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Binding of Oxovanadium(IV) to Guanosine 5′-Monophosphate

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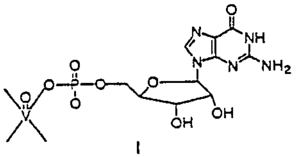
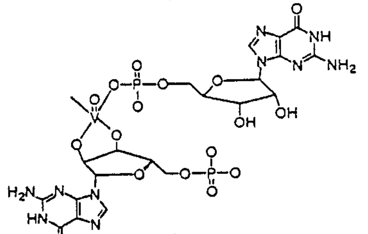
All of the evidence gathered in studies on the VO^{IV} interaction with ribonucleotides is concordant in assigning the phosphate chain and the *cis* couple of C(2′), C(3′) deprotonated hydroxyls as the effective VO^{IV} binding sites.[1−10] In ADP and ATP the former set is active in acidic and the latter in alkaline solution, and both of them may be jointly involved in the intermediate pH range. In the absence of these donor sets, OH^- ions are competitive enough for metal coordination and favor hydrolytic species.

The monophosphate moiety is a less effective donor compared with the di- or tri-phosphate chains of ADP and ATP. Accordingly, at pH values as low as 2−4, monophosphate nucleotides form weak complexes in which the monodentate phosphate group binds the metal ion. They are rather unstable to hydrolysis and transform into hydroxo-bridged polynuclear species at higher pH. Only above pH 7−7.5 do monomeric complexes re-form in which the ribose moieties are chelated to vanadium.

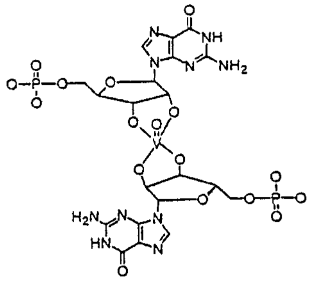
Recently, VO^{IV} complexes formed in aqueous solution by guanosine 5′-monophosphate (GMP), as studied by EPR and ENDOR spectroscopy, have been reported.[11] In contrast to the results presented previously, a bischelated complex formed by the coordination of phosphate groups was claimed at a pH value as high as 8.6. The complex was described apparently in great detail by use of ENDOR, which allowed the authors to conclude that the technique can assign even the molecular conformation of the ligand "at a level of accuracy that is exceeded only by that of X-ray diffraction on single crystals". ENDOR is a rather powerful tool but one should keep in mind that it detects and, in favorable circumstances, locates magnetic nuclei (*e. g.* ^1H) within a certain distance from a paramagnetic center. However, the technique does not establish directly the donor set, particularly if it consists of oxygen atoms. EPR or, preferably, a combination of spectral and equilibrium techniques, assisted by comparative examination of interrelated systems, is often more suitable than ENDOR for the assignment of the metal chromophore.



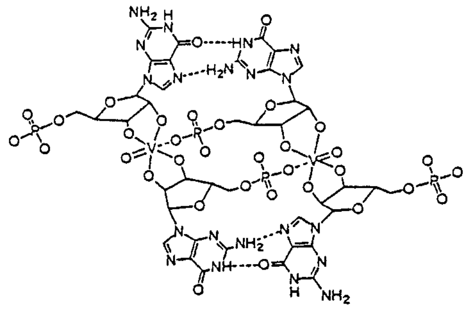
I



II



III



IIIa

In this respect the VO^{IV}−GMP system represents a case study. The reexamination of the VO^{IV}−GMP interaction in aqueous solution at a metal concentration of 5×10^{-3} M, as a function of pH, yields results consistent with the current understanding of these systems. As shown by the sequence of spectra presented in Figure 1, complexation starts with formation of phosphate complexes (**I**)[12] which coexist with the solvated ion, as documented by EPR parameters (see Table 1) and with much more certitude by electronic spectra which exhibit a significant red shift of the d−d electronic absorption maximum typical of $[VO(H_2O)_5]^{2+}$.[1] Precipitates, most likely polymeric compounds in which phosphate groups and heteroatoms of the nucleobases bind the metal,[13] are observed between pH 3 and 7. The equilibrium solution is EPR-silent at room temperature while it exhibits an unresolved broad resonance at $g = 2$ in the anisotropic spectra. The precipitates redissolve above pH 7, yielding, besides a minor species (**II**) in the pH range 7.2−7.5,

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(1) Alberico, E.; Buglyò, P.; Kiss, T.; Micera, G.; Sanna, D. *J. Chem. Res.* **1994**, 42.

(2) Alberico, E.; Dewaele, D.; Kiss, T.; Micera, G. *J. Chem. Soc., Dalton Trans.* **1995**, 425.

(3) Buglyò, P.; Kiss, T.; Micera, G.; Sanna, D. *J. Chem. Soc., Dalton Trans.* **1996**, 87.

(4) Sakurai, H.; Goda, T.; Shimomura, S.; Yoshimura, T. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 1421.

(5) Sakurai, H.; Goda, T.; Shimomura, S. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 474.

(6) Etcheverry, S. B.; Ferrer, E. G.; Baran, E. J. *Z. Naturforsch.* **1989**, *44B*, 1355.

(7) Urretavizcaya, G.; Baran, E. J. *Z. Naturforsch.* **1987**, *42B*, 1537.

(8) Williams, P. A. M.; Baran, E. J. *J. Inorg. Biochem.* **1992**, 48, 15.

(9) Williams, P. A. M.; Baran, E. J. *J. Inorg. Biochem.* **1993**, 50, 101.

(10) Cini, R.; Giorgi, G.; Laschi, F.; Sabat, M.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1989**, 575.

(11) Jiang, F. S.; Makinen, M. W. *Inorg. Chem.* **1995**, *34*, 1736.

(12) Potentiometric measurements carried out, *e.g.*, on the VO^{IV}−cytidine- or adenosine-5′-monophosphate systems indicate that up to 60−70% of VO^{IV} forms PO_3^{2-}-coordinated species in the pH range 2.5−4.5 at a 2:1 ligand to metal molar ratio; see ref 1.

(13) Katsaros, N. *Trans. Met. Chem.* **1982**, *7*, 72.

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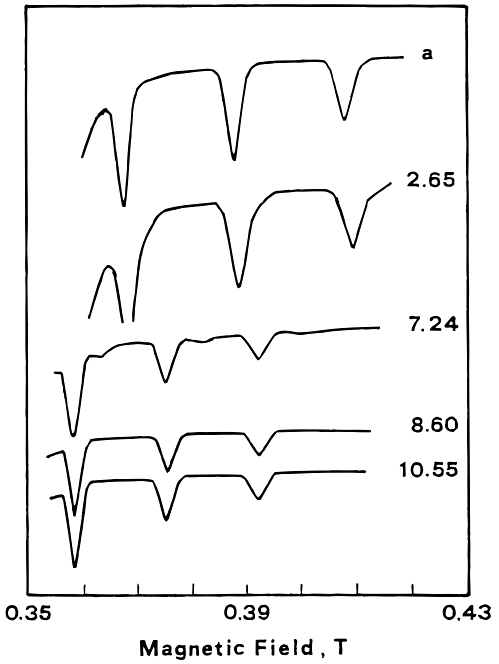

Figure 1. High-field features of X-band frozen-solution EPR spectra recorded at 120 K on the VO^{IV}−GMP system, as a function of pH. VO^{IV} concentration = 4.0×10^{-3} M; ligand-to-metal molar ratio = 10:1. Spectrum a belongs to the solvated ion. Instrumental settings are reported in the Experimental Section (field modulation amplitude 0.1 mT; receiver gain is 4×10^2 in the first two spectra, 2×10^3 in the spectrum at pH 7.24, and 2.5×10^2 in the last two spectra).

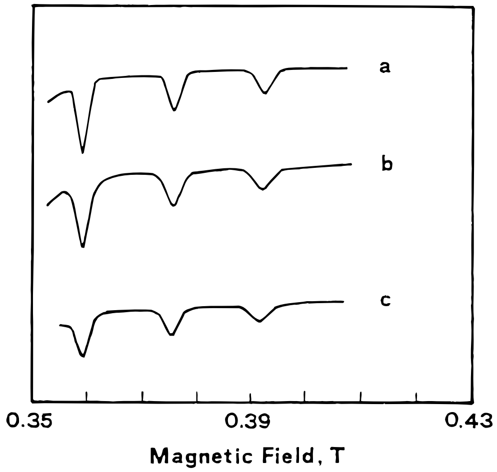

Figure 2. High-field region of X-band frozen-solution EPR spectra recorded on the VO^{IV} complexes of (a) GMP at pH 8.60, (b) guanosine at pH 8.70, and (c) D-ribose at pH 11.14. Spectra were obtained at 120 K on aqueous solutions at VO^{IV} concentration = 4.0×10^{-3} M and ligand-to-metal molar ratio = 10:1. Instrumental settings are reported in the Experimental Section (field modulation amplitude 0.1 mT; receiver gain 10×10^2).

a main complex (**III**) which is a single component up to pH 10. The parameters of **II** are supportive of one metal ion bound to the phosphate chain of a ligand molecule and to the ribose moiety of another. On the other hand, **III** is the usual bis-chelated (O^-,O^-)(O^-,O^-) ribose complex.[14] The assignments are confirmed by examination of the complexing behavior of 2′-deoxyguanosine 5′-monophosphate and guanosine. The former ligand yields only complex **I**, which undergoes hydrolysis leading ultimately to the precipitation of the metal hydrous oxide. In contrast, with guanosine, increasing pH transforms the aquaion directly into hydroxo-bridged species predominating up to pH 7.[15] Pure monomeric species, distinguished by parameters very similar to those of **III**, exist only in basic solution (Figure 2).

The findings substantiate unquestionably the fact that ribose, not phosphate, is involved in the formation of the VO^{IV} complex of GMP at pH 8.6. A comparative analysis of the EPR parameters listed in Table 1[16] supports the presence of strong alcoholate donors in the equatorial plane of the metal and indicates that the data reported by Jiang and Makinen need to be reinterpreted according to a structure basically of type **III**.

Significantly, on increasing the metal concentration to 1×10^{-2} M and maintaining a constant ligand to VO^{2+} molar ratio of 10:1, while the ^{51}V EPR parameters hardly changed, shf splittings attributed to ^{31}P showed up. In the paper of Jiang and Makinen, a triplet feature with 1:2:1 intensity ratio, arising from the interaction of the vanadium nucleus with two equivalent phosphate groups, was assigned to the $+{}^{3}/_{2}$ perpendicular resonance (Figure 4 of ref 11). A careful examination of the EPR patterns reveals that the ^{31}P shf splitting can be more reliably measured on the $+{}^{7}/_{2}$ perpendicular resonance (Figures 3b and 4), in which it appears clearly as a 1:1 doublet with a ^{31}P coupling constant of about 2.15 mT (corresponding to *ca.* 19.9×10^{-4} cm^{-1} or 59.6 MHz and therefore comparable to the value measured by ^{31}P ENDOR in ref 11) requiring the interaction of the vanadium atom with only one phosphate group.[18] This interaction does not produce any gross changes in the equatorial donor set compared to **III**, as substantiated by the *g* and $A(^{51}V)$ magnetic parameters; it occurs therefore in the axial site of vanadium. In such a case, since the phosphate group and the hydroxyls belonging to a ligand molecule cannot interact with the same metal ion, a dimetallic arrangement is likely in which two species of type **III** are joined to each other through intramolecular vanadium−phosphate bonds (**IIIa**). If an equilibrium between **III** and **IIIa** is assumed, the observed trend is consistent with the concentration dependence of the spectra. For a molar ligand excess of 10:1, at 5×10^{-3} M VO^{IV} the dimer formation occurs to a lesser extent than at 1×10^{-2} M. Most likely the training force for the dimerization process is the formation of assemblies (possibly G-quartets, as suggested by Jiang and Makinen) of GMP units held together by hydrogen bonding interactions between the nucleobases, *e. g.* N(1)H- - -O=C(6) and NH_2- - -N(7) as shown in Figure 1 of

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(14) Branca, M.; Micera, G.; Dessì, A.; Sanna, D. *J. Inorg. Biochem.* **1992**, *45*, 169.

(15) A minor monomer **IV** exists between pH 5.5 and 7.5, which on the basis of the EPR parameters most likely involves a ligand bound through the bis(alcoholate) site and the other one through a nitrogen donor. The precise assignment of the species needs further investigation.

(16) It must be observed that, usually, additivity relation in *g* and *A* values facilitates the derivation of ligand types from EPR spectra.[17] All the evidence gathered in our works indicates that the VO^{IV} coordination of phosphate and phosphonate groups to yield 1:1 complexes, which in most cases are easily detected by equilibrium studies, starts with small changes in $A_{\|}$ values and, more noticeably, with a decrease in $g_{\|}$ value, compared to the solvated ion. Only the formation of bis complexes leads to a decrease in $A_{\|}$ and an increase in $g_{\|}$, as expected for "normal" VO^{IV} complexes. Another "anomaly", on which many studies in the field are concordant, is that the 1:1 complexes produce a red-shift of the low-energy electron absorption maximum of the aquaion. This trend is reversed upon formation of bis-complexes. It is difficult to interpret this behavior. However, one can suggest that the coordination of a bulky ligand, like a phosphate or a diphosphate group, can induce some distortion in the metal geometry. The coordination at the metal ion turns more regular when two of such groups are coordinated to the metal.

(17) Chasteen, N. D. In *Biological Magnetic Resonance*; Berliner, L. J., Reuben, J., Eds.; Plenum Press; New York, 1991; Vol. 3, pp 53−119.

(18) The correspondence of the shf values measured by EPR and ^{31}P ENDOR excludes an effect due to the rhombicity of the spectra.

Table 1. EPR Parameters and in-Plane Chelating Sets of VO^{IV} Complexes Formed by Nucleoside−Phosphates and Their Models

ligand		$g_\parallel$	$A_\parallel$[a]	donor set in the metal plane[b,c]	ref
GMP	**I**[d]	1.928	180	$-\mathbf{PO_3}^{2-}$	this work
	II	1.945	168	$[-\mathbf{PO_3}^{2-}, (\mathbf{O}^-,\mathbf{O}^-)]$	this work
	III	1.958	151	$[(\mathbf{O}^-,\mathbf{O}^-)(\mathbf{O}^-,\mathbf{O}^-)]$	this work
	IIIa	1.958	152	$[(\mathbf{O}^-,\mathbf{O}^-)(\mathbf{O}^-,\mathbf{O}^-)] + -\mathbf{PO_3}^{2-}$	this work
guanosine	**IV**	1.948	160	$[(\mathbf{O}^-,\mathbf{O}^-), \mathbf{N?}]$	this work
		1.958	151	$[(\mathbf{O}^-,\mathbf{O}^-)(\mathbf{O}^-,\mathbf{O}^-)]$	this work
adenosine		1.958	150	$[(\mathbf{O}^-,\mathbf{O}^-)(\mathbf{O}^-,\mathbf{O}^-)]$	this work
D-ribose		1.958	149	$[(\mathbf{O}^-,\mathbf{O}^-)(\mathbf{O}^-,\mathbf{O}^-)]$	this work
MP[e]		1.932	179	$-\mathbf{PO_3}^{2-}$	3
ATP		1.930	181	$[(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-O-PO_2^{-}-)]$	2
		1.934	178	$[(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-O-PO_2^{-}-)(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-O-PO_2^{-}-)]$	2
		1.947	166	$[(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-O-PO_2^{-}-)(\mathbf{O}^-,\mathbf{O}^-)]$	2
		1.957	151	$[(\mathbf{O}^-,\mathbf{O}^-)(\mathbf{O}^-,\mathbf{O}^-)]$	2
ADP		1.928	182	$[(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-)]$	2
		1.933	178	$[(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-)(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-)]$	2
		1.947	166	$[(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-)(\mathbf{O}^-,\mathbf{O}^-)]$	2
		1.958	150	$[(\mathbf{O}^-,\mathbf{O}^-)(\mathbf{O}^-,\mathbf{O}^-)]$	2
diphosphate		1.937	176	$[(\mathbf{PO_3}^{2-}-O-\mathbf{PO_3}^{2-})(\mathbf{PO_3}^{2-}-O-\mathbf{PO_3}^{2-})]$	21
triphosphate		1.937	176	$[(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-O-PO_3^{2-})(\mathbf{PO_3}^{2-}-O-\mathbf{PO_2}^{-}-O-PO_3^{2-})]$	21

[a] 10^{-4} cm^{-1} units. [b] Solvent molecules are omitted. [c] Groups taking part in coordination are indicated in bold. [d] Mixture of 1:1 complex and solvated ion. [e] MP = methylphosphonic acid.

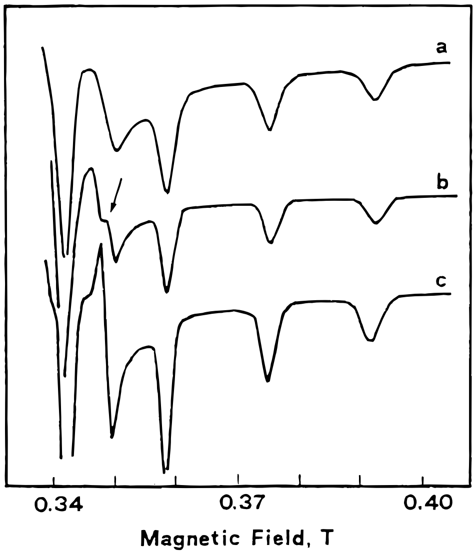

Figure 3. High-field region of X-band frozen-solution EPR spectra recorded on the VO^{IV}−GMP system: (a) 4.0 × 10^{-3} M VO^{IV} and pH 8.60, (b) 1.0 × 10^{-2} M VO^{IV} and pH 8.60, and (c) 1.0 × 10^{-2} M VO^{IV} and pH 11.50. The ligand-to-metal molar ratio is 10:1. The arrow indicates the ^{31}P shf splitting of the $+^7/_2$ perpendicular component of the spectrum. Instrumental settings are reported in the Experimental Section (field modulation amplitude 0.1 mT; gain 10 × 10^2, 2.5 × 10^2, and 4 × 10^2 for a, b, and c, respectively).

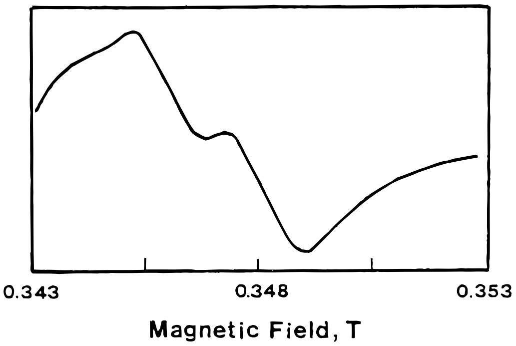

Figure 4. ^{31}P shf splitting of the $+^7/_2$ perpendicular resonance in spectrum b of Figure 3. Instrumental settings are reported in the Experimental Section (field modulation amplitude 0.04 mT; gain 6.3 × 10^2).

ref 11. With increasing concentration, such interactions force monomeric complex species to join together, in the meanwhile bringing a phosphate group from a complex unit next to the vanadium atom of an adjacent unit. The behavior observed with increasing pH is consistent with such an assumption. In fact, upon deprotonation of N(1)H, occurring with $pK_a = 9.46$,[19] hydrogen-bonding contacts are broken. As a consequence, isolated complex units of **III**-type are detected above pH 10.5, as distinguished by the disappearance of ^{31}P shf splitting (Figure 3c) and the slight, although detectable, change in the magnetic ^{51}V parameters of the EPR spectra. In contrast, no similar effect could be detected with guanosine.

An attempt to interpret the ^1H ENDOR data reported by Jiang and Makinen according to structure **IIIa** leads to the following tentative assignments. The presumed axially bound water (with protons distant 3.15 Å from vanadium) could be a molecule interacting with the oxo ligand. The ligand protons located at 3.31 Å from vanadium may be the methine hydrogens on C(2′) and C(3′) atoms of the sugar moiety, which are almost equivalent and occupy positions intermediate between the axial and equatorial in the five-membered chelated ring. The 1.7 MHz coupling has a contribution from protons on the neighboring carbon atoms C(1′) and C(4′). Finally, those at 5.65 Å are the H(8) atoms of guanine, as confirmed by the selective deuteriation experiments of Jiang and Makinen.

In a previous ^1H ENDOR study on the VO^{IV} complex of D-galacturonic acid, which also involved two ligand molecules bound through a couple of deprotonated hydroxyls on C(3) and C(4) atoms, we measured couplings of 3.1 MHz for the magnetic field set parallel to $V{=}O$ axis and 3.4 and 1.7 MHz for the perpendicular setting.[20] These are close to the values of 3.51, 2.82, and 1.58 MHz recorded by Jiang and Makinen on the VO^{IV}−GMP system. In our case too, the 3.1−3.4 MHz signals can be attributed to the protons of the C(3) and C(4) atoms and those at 1.7 MHz to protons on adjacent carbons. In conclusion, structure **IIIa** is consistent with the ^1H ENDOR features and is strongly supported by spectral and chemical evidence.

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(19) Ogasawara, N.; Inoue, Y. *J. Am. Chem Soc.* **1976**, *98*, 7048.

(20) Branca, M.; Micera, G.; Dessì, A.; Kozlowski, H. *J. Chem Soc., Dalton Trans.* **1989**, 1283.

(21) Buglyò, P.; Kiss, T.; Alberico, E.; Micera, G.; Dewaele, D. *J. Coord. Chem.* **1995**, *35*, 105.

Experimental Section

The ligands and $VOSO_4 \cdot H_2O$ were obtained from Aldrich Chemical Co. Aqueous solutions of VO^{2+} (4.0×10^{-3} to 1.0×10^{-2} M) and ligands at varying molar ratios were examined. The pH was adjusted by addition of NaOH. Solutions buffered to pH 8.6 with 0.04 M 2-(*N*-cyclohexylamino)ethanesulfonic acid (Aldrich Chemical Co.) in 1.0 M NaCl yielded the same spectra as those obtained upon adjusting the pH with NaOH. X-Band EPR spectra were recorded at 120 K with a Varian E 9 spectrometer. Instrumental settings were as follows: microwave frequency, 9.15 GHz; incident microwave power, 5 mW; modulation frequency, 100 kHz; field modulation amplitude, 0.04−0.1 mT. DMSO (*ca.* 5% v/v) was added to the samples to ensure good glass formation in acidic solution, whereas it was unnecessary in basic media (however, DMSO presence did not affect the spectral parameters). All solutions were prepared and handled under a nitrogen atmosphere. EPR parameters were derived by applying first-order equations reported in ref 17.

IC9514152

Additions and Corrections

1995, Volume 34

Jaqueline L. Kiplinger, Atta M. Arif, and Thomas G. Richmond*: New Bonding Mode for Cyanoacetylene: A Tungsten(II) Fluoride Complex in Which Cyanoacetylene Serves as a Four-Electron Donor Alkyne Ligand.

Page 399. An earlier report of a spectroscopic characterization of a Mo(II) complex in which cyanoacetylene behaves as a four-electron donor should have been included in the Introduction: Le Berre, N; Kergoat, R.; Kubicki, M. M.; Guerchais, J. E.; L'Haridon, P. *J. Organomet. Chem.* **1990**, *389*, 61−70.

IC9610400

Jeffrey D. Zubkowski,* Dale L. Perry,* Edward J. Valente, and Stevie Lott: A Seven Coordinate Co−EDTA Complex. Crystal and Molecular Structure of Aquo(ethylenediaminetriacetatoacetic acid)cobalt(III) Dihydrate.

Pages 6409−6411. In the original paper, the EDTA ligand was formulated $EDTAH^{3-}$, since only one hydrogen in the crystal structure could be located. This resulted in postulating a cobalt(III) species. On re-examination of the structure, however, the authors were able to locate two half-occupancy hydrogen atoms, which results in the ligand being $EDTAH_2^{2-}$ on average. Thus, the true formula of the complex is $[Co(H_2O)(EDTAH_2)] \cdot 2H_2O$, with the cobalt in the divalent state. The hydrogen atoms are attached as follows: H(2c) to O(2) and H(6c) to O6.

The authors wish to thank Professor K. Miyoshi of Hiroshima University for useful comments and for bringing to their attention a paper [Nesterova, Y. M.; Porai-Koshits, M. A. *Koord. Khim.* **1982**, *8*, 994−1001] relevant to the present work.

atom	*x*	*y*	*z*	*U*(eq), Å^2
H(2c)	0.0000	0.0000	0.0000	80
H(6c)	0.4513	0.5235	0.0056	80

IC960898M

1996, Volume 35

Markus Höpp, Andrea Erxleben, Ingo Rombeck, and Bernhard Lippert*: The Uracil C(5) Position as a Metal Binding Site: Solution and X-ray Crystal Structure Studies of Pt^{II} and Hg^{II} Compounds.

Page 400. In column 1, last line of paragraph 4, the ^{199}Hg NMR shift reported (−2668.5 ppm) was referenced to external $HgCl_2$ in D_2O and not to Me_2Hg. With Me_2Hg as reference, the shift would be −1441 ppm.

IC961984P