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Structures of complexes of cobalt(III) and glucosamine. Applications of molecular mechanics and NMR spectroscopy

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

[a] *Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago 233 (Chile)*
[b] *Department of Chemistry, University of California, Santa Barbara, California 93106 (USA)*

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ABSTRACT

The ^{1}H NMR signals of the hydrochlorides of 2-amino-2-deoxy-α- and -β-D-glucose (α- and β-D-glucosamine) have been assigned and chemical shifts and coupling constants determined from their phase-sensitive COSY spectra in D_2O. Based on the Karplus relation the observed coupling constants fit H-H dihedral angles calculated with MM2 parameters. The COSY spectrum of the Λ isomer of $[Co(en)_2 \cdot$ D-glucosamine$]^{2+}$ shows that it is formed from the α anomer. The coupling constants, except for $J_{2,3}$ and $J_{3,4}$, agree qualitatively with dihedral angles predicted by MM2 parameters. The structure of triammine-Co(III)-L-malate simulated by using MM2 parameters agrees reasonably well with that from X-ray crystallography.

INTRODUCTION

D-Glucosamine (2-amino-2-deoxy-D-glucopyranose) complexes with various metal ions[1], and mixed complexes of it and diamines or ammonia with cobalt(III) have recently been isolated[2]. Two dicationic bis(ethylenediamine) complexes were isolated as solids having the composition $Co(en)_2 \cdot$ D-glucosamine$Cl_2 \cdot 4H_2O$ and either the Λ or Δ configuration at cobalt. The charge on these complexes suggests that the sugar coordinates to Co(III) via an alkoxide residue.

The complex with ammonia has the composition $[Co(NH_3)_2 \cdot H_2O \cdot$ D-glucosamine$]_2 \cdot (SO_4)_3 \cdot 3H_2O$, and it was assumed that one water molecule was coordinated to Co(III). Chromatography of this complex gave only one product[2].

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* Corresponding author.

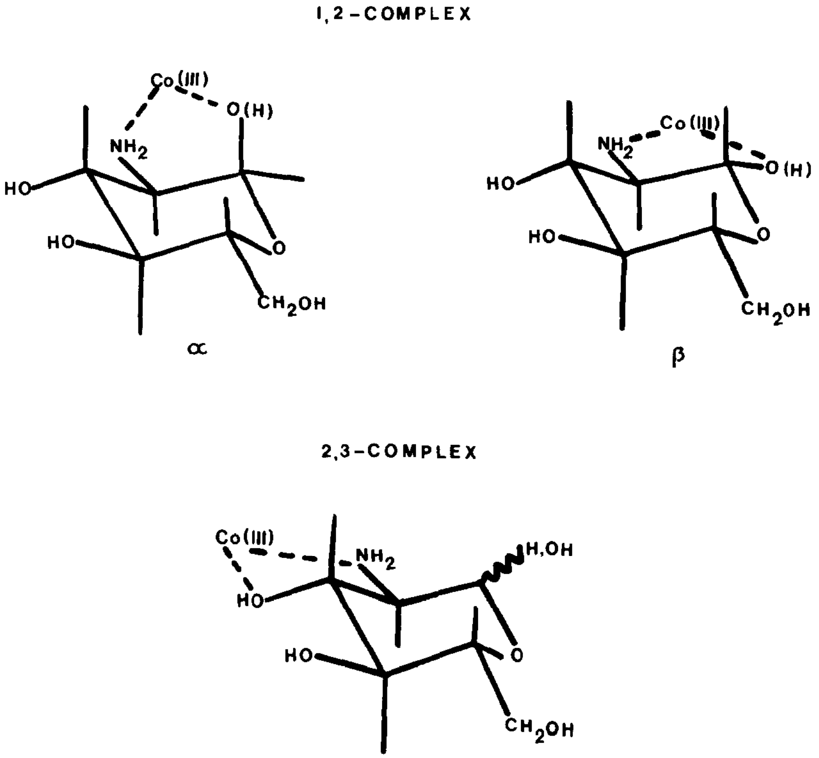
Some structural features of these complexes can be established from their absorbance, circular dichroism (CD), and optical rotatory dispersion (ORD) spectra. For example, configuration at Co(III) may be given by the CD and ORD spectra, based on analogies with the spectra of complexes of known configuration[4–6]. The visible absorbance spectrum of the ammonia complex in the d–d region shows that *cis*-oxygen atoms are coordinated to Co(III). The charge on the ethylenediamine complex establishes coordination of alkoxide oxygen at the 1-position, consistent with the high acidity of the 1-hydroxyl group relative to others in the sugar[3].

There is uncertainty from two standpoints regarding the overall structures of these complexes: (*a*) If coordination involves the 1-alkoxide or hydroxyl and the 2-amino residues of glucosamine, what is the configuration at the anomeric center? The α anomer has *cis*-OH and NH_2 groups (*ax*, *eq*). This arrangement is generally favored over the *eq–eq* relation of the β anomer in complexing to metal ions[7]. (*b*) If coordination is by NH_2 and OH groups of glucosamine it may involve the 2,3- rather than the 1,2-positions[1]. Thus, we cannot assume that the ammonia complex involves coordination by groups at positions 1 and 2 rather than 2 and 3 of the sugar, although the 2-amino and 3-hydroxyl groups are *eq–eq*.

Glucosamine is a bidentate ligand in the ethylenediamine complex and almost certainly so in the ammonia complex because complexing via three groups requires them to have an *ax–eq–ax* arrangement[7], which is very unfavorable with glucosamine. Possible modes of coordination are illustrated in Scheme 1.

Proton NMR spectroscopy is a powerful tool for the study of structures of carbohydrates and their derivatives, and is especially useful for assigning configurations at anomeric centers[8,9]. For example, the chemical shifts of H-1 of α- and β-glucosamine hydrochloride are 5.46 and 4.97 ppm, respectively, and the corresponding coupling constants, $J_{1,2}$ are[9] 3.8 and 8.5 Hz. (Chemical shifts are dependent upon temperature and solvent composition, but not markedly so.) Thus, coupling constants should provide information on dihedral angles of vicinal hydrogen atoms[10]. The ^1H chemical shifts of α-glucosamine have been established by 2D NMR spectroscopy on material prepared enzymically[11], but under conditions in which much of the fine structure was lost, so coupling constants were not measured. There is uncertainty in the quantitative use of the Karplus relation in estimating dihedral angles[10,12], although qualitative values should be reliable and should test the validity of structural simulations as applied to sugar derivatives. Taga and Osaki[13] and Virudachalam and Rao[14] have developed theoretical treatments of conformations of 2-amino-2-deoxy-hexopyranoses and their acetyl derivatives, although they based the treatments on the geometries of chair structures and focused largely on conformations about C-1 and C-2.

The MM2 parameters have been applied to many organic structures, but they are less well established for complexes with heavy metals. As a test of the simulation procedure we first examined the ^1H NMR spectra of α- and β-glucosamine hydrochloride and compared dihedral angles estimated from coupling



Scheme 1.

constants by using the Karplus relation[10,12] with those calculated by using MM2 parameters. As a further test of this procedure we used the MM2 program to predict the structure of triammine–cobalt(III)–L-malate, whose bond lengths and angles are known from X-ray crystallography[15], and whose ^{1}H NMR spectrum is also known. If both these tests agreed we planned to make a similar comparison of NMR data and MM2 simulations of the Co(III) complexes. There are practical problems with this approach. Some of the complexes are labile[2], for example, over time the ammonia complex decomposes, Co(II) is formed, and signals broaden. The ^{1}H NMR spectra of sugars are complex, with overlapping signals[8], so their analysis often requires 2D methods[8b,16] which take time and decomposition may become a problem. Phase-sensitive COSY measurements are particularly useful because they eliminate signals, e.g., of HOD, other than those due to strong vicinal coupling[16], so that signals can be assigned and coupling constants estimated unambiguously, especially if they are based on expanded spectra.

These complexes have exchangeable hydrogens and molecules of water of crystallization, so that there are large HOD signals, which complicate examination of 1D spectra, although they can be reduced by prior exchange in D_2O, which also simplifies the spectra by diminishing signals of NH groups.

RESULTS AND DISCUSSION

NMR spectra.—Crystalline D-glucosamine hydrochloride contains predominantly the α anomer[17], and the ^{1}H spectrum of a freshly prepared solution in D_2O at 25°C (Table I) gives the H-1 chemical shift, $\delta = 5.31$ ppm and coupling constant, $J_{1,2} \approx 4$ Hz (based on the H-1 signal) in agreement with earlier values[9]. Small differences in δ are due to the high concentration (30%) and temperature (70°C) used earlier. The concentration of the β anomer increases with time and its H-1 chemical shift and coupling constant ($\delta = 4.81$ ppm and $J_{1,2}$ 9.5 Hz) agree with

TABLE I

Chemical-shift data for glucosamine hydrochloride and a Co(III) derivative [a]

Shift	αGlcN	βGlcN	Λ-GlcN Co(en)$_2$
H-1	5.313	4.821	4.680
	5.306	4.804	4.672
H-2	3.178	2.896	3.810
	3.170	2.878	3.803
	3.156	2.857	3.796
	3.149		
H-3	3.773	3.617	3.841
	3.755	3.603	3.835
	3.753	3.592	3.832
	3.734	3.576	
H-4	3.366	3.370 [b]	3.573
	3.346		3.571
	3.328		3.554
			3.552
H-5	3.768	[c]	4.005
	3.722		4.000
	3.702		3.994
	3.698		3.988
			3.981
			3.976
			3.970
H-6	3.727	[c]	3.683
	3.722		3.678
	3.702		3.659
	3.698		3.654
H-6′	3.663	[b]	3.560
	3.652		3.550
	3.638		3.536
	3.628		3.525

[a] Values of δ ppm in D_2O at 25°C, relative to HOD, $\delta = 4.630$ ppm; abbreviations, αGlcN and βGlcN, α- and β-D-glucosamine hydrochloride respectively, GlcN-Co(en)$_2$, [Co(en)$_2$·D-glucosamine]$^{2+}$. [b] Multiplet identified in the DQF COSY spectrum. [c] Overlapped with signals of the α anomer.

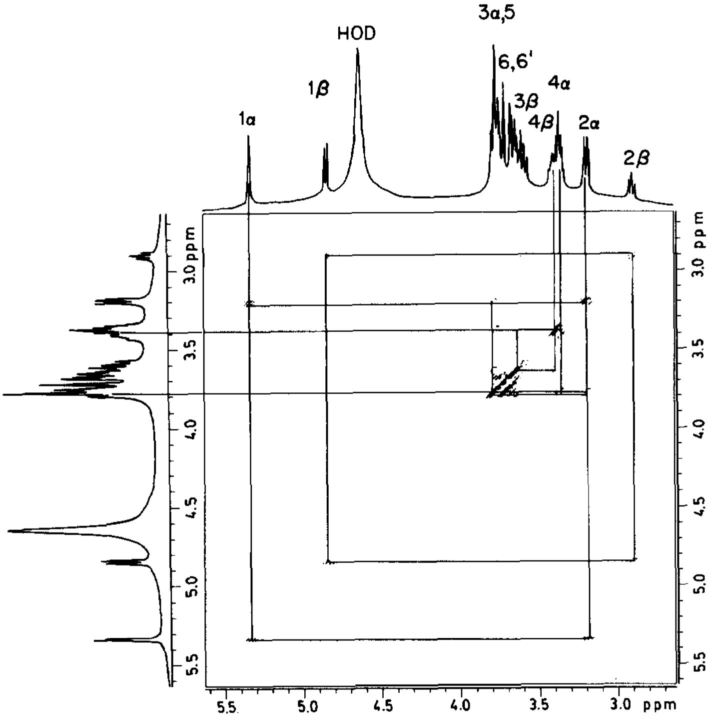


Fig. 1. DQF-COSY ^1H spectrum of 0.05 M D-glucosamine hydrochloride after partial equilibriation (1 day).

earlier values[9]. There are signals of NH_3^+ at 3.538, 3.555, and 3.559 ppm and they decrease by exchange in D_2O during anomerization.

We then obtained a phase-sensitive, double quantum filtered (DQF) COSY spectrum[16] of a freshly prepared solution and established connectivities starting with the H-1 signal of the *α* anomer. We could readily identify the 6,6′ signals of the hydroxymethylene group and also established connectivities starting from that position[8b]. Complete chemical-shift signals are listed in Table I. The sequence is similar to that obtained with an enzymically prepared sample of *α*-glucosamine[11], but values of *δ* differ because of the very different compositions of the solutions.

We next obtained a similar DQF COSY spectrum of a partially equilibriated mixture and measured chemical shifts and coupling constants for H-1–4 (Fig. 1). Chemical shifts of H-5,6,6′ were the same for the *α* and *β* anomers. It is difficult to obtain reliable coupling constants by inspection of high-order spectra, as with these sugars, but we confirmed the coupling constants from expanded DQF COSY spectra, and simulation of the 1D spectra (see Experimental). Coupling constants are consistent with *eq* and *ax* H-1 for *α*- and *β*-glucosamine, respectively, and *ax* H-2–H-5 for both anomers (Table II).

We also obtained good conventional and COSY spectra of the *Λ* form of $[Co(en)_2 \cdot \text{D-glucosamine}]^{2+}$ and established connectivities between all the signals

TABLE II

Coupling constants of glucosamine hydrochloride and its derivatives [a]

Coupling	αGlcN	βGlcN	ΛGlcN Co(en)$_2$
$J_{1,2}$	3.5	8.5	4
$J_{2,1}$	4	8.5	3.5
$J_{2,3}$	11	11	4.5
$J_{3,2}$	11	12	4
$J_{3,4}$	10	8	7.8
$J_{4,3}$	10	10	7.2
$J_{4,5}$	9		9
$J_{5,6}$	2, 2.5		2.5
$J_{6,6}$	12.5		12
$J_{5,6'}$	5.5		5.5
$J_{6,6'}$	12.5		12

[a] Coupling constants in Hz. For abbreviations see Table I.

(Fig. 2). The coupling constants $J_{2,3}$ and $J_{3,4}$ are lower in the complex than in either α- or β-glucosamine, but those at remote positions (4, 5, 6, and 6′) are similar.

We did not attempt to analyze the signals of the ethylenediamine ligands, which are in the region 2.44–2.97 ppm, and fortunately are well separated from signals of

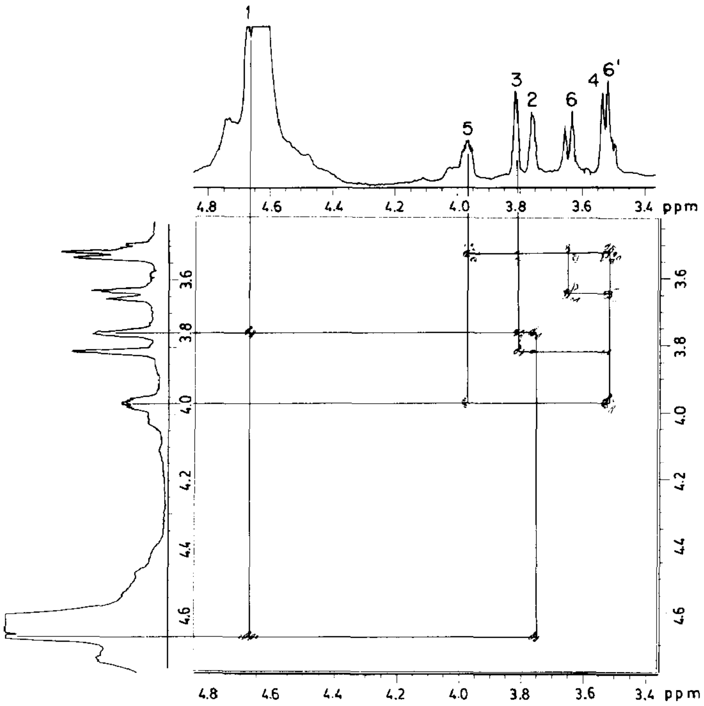


Fig. 2. DQF-COSY ^{1}H spectrum of Λ-[Co(en)$_2$·α-D-glucosamine]$^{2+}$.

the glucosamine residue. There are obvious connectivities between some of the ethylenediamine signals, but we see no simple way of assigning any of them, and the conformation of this ligand is not germane to the structure of the sugar residue. Signals of the NH groups are broad and in the region 4.04–4.12 ppm. They are attenuated by exchange with D_2O.

We could not interpret either the 1D or the COSY spectra of the Δ form of $[Co(en)_2 \cdot \text{D-glucosamine}]^{2+}$, probably because there was more signal overlap than with the Λ isomer. The number of coupled signals seen in the 2D spectra was less than expected from the structure, although the numbers agreed for the Λ isomer.

Chemical shifts of the anomeric glucosamines differ markedly[9] for H-1, less so for H-2 and H-3, and only slightly for H-4 (Table I), but chemical shifts and coupling constants change markedly with formation of the Co(III) mixed complex with ethylenediamine (Table I and II). The value of $\delta = 4.68$ ppm of H-1 is consistent with coordination through alkoxide, which should shield H-1, and there is an increase in δ at H-2 with deshielding, consistent with a change in the inductive effect due to coordination of NH_2 to Co(III) (ref 18). Changes in δ at the other positions are smaller. The value of $J_{1,2}$ is similar for α-glucosamine and the complex (Table II) suggesting that the dihedral angle, ϕ, is relatively small and similar in both compounds. However, the other coupling constants are lower in the complex than in either α- or β-glucosamine, except for those at the remote positions, 4, 5, 6, and 6′. The $J_{1,2}$ coupling constant of the complex points to a dihedral angle of ~50 or 125° (Table III). The lower value is reasonable for coordination of α- rather than β-glucosamine to Co(III), and the higher value is inconsistent with coordination of alkoxide and amino groups with Co(III). Therefore, even though application of the Karplus relation only gives approximate values of dihedral angles[10,12] the results exclude coordination of β-glucosamine.

MOLECULAR MECHANICS SIMULATIONS

α And β-glucosamine.—There is good agreement between H–H dihedral angles calculated by using the MM2 program for the free bases and the ^1H coupling

TABLE III

Observed and predicted dihedral angles [a]

Positions	Glucosamine		Λ-$Co(en)_2 \cdot$D-glucosamine^{2+}	
	α	β	α	β
1–2	49(50–125)	172(20–165)	35(50–125)	173
2–3	172(0–180)	173(0–180)	159(45–125)	175
3–4	169(0–180)	170(10–165)	170(20–145)	168
4–5	175(10–170)	175	178(15–165)	169
5–6	63(55–120)	63	62(55–120)	63
5–6′	174(45–135)	174	174(45–135)	174

[a] Values of $\phi°$ in parentheses are approximate minima and maxima based on the Karplus relation with $J^0 = 10$ Hz and $J^{180} = 11$ Hz (refs 10 and 12), and coupling constants in Table II.

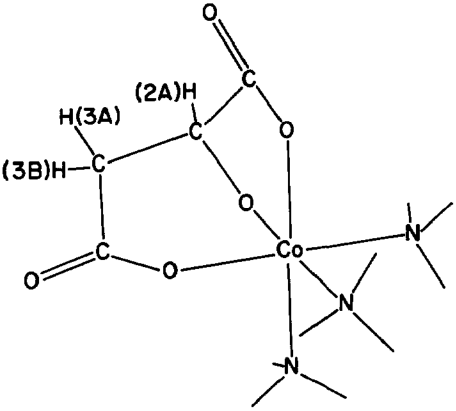


Fig. 3. Simulated structure of triammine Co(III)–L-malate. Ammonia hydrogens are omitted for clarity and methine and methylene hydrogens are labelled as in ref 15.

constants of α- and β-glucosamine hydrochloride (Tables II and III) despite the charge and neglect of hydration in the simulations. The predicted lower enthalpy of the β anomer, by 0.3 kcal mol^{-1}, agrees with the NMR spectrum of a mixture of the free bases (see Appendix) and is consistent with other calculations,[13,14] and the equilibrium constant[19].

Triammine–cobalt(III)–L-malate.—The crystal structure of this Co(III) complex is known, but there are slight differences between it and the structure in solution, based on the NMR spectrum[15] in D_2O. In the crystal, the methine hydrogen [H-(2A) in Fig. 3] is almost perfectly staggered with respect to the methylene hydrogens [H-(3A) and H-(3B)], but ^1H coupling constants show that rotation about the $CH–CH_2$ bond axis reduces one dihedral angle and correspondingly increases the other[15]. Dihedral angles from the MM2 simulation are H-(2A)–H-(3A) 28° and H-(2A)–H-(3B) 91°, which differ from the dihedral angles of 60° in the crystal but agree with values of 20–30° for H-(2A)–H-(3A) and 90–100° for H-(2A)–H-(3B) based on NMR coupling constants[15]. These comparisons show that the molecular mechanics calculation with MM2 parameters fits the geometry of a Co(III) complex reasonably well and they should be useful in predicting the structures of complexes of glucosamine with Co(III).

Glucosamine · Co(III) complexes.—Agreement between the dihedral angles of α- and β-glucosamine estimated from MM2 simulations (Table III) and the ^1H NMR coupling constants, and on the structure of the Co(III)–malate complex (Fig. 3), shows that it is reasonable to extend these comparisons to the Λ isomer of the ethylendiamine complex, especially as regards configuration at the anomeric center.

The MM2 simulation (Fig. 4 and Table III) predicts that bond angles at Co(III) are close to 90°, as expected for an octahedral complex, and $Co–NH_2$ and Co–O bond lengths of 1.919 and 1.898 Å, respectively, are similar to those measured on other Co(III) complexes by X-ray crystallography[15,20]. The H-1–H-2 dihedral angle

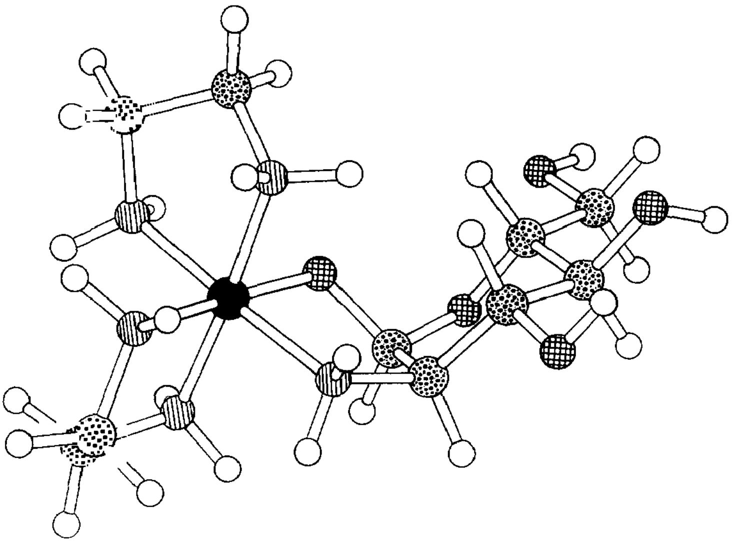


Fig. 4. Simulated structure of Λ-$[Co(en)_2 \cdot \alpha$-D-glucosamine$]^{2+}$; Co, •; C, ⊗; N, ●; O, ⊕; H, ○.

of 35° (Table III) fits the $J_{1,2}$ coupling constant of ~ 4 Hz, as for α-glucosamine (Table II). Values of $J_{2,3}$ and $J_{3,4}$ (Table III) are lower than predicted from the calculated dihedral angle. Rotation about the C-3–C-4 and C-4–C-5 bond axes may flatten that part of the ring and decrease the H-3–H-4 dihedral angle, which reduces interactions between the quasi axial H-3 and an NH of the ethylenediamine ligand (Fig. 4). Simulation suggests that these interactions would also be large in a complex of β-glucosamine, especially if NH_2 groups of the ligand are hydrated. The $J_{5,6}$ and $J_{5,6'}$ coupling constants, depend upon free rotation about the 5,6 bond and should be largely unaffected by complexation (Tables II and III).

Our conclusions regarding experimental values of dihedral angles depend upon the validity of the vicinal Karplus relation[1,12], but it fits the data for α- and β-glucosamine. However, electronegative groups, e.g., coordinated NH_2, may decrease the coupling constants[12].

Simulations predict that Λ and Δ complexes will have similar enthalpies (the value for the Λ complex is lower than that for the Δ complex by ~ 0.2 kcal mol^{-1}), and their relative proportions may be governed by their rates of formation, rather than by their enthalpies, because in the isolated mixture of the complexes[2] $\Delta/\Lambda = 2$. Solvent complicates the simulation. Hydrogen bonding to OH and NH_2 groups is important, although the number of hydrogen bonds should be similar for Λ and Δ complexes, whether derived from α- or β-glucosamine, but possible conformational effects on H-bonding at axial and equatorial positions have been noted[19], see ref 21.

Simulations of structures of the ammonia complex (Fig. 5), with *cis* oxygen atoms at Co(III)[2], indicate that formation enthalpies are insensitive to the location

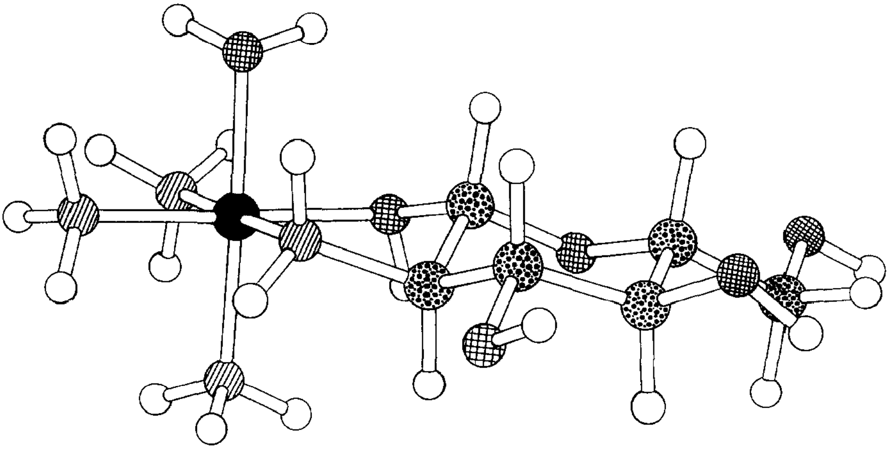


Fig. 5. Simulated structure of an ammonia complex of Co(III) with β-D-glucosamine. Symbols as in Fig. 4.

of a water molecule on Co(III), but that in a 1,2-complex the β is favored over the α anomer, unlike the situation for the ethylenediamine complex. Complexing at the 2,3-positions is slightly less favorable than at the 1,2-positions by ~2 kcal mol^{-1}.

It appears that when coordination to Co(III) is through the 1-OH group the β anomer gives the more stable complex, but that the α anomer is favored when the OH group is deprotonated, as in the ethylenediamine complex. However, there may be interference between NH_2 groups of ethylenediamine and axial hydrogen atoms of glucosamine, especially in a complex of β-glucosamine. The ethylenediamine ligands are relatively immobile, but in ammonia rotation of the complexes about $Co-NH_3$ or $Co-OH_2$ bonds should decrease interference with glucosamine hydrogen atoms. Regardless of the arrangement of NH_3 and OH_2 around Co(III), the hydrogen in 1-OH is predicted to be antiperiplanar to the OH_2 ligands in the ammonia complexes (Fig. 5).

CONCLUSIONS

There is good agreement between H–H dihedral angles of α- and β-glucosamine predicted by MM2 simulations and estimated from ^1H coupling constants (Table III), and simulations correctly predict that β-glucosamine has a lower enthalpy than α-glucosamine (see Appendix[13,19]). The simulation also fits the rigid structure of triammine cobalt(III)-L-malate (Fig. 3 and ref 12).

Agreement between simulated dihedral angles and those from ^1H coupling constants is reasonable except that the dihedral values for H-2 and 3 and H-3 and 4 of the Co(III) ethylenediamine complex are lower than simulated values (Table III). Coordination requirements of Co(III) probably control the conformation of the glucosamine ligand and the endocyclic oxygen makes the pyranose ring more flexible than a corresponding carbocyclic residue, so changes in conformation are probably not energetically very costly[19] and will relieve interference with the diamine ligand.

Predicted enthalpic differences between various structures are small and hydration is a significant factor, especially if water bridges form between amino groups of the ligand and OH groups of glucosamine, and we need objective evidence to test simulations of structures of sugars with heavy metals[1], especially as regards possible interactions between sugar residues and amine ligands (Fig. 4). NMR spectroscopy is useful, but it is of limited value for complexes of paramagnetic ions[1], or when Co(II), for example, is formed by decomposition, as in some of our Co(III) complexes.

Physical evidence and MM2 calculations agree on the structure of triammine Co(III)–L-malate, possibly because it is rigid, but agreement is worse with the more flexible sugar complexes with Co(III) based on the quantitative application of the Karplus equation to the ethylenediamine complex.

EXPERIMENTAL

Spectra.—NMR spectra (500 Mz, ^{1}H) were measured at 25°C on a GN 500 spectrometer. The solvent (D_2O) was deoxygenated and in some experiments the HOD signal was reduced by dissolving samples in 99.6% D_2O and, after isotopic exchange, pumping off the solvent. Samples were redissolved in fresh D_2O. We could not use this procedure with labile complexes. Provided that the solvent ^{1}H signal is not too large it causes no problems in the phase-sensitive COSY experiments[16]. Mutarotation of protonated glucosamine is relatively slow, so we could obtain NMR spectra of a freshly made up solution which contained largely the α anomer and subsequent equilibriation gave extensive amounts of the β anomer.

Phase-sensitive double quantum filtered COSY spectra were recorded with the pulse sequence, $90°–t_1–90°_{\phi 1}–\Delta 90°_{\phi 2}$ acquisition ϕR with a 90° pulse of 25 μs calibrated before the experiment, $\Delta = 8$ μs and an 8-step cycling was used. Spectra were collected into 4K data blocks for 256 t_1 increments with a relaxation delay of 1.5 s. The data matrix was zero filled to 2K and apodized with an exponential function to give a line broadening of 1 Hz in both dimensions.

The ammonia complex contained small amounts of Co(II) derivatives, and, although they did not perturb the absorbance or CD spectra[2], they seriously broadened the NMR signals, so we could not obtain a good NMR spectrum of it. We saw unidentifiable signals with the mixture of the phenanthroline complexes[2], probably because they partially disproportionate in the time required to obtain the NMR spectra.

The signals of H-1 in the Λ-en$_2$ complex are very close to that of HOD and we relied upon phase-sensitive COSY spectra (Fig. 2), which detect only signals that arise from spin–spin coupling. The disadvantage is that data accumulation is slow, so the method could not be used with labile complexes, and we obtained good results only with glucosamine hydrochloride and the Λ-ethylenediamine–glucosamine complex. These Co(III) complexes are soluble in water, but their solubility in convenient NMR solvents, e.g., $(CD_3)_2SO$, C_2D_5OD, and CD_3OD is very low[2], even when some D_2O is present.

The phase-sensitive COSY spectrum provides diagnostic evidence on ^1H couplings, and, with expanded spectra, confirms J values. Agreement between observed and simulated J values is good, within the uncertainties of the measurements.

The 1D NMR spectra were obtained with 5×10^{-3} M solute, but we used higher concentrations ($< 5 \times 10^{-2}$ M) for the 2D spectra, so that the two sets of chemical shifts differ slightly. Values of δ in Table I were obtained with the more-dilute solution. All chemical shifts and coupling constants were obtained with expanded spectra and where possible we estimated J from signals of both hydrogen atoms (Table II).

We used the GEMSIM program (G.E. package) to confirm assignments of chemical shifts and coupling constants of α- and β-glucosamine and the Λ-Co(en)$_2$ complex. This program can be used for up to 7 nuclei of spin $\frac{1}{2}$ and the assumed chemical shifts and coupling constants were the input into an 8K data memory bank with an observed line broadening of 0.25 Hz. This procedure gave simulated spectra with signal patterns and multiplicities in excellent agreement with the original 1D spectra.

Structure simulations.—The MM2 program was run on a CACHE Tektronix computer (Tektronix, August 1991, Version 2.7). The MM2 parameters are: relaxation factor = 1; convergence to 10^{-3} kcal mol^{-1}, and the energy terms include: bond angles and stretch, dihedral angles and improper torsions, intramolecular van der Waals, electrostatic, and hydrogen-bonding interactions. Medium effects and hydrogen-bonding with water are neglected. Simulation procedures may detect false energy-minima, and so we slightly adjusted geometries after initial simulation and found that resimulation gave the initial geometry. We put no restrictions on geometry in the initial simulations except that the sugar derivatives had α- or β-pyranose rings.

The calculations predict angles at Co(III) very close to 90° and lengths similar to those determined for other Co(III) complexes[15,20]. Estimated distances between OH-1 and $NH_2(2)$ are 2.887 and 3.003 Å for α- and β-D-glucosamine respectively, and in the Λ- and Δ-bis(ethylenediamine) complexes they are 2.630 and 2.641 Å for α- and β-glucosamine respectively. Geometry about Co(III) is insensitive to the structure of the glucosamine ligand whose conformation depends upon the geometries of Co(III) and its ammonia or diamine ligands.

Relative enthalpies of the ammonia complex.—The MM2 simulations predict that a 1,2 complex of α-glucosamine and 2,3-complexes of either α- or β-glucosamine and NH_3 will have higher enthalpies than those of 1,2-complexes of β-glucosamine

TABLE IV

Chemical shifts of equilibriated glucosamine in KOD [a]

Position	α-Glucosamine		β-Glucosamine	
	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)
1	5.185(d)	~ 4	4.373(d)	8.5
2	1.747(dd)	9.5	1.613(t) [b]	
3	3.009(t) [b]		3.337(dd)	
4	2.822(m)		2.743(dd)	
5	2.81–2.86(m)		2.72–2.77(m)	
6	3.350(d)	13.5	3.436(d)	15
6′	3.298(dd)		3.200(dd)	8.5; 15

[a] 0.07 M Glucosamine hydrochloride + 0.1 M KOD at 25°C with $\delta_{HDO} = 4.63$ ppm. [b] Pseudo triplet of degenerate dd.

by 2–4 kcal mol^{-1}. The position of the H_2O ligand on Co(III) affects the enthalpy by ~ 0.25 kcal mol^{-1}, and the lowest enthalpy structure is shown in Fig. 5.

APPENDIX

The MM2 simulation and other calculations[13] predict that the β anomer of glucosamine has a lower enthalpy than the α anomer, but in water equilibrium between the hydrochlorides favors the α anomer[9,17,19]. We therefore examined the ^1H NMR (500 MHz) spectrum of 0.07 M glucosamine hydrochloride + 0.1 M KOD in D_2O at 25°C, and for the free bases $\alpha/\beta \approx 0.5$ based on signals of H-1, H-2, H-2, and H-4 + H-5. The value in H_2O is 0.41[19]. The coupling constants, $J_{1,2}$, are similar to those of the hydrochlorides (Tables II and IV), suggesting that deprotonation does not markedly affect conformations of the anomers. Chemical shifts change on deprotonation, as expected[18], and are probably also affected by changes in medium composition. Some of the signals are less well-resolved than with the hydrochlorides, because of overlap, and in basic solution we could only examine the mixture of equilibriated anomers. Therefore, some of the assignments are less certain than for the hydrochlorides and we could not estimate some coupling constants with any degree of certainty, although we could establish multiplicity of the signals, and connectivity of some of them. Exchange of NH_2 groups with D_2O is rapid in basic solution.

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