Vanadium(V) Oxyanions: The Interaction of Vanadate with Pyrophosphate, Phosphate, and Arsenate

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Abstract: The interactions between vanadate and pyrophosphate, phosphate, and arsenate have been studied by vanadium NMR spectroscopy. It was found that all three of the anions studied formed mixed anhydrides with vanadate analogous to pyrophosphate or triphosphate. The mixed anhydrides are one to two orders of magnitude less stable toward hydrolysis than is divanadate under similar conditions, but they are approximately 10^6 times more stable toward hydrolysis than pyrophosphate at pH 7. Formation of the mixed anhydrides is sufficiently fast that they are in rapid chemical exchange with their hydrolysis products on the NMR time scale. With pyrophosphate, a second type of product containing one vanadium atom is formed when the pH is decreased from 8.0 to 7.0. A similar species is not formed with either phosphate or arsenate. A structure in which two pyrophosphate moieties act as bidentate ligands and the central vanadium atom is octahedrally coordinated to six oxygen atoms is proposed for this species. The relevance of this study to various enzymic reactions is discussed.

The chemistry of vanadium(V) oxyanions in aqueous solution is very complex, the predominant behavior being that of oligomerization to form a host of polyvanadate species. When vanadate is combined with oxymetallates, various heteropolyanionic forms are also produced.[1-3] A tool of major importance for the study of vanadate reactions in solution is nuclear magnetic resonance (NMR) spectroscopy, which provides the sensitivity and spectral dispersion to allow identification of minor as well as major components of reaction solutions.

The aqueous chemistry of vanadate is of particular interest in biochemistry since there is considerable evidence which suggests that vanadium is an essential element. It is well-known that vanadium in moderate levels has a significant impact on the function of enzymes and of biological systems.[4,5] It has recently been demonstrated that vanadate-fed diabetic rats are relieved of many of the symptoms of diabetes,[6] and it may be that this behavior lies in the ability of vanadate to spontaneously form a vanadate ester of a tyrosine residue in the insulin receptor, which would normally be phosphorylated in the presence of insulin.[7] It has similarly been suggested that the formation of glucose-6-vanadate is the reason that glucose-6-phosphate dehydrogenase catalyses the formation of gluconic acid in the presence of glucose and vanadate.[8]

The formation of vanadate esters with vanadate in the presence of ethanol has been studied in detail,[9] as has that of vanadate esters of phenol and *N*-acetyltyrosine ethyl ester.[7] Cyclic esters of vicinal diols have also been identified.[10,11] X-ray diffraction and neutron scattering analysis have firmly established the formation of the trigonal-bipyramidal coordination geometry about vanadate in a uridine/vanadate/ribonuclease complex[12] as proposed earlier on the basis of kinetic studies.[13]

It is of interest now to extend the study of the aqueous chemistry of vanadate to the interactions between vanadate and phosphate. Mono-, di-, and triphosphates are ubiquitous components of biological systems, and it may well be that phosphate/vanadate anhydrides are accepted as analogues for the corresponding higher phosphate derivatives and enter via this pathway into biochemical processes. In this paper we report studies of the interaction of vanadate with pyrophosphate, phosphate, and arsenate.

Experimental Section

Materials. Reagent grade chemicals were used without further purifications.

Preparation of Solutions. All solutions were made at 1.0 M ionic strength by adding appropriate amounts of KCl. The necessity for maintaining constant ionic strength when using varying concentrations of charged ligands is apparent from the effect of ionic strength on the equilibrium constants for hydrolysis of tetrameric vanadate to yield divanadate (K_0, eq 2) and for hydrolysis of divanadate to yield vanadate (K_1, eq 3). At pH 8.0 with 20 mM Tris–chloride buffer $K_0 = 2.9 \times 10^{-4}$ M and $K_1 = 5.7 \times 10^{-3}$ M. Under the same conditions but with 0.5 M KCl added, $K_0 = 6.5 \times 10^{-5}$ M and $K_1 = 3.9 \times 10^{-3}$ M. These equilibrium constants were determined from the integrated peak areas of the ^{51}V NMR signals due to monomeric, dimeric, and tetrameric species of vanadate.

pK_a values at 1.0 M ionic strength were determined for $HP_2O_7^{3-}$, $H_2PO_4^-$, and $H_2AsO_4^-$ by preparing 0.1 M solutions of these species 50% in the form of their conjugate bases. Sufficient KCl to bring the solutions 'n 1.0 M ionic strength was included in the solutions, and the pH values, ch under these conditions corresponded to the pK_a values of the above es, where determined with use of a standardized pH meter. The pK_a s thus obtained were 8.22 for $HP_2O_7^{3-}$, 6.47 for $H_2AsO_4^-$, and 6.70 $_2PO_4^-$. Solutions for the ^{51}V NMR spectra were then prepared at sired pH values, ligand concentration, and vanadate concentration, 1.0 M ionic strength was maintained with appropriate concentra- of KCl. This was done by adding a sufficient amount of stock on of ligand at the appropriate ratio of acid and base forms for the desired pH and final concentration to a vessel. Sufficient Tris·HCl for a final concentration of 20 mM and KCl for a final ionic strength of 1.0 M were added and the solution was diluted to near the final volume. Sufficient stock solution of Na_2HVO_4 was then added to give the desired final vanadate concentration, and the pH was adjusted to the desired value with NaOH solution. In this way exposure of the vanadate-containing solutions to acid, which causes formation of decavanadate, was avoided. The stock solution of Na_2HVO_4 was prepared by adding 4 molar equiv of NaOH in the form of 1.0 M NaOH solution to V_2O_5 (Aldrich Gold Label, 99.999%). The resulting yellow solution was stored overnight, after which time it became colorless, and diluted with distilled water to a vanadium atom concentration of 0.1 M.

Spectroscopy. All NMR spectra were obtained with a Bruker WM-400 NMR spectrometer operating at ambient temperature unless spec-

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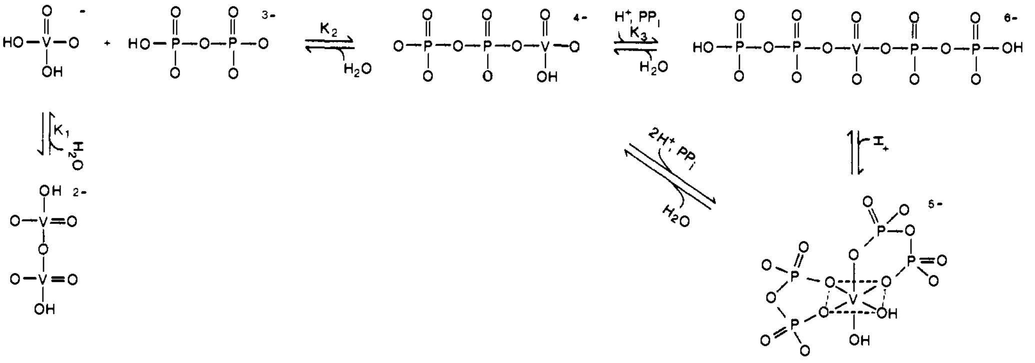

(1)

ified otherwise. ^{51}V NMR spectra were obtained at 105 MHz by using the multinuclear facility of the instrument. Pulse widths of 50°, sweep widths of 20 kHz, and acquisition times of 0.05 s were used throughout. Relative peak areas were obtained with use of the instrument manufacturer's software. A line-broadening of 20 Hz was applied to all spectra before Fourier transforming to the frequency domain with use of a 2K data set zero-filled to 8K.

Results and Discussion

It is well-known that phosphate is readily incorporated into heteropolyvanadate ions such as those formed with tungstate and molybdate.[14,15] On the basis that phosphate undergoes apparently facile reactions with such anionic metallates, it might be predicted that pyrophosphate will undergo similar reactions. Furthermore, vanadate may undergo interactions with pyrophosphate which are analogous to those with diols. In this case, vanadate/pyrophosphate anhydrides may be formed as well as cyclic dianhydrides. A complication encountered with pyrophosphate is that, being a tetrabasic acid, it has four ionization equilibria which must be considered. The first and second pK_a values of 0.85 and 1.49, respectively, indicate that pyrophosphate will carry at least two negative charges throughout the range of pH values utilized in this study (pH 6 to 11). The third and fourth pK_a values are 5.77 and 8.22, which are important within the range of pH variation of this study.

On the basis of the above considerations and in anticipation of results, eq 1 can be written as follows.

If an equilibrium such as this does occur, then as pH is decreased from a high value while maintaining constant vanadate (V_t) and pyrophosphate (PP_t) concentrations the formation of first the anhydride then the cyclic complex should be observed. Other types of cyclic complexes are possible as well. Equation 1 shows only one of these possibilities. Figure 1 shows the effect of changing pH of a 1 mM V_t, 6 mM PP_t, and 10 mM Tris–Cl solution. V_t is the total vanadium atom concentration in the solution, and PP_t is the total concentration of pyrophosphate, both free and complexed. At pH 10.0, a single resonance occurring at −537 ppm and corresponding to VO_4H^{2-} is observed. As the pH is decreased to pH 9.5, a low-intensity signal at −563 ppm and corresponding to the vanadate dimer occurs. With further decrease in pH to pH 8.5, this latter resonance becomes relatively more intense and a third signal at −576.9 ppm, which derives from tetrameric vanadate, arises. At pH 8, broadening in the signal from the monomeric species becomes evident. With continued decrease of pH this latter signal becomes increasingly broad and a further broad resonance appears at −540 ppm. At pH 6.5, this last signal represents the major proportion of the vanadate species present.

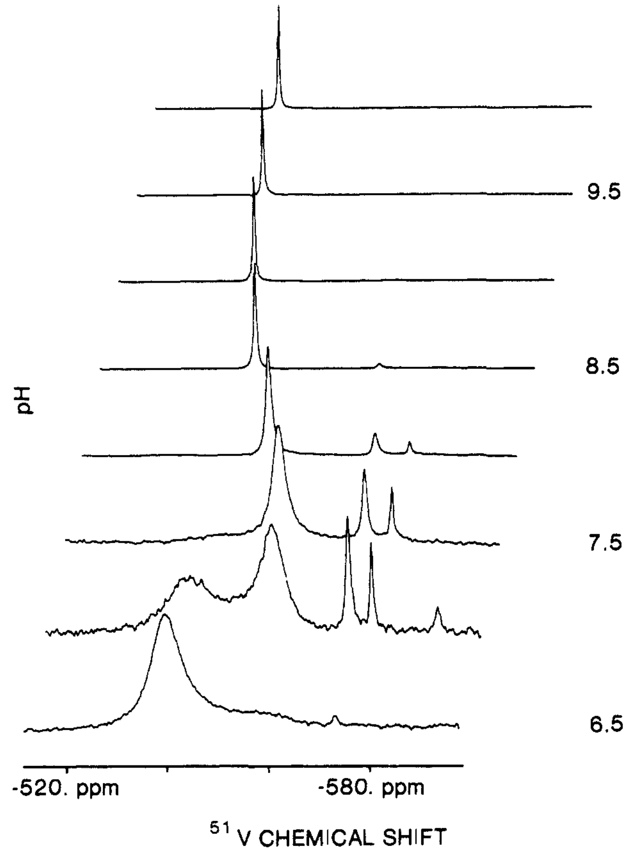

Figure 1. Effect of pH on the formation of pyrophosphate/vanadate complexes. Solutions contained 1.0 mM total vanadate, 6.0 mM pyrophosphate, and 10 mM Tris-chloride and were maintained at 1.0 M ionic strength with KCl. The figure shows the loss of the resonance attributed to the octahedral complex as pH is increased from pH 6.5 to 8.0.

In terms of the equilibration processes of eq 1, there is no resonance that can clearly be ascribed to the species $HO_3POPO_2OVO_3H^{3-}$. The −540-ppm signal is assigned to the octahedral dipyrophosphate complex. With this latter assignment, it must be concluded that the signal occurring near −550 ppm is a superposition of the signal from vanadate, V_i, and that from the vanadate–pyrophosphate anhydride, VP_2, and possibly that from the dipyrophosphate dianhydride, P_2VP_2. This proposal can readily be examined at pH 8 where the signal at −540 ppm is insignificant.

Vanadate, V_i, is in equilibrium with its dimeric, V_2, and tetrameric, V_4, species and, as proposed, with the pyrophosphate derivatives VP_2 and P_2VP_2. The equilibrium expressions are given in eq 2–5, where formation of water is not specifically considered since effects resulting from the small changes in water concentrations must be small, and protons are not considered because the pH was held constant.

$$V_4 \overset{K_0}{\rightleftharpoons} 2V_2 \tag{2}$$

$$V_2 \overset{K_1}{\rightleftharpoons} 2V_i \tag{3}$$

$$V_i + P_2 \overset{K_2}{\rightleftharpoons} VP_2 \tag{4}$$

$$VP_2 + P_2 \overset{K_3}{\rightleftharpoons} P_2VP_2 \tag{5}$$

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The total vanadate, V_t, is distributed throughout the five vanadate species so that eq 6 is obtained. With the establishment of the equilibria of eq 2–5, the concentrations of the various species

$$V_t = 4[V_4] + 2[V_2] + [V_i] + [VP] + [P_2VP_2] \qquad (6)$$

can be written as in eq 7–10.

$$[V_2] = K_0^{1/2}[V_4]^{1/2} \qquad (7)$$

$$[V_i] = K_1^{1/2}[V_2]^{1/2} = K_1^{1/2}K_0^{1/4}[V_4]^{1/4} \qquad (8)$$

$$\begin{aligned}[VP_2] = K_2[P_2][V_i] &= K_2K_1^{1/2}[P_2][V_2]^{1/2} \\ &= K_2K_1^{1/2}K_0^{1/4}[P_2][V_4]^{1/4} \qquad (9)\end{aligned}$$

$$\begin{aligned}[P_2VP_2] = K_3[P_2V][P_2] &= K_3K_2K_1^{1/2}[P_2]^2[V_2]^{1/2} \\ &= K_3K_2K_1^{1/2}K_0^{1/4}[P_2]^2[V_4]^{1/4} \qquad (10)\end{aligned}$$

Equation 8 makes it clear that if a signal derived from V_i has superimposed on it signals from VP_2 and P_2VP_2 and that if either of the equilibrium constants, K_0 or K_1, are known, then the proportion of that signal from V_i can be determined. If both are known, then two independent measurements of the concentration of V_i are available and, in turn, two independent measurements of K_2 and K_3 are provided. Assuming then that the −549.0-ppm signal (pH 8) consists of signals from V_i, VP_2, and P_2VP_2, identical in shift, either fortuitously or by virtue of rapid chemical exchange, eq 11 can be written from a combination of eq 8–10. The relevant concentrations are then provided by signal intensities in the NMR

$$\frac{[V_i] + [VP_2] + [P_2VP_2]}{[V_2]^{1/2}} = K_1^{1/2} + K_1^{1/2}K_2[P_2] + K_1^{1/2}K_2K_3[P_2]^2 \qquad (11)$$

spectra through application of eq 6. A plot of the ratio $([V_i] + [VP_2] + [P_2VP_2])/[V_2]^{1/2}$ vs. $[P_2]$ will give a line with *y*-intercept equal to $K_1^{1/2}$. In the circumstance that no P_2VP_2 is present in solution, the line will be straight with a slope of $K_1^{1/2}K_2$; otherwise, it will have upward curvature. In this latter case, eq 11 can be rewritten as eq 12 and a plot of the ratio on the left of this equation

$$\left(\frac{([V_i] + [VP_2] + [P_2VP_2])}{[V_2]^{1/2}} - K_1^{1/2}\right)\Big/[P_2] = K_1^{1/2}K_2 + K_1^{1/2}K_2K_3[P_2] \qquad (12)$$

vs. $[P_2]$ will provide a line of *y*-intercept $K_1^{1/2}K_2$ and slope $K_1^{1/2}K_2K_3$ so that, from the known K_1, the equilibrium constants K_2 and K_3 can be obtained.

Equations similar to eq 11 and 12 can be written in terms of $[V_4]$ instead of $[V_2]$ so that an independent measure of K_2 and K_3 is provided. Equation 13 is analogous to eq 11.

$$\frac{[V_i] + [VP_2] + [P_2VP_2]}{[V_4]^{1/4}} = K_0^{1/4}K_1^{1/2} + K_0^{1/4}K_1^{1/2}K_2[P_2] + K_0^{1/4}K_1^{1/2}K_2K_3[P_2]^2 \qquad (13)$$

On the basis of the above assumptions concerning equilibria and the corresponding derived equations, concentration studies were done and the appropriate graphs were plotted. The results of the study are provided in Table Is (supplementary material).

Figures 2 and 1s (supplementary material) show the results of experiments done at a constant ionic strength of 1.0 M, where the parameters plotted are those of eq 11 (Figure 1s) and eq 13 (Figure 2). An important feature of these plots is that a linear correlation is obtained and no upward curvature is observable. No upward curvature indicates that very little or no dipyrophosphatovanadate, P_2VP_2, is formed. From the *y*-intercepts and slopes of Figures 1s and 2, the values K_2 = 36.0 and 42.0 M^{-1} were obtained, respectively. The agreement between these two determinations is good and in accord with eq 1. The equilibrium constants which were obtained at a constant ionic strength of 1.0 M with KCl and pH 8 are $K_0 = 6.3 \times 10^{-5}$ M, $K_1 = 2.9 \times 10^{-3}$ M, and $K_2 = 39.0\ M^{-1}$, with an estimated error of less than 10% for each value.

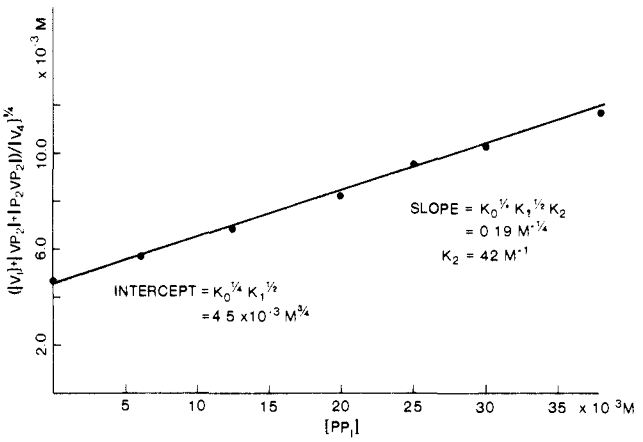


Figure 2. The ratio of the concentration of monomeric vanadate species to the fourth root of the concentration of tetrameric vanadate is shown to be linear with pyrophosphate concentration. The value for the slope divided by that of the intercept provides the constant, K_2, for formation of the vanadate/pyrophosphate anhydride, $HO_3POPO_2OVO_3H^{3-}$.

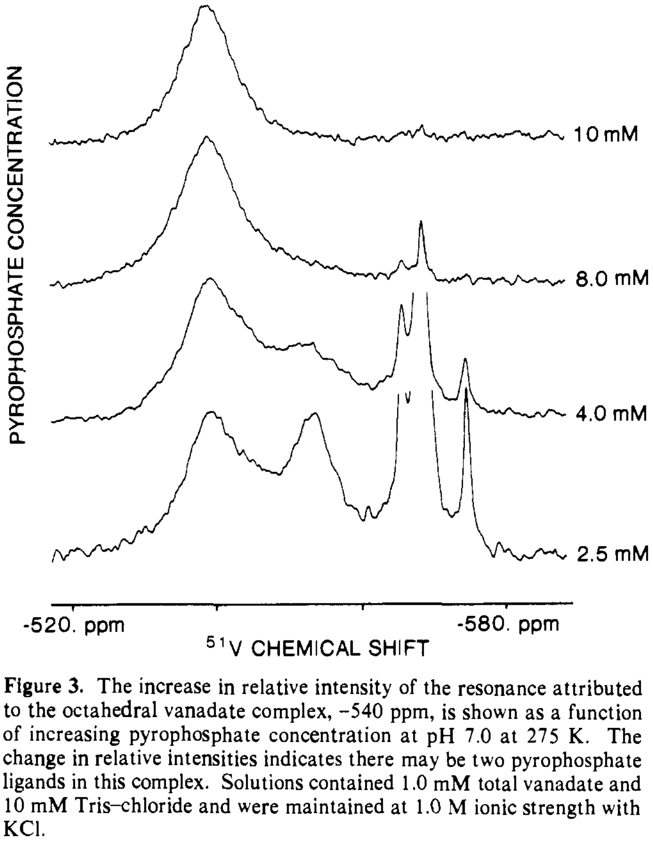


Figure 3. The increase in relative intensity of the resonance attributed to the octahedral vanadate complex, −540 ppm, is shown as a function of increasing pyrophosphate concentration at pH 7.0 at 275 K. The change in relative intensities indicates there may be two pyrophosphate ligands in this complex. Solutions contained 1.0 mM total vanadate and 10 mM Tris-chloride and were maintained at 1.0 M ionic strength with KCl.

As previously discussed for Figure 1, lowering the pH from 8 to 7 promotes the formation of a further vanadate–pyrophosphate derivative. The very broad NMR signals preclude a detailed analysis of this system at pH 7; however, qualitatively, it is readily confirmed that the −540- and −556-ppm resonances arise from species containing the same number of vanadium nuclei. When total vanadate concentration was systematically increased over a threefold range, proportions of the two relevant components were observed to stay constant. A similar study in which pyrophosphate was increased is shown in Figure 3. This figure clearly shows that the formation of the compound giving rise to the −540-ppm resonance is favored at higher pyrophosphate concentration. The changes in the spectra are consistent with the incorporation of two pyrophosphates into this compound. (**Note Added in Proof**: At pyrophosphate concentrations much lower than those utilized here we have found, from UV studies, that only one pyrophosphate is necessary for formation of a cyclic product. This is similar to the case of oxalate.[14] The present study establishes stoichiometry

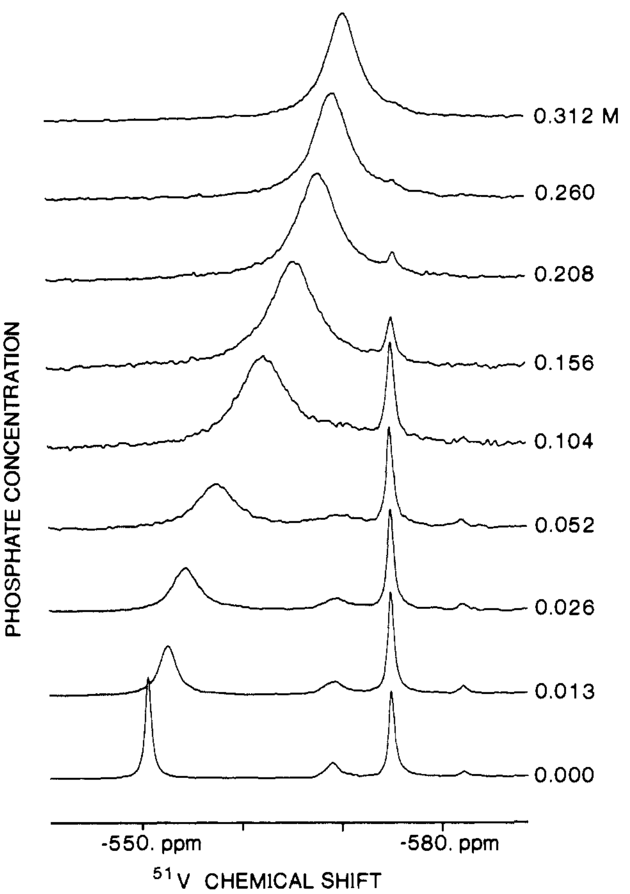
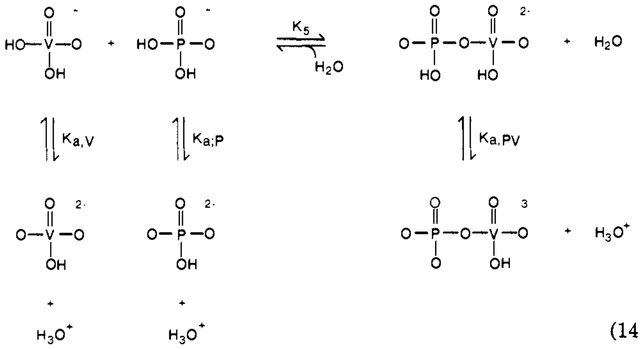


Figure 4. ^{51}V NMR spectra of solutions at pH 7.98, 1.0 mM vanadium atom concentration, 20 mM Tris–chloride, 1 M ionic strength with KCl, and the indicated concentrations of phosphate. Phosphate–vanadate anhydride formation is indicated by the change in chemical shift and increase in relative intensity of the signal which occurs at −550.6 ppm in the absence of phosphate.

only poorly and there may well be a mixture of cyclic products.) This derivative is assigned an octahedral structure since the resonance position of −540 ppm is close to that of octahedral complexes of vanadate with oxalate (−536 ppm)[14] and with lactate (−533 ppm).[14] The absence of a vanadium resonance in this position in solutions containing phosphate (see below) also supports the proposal that the resonance at −540 ppm arises from a species in which pyrophosphate acts as a bidentate ligand.

The formation of the pyrophosphate/vanadate anhydride proceeds readily, and it is, therefore, not surprising that a phosphate/vanadate anhydride is also easily formed. Formation and decomposition of these anhydrides is reasonably fast so that a single broadened line is observed in the NMR spectrum which chemical shift represents a weighted average of the proportions of free vanadate and the vanadate/phosphate anhydride. Figure 4 shows ^{51}V NMR spectra from various solutions at pH 7.98, 1.0 mM total vanadium atom concentration, 1.0 M ionic strength maintained with KCl, and the indicated concentrations of phosphate (P_i). The bottom spectrum in Figure 4 derives from a solution containing no phosphate. The resonances in this spectrum are assigned to vanadate (−550.6 ppm), divanadate (−569.0 ppm), tetravanadate (−574.9 ppm), and an undetermined higher oligomer of vanadate (−582.2 ppm). With increasing P_i concentration, the vanadate (V_i) resonance broadens, shifts progressively to higher field, and increases in relative intensity. This behavior is interpreted in terms of eq 14 which represents the condensation of P_i and V_i to form a mixed anhydride (PV) (analogous to pyrophosphate) and water. The proton ionization constants, indicated in eq 14, are important in the pH range studied here. The anhydride, PV, is in rapid chemical exchange with its precursors so that an average of the chemical shifts from V_i and PV is observed.



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If δ_V and δ_{PV} represent the chemical shifts of V_i and PV then the observed shift is related to the phosphate concentration according to the equation

$$\log [P_i] = \log \frac{\delta - \delta_V}{\delta_{PV} - \delta} - \log K_4 \tag{15}$$

where the equilibrium constant, K_4, is defined in eq 16. The [P], [V], and [PV] of eq 16 represent the total concentration of P_i,

$$K_4 = \frac{[PV]}{[V_i][P_i]} \tag{16}$$

V_i, and PV, respectively, in their various protonated states. Equation 15 shows that a plot of $\log [(\delta - \delta_V)/(\delta_{PV} - \delta)]$ vs. $\log [P_i]$ must have unit slope and intersect the vertical axis at $\log K_4$. Since sufficiently high concentrations of P_i to convert all V_i to PV cannot be obtained, δ_{PV} cannot be determined experimentally. The value of δ_{PV} can, however, be readily obtained by adjusting its value until the required unit slope of the above plot is obtained; this simultaneously provides K_4.

The observed chemical shift values as a function of $\log [P_i]$ are shown in Figure 5 for experiments done both at pH 7.98 and at pH 6.69. The curves are calculated from eq 17 which is a rearranged form of eq 15.

$$\delta = \frac{[P_i]K_4\delta_{PV} + \delta_V}{1 + K_4[P_i]} \tag{17}$$

If K_4 is known for two pH values it is possible, given $K_{a;P}$ and $K_{a;V}$, to calculate values for K_5 and $K_{a;PV}$ of eq 14. The various equilibria of eq 14 are described by eq 18–21 and the conservation equations, eq 22–24. Equation 16 in combination with these equations provides eq 25. From the values of K_4 determined at pH 6.69 and 7.98 and the ionization constants, $K_{a;P} = 10^{-6.70}$ M

$$K_5 = \frac{[H_2PVO_7^{2-}]}{[H_2PO_4^-][H_2VO_4^-]} \tag{18}$$

$$K_{a;P} = \frac{[HPO_4^{2-}][H_3O^+]}{[H_2PO_4]} \tag{19}$$

$$K_{a;V} = \frac{[HVO_4^{2-}][H_3O^+]}{[H_2VO_4]} \tag{20}$$

$$K_{a;PV} = \frac{[HPVO_7^{3-}][H_3O^+]}{[H_2PO_7^{2-}]} \tag{21}$$

$$[P_i] = [H_2PO_4^-] + [HPO_4^{2-}] \tag{22}$$

$$[V_i] = [H_2VO_4^-] + [HVO_4^{2-}] \tag{23}$$

$$[PV] = [H_2PVO_7^{2-}] + [HPVO_7^{3-}] \tag{24}$$

$$K_4 = \frac{K_5[1 + K_{a;PV}/[H_3O^+])}{(1 + K_{a;P}/[H_3O^+])(1 + K_{a;V}/[H_3O^+])} \tag{25}$$

(see experimental section) and $K_{a;V} = 10^{-8.16}$ M (see below), values of K_5 and $K_{a;PV}$ were obtained, using eq 25. The values obtained are $K_5 = 64 \pm 3$ M^{-1} and $K_{a;PV} = 10^{-7.7}$ M.

Table I. A Summary of Equilibrium Constants of Anhydride Formation and Chemical Shifts for Vanadium Species Determined As Described in the Text

	K_{form} = $[XV]/([X_i][V_i])$ (M^{-1})				δ (ppm)			
pH	$X_i = P_i$	$X_i = PP_i$	$X_i = As_i$	$X = V_i$	V_i	P–V	As–V	V_2
6.47			13.0 ± 0.8	$3.4 \pm 0.2 \times 10^2$				
7.85			4.5 ± 0.2	$3.7 \pm 0.2 \times 10^2$	−552.2		−575.0	−569.7
6.69	25.0 ± 1.0			$3.4 \pm 0.2 \times 10^2$	−558.6	−585.1		−571.1
7.98	5.8 ± 0.2	39 ± 3		$3.6 \pm 0.2 \times 10^2$	−550.6	−582.0		−569.0

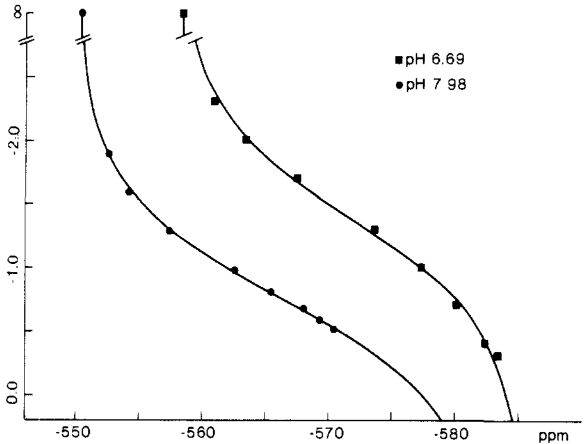

Figure 5. The chemical shift of the resonance from the vanadate and the phosphate–vanadate anhydride as a function of the phosphate concentration at pH 6.69 and 7.98. The points are experimental, and the lines were calculated from eq 17 with the K_4, δ_V, and δ_{PV} shown in Table I.

In the event that rapid protonation/deprotonation occurs it is possible to relate observed chemical shifts to the pK_a of the substrate by an equation analogous to eq 15. This relationship is given by eq 26 and its use has been described.[10] With use of

$$\mathrm{pH} = \mathrm{p}K_a + \log\left(\frac{\delta_l - \delta}{\delta - \delta_h}\right) \tag{26}$$

this equation, the limiting chemical shifts, $\delta_h = -538$ ppm and $\delta_l = -559$ ppm for high and low pH, respectively, and the observed values of chemical shifts, δ, with pH the value of $K_{a;V} = 10^{-8.16}$ M was determined. Conversely, if the pK_a is known, then eq 26 can be used to determine δ_l and δ_h from the chemical shift dependence on pH. Application of $K_{a;PV}$ and utilization of the chemical shift values of δ_{PV} for the two pH values studied allows chemical shifts for $H_2PVO_7^{2-}$ (δ_l) and $HPVO_7^{3-}$ (δ_h) to be determined. The values thus obtained are $\delta_l = -586$ ppm and $\delta_h = -581$ ppm.

An alternative method for determining K_4 is available by utilizing the relative intensities of the signals as they vary with concentration as seen in Figure 4. The signal arising from tetravanadate and the averaged signal from vanadate and the phosphate–vanadate anhydride are well resolved from each other and other signals in the spectra of the solutions at the lower phosphate concentrations. The vanadium atom concentrations in the species causing these two signals can thus be determined, and the data can be analyzed in terms of eq 27, 28, and 16, from which eq 29 is obtained. The relevant concentrations are given

$$K_6 = \frac{[V_4]}{[V_i]^4} \tag{27}$$

$$V_{Tot} = [V_i] + [PV] + 2[V_2] + 4[V_4] \tag{28}$$

$$\frac{[V_i] + [PV]}{[V_4]^{1/4}} = \left(\frac{1}{K_6}\right)^{1/4} + K_4\left(\frac{1}{K_6}\right)^{1/4}[P_i] \tag{29}$$

in Table IIs (supplementary material), and the data were plotted according to eq 29 as shown in Figure 6. The value obtained for K_4 (6.0 M^{-1}) agrees well with the corresponding value (5.7 M^{-1}) obtained from the chemical shift behavior at pH 7.98 as described earlier. A similar analysis was not possible from the spectra obtained at pH 6.69, because the signals were not sufficiently well resolved to allow accurate determinations of their relative intensities. The equilibrium constant obtained from analysis of chemical shifts should be more accurate than that obtained from analysis of relative signal intensities, mainly because fewer data points were available in the latter case. The discrepancy between the two measurements, approximately 5%, is not significant.

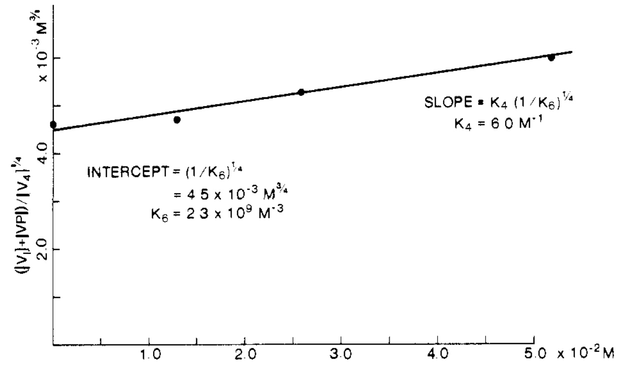

Figure 6. The determination of the equilibrium constant for formation of the phosphate–vanadate anhydride at pH 7.98. Data which were obtained from spectra shown in Figure 5 (Table IIs) were plotted according to eq 29 to obtain the indicated value for K_4.

Vanadate in the presence of arsenate was found to behave similarly to vanadate in the presence of phosphate. Two sets of experiments were carried out (pH 6.47 and 7.85) in the presence of varying concentrations of arsenate. The results were analyzed in terms of the formation of the mixed anhydride (AsV) by using a procedure identical with that described for the formation of PV. From the effects of arsenate on the chemical shifts, equilibrium constants (K_4') for formation of AsV from V_i and As_i were determined to be 13 and 4.4 M^{-1} at pH 6.47 and 7.85, respectively. The limiting chemical shift values used in this analysis were $\delta_V = -559.3$ at pH 6.47, $\delta_V = -552.2$ at pH 7.85, $\delta_{AsV} = -585.0$ at pH 6.47, and $\delta_{AsV} = -575.0$ at pH 7.85. K_4' was also measured at pH 7.85 by analyzing changes in relative signal intensities as a function of arsenate concentration. The value obtained, 4.7 M^{-1}, agrees very well with that ascertained above from analysis of chemical shifts, 4.4 M^{-1}.

With the values of K_4' determined at two pH levels, the pK_a value for $H_2VO_4^-$ (previously discussed) and the pK_a of $H_2AsO_4^-$ (see methods), an equation analogous to eq 25 can be used to determine $K_{a;AsV}$ and K_7 as defined by eq 30 and 31. The values determined were $K_{a;AsV} = 10^{-7.0}$ M and $K_7 = 21 \pm 2$ M^{-1}.

$$K_{a;AsV} = \frac{[HAsVO_7^{3-}][H_3O^+]}{[H_2AsVO_7^{2-}]} \tag{30}$$

$$K_7 = \frac{[H_2AsVO_7^{2-}]}{[H_2VO_4^-][H_2AsO_4^-]} \tag{31}$$

No evidence was obtained in the studies with P_i or As_i for species containing more than one phosphate or arsenate moiety. Increasing the concentration of V_i at fixed P_i or As_i concentrations had no effect on the chemical shift of the V_i–mixed anhydride average signal. This ruled out the presence of more than one vanadium atom in the mixed anhydride.

Table I provides a summary of the equilibrium constants obtained for formation of the mixed anhydrides and divanadate. The chemical shifts obtained for the species are also shown.

Although it is not surprising that the mixed anhydrides of vanadate with pyrophosphate, phosphate, and arsenate reported have formed in aqueous solution, there appear to be no other reports of such species. Complexes of the structure $VOPO_4$ have been studied in nonaqueous phases,[16] and numerous crystal structure determinations of large cluster complexes containing vanadium(V) and phosphorus(V) have been published;[17] but these species have little in common with the simple mixed anhydrides described above.

From the summary in Table I of equilibrium constants for formation of the mixed anhydrides, it is apparent that there is little difference in the stability of the two mixed anhydrides toward hydrolysis, while divanadate is from 10 to 100 times more stable toward hydrolysis. From the free energy of hydrolysis of inorganic pyrophosphate of –8 kcal/mol at pH 7.0,[18] the equilibrium constant for formation of pyrophosphate from phosphate is 1.3×10^{-6} M^{-1}. It is interesting to note that the equilibrium constant for formation of divanadate exceeds by a factor of about 10^8 that for formation of the analogous pyrophosphate. Even formation of the mixed phosphate–vanadate anhydride is more than 10^6 times more favored than is formation of pyrophosphate. At present we can provide no explanation for these large differences in stability toward hydrolysis.

The rapid and relatively favorable formation of mixed anhydrides of phosphate and vanadate makes it reasonable to consider whether such species can interact with biological systems in spite of the normally low physiological vanadium concentrations.[4] In preliminary studies to explore this possibility, it has been observed that when adenosine monophosphate (AMP) is present, added vanadate causes an increase in the rate at which pyruvate kinase converts phosphoenolpyruvate to pyruvate.[19] This result is consistent with the nonenzymic formation of the mixed anhydride AMP–vanadate, which is presumably accepted as a substrate by pyruvate kinase. The formation of mixed phosphate/vanadate anhydrides is also relevant to the inhibition of myosin ATPase by adenosine diphosphate plus vanadate.[20–22]

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Registry No. $HP_2O_7^{3-}$, 42499-21-8; $H_2PO_4^-$, 14066-20-7; $H_2AsO_4^-$, 16518-47-1; Na_2HVO_4, 59826-07-2; $H_2O_4V^-$, 34786-97-5; $O_3POPO_2OVO_3H^{4-}$, 103884-10-2; $(O_3POPO_3)_2V(OH)_2^{5-}$, 103904-05-8; $HO_3VOVO_3H^{2-}$, 103884-11-3; $HO_3VOVO_2OVO_2OVO_3H^{4-}$, 103884-12-4; $HO_3POVO_3H^{2-}$, 103884-13-5; $O_3POVO_3H^{3-}$, 103884-14-6; $HO_3AsOVO_3H^{2-}$, 103903-97-5.

Supplementary Material Available: Figure 1S showing the ratio of concentration of monomeric tetrahedral vanadate species to the square root of vanadate dimer concentration and tables giving the concentrations of various vanadate species determined as a function of pyrophosphate and phosphate concentration (3 pages). Ordering information is given on any current masthead page.

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Activation of Hydrogen by Cationic Cyclopentadienyl Molybdenum Dimers with Sulfido Ligands. 1. Cationic Complexes Derived from Protonation of 1,2-Alkenedithiolate Ligands

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Abstract: The reaction of phenylacetylene with the sulfido ligands in $(CpMo\text{-}\mu\text{-}S)_2S_2CH_2$ ($Cp = C_5H_5$) results in the formation of a phenylacetylene adduct of formulation $(CpMo)_2(S_2CH_2)(SC(Ph)C(H)S)$, **1**. Complex **1** reacts with a protic acid (CF_3CO_2H, HBF_4, $HOSO_2CF_3$) to form a protonated cationic derivative which exists in solution as an equilibrium mixture of isomers, **2A** and **2B**. Single crystals of the triflate salt of **2A** have been grown from an acetonitrile/hexane solution. The molecule crystallizes in space group $P\bar{1}$ with $a = 7.2255$ (14) Å, $b = 11.9950$ (20) Å, $c = 14.6744$ (24) Å, $\alpha = 86.452$ (13)°, $\beta = 77.922$ (15)°, $\gamma = 79.570$ (14)°. The X-ray diffraction study establishes that protonation results in cleavage of one carbon–sulfur bond of the alkenedithiolate ligand to form $[(CpMo)_2(S_2CH_2)(\mu\text{-}S)(\mu\text{-}SC(Ph)CH_2)]^+$. The bridging α-phenylvinylthiolate ligand in **2A** is oriented in an equatorial configuration with the double bond twisted out of the plane of the sulfur atoms. ^1H and ^{13}C NMR data suggest that the structure of **2B** involves a protonated, 1,2-dithiolate ligand in which both carbon–sulfur bonds remain intact, $[(CpMo)_2(S_2CH_2)(\mu\text{-}SC(Ph)CH_2S)]^+$. Complex **2** (**A** and **B**) is deprotonated by triethylamine and stronger bases to form **1**. Less basic nucleophiles, such as methanol or chloride or nitrite ion, react with **2** (**A** and **B**) to cleave the carbon–sulfur bond(s) of the thiolate ligand and form substituted sytrenes. Complex **2** (**A** and **B**) reacts with hydrogen at room temperature to form a neutral molybdenum(III) dimer $(CpMo)_2(S_2CH_2)(S_2C(Ph)Me)$, **4**. Observations on this unusual hydrogen activation process are discussed.

Cyclopentadienylmolybdenum(III) dimers with alkenedithiolate ligands have been found to react with hydrogen under mild conditions to form the cis alkene or alkene adduct as shown in reaction 1.[1] An important feature of this reaction is that it