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Vanadium(V) Oxyanions. Interactions of Vanadate with Methanol and Methanol/Phosphate

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^{51}V nuclear magnetic resonance studies have been utilized to characterize the interactions between methanol and vanadate. Equilibrium constants for the formation of methyl vanadate and dimethyl vanadate were determined as were those for methyl divanadate and dimethyl divanadate. From the variation in vanadate and methanol concentrations at pH 7.5 the following formation constants were determined: $[MeOVO_3H^-][H_2O]/[MeOH][VO_4H_2^-] = 5.2 \pm 0.1$; $[(MeO)_2VO_2^-][H_2O]/[MeOH][MeOVO_3H^-] = 1.2 \pm 0.1$; $[(MeO)O_2VOVO_3H^{2-}][H_2O]/[HO_3VOVO_3H^{2-}][MeOH] = 3.0 \pm 0.3$; $[(MeO)O_2VOVO_2(OMe)^{2-}][H_2O]/[(MeO)O_2VOVO_3H^{2-}][MeOH] = 3.1 \pm 0.3$. From the effect of pH on their chemical shifts the pK_a's of $VO_4H_2^-$ and $MeOVO_3H^-$ were determined as 9.22 ± 0.02 and 9.42 ± 0.02, respectively, for a 7.4 M methanol solution. Phosphate was shown to have a catalytic effect on the formation and hydrolysis of the methyl esters. A 2.5 mM phosphate solution was sufficient to enhance the rate of hydrolysis of dimethyl vanadate to methyl vanadate by a factor of about 50. The relevance of these results to formation of adenosine triphosphate analogues was discussed. Investigation of the water requirements for the formation of tetrameric vanadate indicated that 2 mol of water/mol of product was required. This result is in accordance with an adamantane-like structure for tetravanadate.

Introduction

Vanadium in its higher oxidation states is thought to be an essential element.[1,2] Vanadate in sea water is concentrated as V(III) by various tunicates (sea squirts) to ~1 M concentration from the nanomolar concentration in the sea water itself.[3] Vanadium is found in the enzymes of some nitrogen fixing bacteria[4] and is also found in various mushrooms.[5] In mammals it occurs at about 0.5 μM.[1,2] Vanadium, as V(IV) or V(V), has a large effect on the function of various enzymes, promoting the action of some and inhibiting that of others.[1,2] There is potential for its use in the treatment of diabetes[6-8] and sickle cell anemia.[9] There is also a significant potential for the use of vanadate in preparative chemistry where it can serve to spontaneously provide phosphate analogues for enzymic synthesis.

Despite this importance and unparalleled potential of vanadium, surprisingly little is known about its aqueous chemistry. We have recently undertaken a program to investigate the behavior of vanadate (V(V)) in the presence of various metabolic products with a view to understanding the action of vanadate in biological systems on a molecular basis.

The initial study in this regard concerned the interactions between vanadate and ethanol where the formation of ethyl vanadate on a time scale of about 10^{-3} s was demonstrated.[10] Interest has now shifted to the spontaneous formation of adenosine mono-, di-, and triphosphate analogues, which are readily formed from the appropriate adenosine precursor in the presence of vanadate.[11] This system is complicated somewhat by the presence of several functional groups, and some details of the interactions are lost. We have consequently turned to the simpler models, methanol, and methanol plus phosphate.

The chemistry of vanadate in the presence of methanol, not surprisingly, is not very different from that of vanadate with ethanol. However, in this study the formation of methyl esters of divanadate and mixed anhydride esters of methyl vanadophosphate has been specifically studied as has the effect of phosphate on the formation of the methyl esters.

Methanol was selected for this study principally because there is a larger chemical shift separation in the ^{51}V NMR spectrum between vanadate and its mono- and diesters than there is for the corresponding ethyl esters.

Experimental Section

Materials. Reagent grade chemicals were used without further purification. Methanol, KH_2PO_4, K_2HPO_4, KCl, and HEPES buffer were used as provided. The stock vanadate solution was prepared from V_2O_5 (Aldrich Gold Label, 99.999%).

Solutions. All solutions containing phosphate were prepared at 0.5 M ionic strength by using a 1.0 M KCl stock solution. Ionic strength was not adjusted for the solutions that did not contain added phosphate. These solutions were prepared as described elsewhere for studies using ethanol[10] and phosphate.[13]

Spectroscopy. All NMR spectra were obtained in unlocked mode at 105 MHz at ambient temperature. Spectra widths were 40 kHz, pulse widths, 60°, and acquisition times, 0.025 s. A line-broadening factor of 40 Hz was applied to all spectra. Spectra were acquired into a 2K data set and transformed in an 8K set. The chemical shifts are relative to an external saturated solution of trisodium vanadate where the high-field low-intensity signal of pentavanadate occurs at −572.9 ppm from $VOCl_3$.

Results and Discussion

Vanadate in aqueous solutions reacts rapidly and reversibly with itself to form a variety of oligomeric products, principally the tetrahedrally anhydrides divanadate, T_2, and tetravanadate, T_4 (see the Appendix for a discussion of the structure of this product). Decavanadate is also formed but is favored only below a pH of about 6.

At pH 7.5 both vanadate, T_1, and divanadate have protons available that allow water production to occur so that both these compounds can form methyl esters with methanol. The tetrameric product, T_4, seems relatively inert, and there is no observable reaction with methanol. Indeed, T_4 provides a useful reference compound for investigation of a variety of equilibria when the concentration of T_1 cannot be readily ascertained.[12]

Equations 1–5 describe various equilibria that have been studied here. For brevity the symbol l is used to designate the ligand, methanol (MeOH). At pH 8.5 all the vanadate species indicated

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$$2T_i \underset{H_2O}{\overset{K_1}{\rightleftharpoons}} T_2 \qquad [T_i]^2K_1 = [T_2][H_2O] \tag{1}$$

$$T_i + MeOH \underset{H_2O}{\overset{K_2}{\rightleftharpoons}} T(l) \qquad [T_i][l]K_2 = [T(l)][H_2O] \tag{2}$$

$$T(l) + MeOH \underset{H_2O}{\overset{K_3}{\rightleftharpoons}} T(l)_2 \qquad [T(l)][l]K_3 = [T(l)_2][H_2O] \tag{3}$$

$$T_2 + MeOH \underset{H_2O}{\overset{K_4}{\rightleftharpoons}} T_2(l) \qquad [T_2][l]K_4 = [T_2(l)][H_2O] \tag{4}$$

$$T_2(l) + MeOH \underset{H_2O}{\overset{K_5}{\rightleftharpoons}} T_2(l)_2 \qquad [T_2(l)][l]K_5 = [T_2(l)_2][H_2O] \tag{5}$$

above as well as T_4 can be observed in a single ^{51}V NMR spectrum where they range in chemical shift from about −540 to −580 ppm as shown in Figure 1.

The various equilibria were studied in detail at pH 7.1. Methanol concentration studies at a fixed vanadate concentration of 4.0 mM were carried out as were vanadate concentration studies at a fixed methanol concentration of 7.41 M. The results of these studies are given in Table I.

When the ratios of concentrations $[T(l)][H_2O]/[l]$ and $[T(l)_2][H_2O]/[l]$ were plotted against $[T_i]$ and $[T(l)]$, respectively, the formation constants K_2 and K_3 were provided by the slopes of the resultant lines as indicated by eq 2 and 3. From the data of Table I, the values $K_2 = 5.2 \pm 0.1$ and $K_3 = 1.2 \pm 0.1$ were obtained. These values of K_2 and K_3 are about half of the corresponding values for ethanol, 10.4 and 2.3, respectively.[10]

The equilibrium constants for the formation of $T_2(l)$ and $T_2(l)_2$ from T_2 are not as readily obtained as are those for the mononuclear forms since there is a superposition of NMR signals. One of the signals from $T_2(l)$ is under the signal from T_2 while the other is under that from $T_2(l)_2$.

Summation of eq 1 and 4 then rearrangement gives eq 6, where the factor of 2 is is included since only the vanadium atom concentrations are measured. A plot of the ratio on the left of eq

$$\frac{(2[T_2] + [T_2(l)])[H_2O]}{[T_i]^2} = 2K_1 + K_1K_4\frac{[l]}{[H_2O]} \tag{6}$$

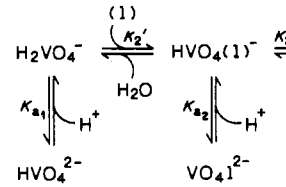
6 vs $[l]/[H_2O]$ should provide a straight line of intercept $2K_1$ and slope K_1K_4. A straight line of intercept $2K_1 = (3.1 \pm 0.2) \times 10^4$ and slope $K_1K_4 = (4.6 \pm 0.4) \times 10^4$ was obtained. The formation of T_2 from monomeric vanadate thus procedes with a formation constant of $K_1 = (1.6 \pm 0.1) \times 10^4$, which leads to a value for formation of $T_2(l)$ from T_2 of $K_4 = 3.0 \pm 0.3$. Thus the formation of $T_2(l)$ from T_2 and methanol is almost as favorable as the formation of T(l) from T_i and methanol.

The formation of $T_2(l)_2$ from $T_2(l)$ can be described by combining eq 1, 4, and 5 to give eq 7. A plot of the relevant pa-

$$\frac{([T_2(l)] + 2[T_2(l)_2])[H_2O]^2}{[T_i]^2[l]} = K_1K_4 + 2K_1K_4K_5\frac{[l]}{[H_2O]} \tag{7}$$

rameters of this equation gave the intercept $K_1K_4 = (5.0 \pm 0.4) \times 10^4$, which agrees well with the value determined as described in the above paragraph. When the previously determined value for K_1 is factored out, a value of 3.1 ± 0.3 is provided for K_4, a value in good agreement with that already determined, $K_4 = 3.0 \pm 0.3$. The slope of the plot, equal to $2K_1K_4K_5$, was $(3.1 \pm 0.2) \times 10^5$ from which $K_5 = 3.1 \pm 0.3$. This last result indicates that the specific vanadium–ligand interaction involved in formation of $T_2(l)_2$ from $T_2(l)$ and methanol is favored by a factor of 4 over that involved in formation of $T_2(l)$ from T_2 since although K_4 and K_5 are essentially equal, there is a statistical factor of 4 that enters into the comparison of K_4 with K_5. It is not clear why the formation of $T_2(l)_2$ should be favored over that of $T_2(l)$.

Hydrogen ion concentration enters into the measured equilibrium constants via the pathway indicated in eq 8. The values of K_2' and K_3' are then pH independent equilibrium constants that



(8)

can be calculated from K_2 or K_3 determined at any pH if the two pKa's are known. At the pH of the above study $H_2VO_4^-$ and $HVO_4(l)^-$ must be by far the predominant species. Protonation of both T_i^{2-} and $T(l)^{2-}$ leads to a substantial change in chemical shift of the ^{51}V NMR signal corresponding to the particular species. The effects of pH on the chemical shifts are given in Table II. If δ_l and δ_h are the limiting chemical shifts at low and high pH, respectively, and δ_{ob} is the observed chemical shift then a simple calculation shows that the pK_a of the entity of interest is related to the chemical shifts and the pH by eq 9. A plot of the

$$\log \frac{\delta_l - \delta_{ob}}{\delta_{ob} - \delta_h} + pK_{a_1} = pH \tag{9}$$

logarithm term versus pH then gives a straight line of slope 1 and y intercept equal to the pK_a. Application of this equation gave the values $pK_{a_1} = 9.22 \pm 0.02$ for T_i^- with the limiting shifts $\delta_l = -560.8$ ppm and $\delta_h = -535.2$ ppm. The corresponding value for $T(l)^-$ was $pK_{a_2} = 9.42 \pm 0.02$ with $\delta_l = -551.0$ ppm and $\delta_h = -528.2$ ppm.

In principle, eq 9 can also be applied to the product T_2^{2-} and $T_2(l)^{2-}$. In this case the chemical shift range is smaller and there is an overlap of NMR signals throughout the pH range. The NMR spectra indicate that the pK_a's of T_2^{2-} and $T_2(l)^{2-}$ are similar; this is not a surprising result since the loss of a single proton affects only one vanadium of the two. Replacement of a hydrogen by a methyl group would not be expected to have a significant affect on the pK_a of the second vanadate. The pK_a determined was 9.69 ± 0.03. The limiting chemical shifts for T_2 were $\delta_l = -574.3$ ppm and $\delta_h = -561.6$ ppm.

The reaction of vanadate with phosphate has been shown to be a highly favorable one with the formation of the vanadate/phosphate (VP) anhydride proceeding rapidly and spontaneously.[13] It seems evident therefore that the PV(l) analogue to $T_2(l)$ should readily be formed. When attempts were made to determine the equilibrium constants for formation of the mixed anhydride ester, it immediately became obvious that this was not possible under the conditions of our experiments. Figure 2 shows the results of adding phosphate to a solution of 4 mM vanadate in 7.41 M methanol at pH 8.35 and 0.5 M ionic strength maintained with added KCl. It is evident that phosphate catalytically promotes the reversible hydrolysis of T(l) and $T(l)_2$ (and possibly $T_2(l)$ and $T_2(l)_2$). The rate enhancement is quite remarkable; at pH 7.46, 2.5 mM phosphate is sufficient to cause coalescence of the NMR signals from T(l) and $T(l)_2$. At higher pH, coalescence occurs with larger amounts of phosphate.

This observation can be put on a more quantitative basis by noting that the $T(l)_2$ signal is approximately 20 Hz wider than the signal from T(l). This extra width could be a consequence solely of quadrupole-induced relaxation of the spin-$^7/_2$ vanadium nucleus. However, close inspection of the NMR signal reveals asymmetry in the signal shape that is indicative of exchange broadening. If this broadening is a result of chemical exchange then the extra width can be used to put an upper limit on the exchange rate[14] at 21 °C in the absence of phosphate. The calculated maximum value for the pseudo-first-order rate constant for hydrolysis is 65 s^{-1}. Of course, the rate could be much slower than this but not significantly faster. At 2.5 mM phosphate the signals from T(l) and $T(l)_2$ are in coalescence. In the absence of phosphate they show a separation of 7.3 ppm (765 Hz). From this separation a rate constant at coalescence of 1.7×10^3 s^{-1} can

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Table I. Vanadium Atom Concentrations of the Vanadate Derivatives Determined as a Function of Vanadate or Methanol Concentration[a]

Methanol Concentration Varied (4.0 mM Total Vanadate)

[MeOH][b]	[H_2O]	[T_i]	[T(l)]	[$T(l)_2$]	[$T_2(l)$] + 2[T_2]	[$T_2(l)$] + 2[$T_2(l)_2$]	4[T_4]
0.00	55.5	1.169	0.000	0.000	0.838	0.000	1.933
1.24	52.9	1.093	1.115		0.846	0.033	1.839
2.47	50.3	1.133	0.295	0.022	0.850	0.071	1.586
3.71	47.7	1.111	0.398	0.044	0.809	0.123	1.469
2.47	50.3	1.090	0.320	0.023	0.868	0.069	1.562
4.94	45.0	1.029	0.631	0.081	0.885	0.236	1.138
6.17	42.4	0.985	0.731	0.132	0.803	0.299	1.050
7.41	39.8	0.944	0.879	0.193	0.803	0.395	0.788
8.64	37.2	0.831	0.916	0.223	0.747	0.490	0.793
9.88	34.5	0.734	1.067	0.356	0.667	0.633	0.541
11.11	31.9	0.558	1.221	0.620	0.562	0.801	0.237
12.34	29.3	0.544	1.210	0.624	0.554	0.794	0.274

Vanadate Concentration Varied (7.41 M Methanol)

[V_t]	[T_i]	[T(l)]	[$T(l)_2$]	2[T_2] + [$T_2(l)$]	2[$T_2(l)_2$] + [$T_2(l)$]	4[T_4]
1.00	0.335	0.383	0.106	0.102	0.057	0.017
2.00	0.550	0.650	0.157	0.307	0.206	0.130
3.00	0.731	0.810	0.210	0.523	0.354	0.371
4.00	0.899	0.966	0.241	0.754	0.473	0.677
5.00	1.002	1.058	0.270	0.971	0.616	1.082

[a] Concentrations were determined from integrated peak areas of spectra such as that shown in Figure 1. Abbreviations are as described in the text. Conditions of the experiments were as follows: pH 7.10; 20 mM HEPES buffer; 21 °C. [b] All concentrations are given in millimolar quantities except those of methanol and water, which are given in molar quantities.

Table II. Effect of pH on the ^{51}V Chemical Shifts of the Various Vanadate Species[a]

pH	T_i	T(l)	$T(l)_2$	T_2	$^1/_2T_2(l)_2$	T_4
6.12	−560.9	−551.2	−544.0	−574.2	−563.7	−579.5
7.16	−560.6	−550.9	−543.3	−574.2	−563.7	−579.2
8.01	−559.3	−550.2	−543.4	−574.0	−563.8	−579.2
8.51	−556.7	−548.6	−543.4	−573.5	−563.8	−579.1
8.94	−551.9	−545.4	−543.5	−572.4	−563.8	−579.0
9.46	−545.0	−539.5	−543.4	−569.7	−563.8	−579.1
10.27	−537.2	−531.0	−543.1	−564.2		

[a] Conditions of the experiments were as follows: 4.0 mM total vanadate; 7.4 M MeOH; 20 mM HEPES buffer; 21 °C. Chemical shifts in ppm are relative to that of $VOCl_3$ at zero ppm.

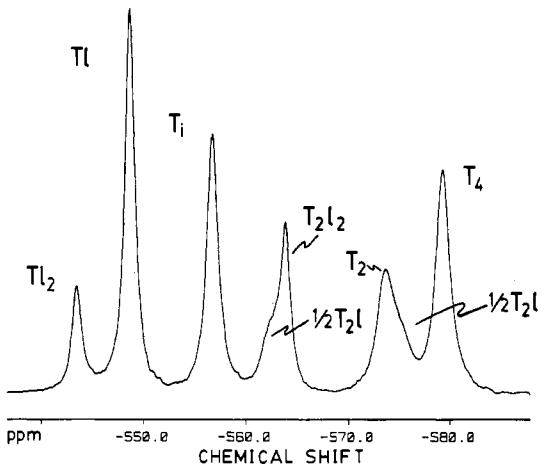

Figure 1. ^{51}V NMR spectrum of vanadate in aqueous methanol at pH 8.51. Signals from the various vanadium derivatives discussed in the text are identified. The conditions of the experiment were as follows: 4.0 mM total vanadate; 7.41 M methanol; 20 mM HEPES buffer; pH 8.51.

be calculated. From the fractional populations of $T(l)_2$ (0.12) and T(l) (0.88) a rate constant for the hydrolysis of $T(l)_2$ of 3.4 × 10^3 s^{-1} can be estimated.[15] Thus, at pH 7.46, 2.5 mM phosphate increased the hydrolysis rate by a factor of at least 50. As is evident from Figure 2 a similar conclusion can be reached for the hydrolysis of T(l) to form T_i. The rates of formation of these

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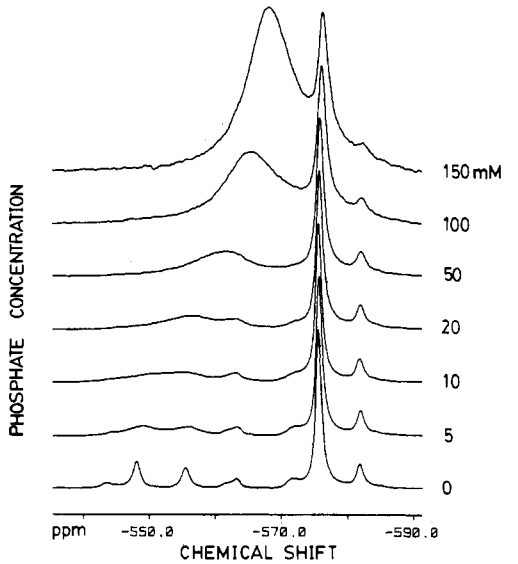
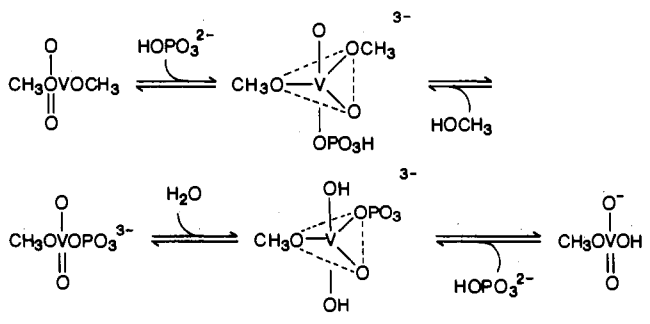


Figure 2. ^{51}V NMR spectra of the aqueous vanadate/methanol system showing the effect of the addition of phosphate anion. Conditions of the experiment were as follows: 4.0 mM vanadate; 7.41 M methanol; 20 mM HEPES buffer; pH 8.35; 0.5 M ionic strength with KCl; phosphate concentrations varied as indicated.

Scheme I



species from their hydrolysis products are of course similarly enhanced.

The proposed mechanism shown in Scheme I for the catalytic effect of phosphate on the reversible hydrolysis of $T(l)_2$ involves

Table III. Equilibrium Equations and Corresponding Equilibrium Constants for the Vanadate Methanol/Water System[a]

equil	equil const
$T_i + l \rightleftarrows T(l) + H_2O$	5.2 ± 0.1
$T(l) + l \rightleftarrows T(l)_2 + H_2O$	1.2 ± 0.1
$T_2 + l \rightleftarrows T_2(l) + H_2O$	3.0 ± 0.3
$T_2(l) + l \rightleftarrows T_2(l)_2 + H_2O$	3.1 ± 0.3
$T_i^- \rightleftarrows T_i^{2-} + H^+$	$10^{-9.22 \pm 0.02}$
$T(l)^- \rightleftarrows T(l)^{2-} + H^+$	$10^{-9.42 \pm 0.02}$
$T_2(l)^{2-} \rightleftarrows T_2(l)^{3-} + H^+$	$10^{-9.69 \pm 0.03}$

[a] All equilibrium constants were determined at pH 7.10 except the K_a's, which required changes in the pH. All conditions were as described in the text.

Table IV. Monomeric and Tetrameric Vanadate Derivatives Compared as a Function of Water Concentration

$[H_2O]^a$	[MeOH]	$[T_i]$	$[T_4]$	$10^{19}[T_i]^4/[H_2O]^4$	$10^{16}[T_i]^4/[H_2O]^2$	$10^{22}[T_i]^4/[H_2O]^6$
55.5	0.00	1.169	0.483	2.0	6.1	0.6
52.9	1.24	1.093	0.460	1.8	5.1	0.7
50.3	2.47	1.133	0.397	2.6	6.5	1.0
47.7	3.71	1.111	0.367	2.9	6.7	1.3
50.3	2.47	1.090	0.391	2.2	5.6	0.9
45.0	4.94	1.029	0.285	2.7	5.5	1.4
42.4	6.17	0.875	0.263	2.9	5.2	1.6
39.8	7.41	0.944	0.197	3.2	5.0	2.0
37.2	8.64	0.831	0.198	2.5	3.4	1.8
34.5	9.88	0.734	0.135	2.0	2.4	1.7
31.9	11.11	0.558	0.059	0.9	1.0	0.9
29.3	12.34	0.544	0.069	1.2	1.0	1.4

[a] See footnote *b* in Table I.

a double displacement reaction. An analogous scheme can be drawn for the reversible hydrolysis of Tl. The reversible hydrolysis or methanolysis of the vanadate/phosphate anhydride linkage of $CH_3OVO_3PO_3^{3-}$ is expected to be fast since the analogous mixed anhydride $HOVO_3PO_3^{3-}$ is known to be in rapid equilibrium with its precursors.[13] This assumes that the substitution of a methyl group for a proton on the oxygen bound to vanadium does not have a large effect on the reaction rate, which appears to be the case.

Increasing the phosphate concentration above 150 mM leads eventually to collapse of all NMR signals except that of T_4 as can be seen in Figure 2. The occurrence of the intense broad signal at the higher concentration represents the formation of the various possible vanadate phosphate anhydrides, all in rapid equilibrium. T_4 does not come into rapid equilibrium presumably because the structure of this compound (see the appendix) requires multiple bond breaking when reaction occurs to give products.

Conclusions

The results of this study show that mono- and dimethyl esters of vanadate are spontaneously formed from vanadate in aqueous methanol solution. Similarly, derivatives of divanadate are also readily formed. The formation constants are summarized in Table III. Of significance here is the finding that formation of methyl vanadate and methyl divanadate proceed essentially equally well. This is perhaps not surprising, but there is a direct relevance to applications in biochemistry or to enzyme-catalyzed organic synthesis. This result suggests, for instance, that adenosine in the presence of vanadate will spontaneously form both adenosine monophosphate and adenosine diphosphate analogues. Either or both of these products might serve as powerful enzyme inhibitors if the enzymic reactions involve adenine nucleotides. Alternately, with other hydroxyl-bearing compounds, vanadate analogues of phosphate substrates for enzymes may be formed that are acted on by the enzyme thus leading to formation of products.

It did not prove possible to measure a formation constant for methyl vanadophosphate although it is apparent it is formed. It seems likely that methyl divanadophosphate is also formed. Phosphate catalyzes the formation and hydrolysis of mono- and dimethyl vanadates. At pH 7.46 a 2.5 mM phosphate solution causes a 50-fold rate enhancement. This actually represents a lower limit, and the rate enhancement might be significantly more than this.

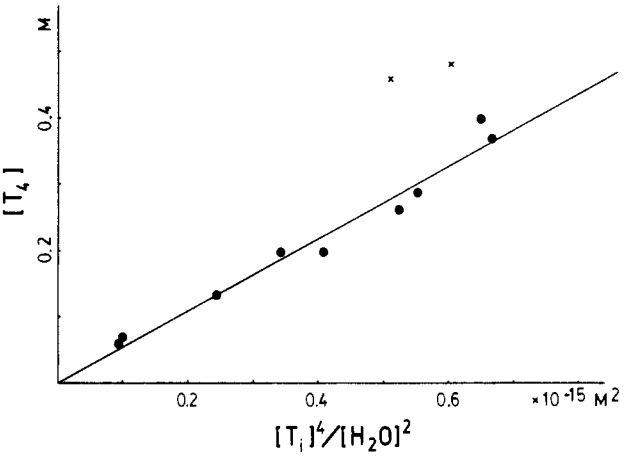


Figure 3. Plot of data shown in Table IV. This graph indicates that the formation of tetravanadate from monomeric vanadate ($VO_4H_2^-$) is accompanied by the production of 2 equiv of water.

In an attempt to gain information concerning the structure of T_4 the amount of water required in forming this product was determined as discussed in the Appendix. The results were not conclusive but were consistent only with uptake of 2 mol of water/mol of product. This is in accord with an adamantane-like structure formed from four pentacoordinate vanadate ions, $H_4V_4O_{14}^{4-}$. It is necessary that similar studies be done in other mixed-solvent systems in order to confirm this result.

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Appendix

The structure of the tetrameric product, T_4, is of interest since, although it has generally been accepted that this compound has a monocyclic ring of alternating oxygen and vanadium nuclei,[16,17] it has recently been suggested on the basis of NMR studies in liquid crystalline solution that the overall symmetry of this compound must be at least tetrahedral.[18] An adamantane-like structure was suggested.

It is possible to obtain structural information concerning T_4 if the amount of water required for its formation can be determined. Water concentation can be varied by replacing water with another solvent. Methanol seems suitable for this procedure.

The condensation of four vanadates to give the tetrameric product is a remarkably favorable one, which proceeds as described in eq 10. This study provides information concerning n, the

$$4T_i \overset{K_0}{\rightleftharpoons} T_4 + nH_2O \qquad [T_i]^4K_0 = [T_4][H_2O]^n \qquad (10)$$

number of water molecules produced as T_4 is formed. If T_4 is monocyclic then n is 4, while if T_4 has an adamantane-like structure, n is 6 unless the product is hydrated. During the attempt to fit the results contained in Table I, Table IV was constructed.

In an attempt to fit the results of Table IV to eq 10 it was clear that a reasonable correlation could only be obtained for a value of n equal to a result inconsistent with a monocyclic product. A plot of $[T_i]^4/[H_2O]^2$ vs $[T_4]$ is shown in Figure 3. A good linear relationship is evident except that the top two points of Table IV do not fit at all well to the graph. This may indicate that the low concentrations of methanol are perturbing the formation of T_4, perhaps by changing the character of the solvent water. Because of the fourth power dependence on vanadate concentration small

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perturbations could account for the discrepancy. The higher methanol concentrations (above 2.47 M, ~10% by volume) apparently have little further adverse effect on the equilibrium. This behavior unfortunately does add a degree of uncertainty to the meaning of this result. The result is, however, consistent with an adamantane-like structure of formula $H_4V_4O_{14}^{4-}$ for the tetravanadate ion. In this structure each of the vanadiums is pentacoordinate and the product tetramer carries a net charge of −4. Such a structure would also be in accord with the results from the study of this ion in liquid crystalline solution.[18]

Registry No. MeOH, 67-56-1; PO_4^{3-}, 14265-44-2; $VO_4H_2^-$, 34786-97-5; $HO_3VOVO_3H^{2-}$, 37353-31-4; $MeOVO_3H^-$, 111291-05-5; $(MeO)_2VO_2^-$, 111291-06-6; $(MeO)O_2VOVO_3H^{2-}$, 111291-07-7; $(MeO)O_2VOVO_2(OMe)^{2-}$, 111291-08-8.

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Proximal Effect of the Nitrogen Ligands in the Catalytic Epoxidation of Olefins by the NaOCl/Manganese(III) Porphyrin System†

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Using synthetic manganese porphyrin complexes with an imidazole or a pyridine ligand covalently attached to the macrocycle, we report the control of the reaction rate, product selectivity, and stereoselectivity by the axial ligand trans to the active site of the catalyst. The results clearly illustrate the proximal effect on nitrogen ligands in this cytochrome P-450 model.

Since the earlier work on metalloporphyrin-catalyzed oxygenation of the hydrocarbons,[1-3] many studies have documented this catalytic transfer of an oxygen atom from an oxidant (PhIO, NaOCl, $KHSO_5$, ROOH, or H_2O_2 or O_2 and an electron source) to an olefin or a saturated hydrocarbon (for recent reviews, see ref 4 and 5). Among the factors that control the oxygen-transfer step, much attention has been paid to the macrocyclic ligand itself: the presence of the bulky substituents at the 2,6-positions of the phenyl groups in the meso positions of the tetrapyrrolic planar structure are able to create a cage around the active metal/oxo species generated during the catalytic cycle. Such a cage effect makes it possible to avoid the dimerization of the catalyst, via the formation of a μ-oxo linkage and the self-destruction of the porphyrin complex ("bleaching effect"), and to control the possible radical species that might be involved in some stages of the catalytic cycle (cage radicals versus free radicals).

Besides this role of the macrocyclic ligand itself, the important effect of an additional nitrogen base acting as the fifth ligand on the rate and the chemo- and stereoselectivity has also been evidenced. The influence of pyridine or N-substituted imidazole has been successively evidenced with NaOCl,[6,7] O_2 and electrons,[8] ROOH,[9] H_2O_2,[10] and peracids[11] as oxygen sources.

These results have to be regarded as the preliminary approach in mimicking the proximal and distal effects that are involved in the chemical and biochemical properties of hemoproteins such as cytochrome P-450, peroxidase, catalase, and hemoglobin, all containing iron protoporphyrin IX as a prosthetic group.[12] The nature of the proximal group in the fifth position is well-established by X-ray structures: an imidazole of an histidine residue in hemoglobin and myoglobin[13] and cytochrome C peroxidase,[14] a phenoxy group from tyrosine in catalase,[15] and a cysteinato ligand in cytochrome P-450.[16]

For most of these proteins, the nature of (the) distal ligand(s) is also known: a histidine is involved in catalase[15] and in myoglobin for the stabilization of the iron–dioxygen complex by hydrogen bonding,[13] while histidine and arginine residues are in distal positions in cytochrome C peroxidase.[14] However, little is known about the electronic effects that are induced by these proximal and distal protein ligands during the different steps of the catalytic cycle and, in particular, about the formation and the reactivity control of the high-valent iron–oxo entities that are the key intermediates in the oxygenase, peroxidase, and catalase systems.

Table I. *cis*-Epoxide:*trans*-Epoxide Ratios in the Manganese Porphyrin Catalyzed Epoxidation of *cis*-Stilbene by NaOCl

run no.	catalyst[e]	extra ligand[e]	% *cis*-epoxide	% *trans*-epoxide
1	Mn(TPP)OAc		35	65
2	Mn(TPP)OAc	py[a]	70	30
3	Mn(TPP)OAc	*N*-Ap-Im[b,c]	85	15
4	**1** (py)[d]		89	11
5	**1**	py	92	8
6	**2** (py)[d]		89	11
7	**2**	py	86	14
8	**3** (Im)[d]		85	15
9	**3**	py	87	13
10	**3**	*N*-Ap-Im	80	20
11	**4** (py)[d]		93	7
12	**4**	py	84	16
13	**4**	*N*-Ap-Im	84	16

[a] A total of 25 equiv of pyridine versus 1 equiv of catalyst is used. [b] A total of 14.5 equiv of *N*-Ap-Im is used. [c] This ligand has a drastic effect on the rate of these catalytic epoxidations.[7] [d] The nature of the attached fifth ligand is indicated in parentheses. [e] Abbreviations: TPP, tetraphenylporphyrin; py, pyridine; Im, imidazole; *N*-Ap-Im, 4-(imidazol-1-yl)acetophenone.

It is expected from these metalloporphyrin-catalyzed oxygenation reactions in the presence of pyridine or imidazoles that a

† This article is dedicated to the memory of Professor Iwao Tabushi of Kyoto University.

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