is detected, and no organic pigment susceptible to oxidation with oxygen is present in the lysate. In the presence of oxygen, cells from the same species on lysis yield acid, vanadium, and oxidizable pigment: the typical Henze solution[5] was obtained. It should be noted that for the deoxygenated lysate indicators gave a lower pH reading than a pH electrode. These experiments were performed with *P. julinea*. Blood, which had been removed by heart puncture using a sterile syringe and had been kept on ice, was gently centrifuged at 500 g for 1 min and the plasma removed under nitrogen. A small volume of deoxygenated distilled water was added to the cells for lysis, and the suspension was bubbled vigorously with nitrogen for 2 h. The pH of the lysate was monitored throughout but did not deviate from 6.5, the original pH of the water. The cell debris was removed by centrifugation, and the pale yellow supernatant was stored under nitrogen at −20 °C. Vanadium concentration was determined by graphite-furnace atomic absorption and the presence of oxidizable pigments was examined by UV–visible spectroscopy. These experiments further verify the absence of any significant concentrations of free acid and would suggest that the vanadium is predominantly present in an insoluble matrix as concluded previously from electron microscopy[23-25] and that the vanadium complex is made soluble by the oxidation process that produces acid.

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Registry No. Vanadium, 7440-62-2; aquaoxovanadium(IV), 15391-95-4.

Department of Chemistry
University of Queensland
St Lucia, Australia 4067

Stephen G. Brand
Clifford J. Hawkins*
David L. Parry

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Articles

Contribution from the Department of Chemistry,
Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Vanadium(V) Oxyanions. Interactions of Vanadate with Oxalate, Lactate, and Glycerate

Alan S. Tracey,* Michael J. Gresser,* and Kevin M. Parkinson

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The interactions between vanadate and oxalate, lactate, and glycerate in aqueous solution have been studied by ^{51}V nuclear magnetic resonance. Two octahedrally coordinated oxalate derivatives were formed, the mono- and bis(oxalato) compounds. Formation of the mono(oxalato) vanadate compound was accompanied by incorporation of a single proton with a second proton being required for the formation of the bis(oxalato) complex. Formation constants were obtained for the various equilibria. Vanadate, in the presence of lactate, forms a variety of derivatives including a tetrahedrally coordinated lactate ester and two trigonal bipyramidally coordinated products, both mononuclear in vanadium but containing either one or two lactate ligands. Also formed are two binuclear vanadium complexes, one with octahedral coordination about each vanadium nucleus and the other with one octahedral and one tetrahedral vanadium atom. Formation of the bipyramidal products does not require proton incorporation; however, formation of the octahedral derivatives does. Equilibrium constants for the formation of the various lactate derivatives were determined. Glycerate was found to behave similarly to lactate with both bipyramidal and octahedral products being formed. Since octahedral coordination is favored by protonation, lowering the pH serves to promote formation of the octahedral products at the expense of the other derivatives. A description of preferred coordination geometry in terms of electron availability is developed.

Introduction

The chemistry of aqueous solutions of vanadate oxyanions is very complex, with the formation of various oligomeric forms as a function both of pH and of concentration. When in combination with alcohols, vanadate spontaneously forms acyclic mono- and diesters,[1] while with vicinal diols, it forms both cyclic and acyclic compounds.[2] The geometry about vanadium is tetrahedral in the acyclic cases and has been proposed to be trigonal bipyramidal in the cyclic esters.[2] Vanadate in combination with oxalate forms a crystalline octahedral *cis*-bis(oxalate),[3] and it has been assumed that this structure is retained in solution.[4]

The facile formation of vanadate esters and the ease by which vanadate adopts different coordination geometries may lie at the source of the biological importance of vanadium. Vanadium is thought to be an essential element and has significant effects on the function of various enzymes.[5,6]

Vanadate can apparently play a role similar to that of phosphate by acting as a phosphate analogue. Vanadate spontaneously esterifies hydroxyl groups, which are then accepted as enzyme substrates in lieu of the normally phosphorylated substrate. This function has been demonstrated for the dehydrogenase activity of glucose 6-phosphate dehydrogenase, where glucose plus vanadate serves as a substrate to produce gluconic acid.[7] A va-

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nadate/uridine/ribonuclease complex has also been studied by neutron and X-ray diffraction.[8] A trigonal-bipyramidal structure about the vanadium in the enzyme complex is clearly evident, in accord with previous predictions concerning the role of vanadium oxyanions as transition-state analogues.[9]

We have recently embarked on a program to investigate the aqueous chemistry of vanadate with a view to identifying the reaction products and the equilibrium constants for the formation of adducts with various hydroxyl group bearing compounds. With this in mind, systems of increasing complexity have been studied from the simple alcohols ethanol[1] and phenol[10] to various vicinal diols[2] and both phosphate and pyrophosphate.[11] In this study the interactions between vanadate and α-hydroxycarboxylates were investigated as were the interactions between vanadate and the only 1,2-dicarboxylate, oxalate. A remarkable versatility has been revealed by these studies as vanadate rapidly, reversibly, and spontaneously adapts its coordination geometry to a variety of structures and stoichiometries.

Experimental Section

Materials. Reagent grade materials were used without further purification. L-(+)-lactic acid, lithium salt, was purchased from Sigma Chemical Co., oxalic acid from BDH, and DL-glyceric acid, calcium salt, from Aldrich. The preparation of aqueous vanadate solutions has been described.[2,7] All solutions, unless otherwise indicated, were prepared at an ionic strength of 1.00 M by including appropriate amounts of KCl. In calculation of the ionic strength of the solutions, it was assumed that all of the carboxylic acid groups were fully deprotonated throughout the pH range used in the experiments reported here.

Spectroscopy. Nuclear magnetic resonance (NMR) spectra were obtained at ambient temperature at 105 MHz with the multinuclear facility of a Bruker 400-MHz NMR spectrometer. Spectral widths of 20 kHz, 0.05-s acquisition times, and 50° pulse widths were used throughout. A line-broadening factor of 20 Hz and zero filling from 2K to 8K data points were applied to all spectra before transforming to the frequency domain. Chemical shifts reported are relative to the external reference standard, $VOCl_3$, which has been assigned to 0 ppm.

Data Analysis. Base-line roll was removed from all spectra before signal intensities were measured. The signal intensities were obtained with the instrument manufacturer's software, and no effort was made to obtain more accurate integrals.

Least-squares fitting of straight lines to the observed data points was carried out by using the least-squares program of a hand calculator. Errors, in all cases, were estimated to be less than 10% of the determined values and were generally much less than this.

Results and Discussion

Oxalate plus Vanadate. Oxalic acid is known to form an octahedral bis(oxalate) with vanadate, its structure having been assigned on the basis of X-ray diffraction studies.[3] The coordination geometry of the oxalate/vanadate complex in aqueous solution has not been established; indeed, it is extremely difficult to do so. It is, however, possible to determine the number of liganding oxalates, the number of protons consumed or released during formation of the complex, and the respective equilibrium constants. The results of this study are fully consistent with the formation of octahedrally coordinated complexes.

Figure 1 shows the ^{51}V NMR spectrum of a 0.5 mM vanadate solution containing 20 mM oxalate at pH 7.04. The two resonances occurring near −536 ppm correspond to oxalate complexes; the remaining signals are obtained from tetrahedral vanadate, T_i, and its dimer, T_2, and tetramer, T_4, as indicated on the figure. The proportions among all species are very sensitive to ionic strength, and consequently all spectra were obtained from solutions at a constant ionic strength of 1.0 M with KCl as the added electrolyte.

It was possible to establish the number of vanadium nuclei in the complexes simply by varying the total vanadate concentration. When this was done, the proportion of vanadium in T_i to vanadium in the −536 ppm signals remained constant (Table 1s, supplementary material). Therefore, the three individual species have the same number of vanadium nuclei, which is 1. On the basis of the structure of the solid it might be supposed that at least one of the oxalate derivatives has two oxalate groups. If so, the formation might proceed in two steps as indicated in eq 1 and 2,

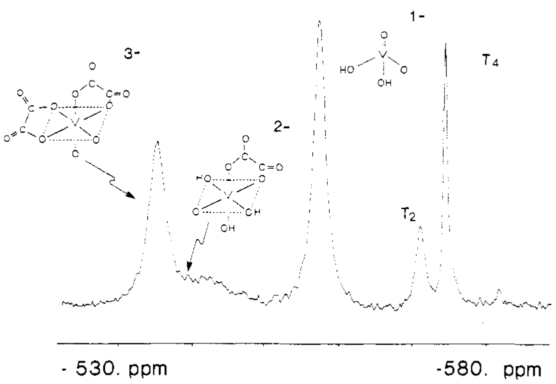

Figure 1. ^{51}V NMR spectrum of the aqueous oxalate/vanadate system. The spectrum was obtained under conditions of 20 mM oxalate, 0.5 mM total vanadate, 20 mM (Tris)Cl, pH 7.04, and 1.0 M ionic strength (KCl). T_2 and T_4 refer to dimeric and tetrameric tetrahedrally coordinated species, respectively. Near −536 ppm a broad signal, almost lost in the base line, corresponds to the mono(oxalato) derivative. Superimposed on this latter resonance is the much sharper signal from the bis(oxalato) complex.

$$T_i + Ox \underset{}{\overset{K_1}{\rightleftharpoons}} VOx \tag{1}$$

$$VOx + Ox \overset{K_2}{\rightleftharpoons} VOx_2 \tag{2}$$

where Ox refers to oxalato, VOx to (oxalato)vanadate, and VOx_2 to bis(oxalato) vanadate. From these equations the product concentrations can be written as eq 3 and 4.

$$[T_i][Ox]K_1 = [VOx] \tag{3}$$

$$[VOx][Ox]K_2 = [T_i][Ox]^2K_1K_2 = [VOx_2] \tag{4}$$

The two resonances in the −536 ppm region of the NMR spectrum consist of a relatively broad signal superimposed on an even broader second signal that is almost lost in the base line. It is difficult to accurately separate the contributions from these two components; however, the signal can be treated as one and a superposition of signals assumed. This allows total integrated intensity to be used when analyzing the results. The corresponding equation, eq 5, is easily obtained from eq 3 and 4. A plot of the

$$\frac{[VOx] + [VOx_2]}{[T_i][Ox]} = K_1 + [Ox]K_1K_2 \tag{5}$$

term on the left of eq 5 vs. oxalate concentration should provide a straight line of intercept K_1 and slope K_1K_2. The changes in concentrations of the various vanadate species as the oxalate concentration was systematically varied were monitored (Table 2s, supplementary material) and the relevant quantities plotted to provide a straight line of slope $K_1K_2 = 2.5 \times 10^3$ M^{-2} and intercept $K_1 = 13$ M^{-1} (Figure 1s, supplementary material). The intercept can be used to calculate the proportion of mono(oxalate) in the solution, and at 20 mM oxalate this is about 20% of total product. This value can be compared with that of about 30% estimated from the NMR integral. Unfortunately, the NMR transition attributed to the mono(oxalate) is so broad and so poorly resolved from that of the bis(oxalate) that sufficiently accurate proportions cannot be obtained directly from the spectrum for use as a reliable check. With use of the intercept and slope of the above plot a value of $K_2 = 2.0 \times 10^2$ M^{-1} can be calculated. Thus at pH 7.04 incorporation of the first oxalate is much less favored than that of the second.

Information concerning the state of protonation of the two products can be obtained by observing the effect of pH on the

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Table I. Concentrations of Various Vanadate Species in Aqueous Oxalate Solution Determined as a Function of pH[a]

pH	$[H^+]$[b]	[VOx] + [VOx$_2$]	[T$_i$]	$[VO_4H_2^-]$[d]	$[VO_4H^{2-}]$[d]	[rem][c]
6.58	2.63	0.808	0.147	0.144	0.003	0.046
6.81	1.55	0.596	0.215	0.208	0.007	0.189
6.91	1.23	0.430	0.262	0.250	0.012	0.308
7.04	0.91	0.327	0.283	0.264	0.019	0.390
7.11	0.78	0.209	0.304	0.280	0.024	0.487
7.18	0.66	0.174	0.315	0.285	0.030	0.511
7.37	0.43	0.085	0.341	0.290	0.051	0.573

[a] Concentrations were determined under conditions of 1.0 mM total vanadate, 20 mM oxalate, and 1 M constant ionic strength (KCl). Tris-HCl (20 mM) was used as the buffer. All concentrations except [H$^+$] are in units of mM. The abbreviations used are as follows: VOx, (oxalato)vanadate; VOx$_2$, bis(oxalato)vanadate; T$_i$, inorganic tetrahedral vanadate. [b] Concentration in units of 10^{-7} M. [c] Total vanadium atom concentration of species not explicitly considered; in units of mM. [d] Concentrations determined from the observed chemical shifts by using eq 3 and the following limiting values: $\delta(VO_4H_2^-)$, 559.8; $\delta(VO_4H^{2-})$, -537.

equilibria. As can be seen from the results given in Table I, the formation of product is quite pH-sensitive, being formed to the extent of about 8% of total vanadate at pH 7.4 and over 80% at pH 6.6; with further decrease in pH the product is formed essentially quantitatively. Hydrogen ion concentration can be incorporated into the equilibrium expression as in Scheme I. This scheme shows some of the species that might be formed, and the proton stoichiometry is indicated. To avoid unnecessary complications, not all of the possible interconversion reactions are depicted in this equation. Within the pH range studied, $VO_4H_2^-$ and VO_4H^{2-} are the only forms of T$_i$ present at significant concentrations since the pH values are all well above the pK_a for fully protonated T$_i$. Forms of VOx carrying more than three negative charges in the pH range studied are ruled out because if they existed at significant fractional levels of the total VOx then increasing pH would favor the formation of VOx. This clearly is not the case as is evident from Table I. This conclusion also implies that VOx is not formed from VO_4H^{2-} since the product would carry four negative charges.

In Table I (also Table 2s) the total T$_i$ as well as T_i^- and T_i^{2-} concentrations are given. The proportion of T_i^- (or T_i^{2-}) in total T$_i$ is provided by the vanadium NMR chemical shift observed (δ(obsd)) compared to the limiting chemical shifts for T_i^- ($\delta(T_i^-)$) and T_i^{2-} ($\delta(T_i^{2-})$), which are -560 and -537 ppm, respectively. Equation 6 expresses the relationship between chemical shifts and concentration.

$$[T_i^-] = \frac{\delta(\text{obsd}) - \delta(T_i^{2-})}{\delta(T_i^-) - \delta(T_i^{2-})}[T_i] \qquad (6)$$

The structure of VOx_2^{3-} in Scheme I corresponds to the structure obtained for the crystalline bis(oxalato)vanadium(V) complex from X-ray diffraction studies.[3] Preliminary correlations of the data in Table I revealed that two protons are taken up in forming the bis(oxalato) complex from T_i^- and two Ox^{2-} ions, in agreement with the observations above. The proton stoichiometry in the individual steps to form the oxalato and bis(oxalato) complex can also be obtained.

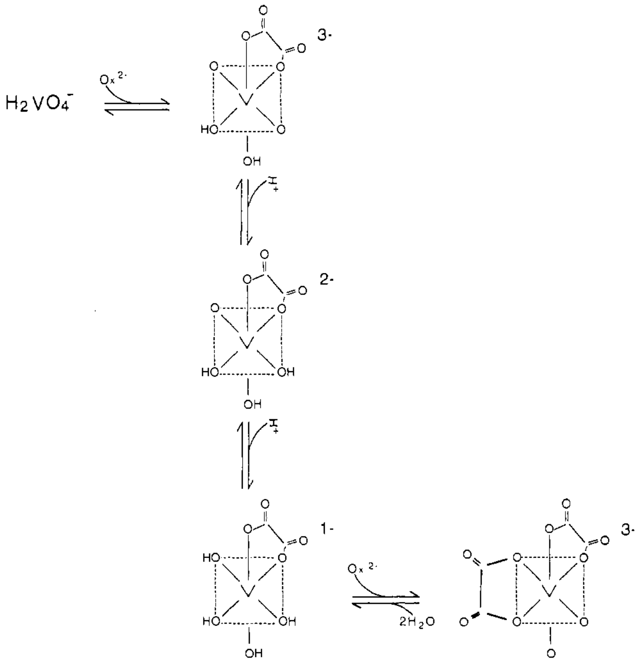
If VOx^{3-} were the only species of VOx present in solution, then the ratio $[VOx]/[T_i^-]$ would be pH-independent. As is clearly evident in Table I, this is not the case. Similarly, if VOx$^-$ were the only species of VOx present, then the ratio $[VOx]/[VOx_2]$ would be pH-independent. Again, as can be seen from Table I, this is not the case. It was, therefore, concluded that VOx^{2-} must constitute a major fraction of the total VOx. This implies that the pK_a values for VOx$^-$ and VOx^{2-} are well outside the range of pH used in this study and that VOx$^-$ and VOx^{3-} are unimportant in Scheme I. If this assumption is made, then the system can be analyzed in terms of eq 7 and 8, from which eq 9 is obtained. If the above assumption were not valid, it would be necessary to also consider reactions of VOx$^-$ and VOx^{3-} and the conservation equation for the total VOx. Fortunately it proved unnecessary to complicate the model in this way.

$$T_i^- + Ox^{2-} + H^+ \underset{}{\overset{K_1'}{\rightleftharpoons}} VOx^{2-} \qquad (7)$$

$$VOx^{2-} + Ox^{2-} + H^+ \overset{K_2'}{\rightleftharpoons} VOx_2^{3-} \qquad (8)$$

$$\frac{[VOx^{2-}] + [VOx_2^{3-}]}{[T_i^-][Ox^{2-}][H^+]} = K_1' + K_1'K_2'[Ox^{2-}][H^+] \qquad (9)$$

Scheme I



On the basis of eq 9 it is predicted that if the ratio ([VOx] + [VOx$_2$])/[T$_i$][Ox][H$^+$] is plotted against [H$^+$][Ox] a straight line of intercept K_1' and slope $K_1'K_2'$ will be obtained. When the appropriate quantities from Table I were plotted, a straight line of intercept $K_1' = 1.4 \times 10^8$ M^{-2} and slope $K_1'K_2' = 2.5 \times 10^{17}$ M^{-4} was obtained (Figure 2s, supplementary material), from which $K_2' = 1.8 \times 10^9$ M^{-1}. This slope and intercept are related to the slope, K_1K_2, and intercept, K_1, of eq 5 (Figure 1s) by the hydrogen ion concentration, so that $K_1K_2 = K_1'K_2'[H^+]^2$ and $K_1 = K_1'[H^+]$. From the above K_1' and $K_1'K_2'$, values of 13 M^{-1} and 2.1 × 10^3 M^{-2} were calculated for K_1 and K_1K_2, respectively, at pH 7.04. These values agree well with those obtained from the oxalate concentration study, where 13 M^{-1} and 2.5 × 10^3 M^{-2} were obtained for the intercept and slope, respectively. The discrepancy between the two studies can be accounted for by an error of 0.04 in pH calibration for the pH 7.04 study, i.e. pH 7.00 instead of pH 7.04.

Addition of oxalate to vanadate is thus seen to proceed readily. The addition, however, is accompanied by protonation so that lower pH favors formation of the products. Since overall the formation of the bis(oxalato) complex requires two protons while that of the mono(oxalato) derivative requires one, the former product is increasingly favored over the latter as the pH is decreased. The ratio of the two formation constants K_2'/K_1' ($=K_2/K_1$) is, however, a constant independent of pH. This ratio indicates that incorporation of an oxalate ligand into (oxalato)vanadate is about 14 times more favored than incorporation of an oxalate into free

Table II. Concentrations of Various Vanadate Derivatives in Aqueous Lactate Solution Determined as a Function of Lactate Concentration[a]

[l]	[BP(t)]	$[Oc_2] + {}^1/_2[TO]$[b]	$[T_i] + [Tl]$	${}^1/_2[TO]$[b]	$[T_2]$	$[T_4]$	[rem][c]
25	0.079	0.015	0.322		0.112	0.424	0.048
50	0.152	0.065	0.314	0.003	0.104	0.320	0.042
100	0.287	0.170	0.269	0.015	0.072	0.172	0.015
150	0.350	0.262	0.216	0.017	0.041	0.069	0.045
200	0.404	0.303	0.185	0.025	0.029	0.032	0.022
300	0.456	0.305					0.239
400	0.510	0.325					0.165
600	0.584	0.305					0.111
800	0.609	0.274					0.117

[a] Concentrations were determined under conditions of 1.0 mM total vanadate, 20 mM Tris-HCl, 1 M constant ionic strength (KCl), and pH 7.35. All concentrations are quoted as vanadium atom concentrations in millimolar quantities. The abbreviations used are as follows: l, lactate; BP(t), total species of bipyramidal coordination; Oc(t), total species of octahedral coordination; TO, binuclear vanadate species of mixed tetrahedral–octahedral coordination; T_i, tetrahedral inorganic vanadate; Tl, lactate ester of tetrahedral vanadate; T_2, dimeric tetrahedral vanadate; T_4, tetrameric tetrahedral vanadate. [b] One signal occurs under the octahedral signal and the other as a shoulder on the tetrahedral signal. [c] Concentrations corresponding to NMR signals from vanadium species not explicitly considered.

vanadate. This phenomenon of positive cooperativity apparently is a general one for the reaction of oxyanionic metalates with bidentate oxy ligands. An explanation suggesting that kinetic lability of the mono complex is induced by the presence of a hydroxo ligand in the mono complex has been based on a study of the interaction of molybdate with catechol.[12]

Lactate plus Vanadate. Lactate in many respects is a considerably more complex molecule than oxalate, having both carboxyl and hydroxyl functional groups. The possibility of increased complexity in its reactions with vanadate is afforded and clearly observed as seen in Figure 2. The signals occurring near −517 ppm in the NMR spectrum are ascribed to trigonal-bipyramidal structures similar to those formed with vicinal diol ligands.[2] Similarly, those signals near −536 ppm are attributed to octahedral species as for oxalate. The remaining signals at higher field are from tetrahedral compounds, inorganic vanadate, T_i, occurring at −556 ppm, divanadate at −571 ppm, and tetravanadate at −575 ppm.

An interesting aspect of Figure 2 is the occurrence of at least three signals in the region of the NMR spectrum that are derived from octahedral species. Under conditions of 1 M constant ionic strength maintained with KCl, these resonances are no longer resolved. A further observation concerning Figure 2 is that a broad low-intensity resonance occurs near the low-field side of the T_i peak. Under conditions of 1 M ionic strength this signal is more clearly resolved and occurs at −550 ppm at pH 7.35. With either increasing lactate concentration or increasing vanadate concentration this signal increases in intensity relative to that of T_i. This behavior shows that the product corresponding to this resonance cannot be a simple lactate ester containing only a single vanadium atom. It is possible to obtain a formation constant for this product from T_i. However, it is possible that the signal attributed to T_i is superimposed on a signal from a lactate ester.

There are two resonances in the NMR spectra that depend on T_i concentration in a direct manner. These resonances are those from dimeric vanadate, T_2, and tetrameric vanadate, T_4. Either of these resonances can be utilized to determine if the −556 ppm signal derives from the single species, T_i. To this end eq 10–12

$$2T_2 \overset{K_0}{\rightleftharpoons} T_4 \qquad (10)$$

$$2T_i \overset{K_1}{\rightleftharpoons} T_2 \qquad (11)$$

$$T_i + l \overset{K_2}{\rightleftharpoons} Tl \qquad (12)$$

can be written, where l refers to lactate and Tl to the tetrahedrally coordinated lactate ester. Tetrahedral coordination is assigned to this product since it has been shown that esterification of alcohols is accompanied by the elimination of water so that the coordination sphere is not expanded.[1,2] From these equations, eq 13 can be obtained and, as well, an analogous equation in T_4. In practice the total concentration, $[T_2] + [T_4]$, is more accurately determined than the individual concentrations because these resonances are close in frequency. It is better to first determine the equilibrium constant, K_0, as defined by eq 10, and then from $[T_2] + [T_4]$ and K_0 calculate $[T_2]$, which can be utilized in eq 13. This process aids in obtaining a more accurate intercept, which defines K_1 in eq 13.

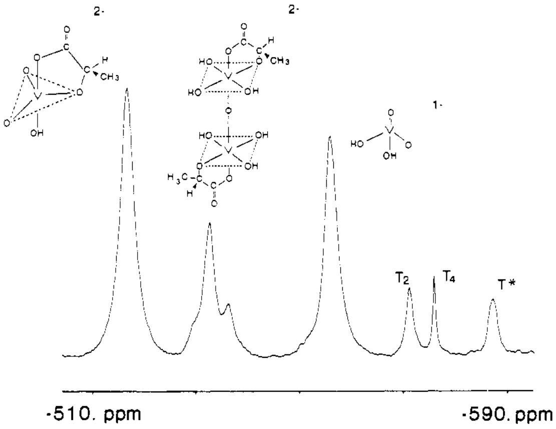


Figure 2. ^{51}V NMR spectrum of an aqueous solution of vanadate with lactate at pH 7.5, 1.0 mM total vanadate, 8 mM (Tris)Cl, and 90 mM lactate. T_2 and T_4 refer to dimeric and tetrameric vanadate, respectively, and T* refers to an inorganic vanadate derivative of undetermined structure (probably T_5), for which no other NMR signal was observed.

$$\frac{[T_i] + [Tl]}{[T_2]^{1/2}} = K_1^{-1/2} + [l]K_1^{-1/2}K_2 \qquad (13)$$

Table II gives the results of a lactate concentration study at 1 mM total vanadate, pH 7.35, and 1 M ionic strength maintained with KCl. A plot of $([T_i] + [Tl])/[T_2]^{1/2}$ vs. [l] gave a straight line of intercept $K_1^{-1/2} = 0.042$ $M^{1/2}$ and slope $K_1^{-1/2}K_2 = 0.023$ $M^{-1/2}$ (Figure 3s, supplementary material), which provided the values $K_1 = 5.6 \times 10^2$ M^{-1} and $K_2 = 0.54$ M^{-1}. From this value of K_2 and with [l] = 0.10 M, it is seen that about 5% of the −556 ppm signal is derived from the lactate ester. This value of K_2 is comparable to the corresponding value obtained for the esterification of ethanol, a primary alcohol, by vanadate, ~0.2 M^{-1}.[1]

Having ascertained the equilibrium constant for formation of the tetrahedral lactate ester, it is now possible to determine the equilibrium constant for formation of the unknown tetrahedral product, TO. It is clear from the results of the lactate and vanadate concentration studies that formation of this product is favored by both increasing lactate and increasing vanadate. On the basis of this, it is possible that this product contains two vanadium atoms and two lactate ligands. This proposal leads to eq 14, from which eq 15 is obtained. Using the information in

$$2T_i + 2l \overset{K_{10}}{\rightleftharpoons} TO \qquad (14)$$

$$[T_i]^2[l]^2K_{10} = [TO] \qquad (15)$$

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Table III. Concentrations of Various Vanadate Derivatives in Aqueous Lactate Solution Determined as a Function of Vanadate Concentration[a]

0.1 M Lactate

V_t	[BP]	$[Oc_2] + {}^1/_2[TO]$	$[T_i]^b$	$[Tl]^b$	${}^1/_2[TO]^b$	$[rem]^c$
0.5	0.175	0.076	0.149	0.008	0.005	0.087
1.0	0.287	0.170	0.259	0.013	0.014	0.259
1.5	0.340	0.272	0.321	0.017	0.021	0.529
2.0	0.417	0.420	0.369	0.020	0.027	0.747

0.4 M Lactate

V_t	[BP]	$[Oc_2] + {}^1/_2[TO]$	$[rem]^c$	V_t	[BP]	$[Oc_2] + {}^1/_2[TO]$	$[rem]^c$
0.5	0.294	0.106	0.100	2.5	1.02	1.06	0.420
1.0	0.514	0.324	0.162	3.0	1.09	1.39	0.520
1.5	0.616	0.542	0.342	3.5	1.14	1.46	0.900
2.0	0.864	0.842	0.294	4.0	1.28	2.00	0.720

[a] Concentrations were determined under conditions of 20 mM Tris-HCl, 1 M constant ionic strength (KCl), and pH 7.35. Lactate concentrations were as shown. Concentrations are vanadium atom concentrations in mM/L. V_t refers to total vanadate in solution while the remaining abbreviations are as for Table II. [b] The proportions of the various components were calculated by using the appropriate equilibrium constants, determined as discussed in the text. [c] Total vanadium atom concentrations in compounds not explicitly considered.

Table II and plotting $[TO]/[T_i]^2$ vs. $[l]^2$ should give a straight line of slope K_{10} if eq 14 is correct. The result of this plot was a straight line of slope $K_{10} = 2.0 \times 10^4\ M^{-3}$ (Figure 4s, supplementary material). It therefore seems that the assumption that this product contains two lactates and two vanadates is correct. Since this product contains two vanadium atoms, it is necessary to establish their identity. It cannot be assumed that both vanadium nuclei give rise to resonances at the same chemical shift. The resonance for the second vanadium may indeed lie under that of the first, at −550 ppm, or under the signals from the bipyramidal or octahedral derivatives. There are three reasonable possibilities for the structure of TO. It may be a ditetrahedral anhydride, a tetrahedral–octahedral anhydride, or a tetrahedral–bipyramidal anhydride. The choice among these three possible structures relies partly upon observations of the effect of pH on the relative intensities of signals from the various vanadate derivatives. Lactate and vanadate concentration studies also provide details that aid in the assignment of a structure to TO.

The effects of changing vanadate concentration at fixed 0.10 M lactate on the concentration of the various species is given in Table III. It is evident from this table that the NMR signal ascribed to the bipyramidal derivative maintains a constant proportionality with the signal from inorganic vanadate, T_i, while the signal that is derived from octahedral species increases strongly relative to that of the inorganic vanadate as total vanadium atom concentration is increased (Figure 5s, supplementary material). These observations indicate that the bipyramidal derivative, BP, contains one vanadium nucleus while the octahedral derivative, Oc_2, contains at least two. On this basis eq 16 and 17 can be written. Equation 16 leads directly to eq 18, from which K_3^0 can be obtained by plotting $[BP]/[l]$ against $[T_i]$ to yield a straight line of slope K_3^0. When the experimental results corresponding to the vanadate concentration study of Table III (Figure 5s) were plotted, a straight line was obtained with a slope of $K_3^0 = 11.0\ M^{-1}$. The linear relationship establishes that the trigonal-bipyramidal complex has only one vanadium nucleus per molecule. It also indicates that no signal that is derived from the unknown compound, TO, lies under the BP resonance.

$$T_i + l \overset{K_3^0}{\rightleftharpoons} BP \tag{16}$$

$$2BP \overset{K_5^0}{\rightleftharpoons} Oc_2 \tag{17}$$

$$[T_i][l]K_3^0 = [BP] \tag{18}$$

There is no simple way to prove that the second resonance from TO lies under the NMR signal from Oc_2; however, analysis of the data in terms of this assumption yields a set of equilibrium constants whose internal consistency is very good. Furthermore, the formation of TO is strongly favored by increasing hydrogen ion concentration (see Figure 4, resonance at −550 ppm). There is no reason for a ditetrahedral species to be favored by decreasing pH since it cannot reasonably be expected to have any sites capable of being protonated in the pH range used here. Also, as seen in Figure 2, there are at least three resonances observed in the region of the NMR spectrum arising from octahedral derivatives. One of these very likely arises from TO. The octahedral derivative, Oc_2, is in equilibrium with TO according to eq 19. The equilibrium constant determined from the results in Table II for this equilibrium is $K_7 = 5.9$.

$$TO \overset{K_7}{\rightleftharpoons} Oc_2 \tag{19}$$

Table III gives the results obtained from vanadate concentration studies at both 0.1 and 0.4 M lactate. In the latter study, only a relatively small proportion of total vanadate occurs as T_i and its concentration is not well-determined.

Equation 20 follows directly from eq 17. K_5^0 can be obtained by plotting $[Oc_2]$ against $[BP]^2$ with use of the data of Table III. When this was done for the 0.1 M lactate concentration, a straight line of slope $1.13 \times 10^3\ M^{-1}$ was obtained. A similar plot for the 0.4 M lactate concentration gave a straight line of slope $0.58 \times 10^3\ M^{-1}$. That these two plots are linear shows that the octahedral derivatives does contain two vanadium nuclei. However, the fact that the slopes of the two graphs are different shows that eq 16 and 17 are not complete. The lactate concentration study, done at constant total vanadate concentration of 1.0 mM at pH 7.35 and 1 M ionic strength maintained with KCl, allows resolution of this problem. A series of spectra from this study are displayed in Figure 3. A particularly important observation to be made from this concentration study is that the proportion of octahedral species, relative to bipyramidal derivatives, initially increases strongly with concentration of lactate, levels off, and then begins to decrease. This result supports the previous conclusion that eq 16 and 17 must be expanded. It is important first to confirm the presence of only one octahedral species other than TO. Considering the possibility of the presence of an octahedral species containing only one vanadium atom, eq 21 can be written, which, in combination with eq 16 and 17, provides eq 22. The factor of 2 is included since a sum of superimposed signals from Oc_1 and Oc_2 requires the use of vanadium atom concentration for Oc_2 rather than its molar concentration. In this expression K_3^0 cannot be zero ($K_3^0 = 11.0\ M^{-1}$) so that a plot of $([Oc_1] + 2[Oc_2])/[T_i][l]$ vs. $[T_i][l]$ will give a straight line of slope $2(K_3^0)^2K_5^0$ and intercept $K_3^0K_5'$. If no monomeric species is present, $K_5' = 0$ and a zero intercept will be obtained. The data of Tables II and III in conjunction with the equilibria involving K_1 (eq 11) and K_{10} (eq 14) were used in order to obtain the relevant quantities to be plotted. The graph (Figure 6s, supplementary material) clearly

$$[BP]^2K_5^0 = [Oc_2] \tag{20}$$

$$BP \overset{K_5'}{\rightleftharpoons} Oc_1 \tag{21}$$

$$[Oc_1] + 2[Oc_2] = [T_i][l]K_3^0K_5' + 2[T_i]^2[l]^2(K_3^0)^2K_5^0 \tag{22}$$

Table IV. Concentrations of Various Vanadate Derivatives in Aqueous Lactate Solution Determined as a Function of pH[a]

pH	$[H^+]$[b]	[BP]	[BPl]	$[Oc_2]$	$^1/_2$[TO][c]	$[VO_4H_2^-]$[d]	$[VO_4H^{2-}]$[d]	[Tl][c]	[rem][e]
7.91	1.23	0.226	0.029	0.020	0.002	0.227	0.143	0.019	0.331
7.75	1.78	0.240	0.031	0.038	0.003	0.226	0.105	0.018	0.335
7.62	2.40	0.235	0.030	0.048	0.004	0.238	0.082	0.018	0.341
7.51	3.09	0.237	0.031	0.094	0.008	0.237	0.061	0.016	0.307
7.35	4.47	0.252	0.033	0.153	0.013	0.218	0.039	0.014	0.265
7.20	6.31	0.231	0.030	0.233	0.021	0.202	0.027	0.013	0.222
7.11	7.76	0.230	0.030	0.322	0.028	0.186	0.021	0.011	0.144
6.79	16.22	0.182	0.023	0.548	0.048	0.113	0.008	0.006	0.030

[a] Concentrations were determined under conditions of 1.0 mM total vanadate, 20 mM Tris-HCl, 1 M constant ionic strength (KCl), and varying pH. Concentrations were vanadium atom concentrations in millimolar quantitites except $[H^+]$, which is in units of 10^{-8} M. BPl refers to a bis(lactato)vanadate derivative of bipyramidal coordination while the other abbreviations are as for Table II. [b] Concentrations given are in units of 10^{-8} M. [c] Concentrations given are calculated by assuming the appropriate equilibrium constants, determined as discussed in the text, are pH-independent. [d] Concentrations were calculated from the total concentration of T_i and the chemical shift of the T_i resonance by using the limiting chemical shifts $\delta(VO_4H_2^-) = -559.8$ and $\delta(VO_4H^{2-}) = -537$ and eq 6. [e] Vanadium atom concentration in species not explicitly considered.

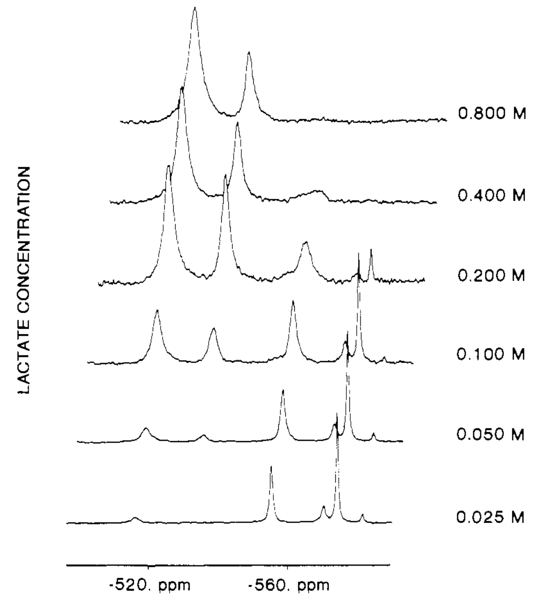


Figure 3. ^{51}V spectra showing the change in relative intensities of the various vanadate resonances as lactate concentration is increased. Of major interest is the change in proportion of the signal intensity from the bipyramidal derivative (−517 ppm) compared to the octahedral product (−533 ppm). The proportion of the octahedral compound is initially seen to gain rapidly on that of the bipyramidal derivative but at the higher lactate concentrations starts to decrease. This results because the formation of the bipyramidal bis(lactate) derivatives becomes increasingly important at the higher lactate concentrations. The conditions of the experiments were 1.0 mM total vanadate, 20 mM (Tris)Cl, 1.0 M ionic strength (KCl), pH 7.35, and varying amounts of lactate.

had an intercept very close to zero in accordance with our previous suggestion that no significant proportion of octahedral monomer is present in solution. The zero intercept means that eq 17 is valid. Equation 16 must therefore be incorrect, and this must be the reason for the discrepancy in K_5^0 values obtained from the two data sets of Table III. The observation of at least two resonances (other than from TO) near −553 ppm (Figure 2) must therefore indicate the presence of geometrical isomers of the octahedral product.

Although it has been shown that all of the intensity in the signal at −517 ppm is from species containing only one vanadium nucleus, there is the possibility that a portion of the total BP resonance is derived from a species containing two lactate ligands. In this case K_3^0 of eq 16 is replaced by K_3' and eq 23 is written, from which eq 24 is obtained. In turn, eq 24 can be combined with

$$BP + l \xrightleftharpoons{K_4} BPl \qquad (23)$$

$$[BP][l]K_4 = [BPl] \qquad (24)$$

eq 20 (where K_5 now replaces K_5^0) to give the sum, eq 25. When

$$[BP] + [BPl] = [Oc_2]^{1/2}K_5^{-1/2} + [Oc_2]^{1/2}[l]K_4K_5^{-1/2} \qquad (25)$$

the ratio $([BP] + [BPl])/[Oc_2]^{1/2}$ is plotted against lactate concentration, a straight line with intercept $K_5^{-1/2}$ and slope, $K_4K_5^{-1/2}$ should be obtained. When the appropriate quantities were plotted (Figure 7s, supplementary material), a straight line of intercept 0.027 $M^{1/2}$ and slope 0.036 $M^{-1/2}$ was obtained, which provides the equilibrium constants $K_5 = 1.37 \times 10^3\ M^{-1}$ and $K_4 = 1.34\ M^{-1}$. From this value of K_4 it can be calculated that at 0.10 M lactate and pH 7.35 about 88% of the signal intensity corresponding to bipyramidal compounds is derived from the mono(lactate) derivative. From the value of 11.0 M^{-1} previously obtained for K_3^0, a value for K_3' of 9.7 M^{-1} is calculated for the formation of BP from T_i and lactate. With this value for K_3' and the slope ($2.6 \times 10^5\ M^{-3}$) of the line in Figure 6s (from eq 22 in which K_3^0 is now replaced by K_3') a second value for K_5 of $1.39 \times 10^3\ M^{-1}$ can be obtained. The correpondence between this and the above value for K_5 is excellent.

There is an alternative means of determining K_3' and K_4. Combination of eq 18 (K_3' replacing K_3^0) with eq 24 and rearrangement provide eq 26. From the results given in Table II,

$$\frac{[BP] + [BPl]}{[T_i][l]} = K_3' + [l]K_3'K_4 \qquad (26)$$

the ratio of concentrations plotted vs. [l] gives a straight line of intercept $K_3' = 9.6\ M^{-1}$ and slope $K_3'K_4 = 13.5\ M^{-2}$ from which $K_4 = 1.41\ M^{-1}$. The close correspondence between these values and those determined in the preceding paragraph provides a valuable confirmation of the internal consistency of the model used to analyze the data.

The effect of pH on the equilibria is quite remarkable. A change of about 1.2 pH units is sufficient to convert most of the vanadate from tetrahedral reactants to octahedral products. Figure 4 shows various NMR spectra obtained as a function of pH. It is clear from this figure that the octahedral derivatives are stabilized, relative to the other vanadates, by increasing hydrogen ion concentration. An analytical solution to the entire system is not possible when all states of protonation must be considered since there is superposition of too many signals. It is, however, possible to obtain information concerning the state of protonation of the octahedral dimer by using the resonance from the tetrahedral species $VO_4H_2^-$. The two tetrahedral derivatives $VO_4H_2^-$ (T_i^-) and VO_4H^{2-} (T_i^{2-}) are rapidly equilibrating in solution. The proportions between the two species are provided by the averaged chemical shift, which is observed, through utilization of eq 6.

Table IV gives the concentrations of the two compounds, T_i^- and T_i^{2-}, as a function of pH, the proportions being determined by the observed shift. The concentration of inorganic tetrahedral vanadate, T_i, is determined from total monomeric tetrahedral vanadate by assuming that the equilibrium constant for formation of Tl from T_i is pH-independent in the pH range studied. The concentration of TO is calculated from that of total octahedral products by assuming pH invariance of its formation constant from Oc_2. In this latter case it is possible to directly observe one of the TO signals at pH 6.79. The calculated concentration agrees to within 25% of the observed concentration, which itself has a large error associated with its determination at this low pH. Even

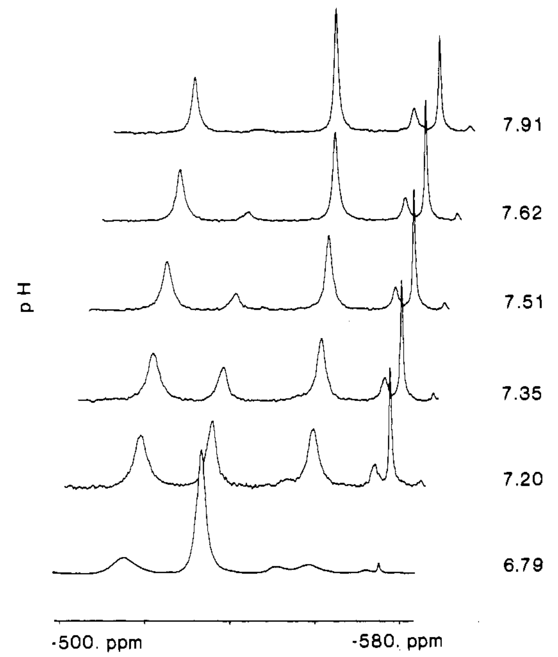


Figure 4. Effect of pH on the various equilibria of the aqueous vanadate/lactate system. The rapid decrease in NMR signal intensity from the octahedral product (−533 ppm) as the pH is increased indicates that incorporation of a proton is required for formation of this product. At the lowest pH the signal at −550 ppm, ascribed to the tetrahedral vanadate of the tetrahedal–octahedral mixed anhydride (TO), is clearly observed.

at the lowest pH the signal from TO represents only 25% of total signal intensity from *tetrahedral* species and the contribution decreases rapidly as the pH is raised. As a consequence errors associated with the assumption of pH invariance of the above equilibrium constants are assumed negligible.

The equilibria expressed in eq 27 and 28 may be used to represent the observation that formation of the octahedral derivative is favored at lower pH. These two equilibria provide eq 29 and 30. Addition of eq 29 to eq 30 followed by rearrangement gives eq 31. The result of plotting the experimental data according to this equation provided a linear relationship of non-zero intercept that supports the occurrence of the equilibria expressed in eq 27 and 28 and clearly demonstrates the incorporation of first one and then a second proton into the octahedral product. The equilibrium constants K_{12} = 2.9 × 10^{11} M^{-4} and K_{13} = 2.7 × 10^{8} M^{-1} are determined from the slope and intercept (Figure 8s, supplementary material). Equation 28 represents a protonation step so that a pK_a of 8.4 is obtained from dissociation of a proton from $Oc_2H_2^{2-}$ to yield Oc_2H^{3-}. Examination of the experimental data provided no evidence for formation of nonprotonated product. This indicates that formation of the octahedral product from $VO_4H_2^-$ requires the uptake of at least one proton. In a subsequent step a second proton is incorporated into the product.

$$2T_i^- + 2l^- + H^+ \overset{K_{12}}{\rightleftharpoons} Oc_2H^{3-} \quad (27)$$

$$Oc_2H^{3-} + H^+ \overset{K_{13}}{\rightleftharpoons} Oc_2H_2^{2-} \quad (28)$$

$$[T_i^-]^2[l]^2[H^+]K_{12} = [Oc_2H^{3-}] \quad (29)$$

$$[Oc_2H^{3-}][H^+]K_{13} = [Oc_2H_2^{2-}] \quad (30)$$

$$\frac{[Oc_2H^{3-}] + [Oc_2H_2^{2-}]}{[T_i^-]^2[l^-]^2[H^+]} = K_{12} + K_{12}K_{13}[H^+] \quad (31)$$

Table V. Selected Chemical Equilibria and Corresponding Equilibrium Constants for the Lactate/Vanadate Aqueous System[a]

equation	equil const
pH 7.35	
$2T_2 \overset{K_0}{\rightleftharpoons} T_4$	3.2 × 10^{4} M^{-1}
$2T_i \overset{K_1}{\rightleftharpoons} T_2$	5.6 × 10^{2} M^{-1}
$T_i + l \overset{K_2}{\rightleftharpoons} Tl$	0.54 M^{-1}
$T_i + l \overset{K_3'}{\rightleftharpoons} BP$	9.7 M^{-1}
$Tl \overset{K_3}{\rightleftharpoons} BP$[b]	18
$BP + l \overset{K_4}{\rightleftharpoons} BPl$	1.4 M^{-1}
$2BP \overset{K_5}{\rightleftharpoons} Oc_2$	1.4 × 10^{3} M^{-1}
$Tl + BP \overset{K_6}{\rightleftharpoons} TO$[c]	3.8 × 10^{3} M^{-1}
$TO \overset{K_7}{\rightleftharpoons} Oc_2$	5.9
$2T_i + 2l \overset{K_{10}}{\rightleftharpoons} TO$	2.0 × 10^{4} M^{-3}
$2T_i + 2l \overset{K_{11}}{\rightleftharpoons} Oc_2$	1.3 × 10^{5} M^{-3}
pH Varied	
$2VO_4H_2^- + 2l^- + H^+ \overset{K_{12}}{\rightleftharpoons} Oc_2H^{3-}$	2.9 × 10^{11} M^{-4}
$Oc_2H^{3-} + H^+ \overset{K_{13}}{\rightleftharpoons} Oc_2H_2^{2-}$	2.7 × 10^{8} M^{-1}
$VO_4H_2^- + l^- \overset{K_{14}}{\rightleftharpoons} BP^{2-}$	9.3 M^{-1}
$BP^{2-} + H^+ \overset{K_{15}}{\rightleftharpoons} BPH^-$	4.5 × 10^{6} M^{-1}

[a] Estimated errors in equilibrium constants for all equilibria are less than 10% of the reported values; abbreviations are as for Tables II and III. [b] Calculated from K_3'/K_2. [c] Calculated from K_2K_3'/K_{10}.

It is possible that the bipyramidal derivative, BP, also requires protons for its formation. Since it is not favored relative to the octahedral product as the pH is decreased, it may be that only one proton is incorporated during its formation. In this case eq 32 and 33 may apply. These two equations provide eq 34 and 35, which when combined lead directly to eq 36. When the values of Table IV are plotted according to eq 36, a straight line of intercept 9.31 M^{-1} and slope 4.15 × 10^{7} M^{-2} is obtained (Figure 9s, supplementary material). It is clear from this result that the bipyramidal derivative does not require protonation for its formation, which occurs from T_i^- with an equilibrium constant, K_{14} = 9.3 M^{-1}. Subsequent protonation can occur, however, and the equilibrium constant, K_{15}, for protonation of this product is 4.5 × 10^{6} M^{-1}, from which a pK_a of 6.65 is calculated for the dissociation of a proton from BPH$^-$ to yield BP^{2-}.

$$T_i^- + l^- \overset{K_{14}}{\rightleftharpoons} BP^{2-} \quad (32)$$

$$BP^{2-} + H^+ \overset{K_{15}}{\rightleftharpoons} BPH^- \quad (33)$$

$$[T_i^-][l^-]K_{14} = [BP^{2-}] \quad (34)$$

$$[BP^{2-}][H^+]K_{15} = [BPH^-] \quad (35)$$

$$\frac{[BP^{2-}] + [BPH^-]}{[T_i^-][l^-]} = K_{14} + K_{14}K_{15}[H^+] \quad (36)$$

The equilibrium constant (K_3') for formation of BP from T_i was previously determined as 9.7 M^{-1} at pH 7.35. From the proportion of $VO_4H_2^-$ (T_i^-) to VO_4H^{2-} (T_i^{2-}) given in Table IV and by use of the slope and intercept for eq 36 (Figure 9s), a value for K_3' of 9.5 M^{-1} can be obtained for pH 7.35. The agreement between this latter determination and the previous one is excellent, which lends strong support to this interpretation of the results.

In an effort to draw together the various equilibria of this lactate system, Table V was constructed. The table lists various chemical

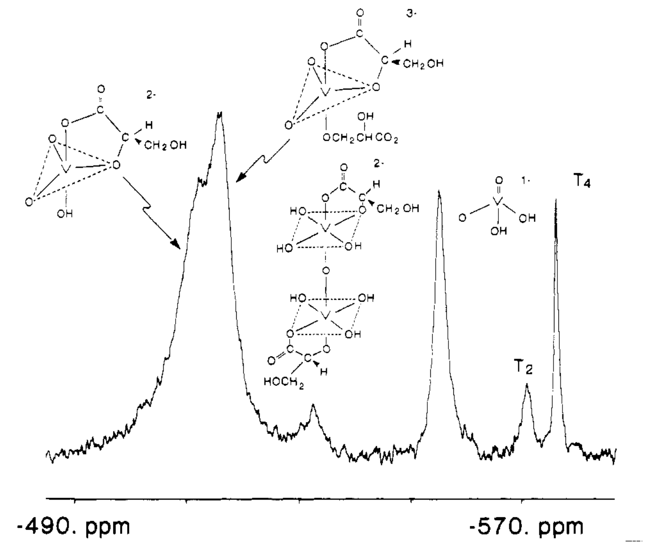


Figure 5. ^{51}V NMR spectrum of vanadate in aqueous solution with glycerate. Mono- and bis(glycerato) trigonal-bipyramidal products provide partially resolved NMR signals near −516 ppm. Octahedral products in low concentrations are also observed. The spectrum was obtained under conditions of 20 mM (Tris)Cl, 70 mM glycerate, 0.54 M sodium acetate, 2 mM total vanadate, and pH 7.5.

equilibra for this system and the corresponding equilibrium constants.

Glycerate plus Vanadate. The interactions between glycerate and vanadate represent a stage of complexity even greater than that between lactate and vanadate. With glycerate there exists the possibility of bipyramidal complexes that involve, in a one case, the vicinal 2,3-hydroxyls or, in the second case, the 2-hydroxyl and the carboxylate. The first situation is analogous to that of the reaction of vanadate with ethylene and propylene glycol[2] while the second situation is similar to that of its reaction with lactate.

Figure 5 shows an NMR spectrum obtained for a 2 mM vanadate, 70 mM glycerate solution at pH 7.5 and 0.54 M in sodium acetate. The spectrum clearly shows the presence in solution of two components, which give signals in the region of the spectrum at −513 and −517 ppm, ascribed to bipyramidal derivatives. Signals are also observed at −533 ppm, which as for lactate are assigned to octahedral compounds. The relative intensity of this latter resonance is favored with decrease in pH and with increase in vanadate. The major compound giving rise to this signal is assigned a structure similar to that of the analogous lactate; that is, it is the anhydride of (glycerato)trihydroxooxovanadate(2−) as indicated in Figure 5. The possibility of superposition of resonances from other compounds cannot be ruled out.

In the region of the NMR spectrum corresponding to tetrahedral vanadates only one signal is observed (−556 ppm) in addition to those from dimeric (−571 ppm) and tetrameric (−575 ppm) tetrahedral vanadates. If acyclic glycerate esters of vanadate are formed, they must give rise to signals superimposed on that from free vanadate, providing the signal identified as T$_i$(t). These species all contain one vanadium nucleus so that as the amount of vanadate in solution is varied their proportions remain constant. As a consequence of this, a vanadate concentration study can be used to define the number of vanadium nuclei in the bipyramidal species, which give rise to the −513 and −517 ppm resonances. Equilibrium constants for individual reactions, of course, cannot be obtained.

When a vanadate concentration study was done, the proportion of the resonances from T$_i$(t) to BP(t) remained constant over an eightfold change in total vanadate concentration, 0.25 to 2.0 mM (Figure 10s, supplementary material). Thus, there is no evidence of bipyramidal species containing more than one vanadium atom throughout the vanadate concentration range studied. This is an interesting result since a study of the ethylene glycol/vanadate system showed that in that case the bipyramidal derivative was a dimer with no detectable monomeric species.[2] This suggests that the two bipyramidal derivatives observable with glycerate are formed via coordination with the carboxylate and 2-hydroxyl groups as with lactate rather than between the vicinal hydroxyl groups of the 2- and 3-positions as for ethylene glycol. Such a conclusion is not surprising since, if the equilibrium constant for formation of the bipyramidal derivative with ethylene glycol[2] were even approximately correct for the 2,3-hydroxyls of glycerate, there must be much less than 1% (compared to T$_i$) of the corresponding glycerate product in solution.

If the analogy with lactate can be pursued further, then both mono- and bis(glycerate) bipyramidal species can be expected. When the vanadate concentration was held constant at 1 mM and glycerate concentration increased from 17.5 to 70 mM, it was observed that the proportion of the signal at lower field (−513 ppm) decreased relative to that at higher field (−517 ppm). This shows that the −513 ppm resonance is derived from a mono(glycerate) species while that of the −517 ppm resonance is from a bis(glycerate). These compounds are therefore identified as (glycerato)hydrooxovanadate(2−) and (glycerato-O^3)(glycerato)dioxovanadate(2−) as depicted in Figure 5 for the −513 and −517 ppm resonances, respectively. The linkage in the latter compound is shown in the figure as occurring through the primary hydroxyl oxygen, but it must be recognized that it may be through the secondary position; indeed, there may be a mixture of the two.

Conclusions

Addition of oxalate to vanadate, $VO_4H_2^-$, was found to proceed readily. The addition is accompanied by protonation so that lower pH favors formation of the product. The addition occurs in two steps with the formation of first a mono(oxalato) complex and then with a further oxalate a bis(oxalato) derivative. The mono(oxalate), trihydroxo(oxalato)oxovanadate(2−), requires a single proton for its formation. Incorporation of a second oxalate to form bis(oxalato)dioxovanadate(3−) requires an additional proton. The equilibrium constant, K_1', for formation of trihydroxo(oxalato)oxovanadate(2−) from oxalate, tetrahedral vanadate, and a proton was found to be 1.4×10^8 M^{-2} while the formation constant for bis(oxalato)dioxovanadate(3−) from the mono(oxalato) derivative, oxalate, and a proton was found to have an equilibrium constant, K_2', of 1.8×10^9 M^{-2}. At pH 7.04 and from an oxalate concentration study the corresponding K_1 and K_2 values of 13 M^{-1} and 2.0×10^2 M^{-1}, respectively, for the formation of the mono- and bis(oxalates) were measured. These results are in accord with the formation of products with octahedral coordination.

A complex system of equilibrium reactions is set up when lactate and vanadate are combined in aqueous medium. Tetrahedral, octahedral, and trigonal-bipyramidal structures have been assigned to the (lactato)vanadate derivatives that are formed in various states of substitution and protonation.

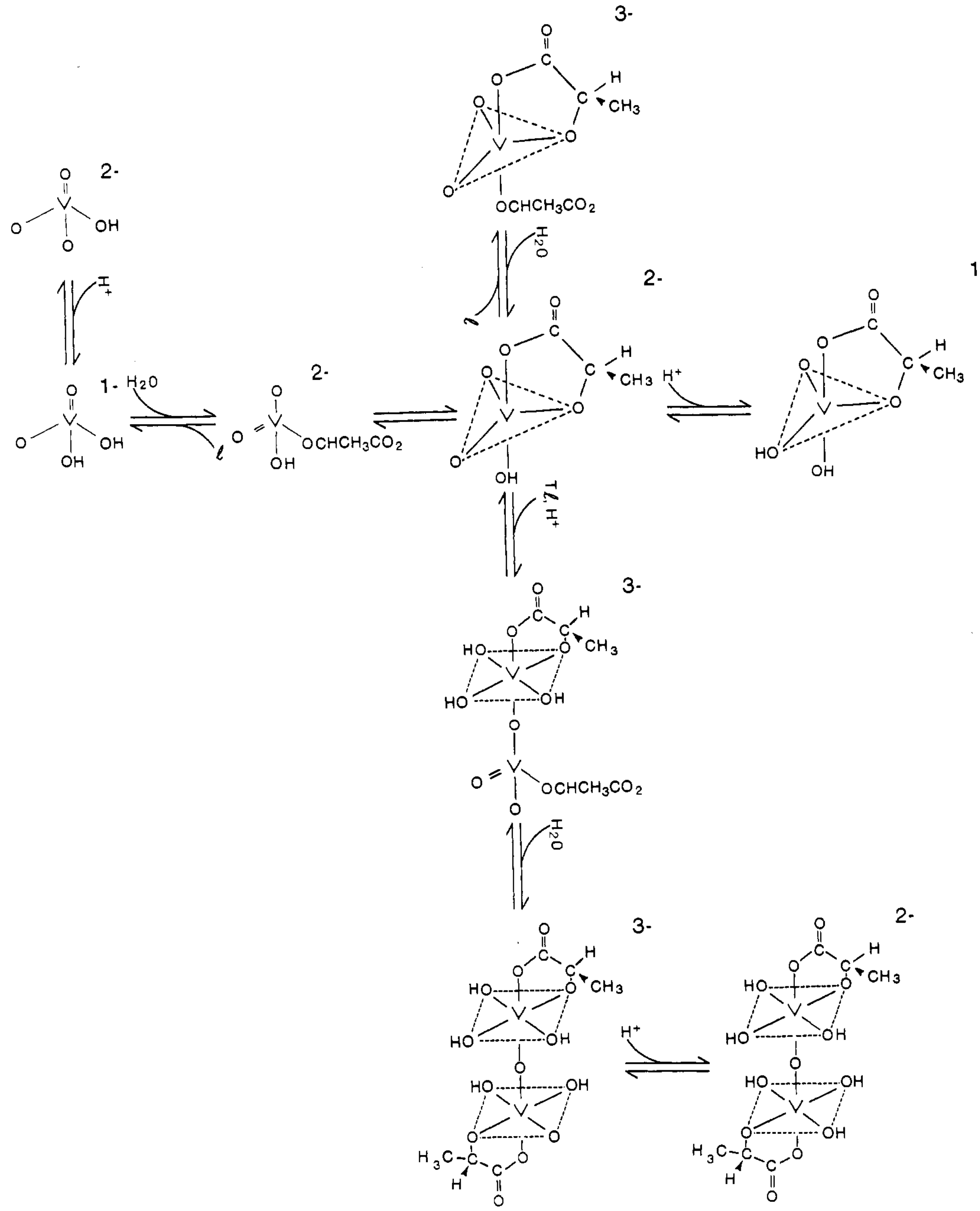
The octahedral product was formed as an anhydride, binuclear in vanadium, with no evidence for a significant proportion of the monomeric derivative. Formation of the octahedral complex was accompanied by protonation, and thus its formation is favored by decrease in pH. This product could be further protonated with a pK_a of 8.4.

The bipyramidal derivatives were formed as monomeric compounds. Both mono- and bis(lactate) derivatives were formed. Protonation was not required for formation of these products. The mono(lactate) was, however, protonated at lower pH. The pK_a for dissociation of this latter product was found to be 6.6.

A tetrahedral–octahedral mixed anhydride was also identified, and formation of this product like that of the octahedral derivative is favored by decrease in pH. Protonation of this product must occur on the octahedral residue since, in the pH range studied here, there are no available sites for protonation on the tetrahedral half of this compound. Unfortunately it was not possible to determine a formation constant as a function of pH.

One further product was identified as a tetrahedral vanadate ester with esterification occurring at the hydroxyl of the lactate. The formation of this product was not favored when compared to the other products.

Scheme II



In an attempt to combine the various equilibria into a coherent system of chemical reactions Scheme II was written. Table V provides various equilibrium constants of interest for this system.

Glycerate was found to behave similarly to lactate with the formation of both trigonal-bipyramidal and octahedral products. As for lactate, the bipyramidal products were found to be mononuclear in vanadium while the octahedral products were formed as binuclear anhydrides. Two bipyramidal products were observed and identified as mono- and bis(glycerates). No evidence for cyclic derivatives involving the vicinal hydroxyl groups of the glycerate moiety, similar to those observed for ethylene glycol,[2] was found.

There is an apparent progression in coordination geometry of the vanadate as the oxidation state of the ligand is increased. Vanadate in the presence of ethanol forms only the tetrahedral mono- and diethylvanadates in observable amounts.[1] In the presence of ethylene glycol not only are the esters, mono- and bis(2-hydroxyethyl) vanadates, formed, but also a trigonal-bipyramidal derivative that exists as an anhydride, binuclear in vanadium, in which ethylene glycol is a bidentate ligand.[2] In the presence of lactate (2-hydroxypropanoate), octahedral products are observed as well as the bipyramidal and tetrahedral species. In this case the octahedral product is formed as a binuclear anhydride while the bipyramidal product is monomeric. Furthermore, formation of the octahedral product requires at least one proton while that of the bipyramidal derivative does not require a proton.

In the presence of the highly oxidized compound oxalate, the only observed products are monomeric octahedral compounds formed as both mono- and bis(oxalates). Incorporation of each oxalate requires uptake of a single proton.

The coordination geometry thus seems to be determined by a delicate balance of charge at the vanadium nucleus. The uncharged bidentate ligand, ethylene glycol, promotes the expansion of the coordination geometry from tetrahedral to trigonal bipyramidal. This latter product apparently is electron-rich. Electron density at the nucleus could be reduced by protonation, but the preferred pathway is anhydride formation. Other things being equal, anhydride formation decreases the number of electrons on oxygen that are available for interaction with vanadium by one electron per vanadium nucleus to be compared with a reduction of two electrons per nucleus if protonation of the monomeric unit occurred. The electrons on the oxygen atoms presumably are involved in bonding through the d orbitals of the vanadium nucleus.

Lactate is a more strongly electron withdrawing group than ethylene glycol. The bipyramidal product formed with lactate is now not electron-rich, and anhydride formation does not occur; indeed, there is a tendency to accept more electrons by expanding the coordination shell by addition of water. This leads to an octahedral product that now is electron-rich. This compound reduces the electron density by anhydride formation accompanied by uptake of a single proton. A further proton can be taken up in a subsequent reaction.

Oxalate is a strongly electron withdrawing group. No bipyramidal product is observed with this ligand. Formation of the octahedral product is accompanied by protonation, but a futher decrease in electron density through anhydride formation is not observed. In this case incorporation of a second oxalate accompanied by uptake of a further proton can and does occur, leading to the major product.

If this description of coordination geometry in terms of electron availability is valid, then it can be predicted that if highly electron withdrawing alcohols are used with vanadate in an effort to form tetrahedral derivatives, bipyramidally or even octahedrally coordinated products may be formed. Sufficiently electron withdrawing diols may lead to the formation of bipyramidal monomeric and octahedral forms as well as the expected bipyramidal anhydrides. Similarly, α-hydroxy acids containing electron-withdrawing groups may form only minimal proportions of bipyramidal products. Conversely, the presence of electron-donating substituents should force the equilibria toward less substituted products.

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Supplementary Material Available: Listings of concentrations of various vanadate species determined as a function of total vanadate and of oxalate concentration (Tables 1s and 2s) and various plots concerning vanadate complex formation (Figures 1s-10s) (12 pages). Ordering information is given on any current masthead page.

Contribution from the Institute of Inorganic Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland,
and Chemistry Department, University of Virginia, Charlottesville, Virginia 22903

Ternary Complexes in Solution.[1,2] Intramolecular Equilibria in Metal Ion Complexes of Adenosine 5′-Triphosphate (ATP^{4-}): Coordination of Ammonia or Imidazole to $M(ATP)^{2-}$ Releases N-7 from the Metal Ion Coordination Sphere

Roger Tribolet,[3a] R. Bruce Martin,*[3b] and Helmut Sigel*[3a]

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By ^1H NMR shift measurements in D_2O at 27 °C under conditions where the monomeric complexes dominate, it is shown by monitoring the shifts of H-2, H-8, and H-1′ of $Cd(ATP)^{2-}$ and $Zn(ATP)^{2-}$ (ATP^{4-} = adenosine 5′-triphosphate) that upon formation of ternary complexes with ammonia or imidazole N-7 is released from the coordination sphere of the metal ions. These results together with earlier observations show that mixed-ligand complex formation between $M(ATP)^{2-}$ and a unidentate (e.g., OH^-, NH_3, imidazole) or bidentate ligand (e.g., 2,2′-bipyridyl, tryptophanate) leads to release of the adenine moiety from the coordination sphere. A careful analysis of stability data for ternary systems consisting of Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}/ATP, UTP/imidazole, OH^- confirms the results obtained by NMR spectroscopy. For $Ni(ATP)^{2-}$ and $Cu(ATP)^{2-}$ the analysis shows that addition of imidazole at least reduces (and possibly also eliminates) the extent of N-7 back-bonding. These results are utilized to calculate the percentage (or its lower limit) of the macrochelated isomer of several $M(ATP)^{2-}$ complexes by employing the difference in complex stability between the ternary $M(ATP)(imidazole)^{2-}$ and $M(UTP)(imidazole)^{2-}$ complexes (UTP^{4-} = uridine 5′-triphosphate). This evaluation is possible because in $M(UTP)^{2-}$ no metal ion/base interaction occurs, allowing an easier access of imidazole (or other ligands, such as NH_3 and OH^-) to the metal ion than in $M(ATP)^{2-}$, where in the back-bound isomer N-7 has to be substituted. The probable consequences of such structural alterations for enzymic systems are briefly indicated.

Adenosine 5′-triphosphate (ATP^{4-}) in the form of metal ion complexes is the substrate of many enzymic reactions.[4] Evidently, during the reaction process at least, ternary complexes of the kind enzyme/metal ion (M^{2+})/nucleotide must be formed.[4-6] Consequently, mixed-ligand complexes of ATP are receiving increasing attention.[7-12]

For the metal ions of the second half of the first transition series and Zn^{2+}, as well as Cd^{2+}, it is known[13,14] that in the binary $M(ATP)^{2-}$ complexes considerable base back-binding occurs.[15] Thus, a significant amount of these complexes contains in equilibrium a species in which the metal ion is bound not only to the triphosphate chain but also to N-7 of the adenine moiety, thus forming a macrochelate. A question arises upon ternary complex formation in such systems: Does this metal ion/N-7 interaction still exist in mixed-ligand complexes? For bidentate ligands such as 2,2′-bipyridyl, 1,10-phenanthroline, and tryptophanate, N-7

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