complex began to decompose to give Co(III) ions[8] and then Co(II) and extensive line broadening. Formation of Co(II) was not a problem at lower pD.

Relative amounts of the Co(III) complex and free fructose were estimated from the areas of signals at low chemical shift, which are of the complex, and at $\delta > 3.4$ ppm which are those of fructo-pyranose and -furanose + complex. Relative amounts of the Λ- and Δ-complexes were estimated from ^1H signals at $\delta > 2.6$ ppm and $\Lambda/\Delta \approx 10$, which is similar to the spectral value.

Phase-sensitive double quantum filtered COSY (DQF-COSY) spectra[24] were recorded as described [7]. The ^1H chemical shifts and, where possible, coupling constants were measured with expanded spectra and chemical shifts are referred to sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS). The ^{13}C chemical shifts were referred to external Me_4Si.

Estimation of the effect of deprotonation at 2-OH was made from the chemical shifts of fructose in D_2O, pD 13.4 with KOD, i.e., above the pK_a of fructose in H_2O [13b], but deprotonation is incomplete, because for dissociation of these very weak acids $K_a^H/K_a^D \approx 5$ [25]. The signals were broad because of electrolyte but δ was decreased by ca. 0.1 ppm for hydrogens at positions adjacent to the anomeric center and to a lesser extent at remote positions.

The ^1H-decoupled ^{13}C spectra were examined in GN-500 or Gemini 200 spectrometers in D_2O at 25°C. The one-dimensional spectra were obtained with 0.2 M complex and sugar, and these solutions were used for the attached proton test [21].

Structure simulation.—The MM2 program was run on a CaChe Tektronix computer as described earlier for simulations of structures of other Co(III) complexes with sugar or hydroxyacidate ligands [7]. The energy terms include: bond angles and length, dihedral angles and improper torsions, intramolecular van der Waals, electrostatic and hydrogen-bonding interaction. Bonding with Co(III) at position 2 involves alkoxide oxygen based on the charge of the complex. The predicted structure of the Λ-complex with coordination at OH-3 is shown in Fig. 4A. This complex is predicted to be more stable than the Δ-complex by ca. 4 kcal/mol, which is much larger than the difference from spectral data, probably because molecular modeling does not adequately allow for hydration. The structure of the Λ-complex with coordination at positions 1 and 2 is shown in Fig. 4B.

Acknowledgements

Support of this work by FONDECYT, Project No. 1931009 and the National Science Foundation, International and Organic Chemical Dynamics Programs, is gratefully acknowledged.

References

[1] (a) S.J. Angyal, *Chem. Soc. Rev.*, 9 (1980) 415–428; (b) S.J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 1–43.

[2] (a) S.J. Angyal, *Carbohydr. Res.*, 200 (1990) 181–188; (b) M.C.R. Symons, J.A. Benbow, and H. Pelmore, *J. Chem. Soc. Faraday Trans. 1*, 80 (1984) 1999–2016.

[3] H. Kozlowski, P. Decock, I, Olivier, G. Micera, A. Pusino, and L.D. Pettit, *Carbohydr. Res.*, 197 (1990) 109–117.

[4] S. Bunel, C. Ibarra, E. Moraga, V. Calvo, A. Blaskó, and C.A. Bunton, *Carbohydr. Res.*, 239 (1993) 185–196.

[5] W.E. Keyes and J.I. Legg, *J. Am. Chem. Soc.*, 98 (1976) 185–196.

[6] (a) L.I. Katzin and I. Eliezer, *Coord. Chem. Rev.*, 7 (1972) 331–343; (b) R.G. Gillard, in H.A.O. Hill and P. Day (Eds.), *Physical Methods in Advanced Inorganic Chemistry*, Interscience, New York, 1968, Chap. 5.

[7] S. Bunel, C. Ibarra, E. Moraga, A. Blaskó, and C.A. Bunton, *Carbohydr. Res.*, 244 (1993) 1–14.

[8] F.A. Cotton and G. Wilkinson, *Basic Inorganic Chemistry*, Wiley, New York, 1976, p 404.

[9] (a) J.H. Bradbury and J.G. Collins, *Carbohydr. Res.*, 71 (1979) 15–24; (b) J. Keller, *Chem Soc. Rev.*, 19 (1980) 381–406.

[10] (a) D. Horton and Z. Walaszek, *Carbohydr. Res.*, 105 (1982) 145–153; (b) W. Funcke, C. von Sonntag, and C. Triantaphylides, *ibid.*, 75 (1979) 305–309; (c) A. de Bruyn, M. Anteunis, and G. Verhegge, *ibid.*, 41 (1975) 295–297; (d) P. Dais and A.S. Perlin, *ibid.*, 169 (1987) 159–169; (e) P. Dais and A.S. Perlin, *ibid.*, 136 (1985), 215–223.

[11] M. Jaseja, A.S. Perlin, and P. Dais, *Magn. Reson. Chem.*, 28 (1990) 283–289.

[12] F. Basolo and R.G. Pearson, *Mechanisms of Inorganic Reactions*, Wiley, New York, 1958, p 387.

[13] (a) B. Capon and W.G. Overend, *Adv. Carbohydr. Chem.*, 15 (1960) 11–51; (b) A.E. Martell and R.M. Smith, *Critical Stability Constants*, Vol. 3, Plenum Press, New York, 1977, p 274.

[14] A. Tatehata, *Inorg. Chem.*, 21 (1982) 2496–2499.

[15] J.A. Chambers, R.D. Gillard, P.A. Williams, and R.S. Vagg, *Inorg. Chim. Acta*, 72 (1983) 263–268.

[16] A.P.B. Lever, *Inorganic Electronic Spectroscopy*, Elsevier, Amsterdam, 1968, p 309.

[17] A. Decinti, P. Aguirre, and G. Larrazabal, *Polyhedron*, 12 (1993) 1515–1522.

[18] (a) S. Bunel, C. Ibarra, V. Calvo, and L. Larrera, *Bol. Soc. Chil. Quim.*, 35 (1990) 137–145; (b) S. Bunel and C. Ibarra, *Polyhedron*, 4 (1985) 1537–1542.

[19] L.M. Jackman and S. Sternhell, *Applications of NMR Spectroscopy in Organic Chemistry*, 2nd ed., Pergamon, New York, 1969, pp 281– 283.

[20] R.M. Silverstein, G.C. Bassler, and T.C. Morrill, *Spectrometric Determination of Organic Compounds*, 4th ed., Wiley, New York, 1981, Chap. 4.

[21] S.L. Patt and J.N. Shoolery, *J. Magn. Reson.*, 46 (1982) 535–539.

[22] (a) R.D. Gillard, N.C. Payne, and G.B. Robertson, *J. Chem. Soc. Sect. A*, (1970) 2579–2586; (b) S. Bunel, C. Ibarra, V. Calvo, A. Blaskó, C.A. Bunton, and N.L. Keder, *Polyhedron*, 10 (1991) 2495–2500.

[23] A.V. Ablov, *Russ. J. Inorg. Chem.*, 6 (1961) 157–161.

[24] G.E. Martin and A.S. Zektzer, *Two Dimensional NMR Methods for Establishing Connectivity*, VCH, New York, 1988, pp 99–101.

[25] C.A. Bunton and V.J. Shiner, *J. Am. Chem. Soc.*, 83 (1961) 42–47.