expected for C_2 symmetry. The sharpness of these peaks argues strongly against this pattern resulting from the overlap of two peaks of opposite sign.

The [Co(edta-type)]$^-$ complexes with C_2 symmetry can be related to [Co(en)(mal)$_2$]$^-$, which can be correlated with *cis*-[Co(en)$_2$X$_2$]$^{n+}$ complexes.[25] The A(C_2) component for these complexes was considered[25] to retain the sign of the E$_a$(D_3) component for [Co(en)$_3$]$^{3+}$. The empirical correlation has weaknesses because the *cis*-[Co(N)$_2$(O)$_4$]-type complex has the weak- and strong-field ligands reversed compared to the case for *cis*-[Co(N)$_4$(X)$_2$].

For the [Co(edta-type)]$^-$ complexes, the empirical correlations are consistent with the results from D_{4h} symmetry when there are two CD peaks, one on each side of the absorption maximum, or from C_2 symmetry when there are three CD peaks or only two peaks, both on the low-energy side of the absorption maximum.

The geometry of the *trans*(O_5O_6)-[Co(eda3p)]$^-$ complex was assigned on the basis of its ^{1}H NMR spectrum, which indicated an R (out-of-plane) glycinate ring. The CD spectrum of (+)-[Co(eda3p)]$^-$ is compared with spectra of other related complexes (Table I) of known Λ configuration. All complexes shown have positive lowest energy CD peaks (A(C_2)), indicating the same Λ absolute configuration. The CD data obtained for the (+)-[Co(eda3p)]$^-$ complex (see Figure 3 and Table I) are more similar to those of the Λ-[Co(edtp)]$^-$ complex than to those of the Λ-[Co(edta)]$^-$ complex. The positive lowest energy CD peak of this complex is presumed to be the A(C_2) component of E$_g$(D_{4h}) parentage, and the (+)-[Co(eda3p)]$^-$ isomer is also assigned the Λ configuration.

In the Λ-(+)-[Co(eda3p)]$^-$ complex there are possible contributions to the CD intensities from one asymmetric nitrogen atom, as well as from the configurational effect. The latter effect, however, is thought to dominate.[10-13] The contribution of the asymmetric nitrogen of Λ-[Co(eda3p)]$^-$ is *R* for the trans(O_5O_6) isomer (Figure 1, isomer I). The similarity in shapes of the CD curves of the Λ-[Co(edtp)]$^-$ complex (in which case there is no contribution due to asymmetric nitrogens) and the Λ-[Co(eda3p)]$^-$ complex and the relative intensities of their lowest energy CD components (Figure 3 and Table I) support the idea that the contribution due to the asymmetric nitrogen is not great in hexadentate edta-type complexes. Thus, the difference in CD spectra of these complexes is mostly due to different configurational effects associated with the distribution of the five- and six-membered chelate rings. In the case of these two complexes only one chelate ring in an axial position (3-propionate for the Λ-[Co(edtp)]$^-$ complex and (*R*)-glycinate for the Λ-[Co(eda3p)]$^-$ complex) makes a difference in their CD spectra. For clarification of the relative contributions of chelate rings and the dependence on their size and position about an octahedron, more examples with known structure are needed.

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Registry No. H$_4$eda3p, 112988-86-0; H$_4$edma·2HCl, 25240-38-4; K[Co(edtp)], 113008-33-6; *trans*(O_5O_3)-K[Co(eda3p)], 113085-23-7; (−)$_{589}$-[Colen)$_2$(ox)]Br, 31126-57-5; (−)$_{589}$-[Co(en)$_2$(ox)]-(−)$_{546}$-[Co(edtp)], 113158-26-2; (+)$_{589}$-[Co(en)$_2$(ox)]I, 40028-98-6; (−)$_{546}$-Li[Co(edtp)], 113158-24-0; (+)$_{546}$-Li[Co(edtp)], 113083-09-3; (−)$_{589}$-[Co(en)$_2$(ox)]-(+)$_{546}$-[Co(eda3p)], 113008-32-5; (−)$_{589}$-[Co(en)$_2$(ox)]-(−)$_{546}$-[Co(eda3p)], 113084-39-2; (+)$_{546}$-*trans*(O_5O_6)-K[Co(eda3p)], 113008-30-3; (−)$_{546}$-*trans*(O_5O_6)-K[Co(eda3p)], 113084-40-5; 3-chloropropionic acid, 107-94-8.

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Vanadium(V) Oxyanions: Interactions of Vanadate with 1,1,1-Tris(hydroxymethyl)ethane and with the Buffer Tris(hydroxymethyl)aminomethane

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^{51}V NMR spectroscopy has been used to study the interactions of vanadate with 1,1,1-tris(hydroxymethyl)ethane (tris-eth) and with the buffer tris(hydroxymethyl)aminomethane. The reaction of vanadate with tris-eth was found to proceed readily at pH 7.5 to give a variety of products. From variation of vanadate and ligand concentration it was possible to obtain the ligand stoichiometry and formation constants for the various products. The products identified included the monoester of vanadate, the mono- and diesters of divanadate, and a binuclear product for which a bipyramidal coordination geometry about each vanadium nucleus was proposed. The reaction of Tris buffer with vanadate was similar to that of tris-eth. However, a variety of products, which could not be properly studied, were found to occur below pH 7.6. Studies at pH 7.6 and 9.0 showed the formation of vanadate esters of tetrahedral coordination, a bis(ligand) product, assigned an octahedral coordination, and, as well, two binuclear products, one with a single Tris ligand and the second with two ligands. The results were consistent with a bipyramidal/tetrahedral mixed anhydride in the first case and a symmetrical bipyramidal anhydride analogous to that formed with tris-eth in the second case. A rather surprising observation was that these two products exhibited a large chemical shift dependence on pH, presumably reflecting the protonation/deprotonation of the amino group of Tris. The pK_a determined for this reaction was 7.33, which is considerably smaller than that normally associated with tetrahedral products such as the 8.64 ± 0.02 value determined here for the vanadate ester of tris-eth. Investigation of vanadate in the presence of *cis*- or *trans*-1,2-cyclohexanediol and with or without Tris buffer revealed a strong synergistic reaction of Tris with the products of the reaction of vanadate with either of the diols. This result suggests that Tris buffer should be used with extreme caution when vanadate/ligand interactions are studied. Preliminary studies indicated that HEPES buffer is much less reactive toward vanadate and vanadate derivatives.

Introduction

Over the past few years the aqueous chemistry of vanadium has been a subject of developing interest not only because vanadium may be an essential element but also because vanadium has a significant effect on the function of a variety of enzymes.[1,2] Furthermore, it is possible that vanadium may prove to be useful as a therapeutic agent for the treatment of various diseases such as diabetes[3,4] and sickle cell anemia.[5] There may also be ap-

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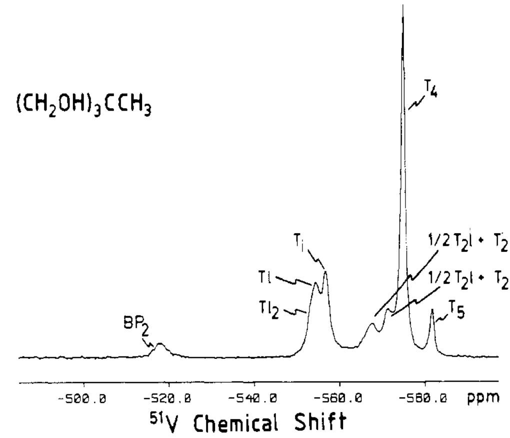


Figure 1. ^{51}V NMR spectrum of vanadate in the presence of 1,1,1-tris(hydroxymethyl)ethane. Conditions of the experiment were as follows: 3.0 mM vanadate; 1.66 M tris-eth; 20 mM HEPES buffer; pH 7.51; 1.0 M ionic strength obtained with added KCl.

plication in cancer treatment.[6]

Much of the available evidence suggests that many of the biological effects of vanadium(V) (vanadate) derive from its capability of mimicking the behavior of phosphate both in its ability to form vanadate esters analogous to phosphate esters[4,7] and in its ability to readily form five-coordinate complexes that are analogous to the transition state of phosphate.[8,9]

Many of the studies of vanadate chemistry have relied on the use of Tris (tris(hydroxymethyl)aminomethane) as pH buffer for the solutions being studied. It, implicitly, has been assumed that Tris, at the concentrations generally employed, has negligible effect on the system being studied. However, Tris contains both hydroxyl and amino functions, either of which might react with vanadate to form V(V) complexes. Furthermore, even if the reactions of Tris with tetrahedral vanadate are not favored, it does not necessarily follow that the same is true for the reaction of Tris (or indeed any other ligand) with the products of equilibrium reactions in which penta- or hexacoordinated products are formed with alternative ligands.

In view of the above considerations we have carried out an investigation of the reactions of tris(hydroxymethyl)aminomethane and of 1,1,1-tris(hydroxymethyl)ethane with vanadate in aqueous solution. The effect of Tris on the equilibria established by vanadate in the presence of *cis*- and *trans*-1,2-cyclohexanediols was also studied.

Experimental Section

Materials. 1,1,1-Tris(hydroxymethyl)ethane (Aldrich, 97%), tris(hydroxymethyl)aminomethane (Sigma, TRIZMA BASE) and *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES, Aldrich, 99%) were used as provided by the suppliers. Stock vanadate solutions were prepared from vanadium pentoxide (Aldrich, Gold Label (99.999%)). All stock solutions and samples were prepared by using previously described procedures.[10]

NMR Spectra. ^{51}V NMR spectra were obtained at 105 MHz by using the broad-banded facility of a Bruker WM-400 NMR spectrometer. All spectra were obtained at ambient temperature. Pulse widths of 60° and acquisition times of 0.05 s were used for all spectra. Spectra were acquired in a 2K data set and transformed in an 8K set. A line broadening of 40 Hz was applied to all spectra. Relative signal intensities were obtained by using the integration routine of the instrument manufacturer.

Methods. Analysis of the results was accomplished by writing the equations in the appropriate linear form as outlined in the text. The appropriate parameters were then plotted to confirm the proposed linearity. Linear correlations were then obtained by using the least-squares routine of a hand calculator. Errors in the least-squares parameters were estimated from the graphs.

Table I. Concentrations of Vanadate Species Determined in the Presence of Varying Concentrations of 1,1,1-Tris(hydroxymethyl)ethane or as a Function of Total Vanadate Measured at pH 7.5[a,b]

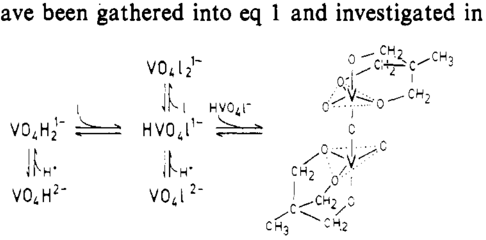
$[V_t]$	$[\ell]$	$[T_i(t)]$	$[T_2(t)]$	$[T_4]$	$[T_5]$	[−518]
0.5	1.66	0.363	0.077	0.034		0.025
1.0	1.66	0.523	0.167	0.248	0.022	0.040
2.0	1.66	0.795	0.380	0.643	0.077	0.104
3.0	0.00	0.502	0.239	1.918	0.340	
3.0	0.10	0.509	0.223	1.939	0.328	
3.0	0.20	0.541	0.261	1.879	0.318	
3.0	0.30	0.550	0.292	1.849	0.308	
3.0	0.40	0.579	0.296	1.800	0.297	0.021
3.0	0.83	0.699	0.346	1.639	0.271	0.044
3.0	1.25	0.787	0.494	1.420	0.219	0.081
3.0	1.66	0.917	0.644	1.140	0.172	0.126

[a] The concentrations of all vanadate species are given as vanadium atom concentrations in millimolar units and were obtained from the NMR integrals. The ligand concentrations are given in molar units. Conditions of the experiments were pH 7.50 and 1.0 M ionic strength maintained with KCl. [b] Abbreviations: V_t, total vanadate; $T_i(t)$, total tetrahedral species; $T_2(t)$, total dimeric tetrahedral species; T_4, tetrameric vanadate; T_5, pentameric vanadate; −518, product giving rise to a ^{51}V NMR signal at −518 ppm; ℓ, 1,1,1-tris(hydroxymethyl)ethane.

Results and Discussion

1,1,1-Tris(hydroxymethyl)ethane. On examination of the ^{51}V NMR spectra of vanadate in the presence of 1,1,1-tris(hydroxymethyl)ethane (tris-eth) it is evident as seen in Figure 1 that a rather limited number of products is formed. Observations of the changes in signal intensities as a function of ligand and vanadate concentration and of the changes in peak positions as a function of pH allowed assignments of the signals to products to be made. Detailed correlations confirmed the assignments. A minor product identified as the vanadate diester ($T\ell_2$) gives rise to a signal at −553 ppm. The chemical shift of this signal is independent of pH, as expected for a product with no ionizable protons. A second product signal occurs at −554.4 ppm. The chemical shift of this signal is pH-dependent. This signal has been assigned to the vanadate monoester $T\ell$. Two further tetrahedral products are formed. These products give signals at −567.4 and −571.2 ppm and are derived from dimeric vanadate, T_2. These signals are considered to arise from a mixture of $T_2\ell$ and $T_2\ell_2$.

The remaining product formed gives rise to an NMR signal at −518.4 ppm, is binuclear in vanadium, and contains two ligands. This chemical shift is near that observed for pentacoordinate products formed from ethylene glycol (−522 ppm),[8] uridine (−519 ppm),[11] and lactate (−517 ppm).[12] Various equilibria of this system have been gathered into eq 1 and investigated in detail



(1)

at pH 7.5. In eq 1, ℓ refers to 1,1,1-tris(hydroxymethyl)ethane and the pentacoordinate product is shown as being trigonal bipyramidal, as this is thought to be the structure of the products formed with ethylene glycol[8] and uridine.[11,13] The water stoichiometry was not obtained in this study, so it was not possible to determine if the three hydroxyls of each ligand were involved in bonding. Under similar conditions it was not possible to form an analogous product with 1,3-propanediol even at much higher

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concentrations, so it is likely that tris-eth is acting here as a tridentate ligand.

Table I gives the concentrations of the various vanadate species obtained as a function of ligand concentration and of vanadate concentration. At pH 7.5 the tetrahedral esters $T\ell$ and $T\ell_2$ do not give well-resolved NMR signals. However, tetrameric vanadate, T_4, serves as a good reference compound for determining the various equilibria as expressed in eq 2–11. Since the water stoichiometry cannot be determined, the formation of water is not considered in any of these equilibria. In eq 9–11, BP refers to bipyramidal products, which are either monomeric, BP_1, or dimeric, BP_2.

$$4T_i \xrightleftharpoons{K_0} T_4 \qquad [T_i]^4K_0 = [T_4] \tag{2}$$

$$2T_i \xrightleftharpoons{K_1} T_2 \qquad [T_i]^2K_1 = [T_2] \tag{3}$$

$$2T_2 \xrightleftharpoons{K_2} T_4 \qquad [T_2]^2K_2 = [T_4] \tag{4}$$

$$T_i + \ell \xrightleftharpoons{K_3} T\ell \qquad [T_i][\ell]K_3 = [T\ell] \tag{5}$$

$$T\ell + \ell \xrightleftharpoons{K_4} T\ell_2 \qquad [T\ell][\ell]K_4 = [T\ell_2] \tag{6}$$

$$T_2 + \ell \xrightleftharpoons{K_5} T_2\ell \qquad [T_2][\ell]K_5 = [T_2\ell] \tag{7}$$

$$T_2\ell + \ell \xrightleftharpoons{K_6} T_2\ell_2 \qquad [T_2\ell][\ell]K_6 = [T_2\ell_2] \tag{8}$$

$$T_i + \ell \xrightleftharpoons{K_7} BP_1 \qquad [T_i][\ell]K_7 = [BP_1] \tag{9}$$

$$2BP_1 \xrightleftharpoons{K_8'} BP_2 \qquad [BP_1]^2K_8' = [BP_2] \tag{10}$$

$$2T_i + 2\ell \xrightleftharpoons{K_8} BP_2 \qquad [T_i]^2[\ell]^2K_8 = [BP_2] \tag{11}$$

As mentioned above, at pH 7.5 the NMR signals from monomeric vanadate, T_i, and its esters $T\ell$ and $T\ell_2$ are not resolved; however, the equilibrium equation for the sum of these products can be written by combining eq 2 with eq 5 and 6 to give eq 12.

$$\frac{[T_i] + [T\ell] + [T\ell_2]}{[T_4]^{1/4}} = K_0^{-1/4} + K_0^{-1/4}K_3[\ell] + K_0^{-1/4}K_3K_4[\ell]^2 \tag{12}$$

A plot of the ratio on the left of this equation versus $[\ell]$ gave a line with upward curvature and a y intercept equal to $K_0^{-1/4} = (3.28 \pm 0.06) \times 10^{-3}\ M^{3/4}$. With this value of $K_0^{-1/4}$, eq 12 can be rewritten as eq 13. A plot of the relevant quantities of eq 13

$$\left(\frac{[T_i] + [T\ell] + [T\ell_2]}{[T_4]^{1/4}} - K_0^{-1/4}\right)\Bigg/ K_0^{-1/4}[\ell] = K_3 + K_3K_4[\ell] \tag{13}$$

from the data of Table I gave a straight line of intercept $K_3 = 0.51 \pm 0.10\ M^{-1}$ and slope $K_3K_4 = 0.09 \pm 0.03\ M^{-2}$, from which $K_4 = 0.18 \pm 0.12\ M^{-1}$. In fact, the error in K_4, which is very sensitive to the error in $K_0^{-1/4}$, must be much smaller than that indicated above since the calculated amount of $T\ell_2$ at pH 6.44, where the spectrum is better resolved, agrees well with that observed. This equilibrium should not be very sensitive to a decrease in pH, since it is expected, by analogy with other studies, that none of the species involved has a pK_a value near the pH range used. The value of K_3 is similar to the equivalent value determined for ethanol, 0.20 M^{-1} when the statistical factor of 3 is considered.

The equilibrium constants for the formation of esters of divanadate can be obtained by an equation similar to eq 12 except that it is derived from eq 4, 7, and 8 to give eq 14. Actually at pH 7.5 at least one NMR signal from the product is separated in chemical shift from T_2; however, the transitions are rather broad and the total of the signal intensities was used. When the ratio of the term on the left of eq 14 is plotted vs $[\ell]$, with use of the concentrations in Table I, a line with a small upward curvature was obtained. The intercept of the plot gave $K_2^{-1/2} = (5.14 \pm 0.11) \times 10^{-3}\ M^{-1}$. Rearranging eq 14 to eq 15 and replotting provided the remaining equilibrium constants K_5 and K_6 with the values 0.88 ± 0.11 and 0.34 ± 0.08 M^{-1}, respectively.

$$\frac{[T_2] + [T_2\ell] + [T_2\ell_2]}{[T_4]^{1/2}} = K_2^{-1/2} + K_2^{-1/2}K_5[\ell] + K_2^{-1/2}K_5K_6[\ell]^2 \tag{14}$$

$$\left(\frac{[T_2] + [T_2\ell] + [T_2\ell_2]}{[T_4]^{1/2}} - K_2^{-1/2}\right)\Bigg/ K_2^{-1/2}[\ell] = K_5 + K_5K_6[\ell] \tag{15}$$

Table II. Chemical Shifts of Inorganic Vanadate and Its Monoester Determined as a Function of pH[a]

	chem shift			chem shift	
pH	T_i	$T\ell$	pH	T_i	$T\ell$
6.44	−559.7	−555.9	8.43	−546.2	−548.0
7.51	−556.6	−554.4	9.19	−539.3	
8.00	−551.5		9.57	−537.6	−534.9

[a] All chemical shifts were determined for solutions of 3.0 mM vanadate, 1.25 M 1,1,1-tris(hydroxymethyl)ethane, 20 mM HEPES buffer, and ionic strength 1.0 M maintained with KCl. The shifts are relative to an external reference of $VOCl_3$ at 0.0 ppm. T_i and $T\ell$ refer to inorganic vanadate and its monoester, respectively.

The remaining ^{51}V NMR signal occurs at −518 ppm. This chemical shift is similar to that observed for one of the products formed from the reaction of vanadate with ethylene glycol. A trigonal-bipyramidal structure has been assigned to that product[8] and to similar products formed with lactate[12] and uridine.[11,13] A similar structure is proposed for the product formed here. The formation of this product (or products) can be anticipated to proceed as indicated in eq 1 as summarized by eq 9 and 10, where the possibility of forming both monomeric and dimeric products is considered. When these equations are summed and combined with eq 2, and then rearranged, eq 16 is obtained, where the factor

$$\frac{[BP_1] + 2[BP_2]}{K_0^{-1/4}[T_4]^{1/4}[\ell]} = K_7 + 2K_0^{-1/4}K_7^2K_8'[T_4]^{1/4}[\ell] \tag{16}$$

of 2 is incorporated since only vanadium atom concentrations are measured and the proportions between BP_1 and BP_2 are not known. A plot of the ratio on the left of eq 16 versus $2K_0^{-1/4}[T_4]^{1/4}[\ell]$ gave a line of intercept $K_7 = (2.3 \pm 2.4) \times 10^{-2}\ M^{-1}$ and slope $K_7^2K_8' = K_8 = (1.2 \pm 0.2) \times 10^2\ M^{-3}$. The value of K_7 is very small and is not distinguishable from zero, for which case K_8 of eq 11 is equal to $(1.4 \pm 0.1) \times 10^2\ M^{-3}$.

In an effort to gain further information concerning this system, a pH study at constant ligand concentration and total vanadate concentration was done. From this study it was possible to obtain pK_a values for vanadate, $H_2VO_4^-$, and its monoester $HVO_4\ell^-$. For the rapid equilibrium reaction of eq 17 (where R equals H or ℓ)

$$HVO_4R^- \xrightleftharpoons{K_a} VO_4R^{2-} + H^+ \tag{17}$$

the NMR spectrum shows only a single resonance. The observed chemical shift position is a weighted average dependent on the proportion between reactant and product. If δ_l and δ_h are the limiting chemical shifts at low and high pH, respectively, then the observed shift, δ, is related to the pK_a by eq 18. A plot of

$$\mathrm{pH} = \log\left(\frac{\delta_l - \delta}{\delta - \delta_h}\right) + \mathrm{p}K_a \tag{18}$$

pH versus $\log((\delta_l - \delta)/(\delta - \delta_h))$ should give a straight line of slope 1 and y intercept equal to the pK_a. From the results of Table II, the value obtained for the pK_{a2} value of the ester was 8.65 ± 0.02 with $\delta_l = -556.1$, $\delta_h = -532.4$, and slope 0.999. The corresponding values obtained for the vanadate anion were pK_{a2} = 8.32 ± 0.02, $\delta_l = -560.0$, and $\delta_h = -536.3$ with a calculated slope of 1.00.

Tris(hydroxymethyl)aminomethane. Preliminary studies of the reaction between vanadate and tris(hydroxymethyl)aminomethane (Tris) revealed a rather complex series of equilibrium reactions with the formation of a variety of products, particularly at pH below 7.5. At higher pH the situation was more tractable and

Table III. Concentrations of Vanadate Species Determined in the Presence of Varying Concentrations of Tris(hydroxymethyl)aminomethane or as a Function of Total Vanadate[a,b]

$[V_t]$	[Tris]	$[T_i(t)]$	$[T_2(t)]$	$[T_4]$	$[T_5]$	[−535]	[−500]
0.5	100	0.279	0.058	0.120	0.008	0.035	
1.0	100	0.365	0.107	0.445	0.043	0.040	
4.0	100	0.544	0.298	2.657	0.448	0.059	
0.5	300	0.239	0.044	0.043		0.130	0.044
1.0	300	0.369	0.097	0.195	0.020	0.242	0.077
1.5	300	0.474	0.126	0.467	0.055	0.305	0.074
2.0	300	0.549	0.179	0.727	0.092	0.347	0.105
2.5	300	0.616	0.217	1.014	0.145	0.420	0.086
3.5	300	0.711	0.255	1.717	0.279	0.443	0.094
4.0	300	0.750	0.258	1.972	0.346	0.554	0.119
2.0	10	0.430	0.162	1.217	0.190		
2.0	20	0.416	0.156	1.220	0.184	0.024	
2.0	30	0.446	0.186	1.184	0.170	0.014	
2.0	50	0.442	0.166	1.195	0.172	0.025	
2.0	75	0.439	0.177	1.181	0.150	0.053	
2.0	100	0.475	0.184	1.140	0.149	0.052	
2.0	150	0.498	0.187	1.090	0.137	0.088	
2.0	200	0.482	0.178	1.082	0.135	0.121	
2.0	300	0.545	0.163	0.792	0.083	0.347	0.069
2.0	400	0.576	0.222	0.515	0.060	0.488	0.139
3.0	250	0.636	0.222	1.545	0.234	0.309	0.054
3.0	300	0.665	0.197	1.408	0.266	0.374	0.090
3.0	350	0.684	0.215	1.283	0.205	0.476	0.136
3.0	400	0.719	0.230	1.155	0.169	0.560	0.166
3.0	450	0.739	0.213	1.035	0.135	0.692	0.183

[a] The concentrations of all vanadate species are given as vanadium ion concentrations in millimolar units as are total ligand concentrations. All spectra were obtained at pH 7.50 under conditions of 1 M ionic strength maintained with KCl. [b] Abbreviations: V_t, total vanadate; $T_i(t)$, total monomeric tetrahedral species; $T_2(t)$, total tetrahedral divanadate species; T_4, tetrameric vanadate; T_5, pentameric vanadate; −535, product giving rise to a ^{51}V signal at −535 ppm; −500, product giving rise to a ^{51}V signal at −500 ppm.

it proved possible to obtain equilibrium constants for the products observable at pH 7.6.

Figure 2 shows a ^{51}V NMR spectrum obtained at pH 7.6, 400 mM Tris, and 2 mM vanadate (V_i) at an ionic strength of 1.0 M obtained with added KCl. The resonances at −551.3, −569.3, −575.2, and −582.8 are derived predominantly from inorganic vanadate (T_i), divanadate (T_2), tetravanadate (T_4), and pentavanadate (T_5), where the T is used to represent the presumed tetrahedral coordination geometry. There is no chemical shift separation of vanadate esters from their precursors. It is possible, however, to show that they are formed.

The resonance at −534 ppm is superimposed on a broader resonance at slightly lower field. The signal at −500 ppm is apparently derived from a single compound. Detailed analysis of a series of ^{51}V spectra obtained from solutions in which vanadate and Tris concentration as well as pH were varied allow the coordination number and state of protonation to be assigned to these three products.

On the basis of the interactions of vanadate with tris-eth and other alcohols[4,7,8] it can reasonably be expected that Tris also forms vanadate esters. Since signals ascribable to these esters are not observed, either as seen in Figure 1 or, indeed, even when the pH is changed, it may be that they are in rapid equilibrium with their hydrolysis products, a result, perhaps, of the presence of the primary amine moiety. The structurally analogous tris-eth, which instead of the amino group has a methyl group, does show clearly observable signals for $T\ell$ and $T\ell_2$.

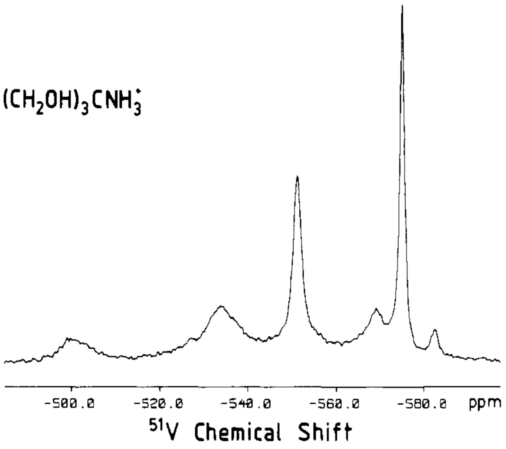

Figure 2. ^{51}V NMR spectrum of vanadate in the presence of tris(hydroxymethyl)aminomethane. Conditions of the experiment were as follows: 2.0 mM vanadate; 400 mM Tris; pH 7.6; 1.0 M ionic strength obtained with added KCl.

The analysis of the results of the concentration studies proceeds identically with that discussed for the tris-eth solutions. Table III gives the vanadium atom concentrations of the various components that were obtained as a function of Tris or vanadate concentration at pH 7.6. Application of eq 12 should then provide the parameters K_0, K_3, and K_4 of eq 2–4. A point to be made about eq 12 is that at a fixed ligand concentration the ratio on the left of the equation is a constant, independent of vanadate concentration. When this ratio was determined experimentally for the various concentrations of the vanadate concentration study (Table III), it was found to vary well outside the experimental error in a systematic manner. This result indicated that the analysis of the NMR spectra according to eq 12 is incorrect, and it was evident that a further product, identified in the text as $B\ell_n$, was formed. Further analysis showed that this product contains two vanadium nuclei, at least one of which gives an NMR signal under the T_i resonance. The formation of this product might then proceed as in eq 19.

$$2T_i + n\ell \overset{K_9}{\rightleftharpoons} B\ell_n \qquad [T_i]^2[\ell]^nK_9 = [B\ell_n] \tag{19}$$

Bearing in mind that the proportions T_i:$T\ell$:$T\ell_2$ are independent of vanadate concentration, eq 20, which is valid only at constant $[\ell]$, can be written and the formation constant for the unknown compound determined, but only to a power, n, of ligand concentration. In this equation, K is the overall formation constant of

$$\frac{[T_i] + [T\ell] + [T\ell_2] + [B\ell_n]}{[T_4]^{1/4}} = K^{-1/4} + K_0^{-1/2}K_9'[T_4]^{1/4}[\ell]^n \tag{20}$$

T_4 from $T_i + T\ell + T\ell_2$. It is immaterial at this juncture whether the signals from one or both vanadiums of $B\ell_n$ are under the T_i signal, and this is represented by using K_9'. Figure 3 shows the

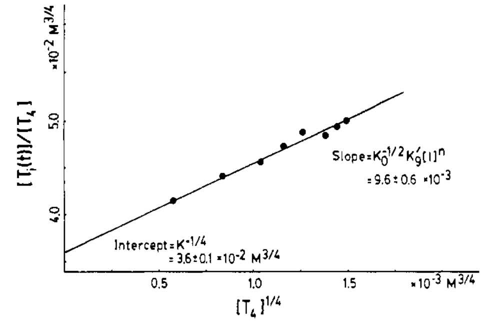

Figure 3. Correlation, utilizing eq 20, establishing the occurrence of a Tris derivative binuclear in vanadium, which has at least one ^{51}V NMR signal under the resonance of the vanadate ion. Experimental conditions were as described in the text.

result of plotting the ratio on the left of eq 20 vs $[T_4]^{1/4}$. A good straight line with intercept $K^{-1/4} = (3.6 \pm 0.1) \times 10^{-3}$ M$^{3/4}$ and slope $K_0^{-1/2}K_9'[l]^n = (9.6 \pm 0.6) \times 10^{-3}$ was obtained.

A plot of the ratio $[T_i(t)]/[T_4]^{1/4}$ vs $[\ell]$, where $[T_i(t)]$ is defined by eq 21 (assuming that only one of the vanadium atoms in $B\ell_n$ gives rise to a signal in this region of the spectrum), gives a line of upward curvature with y intercept equal to $K_0^{-1/4}$. The plot

$$[T_i(t)] = [T_i] + [T\ell] + [T\ell_2] + [B\ell_n] \tag{21}$$

provided a value of $(3.15 \pm 0.05) \times 10^{-3}$ M$^{3/4}$ for this intercept, from which $K_0 = (1.02 \pm 0.06) \times 10^{10}$ M^{-3}, a value in good agreement with our determination of this equilibrium constant for the tris-eth system. From the values of the slope of the straight line of Figure 3 and $K_0^{-1/2}$, $K_9' = (9.7 \pm 0.6) \times 10^2[\ell]^{-n}$, where $[\ell]$ was 300 mM for this study.

The value of n can be determined most directly by doing a second vanadate concentration study at a second ligand concentration. It turns out in this case that a second concentration study was not necessary. With the equilibrium constant K_9' and the measured concentrations of T_4, T_i can be calculated for all concentrations of ligand or vanadate by using eq 2. The concentration of $B\ell_n$ is then given from eq 19 by using the K_9' value determined above with assumed values of n. Since $[T_i(t)]$, $[T_i]$, and $[B\ell_n]$ of eq 21 are known, $[T\ell] + [T\ell_2]$ is given by difference. These calculations showed that $n = 1$, since only with this value is there a consistent increase in $[T\ell] + [T\ell_2]$ relative to $[T_i]$ as the ligand concentration is increased. Thus, the compound $B\ell$ is binuclear in vanadium, contains one Tris ligand, and can be formed from $2T_i$ and a ligand with a formation constant of $(3.2 \pm 0.3) \times 10^3$ M^{-2}.

In the above analysis it was necessary to calculate both the concentrations of T_i and the sum of the concentrations of $T\ell$ and $T\ell_2$. A plot of $([T\ell] + [T\ell_2])/[T_i]$ vs [Tris] should give a line of zero y intercept and, if K_4 is sufficiently large, with upward curvature. The results did not allow a meaningful determination of curvature, so K_4 was not measured. Normally we have found the formation of diesters to be less favored than that of monoesters by a factor of 3–5 in their respective formation constants.[7,8] A value for K_3 of 0.55 ± 0.17 M^{-1} was obtained. This value of K_3, when the statistical factors are taken into account, is similar to that measured for ethanol, 0.19 M^{-1},[7] ethylene glycol, 0.34 M^{-1},[8] and to the value measured here for 1,1,1-tris(hydroxymethyl)ethane, 0.51 M^{-1}.

From the above analysis it is clear that at least one of the NMR signals from the $B\ell$ derivative occurs under the T_i signal. In order to ascertain the location of the second signal from the $B\ell$ derivative, it is necessary to examine the remaining signals in detail.

In addition to the interaction of Tris with T_i there is the possibility of its reacting with T_2 to form $T_2\ell$ and $T_2\ell_2$ with the formation constants K_5 and K_6, respectively, as described by eq 7 and 8. Application of eq 14 can then provide the equilibrium constants. When the appropriate quantities were plotted, a line of y intercept equal to $K_2^{-1/2} = (4.7 \pm 0.2) \times 10^{-3}$ M$^{1/2}$ was obtained, from which $K_2 = (4.4 \pm 0.2) \times 10^4$ M^{-1}. No upward curvature was observed in this plot, indicating that K_6 is small. The slope of the line gave $K_2^{-1/2}K_5$, from which $K_5 = 0.97 \pm 0.05$ M^{-1}. For the concentration range utilized in this study a value of K_6 equal to or larger than K_5 should have been readily observed. K_6 must then be considerably smaller than K_5. The formation constant of $T_2\ell$ from T_2 in conjunction with the formation constants of T_4 from T_i and T_2 can be used to calculate the formation constant of $T_2\ell$ from two vanadates and a ligand as shown in eq 22. The value obtained for K_5' ($=4.6 \times 10^2$ M^{-2}) is about a factor

$$2T_i + \ell \xrightleftharpoons{K_5'} T_2\ell \tag{22}$$

of 6 smaller than K_9, the value obtained for the formation of $B\ell$ from two vanadates and a Tris ligand. As a consequence, $B\ell$ cannot be the same as $T_2\ell$ and it seems that unless both of the vanadium signals from $B\ell$ are under the T_i signal, then at least one of the vanadiums of $B\ell$ must have a nontetrahedral coordination geometry. Such behavior has been observed in the case of lactate, where a mixed octahedral/tetrahedral anhydride is formed.[2]

In previous studies of the interactions of vanadate in aqueous solution we have not observed resonances occurring at −500 ppm. The compound giving rise to this signal occurs at relatively low concentrations compared to those of most other products. Correlations of the ^{51}V signal intensity with the concentration of vanadate at fixed Tris concentration showed that this product is mononuclear in vanadium. Having found this, ligand concentration studies at fixed total vanadate concentration showed that this product contains two Tris ligands and is therefore formed according to eq 23. The formation constant for this product can

$$T_i + 2\ell \xrightleftharpoons{K_{11}} [-500] \tag{23}$$

be written in terms of the T_4 concentration (eq 2) to give eq 24.

$$[-500] = K_0^{-1/4}K_{11}[T_4]^{1/4}[\ell]^2 \tag{24}$$

From the known value of $K_0^{-1/4}$ and the slope ($(8.1 \pm 0.4) \times 10^{-3}$ M$^{-5/4}$) from the plot of $[-500]/[T_4]^{1/4}$ vs $[\ell]^2$ a value for K_{11} of 2.6 ± 0.2 M^{-2} was determined.

The remaining signals in the NMR spectra are centered around −535 ppm. Although not obvious in Figure 2, it can be seen that there is a rather broad signal to the low-field side of the more obvious peak at −535 ppm. Investigation of these signals indicated that the broader component was mononuclear in vanadium and required one ligand for its formation; the second component was a binuclear product that contained two ligands. Because of the presence of the mononuclear product, complicated by the rather broad signals, it was not possible to determine whether the binuclear product is only a single product with two ligands or whether there is also a minor second product that is binuclear in vanadium but contains only one ligand, i.e. the binuclear product, $B\ell$, previously observed.

In an attempt to gather further information concerning this system, a pH study was done at 3.0 mM V_i, 300 mM Tris, and 1.0 M ionic strength maintained with appropriate quantities of added KCl. At pH 6 several products, in addition to those already identified, were observed. All gave rise to broad poorly resolved NMR resonances throughout the range −515 to −555 ppm. Their formation evidently is favored by acid conditions since, as indicated by the NMR spectra, they decreased in concentration with increase in pH until at pH 7.6 only the products previously discussed were present at significant concentrations. At pH 9 the products corresponding to the −500 and −535 ppm signals were still observed. The chemical shift of the signal occurring at −500 ppm was independent of pH, while that at −535 ppm corresponding to the product identified subsequently as BP_2 was pH-dependent, occurring at −555 ppm at pH 6.35, −527.3 ppm at pH 8.8, and, of course, at −535 ppm at pH 7.6. It is evident that the effect of pH on this latter chemical shift is derived from a protonation/deprotonation reaction since the shift is dependent neither on vanadate concentration nor on ligand concentration. Furthermore, the formation of this product is not strongly disfavored with increase in pH, indicating that its formation involves either the neutral amino group or the hydroxymethyls of Tris rather than

Table IV. Concentrations of Vanadate Species in the Presence of Varying Concentrations of Tris(hydroxymethyl)aminomethane at pH 9.0[a,b]

[Tris]	[T_i(t)]	[T_2]	[−570]	[T_4]	[T_5]	[−527]	[−500]
20	2.98	0.999	0.542	1.259	0.174	0.047	
100	3.21	0.821	0.450	1.084	0.143	0.295	
150	3.15	0.866	0.555	0.835	0.126	0.425	0.045
200	3.08	0.90	0.54	0.741	0.090	0.570	0.071
250	3.07	0.89	0.47	0.576	0.085	0.787	0.162
300	3.12	0.90	0.46	0.488	0.04	0.908	0.136
350	3.03	1.46		0.404		0.929	0.172
400	3.08	1.34		0.265		1.081	0.235

[a] All concentrations of vanadate species are given as vanadium ion concentrations in millimolar units as are total ligand concentrations. All spectra were obtained at pH 9.0 under conditions of 1 M ionic strength maintained with KCl. The total vanadate concentration was 6.0 mM. [b] Abbreviations are as for Table I. −570, −527, and −500 refer to the compounds giving rise to ^{51}V signals at these chemical shifts.

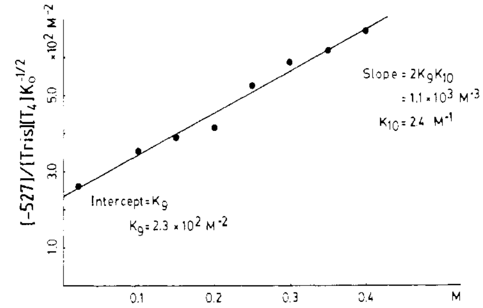

Figure 4. Demonstration of the formation of two binuclear vanadate products at pH 9. Tris ligand and tetrameric vanadate concentrations as required for eq 26 were taken from Table IV.

the positively charged ammonium moiety.

The change in chemical shift as a function of pH is very simply related to the pK_a of BP_2 by eq 18. With the limiting values $\delta_l = -557.7$ and $\delta_h = -526.0$ an excellent line of slope 1.002 and intercept 7.33 ± 0.01 (=pK_a) was obtained for a plot of the above expression. Equation 18 requires a slope of 1, which was obtained to reasonable accuracy.

The pH study gave no indication that the NMR signal from the product BP_2 concealed a signal from a second product. It was possible to show that there was a second product by doing a ligand concentration study at pH 9. Table IV gives the concentrations of the various products observed at pH 9 and constant total vanadate concentration of 6 mM as the Tris concentration was varied. Application of eq 20, by a plot of $[T_i(t)]/[T_4]^{1/4}$ vs $[\ell]$, gave a line of y intercept equal to $K_0^{-1/4}$. This value, $K_0^{-1/4}$ (=(2.25 ± 0.10) × 10^{-2} $M^{3/4}$), will vary with pH and consequently is different from that previously determined at pH 7.6.

Combination of eq 25 with eq 2 and eq 19 ($n = 1$) followed by rearrangement gave eq 26. Figure 4 shows a plot of the

$$B\ell + \ell \overset{K_{10}}{\rightleftharpoons} BP_2 \qquad [B\ell][\ell]K_{10} = [BP_2] \tag{25}$$

$$\frac{[B\ell] + 2[BP_2]}{[\ell][T_4]^{1/2}K_0^{-1/2}} = K_9 + 2K_9K_{10}[\ell] \tag{26}$$

relevant quantities of eq 26 with the data of Table IV. A good linear relationship clearly is obtained. From the slope and intercept, values of $K_9 = (2.3 \pm 0.1) \times 10^2$ M^{-2} and $K_{10} = 2.4 \pm 0.2$ M^{-1} were obtained. From these equilibrium constants and at a ligand concentration of 0.4 M Tris the concentration of $B\ell$ is 0.39 mM, as is the concentration of BP_2. On this basis, 66% of the ^{51}V NMR signal at −527 ppm is derived from BP_2 while the remaining 33% arises from $B\ell$. The other ^{51}V signal from $B\ell$ occurs under the T_i signal and is about 12% of the total intensity of that signal at pH 9. Knowledge of the equilibrium constants K_9 and $K_0^{-1/4}$ allows $B\ell$ concentrations to be calculated for all ligand concentrations so that from the measured quantity $[T_i(t)]$ of Table IV and eq 21 the quantity $[T_i] + [T\ell] + [T\ell_2]$ can be obtained, which can be utilized in eq 12 with $K_0^{-1/4} = 2.25 \times 10^{-2}$ M^{-1} as previously determined. The resultant plot gave a straight line. From the slope of the line $K_3 = 0.56 \pm 0.13$ M^{-1}. This value is close to that which might be expected since its value at pH 7.5 was determined to be 0.55 ± 0.17 M^{-1}. The value for K_3 will change significantly with pH only if the pK_a of $H_2VO_4^-$ is significantly different from the pK_a of its ester, $HVO_4\ell^-$. There is no indication that there is such a difference. The above analysis indicates quite clearly that the binuclear product $B\ell$ has attached to one vanadium center a single Tris ligand. The ^{51}V NMR spectrum then gives rise to two signals, one close in frequency to that of unsubstituted vanadate and the other close to that of the disubstituted binuclear product BP_2. The coincidence of the chemical shift suggests that BP_2 is formed by the addition of Tris to the asymmetrical derivative, $B\ell$, to give a symmetrical product. The pK_a of the moiety giving rise to the low-field signal appears to be insensitive to the nature of the attached group, as is the chemical shift. These observations also support the conclusion that the two compounds $B\ell$ and BP_2 are closely related derivatives, one being a symmetrical product while the other lacks a Tris ligand on the second vanadium nucleus, which has a tetrahedral coordination as indicated by its ^{51}V chemical shift.

From the information available it is not possible to assign other than a tentative structure to the product BP_2. In view of the fact that a trigonal-bipyramidal structure has been proposed for one product of the reaction of tris-eth with vanadate, a similar structure is proposed for this Tris derivative. The hydroxyls of the ligand are proposed to be coordinated with the five-coordinate vanadium atoms, and the change in chemical shift with pH results from protonation/deprotonation of the free amino function. This structure is consistent with the observation of a single pK_a of 7.33 for the mixture of products $B\ell$ and BP_2.

Unlike that of BP_2 the chemical shift of the signal from the product giving rise to the −500 ppm resonance is independent of pH. The formation of this product, as is evident from Table IV, is not strongly disfavored at pH 9 as compared to pH 7.6. Application of eq 24 to the results of the pH 9 study provided a value for K_{11} of 0.69 ± 0.04 M^{-2}, which is only a factor of about 4 less than the 2.6 ± 0.2 M^{-2} value measured at pH 7.6.

The pH study indicates strongly that the formation of this latter product requires the presence of the amine functionality since at pH 6 no signal at −500 ppm was observed. At pH 6, Tris should be essentially fully protonated. With an increase in pH the formation of the product initially is increasingly favored and then is less favored as reflected in the formation constants for pH 7.6 and pH 9.0. This result may indicate the reaction of the amine with $VO_4H_2^-$ rather than with VO_4H^{2-}, since the pK_a of $VO_4H_2^-$ is about 8.3 under the 1.0 M ionic strength conditions of this study.

It is difficult to assign a coordination geometry to this product. It does not appear that Tris is acting as a bidentate ligand since a similar product is not formed with ethanolamine even at much higher concentrations than those employed here. Also, as we have just seen, no similar product is formed with tris-eth. It thus appears that Tris is acting as a tridentate ligand with binding through two hydroxyl groups and an amino group. If this is true, then the product probably has octahedral coordination since two ligands are required to form the product.

An interesting aspect of the chemistry of the Tris buffer is its interaction with pentacoordinate vanadate complexes formed from cyclic diols. This is clearly demonstrated in Figure 5, where the top trace shows a ^{51}V spectrum of vanadate in aqueous *cis*-1,2-cyclohexanediol solution with HEPES buffer at pH 7.5. The

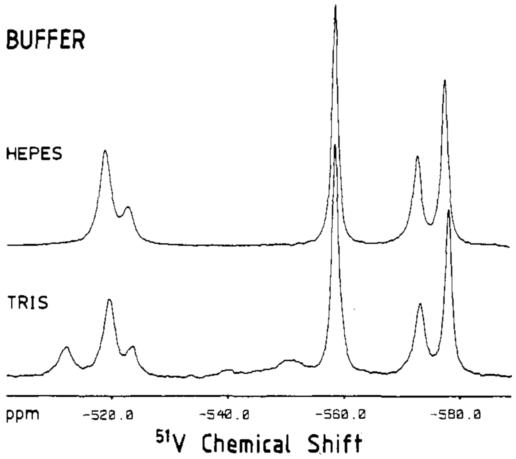

Figure 5. ^{51}V NMR spectra of vanadate in the presence of *cis*-1,2-cyclohexanediol either with (bottom trace) or without (top trace) added Tris buffer. The conditions of the experiments were as follows: 3.0 mM vanadate; 0.172 M *cis*-1,2-cyclohexanediol; pH 7.5; 20 mM HEPES buffer (trace A) or 20 mM Tris buffer (trace B).

bottom trace shows a spectrum obtained under similar conditions but with HEPES buffer replaced by Tris buffer. The predominant new features of the bottom spectrum are the signals at −511 and −550 ppm. When concentrations of vanadate and the diol were varied, it was found that these signals correlate well with each other in intensity, indicating they are derived from the same compound. The behavior of either signal under varying experimental conditions can be used to show that this product is binuclear in vanadium and contains a Tris ligand and a single cyclohexanediol. The ^{51}V chemical shift of the −511 ppm signal is independent of pH. The resonance position and the pH independence of chemical shift indicate that the vanadium nucleus giving rise to this signal is pentacoordinate and is presumably the vanadate to which the diol is complexed. The chemical shift of the rather broad signal at −550 ppm might reasonably be taken to indicate that the vanadate giving rise to this signal is tetrahedrally coordinated and has a Tris ligand attached. Unfortunately, the absence of a similar binuclear product formed with one unsubstituted vanadate does not provide much support to this hypothesis. However, a similar binuclear mixed anhydride has been proposed for the lactate system and there, also, no product with only one ligand incorporated was observed. In the lactate case it was shown that a mixed octahedral/tetrahedral product was formed, which did not depend on the presence of Tris.

In the case of lactate the pentacoordinate product is mononuclear in vanadium and no reaction of this latter compound with Tris buffer was observed.[12] Interestingly enough it can be shown that with *trans*-1,2-cyclohexanediol a second diol ligand can be incorporated into the mixed anhydride.

The formation of the mixed-ligand product was studied in detail in an initial investigation of the interactions of vanadate with cyclohexanediols. Only afterwards was it appreciated that a major product of the reaction was formed as a consequence of the presence of Tris buffer. The formation of products can be written as in eq 27 and 28, where CHD refers to cyclohexanediol and

$$\begin{gathered} 2\mathrm{T_i} + \mathrm{CHD} + \mathrm{Tris} \overset{K_{12}}{\rightleftharpoons} \mathrm{T_1BP_1} \\ [\mathrm{T_i}]^2[\mathrm{CHD}][\mathrm{Tris}]K_{12} = [\mathrm{T_1BP_1}] \end{gathered} \tag{27}$$

$$\begin{gathered} \mathrm{T_1BP_1} + \mathrm{CHD} \overset{K_{13}}{\rightleftharpoons} \mathrm{T_1'BP_1} \\ [\mathrm{T_1BP_1}][\mathrm{CHD}]K_{13} = [\mathrm{T_1'BP_1}] \end{gathered} \tag{28}$$

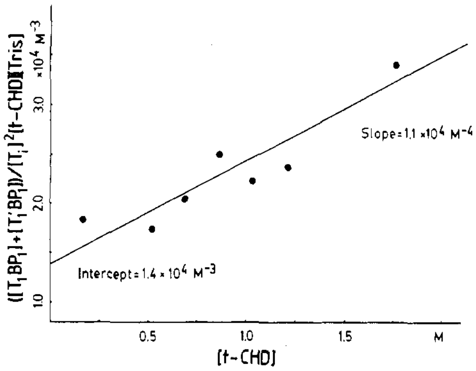

Figure 6. Incorporation of a second *trans*-1,2-cyclohexanediol ligand into the mixed binuclear anhydride formed with Tris and *trans*-1,2-cyclohexanediol as described by eq 29. The conditions of the experiments were as follows: 0.5 mM vanadate; 20 mM Tris buffer; pH 7.5; varying amounts of the trans diol.

T_1BP_1 and $T_1'BP_1$ denote the two binuclear products considered. Combination and rearrangement of eq 27 and 28 gives eq 29. A

$$\frac{[\mathrm{T_1BP_1}] + [\mathrm{T_1'BP_1}]}{[\mathrm{T_i}]^2[\mathrm{CHD}][\mathrm{Tris}]} = K_{12} + K_{12}K_{13}[\mathrm{CHD}] \tag{29}$$

plot of the ratio on the left of this equation versus [CHD] should give a straight line of intercept K_{12} and slope $K_{12}K_{13}$. When the results of the concentration study of *trans*-1,2-cyclohexanediol were plotted according to eq 29, with the signal intensity of the −511 ppm peak used to provide concentrations, the graph shown in Figure 6 was obtained. The values obtained were $K_{12} = (1.4 \pm 0.2) \times 10^4\ \mathrm{M^{-3}}$ and $K_{13} = 0.8 \pm 0.2\ \mathrm{M^{-1}}$. With *cis*-1,2-cyclohexanediol the corresponding values were $K_{12} = (7.5 \pm 0.8) \times 10^4\ \mathrm{M^{-3}}$ and $K_{13} < 0.1\ \mathrm{M^{-1}}$. The formation of the binuclear product is, as indicated by K_{12}, a favorable process. The observable uptake of a second diol in the case of *trans*- but not *cis*-1,2-cyclohexanediol is a behavior similar to that observed in the formation of acyclic esters of vanadate with these diols, where it was found that the product with the trans diol added a second ligand much more readily than the product formed with the cis diol.[14]

The magnitude of the formation constant for this second reaction, $K_{13} = 0.8\ \mathrm{M^{-1}}$ for *trans*-CHD and an unobservably small value for the cis derivative, is consistent with the cyclohexanediol acting as a monodentate ligand, as expected if the second vanadate of the mixed anhydride has tetrahedral coordination.

It is proposed that the binuclear product T_1BP_1 is formed from a pentacoordinate (possibly trigonal bipyramidal) vanadate complexed with the cyclohexanediol (acting as a bidentate ligand) and with a Tris ligand. It is also linked through an oxygen to a second vanadate of tetrahedral coordination. This structure is proposed to be similar to that found in Bℓ as previously discussed. A subsequent reaction allows a cyclohexanediol to be incorporated into the tetrahedral moiety to yield $T_1'BP_1$.

A detailed study of the interactions of vanadate with *cis*- and *trans*-1,2-cyclohexanediols and with some monosaccharides has been reported elsewhere.[14]

In the above study the buffer *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid, HEPES, has been used. This buffer can be expected to form esters with vanadate since it does have a hydroxyl group. Ester formation with alkyl alcohols tends to occur with a formation constant of about 0.2 M^{-1}, so even at concentrations of 100 mM HEPES only about 2% of the vanadate will exist as the ester. At concentrations as high as 200 mM no formation of unusual vanadate products was observed. This does not mean that HEPES will be an innocuous additive under all circumstances. Conditions may well occur that will promote active participation of HEPES in reactions to provide unexpected products in a manner analogous to the reaction of Tris with vanadate derivatives of cyclohexanediols.

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Registry No. HEPES, 7365-45-9; $VO_4H_2^-$, 34786-97-5; VO_4H^{2-}, 26450-38-4.

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(14) Tracey, A. S.; Gresser, M. J., unpublished results, 1988.