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Supplementary Material Available: Crystal packing diagram, table of coordinates and equivalent isotropic temperature factors for carbon and oxygen atoms, table of molecular dimensions, and tables of positional parameters for carbon and oxygen atoms and hydrogen atoms of 4 (6 pages). Ordering information is given on any current masthead page.

Substituent Effects in Organic Vanadate Esters in Imidazole-Buffered Aqueous Solutions

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The spontaneous formation of vanadate esters and diesters in imidazole-buffered aqueous solutions was observed by using ^{51}V NMR spectroscopy. The ^{51}V NMR chemical shifts of monoesters varied as follows: primary alcohols > vanadate monomer $\simeq$ primary alcohols substituted with electronegative substituents > secondary alcohols > tertiary alcohols. Vanadate esters formed from primary alcohols were more stable than the vanadate esters formed from secondary or tertiary alcohols. This order of stability in solution containing high ionic strength and imidazole is contrary to the order of stability observed in low ionic strength solutions containing Hepes. The role of imidazole in vanadate ester reactions was examined by using relaxation times and 1D and 2D ^{51}V NMR spectroscopy. The effects of imidazole on the relaxation times and the concentration of vanadate oligomers suggest that imidazole may interact directly with vanadium. The presence of imidazole or salts significantly affects the reactions of vanadate with organic molecules and may be important for the observed catalytic and biological effects of vanadate derivatives.

Introduction

Spontaneously formed organic vanadate esters have recently been found to have substrate activity and consequently possible synthetic utility[1] and biological activity.[2] The spontaneously formed organic vanadates are structural analogues of the corresponding organic phosphates, and this similarity presumably explains the substrate activity with enzymes that convert organic phosphates.[3] The structural analogies do not extend to the kinetic properties of these compounds. Vanadate esters form with rate constants 10^{10} times that of the corresponding organic phosphates in aqueous solutions.[4] Solutions of vanadate and alcohols reach equilibrium within milliseconds as would organic vanadates prepared by other methods on addition to aqueous solutions. Vanadate esters have been reported to form from alcoholic solutions containing ethanol,[5] ethylene glycol,[6] lactate,[7] glyceric acid,[7] glucose,[8] citrate,[9] and glycosides.[10] The vanadate–alcohol complexes have been characterized by using ^{51}V NMR spectroscopy and UV spectroscopy, but no other structural information is available on these derivatives. In this paper we will describe the complexes between an alcohol and vanadate as organic vanadate esters in analogy with organic phosphates, although the structure of these vanadium complexes in aqueous solution at high alcohol concentrations are not known.

Table I. $K_{eq\,1}$ and $K_{eq\,2}$ for a Series of Alcohols[a]

compound	$K_{eq\,1}$	$K_{eq\,2}$	compound	$K_{eq\,1}$	$K_{eq\,2}$
CH_3CH_2OH	9.4	7.2	$(CH_3)_3COH$[b]	3.4[b]	1.4[b]
$CH_3(CH_2)_2OH$	6.1	2.8	$HOCH_2CH_2OH$[c]	2.3[c]	19
$(CH_3)_2CHOH$	5.0	3.5			

[a] Samples contained 10 mM vanadate, 0.35 M KCl, 0.10 M imidazole, and various concentrations of alcohol and had a final pH of 7.00 ± 0.05. [b] These equilibrium constants were determined in samples containing 0.1 mM vanadate, 0.35 M KCl, 0.10 M imidazole, and various concentrations of alcohol and had a final pH of 7.00 ± 0.05. [c] Chemical shift for the monoester could not be distinguished from the chemical shift of the vanadate monomer.

The reaction of a simple alcohol at neutral pH with vanadate is presumed to be a reaction between the monomeric vanadate species (diprotonated or monoprotonated vanadate anion) and the alcohol, ROH, although in a solution of several equilibrating vanadate species other reaction pathways are possible. The reaction generates a vanadate monoester (eq 1) and a diester (eq 2).[5-8,10]

$$ROH + H_2VO_4^- \xrightleftharpoons{K_{eq\,1}} ROVO_3H^- + H_2O \qquad (1)$$

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$$\mathrm{ROH} + \mathrm{ROVO_3H^-} \xrightleftharpoons{K_{eq2}} \mathrm{(RO)_2VO_2^-} + \mathrm{H_2O} \qquad (2)$$

Vanadate esters formed from primary, secondary, and tertiary alcohols have similar formation constants at low ionic strength in Hepes.[5,6] We are interested in the reactions of such spontaneously formed vanadate esters as analogues of organic phosphates and their conversion by the appropriate enzymes. Since most enzyme reactions are studied at neutral pH, in buffered solution at fairly high ionic strength, vanadate ester formation under these conditions are of interest.[11] Vanadate oligomerization reactions are known to be very sensitive to the reaction medium and differences in buffers, ionic strength, and pH.[12] The vanadate ester formation constants at low ionic strength in Hepes are therefore likely to change considerably at higher ionic strength and in other buffers.

Imidazole has been found to be the buffer of choice for biological studies with vanadate because complicating redox reactions involving vanadate occur less in imidazole than in Hepes, Tris, or other buffers.[13,14] If biological studies are to be conducted with organic vanadate esters, determination of formation constants should therefore also be done in imidazole at neutral pH and high ionic strength. The compounds forming organic vanadate esters with enzyme activities contain several hydroxyl functionalities.[1,2] Differences in the ^{51}V NMR chemical shifts of vanadate esters of primary, secondary, or tertiary alcohols would therefore allow convenient assignment of the ^{51}V NMR resonances and simplify the determination of the concentration of specific organic vanadates. Organic vanadate esters from primary, secondary, and tertiary alcohols in solutions containing 20 mM Hepes have varying chemical shifts,[15] and such observation may also be observed in solutions of higher ionic strength and in other buffers. In this paper we describe the determination of formation constants and chemical shifts of a series of organic vanadate esters at 0.35 M KCl and 0.10 M imidazole. Unexpectedly, the size *and* the order of the stability constants obtained in this study were different than those previously obtained in Hepes.[16] The linear free energy relationship between log $(1/K_{\mathrm{eq}\,1})$ and pK_a again had a $\rho \approx 0$, but the ρ was of opposite sign in our experiments. We feel that further interpretation of these results is not warranted at the present time. These observations suggest that ionic strength and/or imidazole directly affect the vanadate reactions. Since the stability of the vanadate esters appear to change in the presence of salts and/or imidazole, it is possible that either salt and/or imidazole interacts with vanadate to stabilize the complexes. Alternative possibilities exist and should be considered in an exhaustive investigation. The effects of imidazole on the relaxation times and exchange rates of various vanadate derivatives were examined in this study. The exchange rates were measured by using 2D ^{51}V NMR EXSY spectroscopy with and without imidazole present in low ionic strength solutions. Vanadium-51 is a 7/2 spin, quadrupolar nucleus of 99.75% abundance and has been a convenient tool for 1D studies of vanadate reactions. 2D ^{51}V NMR EXSY spectroscopy was only recently introduced in kinetic studies of the vanadate oligomerization reactions at pH 8.6 in 1 M KCl.[17] Since this study represents the only quantitative kinetic study conducted with vanadate solutions containing more than two species, and since the vanadate speciation varies significantly with pH, ionic strength, and total vanadate concentrations, an exhaustive study of the vanadate ester reactions and the role of salts and imidazole in such reactions under various conditions is not presented here. However, novel qualitative and quantitative conclusions concerning the exchange rates and exchange mechanisms of monoesters and diesters in solutions with and without imidazole are presented.

Experimental Section

General. Chemicals were reagent grade. Water was distilled and deionized. A vanadate stock solution was prepared by dissolving vanadium pentoxide with 2 equiv of sodium hydroxide to generate 0.25 M vanadate (pH = 8.6). This solution was stored at 4 °C in order to minimize formation of precipitates. The concentration of the vanadate stock solution was monitored at pH 13 by UV spectroscopy using the absorption at 260 nm and an extinction coefficient of 3.55×10^3 M^{-1} cm^{-1}. No changes in vanadate concentration were observed over the course of 6 months. Stock solutions of imidazole were prepared (0.48 and 1.41 M) at pH 7.20. A stock solution of 4.0 M KCl was prepared from distilled-deionized water. Stock solutions of 10 M alcohol were prepared by measuring an appropriate weight of alcohol and adding distilled-deionized water to the required volume.

1D ^{51}V NMR Spectroscopy. ^{51}V spectra were recorded at ambient temperatures, at 131.5 MHz, on a Bruker AM-500 spectrometer (11.7 T). We typically used spectral windows of 80 ppm, a 90° pulse angle, and an acquisition time of about 0.1 s with no relaxation delay between pulses. An exponential line broadening of 10 Hz was applied to the FID prior to Fourier transformation. Chemical shifts are reported relative to an external reference of VOCl$_3$ (0 ppm), but in practice an external reference solution containing 10 mM vanadium and diethanolamine at pH 8.8 (−490 ppm) was used.[14]

T_1s were measured using modified Freeman–Hill inversion recovery experiments.[18] The recovery times varied from 0.003 ms to 100 ms. Plotting ln (M_0-M_t) as a function of t gave linear relationships that yielded the T_1s. At no time was a biexponential decay, indicating more than one species for a given resolved signal, observed in the vanadate–ethanol mixtures. We estimate that the uncertainty in the determined T_1s do not exceed 15%.

Concentration Calculations. The concentrations of each vanadate species were determined by using the mole fraction for each resonance calculated from the spectral integration and the known total vanadium concentration. The concentrations of vanadate monomer, dimer (when appropriately resolved), and tetramer were determined and their relationships explored. Vanadate monomer and oligomers exchange rapidly in aqueous solutions and their exchange reactions are related as shown n eqs 3–5 in dilute aqueous solutions.[5–10,14,15] The monomeric vanadate concentration is therefore related to the dimer concentration and tetramer concentration as shown in eqs 6 and 7.

$$2\mathrm{V_1} \xrightleftharpoons{K_{12}} \mathrm{V_2} \qquad (3)$$

$$4\mathrm{V_1} \xrightleftharpoons{K_{14}} \mathrm{V_4} \qquad (4)$$

$$2\mathrm{V_2} \xrightleftharpoons{K_{24}} \mathrm{V_4} \qquad (5)$$

$$[\mathrm{V_2}] = K_{12}[\mathrm{V_1}]^2 \qquad (6)$$

$$[\mathrm{V_4}] = K_{14}[\mathrm{V_1}]^4 \qquad (7)$$

Studies of the reactions shown in eqs 1 and 2 require that the added alcohol concentrations do not affect the vanadate equilibria

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by changing the ionic strength. Since we use high alcohol concentrations, such requirements are nontrivial, and we carefully checked that the relationship described by eq 7 was fulfilled for ethanol and *n*-propanol; a constant $[V_4]/[V_1]^4$ ratio was observed. Only at concentrations above 8 M alcohol did we observe a small deviation for *n*-propanol. We therefore assume that corresponding relationships also hold in solutions containing up to 6 M isopropanol, ethylene glycol, and chloroethanol and that the concentrations of vanadate oligomers can be used to calculate the concentrations of the oligomers with overlapping and poorly resolved signals. We also examined the reactions of 2,2,2-trifluoroethanol with vanadate, but since the concentrations of vanadate oligomers did not show the required linear relationships shown in eqs 3 to 5, the approach described in this paper and elsewhere cannot be used for this alcohol in 0.35 M KCl and 100 mM imidazole.

2D ^{51}V EXSY NMR Spectroscopy.[17,19] The phase-sensitive ^{51}V homonuclear 2D EXSY experiments were done at 131.5 MHz on a Bruker AM-500 spectrometer at 23 °C. The standard three-pulse NOESYPH pulse sequence ($90°-t_1-90°-\tau_m-90°$) supplied with the Bruker software was used to perform the experiment. This pulse sequence utilizes the time-proportional phase incrementation (TPPI) to obtain pure phase line shapes. A mixing time of 8 ms was used in a 100 ppm window for solutions containing no imidazole, 5 mM, and 50 mM imidazole. The acquisition time was 40 ms and a relaxation delay of 40 ms extended the time between transients to approximately 80 ms, which was at least $4T_1$. Using 1000 accumulations for each of 256 t_1 increments gave a spectral acquisition and accumulation time of 4 h. A $\pi/2$ shifted sine-bell weighting function was applied to both F_1 and F_2 domains, and they were zero-filled to 512 and 1024 points respectively, prior to Fourier transformation. This gave a final resolution of about 60 and 30 Hz along F_1 and F_2, respectively.

Integration of the peak volumes was carried out by integrating the individual row and column slices of the 2D spectral matrix using the standard Bruker software, DISNMR for process controller and Glinfit. The volume elements obtained in this manner were normalized by using the relative peak integrals from a 1D ^{51}V NMR spectrum. The normalized intensity matrix was diagonalized by an adapted routine from EISPAC for solving real and general matrices. The exchange matrix calculated in this manner contains specific rate constants for exchange of magnetization between sites. The rate matrix can be obtained from the rate constants by multiplying each row by the concentration of the specific oligomer.

Spectral Simulation. The ^{51}V NMR spectra used for the 2D ^{51}V EXSY NMR experiment were simulated by using the program Glinfit (by Alex D. Bain) supplied with the Bruker software. Our simulations were accurate within ±3%.

NMR Sample Preparation. All NMR samples for 1D ^{51}V NMR studies were prepared at ambient temperature and contained 0.35 M KCl, 0.10 M imidazole,[11,14] 0.010 M vanadate, 10% D_2O, and alcohol concentrations varying from 0 to 6.0 M unless otherwise specified. The sample pH was checked before and after the ^{51}V NMR spectra were recorded and was between 6.95 and 7.05 for all samples. Correction of the pH determination for the presence of the alcohols was not done.

Uncertainties. The 1D spectra were reproducible to within ±5% and the concentrations obtained from one spectrum reproducible to within ±3%. We estimate that our formation constants are accurate to within ±30%. Detailed description of error analysis for the 2D EXSY experiments has been described previously.[17,19] The errors arise from the limited *S*/*N* ratios of the computed spectra, instrumental drift during the experiment, or errors in the 2D signal integration. We were able to reproduce the rate matrix with less than 3% deviation on the most intense peaks and less than 20% deviation on the smallest peaks. We estimate that the largest rate constants are determined within a 20% accuracy and the smallest rate constants are determined within a factor of 2.

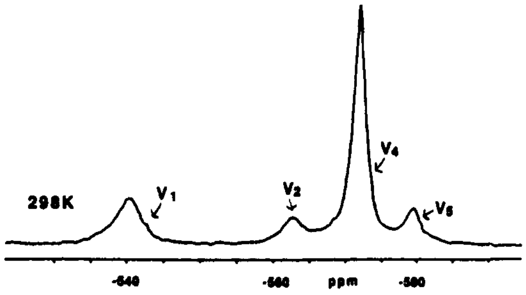

Figure 1. ^{51}V NMR spectrum at 52.6 MHz (4.7 T) of a solution containing 10 mM vanadate, at pH 8.6 (I = 0.4). Signals for vanadate monomer (V_1; −541 ppm), dimer (V_2; −563 ppm), tetramer (V_4; −573 ppm), and pentamer (V_5; −580 ppm) are marked.

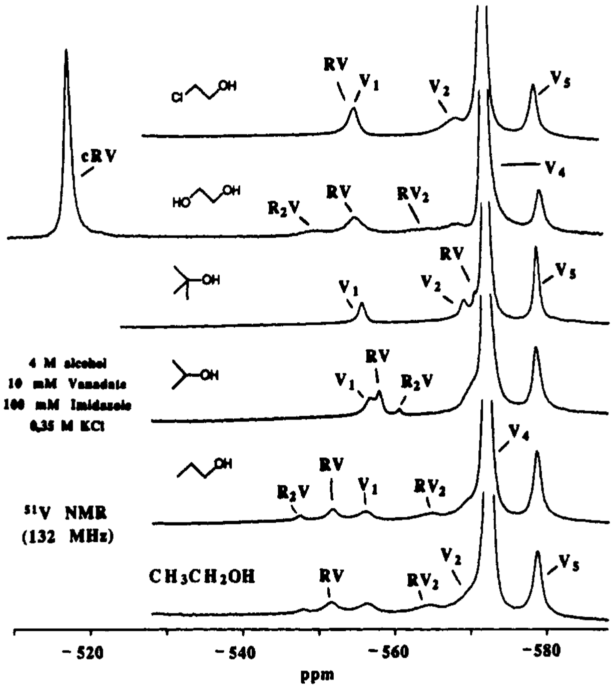

Figure 2. ^{51}V NMR spectra at 131.5 MHz (11.7 T, 500-MHz ^{1}H) are shown of various solutions containing 4 M alcohol (ethanol, *n*-propanol, 2-propanol, *tert*-butyl alcohol, ethylene glycol, and chloroethanol) in the presence of 10 mM vanadate, 100 mM imidazole, and 0.35 M KCl. Signals for monoester (RV), diester (R_2V), vanadate dimer-derived esters (RV_2 and R_2V_2), cyclic ester (cRV), monomeric vanadate (V_1), dimeric vanadate (V_2), tetrameric vanadate (V_4) and pentameric vanadate (V_5) are marked.

Results and Discussion

Vanadate Oligomerization Reactions. Vanadate monomer oligomerizes in aqueous solutions to form vanadate dimer, tetramer, and pentamer. The various vanadate oligomers can be identified by ^{51}V NMR spectroscopy and their concentration and protonation state can be determined. The vanadate monomer is assigned to the resonance at −541 ppm, the vanadate dimer at −563 ppm, the vanadate tetramer at −574 ppm, and the vanadate pentamer at −580 ppm at pH 8.6 (Figure 1).[5-10,12,14] The position of the equilibria are very sensitive to ionic strength, pH, temperature, buffers, and additional compounds present in solution. At pH 7.0 the vanadate monomer is assigned to the resonance at −557 ppm, the vanadate dimer at −570 ppm, the vanadate tetramer at −574 ppm, and the vanadate pentamer at −581 ppm (Figure 2). The changes in the chemical shift for the monomer from −541 to −557 ppm indicate a shift from the monoprotonated monomer, HVO_4^{2-}, at pH 8.6 to the diprotonated monomer, $H_2VO_4^-$, at pH 7.0. These assign-

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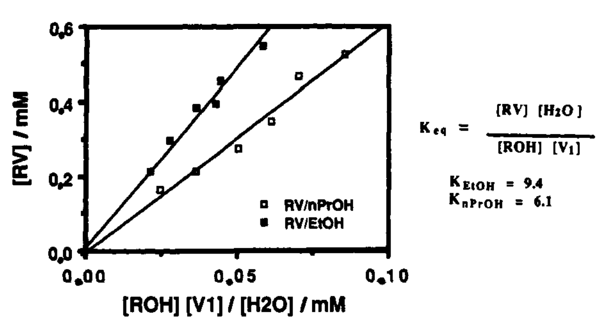


Figure 3. Concentration of monoesters from ethanol and *n*-propanol are plotted against $[EtOH][H_2VO_4^-]/H_2O$ and $[n\text{-}PrOH][H_2VO_4^-]/[H_2O]$, respectively. Correlation coefficients were 0.99.

ments are in accord with a pK_a around 8.2 for $H_2VO_4^-$ under these conditions.[14]

We have chosen to study the reactions at pH 7.00 ± 0.05 in 100 mM imidazole because these conditions are compatible with enzymatic studies of glycolytic and pentose phosphate shunt enzymes.[2,8,11] Imidazole is commonly used as a buffer in biological studies with vanadate because this buffer does not catalyze the reduction of NAD or NADP in the presence of vanadium.[13,14] The chemical studies reported in this paper were carried out at 0.35 M KCl and an approximate ionic strength of 0.4. Changes in vanadate oligomer populations or charged lipid concentrations should not significantly change the overall ionic strength.

Stability of Organic Vanadate Monoesters and Diesters. The equilibrium constants for eqs 1 and 2 are defined as shown in eqs 8 and 9. At pH 7.00 ± 0.05

$$K_{eq\,1} = \frac{[ROVO_3H^-][H_2O]}{[ROH][H_2VO_4^-]} \tag{8}$$

$$K_{eq\,2} = \frac{[(RO)_2VO_2^-][H_2O]}{[ROH][ROVO_3H^-]} = \frac{[(RO)_2VO_2^-][H_2O]^2}{[ROH]^2[H_2VO_4^-]K_{eq\,1}} \tag{9}$$

monomeric vanadate is almost exclusively present as $H_2VO_4^-$.[14] Correspondingly, the monoester and diester are also almost exclusively present as monoanions under these conditions.[5] The concentrations were calculated by integration of the spectrum and using the mole fraction to determine the relative amounts of the various species. The ^{51}V NMR spectra were recorded at several alcohol concentrations, and the vanadate monomer and esters concentrations were determined at each alcohol concentration. Plots of monoesters as a function of $[ROH][H_2VO_4^-]/[H_2O]$ and diesters as a function of $[ROH]^2[H_2VO_4^-]/[H_2O]^2$ were then used to determine $K_{eq\,1}$ and $K_{eq\,2}$ (Figure 3).

Primary Alcohols. Three primary alcohols, ethanol, *n*-propanol, and 2-chloroethanol, were mixed with vanadate in 100 mM imidazole at pH 7.00 ± 0.05 and an ionic strength of 0.4. The vanadate monoesters of both ethanol and *n*-propanol have a chemical shift of −552 ppm (Figure 2). The vanadate diesters of ethanol and *n*-propanol have chemical shifts of −549 and −548 ppm, respectively. The $K_{eq\,1}$ for the ethanol complex was determined from the plot of $[EtOVO_3H^-]$ as a function of $[EtOH][H_2VO_4^-]/[H_2O]$ to be 9.4 with a correlation coefficient of 0.99 (Figure 3). Similar calculations on *n*-propanol yield a $K_{eq\,1}$ of 6.1 (Figure 3). The equilibrium constants are summarized in Table I. $K_{eq\,2} = 7.2$ for ethanol was determined by plotting $[(EtO)_2VO_2^-]$ as a function of $[EtOH][EtOVO_3H^-]/[H_2O]$. $K_{eq\,2}$ for the *n*-propyl diester was 2.8. The addition of a methyl group to ethanol did not affect the chemical shifts of the vanadate mono- and diesters. $K_{eq\,1}$ was decreased by a factor of 1.5, whereas $K_{eq\,2}$ was decreased by a factor of 2.6 by the addition of a methyl group.

The reaction of vanadate with 2-chloroethanol did not generate a monoester at −552 ppm. The spectrum for the solution containing vanadate and 2-chloroethanol illustrated in Figure 2 shows resonances at −557, −570, −574, and −581 ppm and these signals are assigned to vanadate monomer, dimer, tetramer, and pentamer. However, using eqs 4 and 7, the expected concentration of vanadate monomer can be calculated from the tetramer concentration. The expected concentration of monomeric vanadate is 0.74 mM, whereas 0.83 mM is observed at the −557 ppm signal in the 1 M 2-chloroethanol sample. At 6 M 2-chloroethanol 0.72 mM monomeric vanadate is expected, whereas 1.23 mM is observed in the −557 ppm signal. This sample does not give a constant $[V_4]/[-557\text{ ppm}]^4$ because the peak at −557 ppm contains both vanadate and vanadate 2-chloroethanol esters. The difference between the expected concentration for vanadate monomer and the observed concentration of the signal at −557 ppm was therefore assigned to the vanadate mono- and diesters of 2-chloroethanol. Since we cannot separate the possible contributions of the monoester from the diester (we expect that both are formed), we have not calculated formation constants for these esters.

Secondary Alcohols. The two complexes formed between isopropanol and vanadate had chemical shifts of −559 ppm and −562 ppm for the monoester and diester, respectively. Since the vanadate isopropyl ester and vanadate monomer signal overlap, we used eq 7 to calculate the monomeric vanadate concentration. The concentration of vanadate isopropyl ester is therefore calculated as the difference between the concentration of the overlapping signals at −557 and −559 ppm and the calculated concentration of monomer. Calculations for $K_{eq\,1,i\text{-}PrOH}$ were made by using eq 10. $K_{eq\,1,i\text{-}PrOH}$ and $K_{eq\,2,i\text{-}PrOH}$ were calculated to be 5.0 and 3.5 for this secondary alcohol. $K_{eq\,1,i\text{-}PrOH}$ is about half that of $K_{eq\,1,EtOH}$.

$$K_{eq\,1} = \frac{([-557 + -559\text{ ppm resonance}] - [V_1]_{cal})}{[ROH][V_1]_{cal}} \tag{10}$$

Tertiary Alcohols. The chemical shifts for the *t*-BuOH monoester and diester were −572 ppm and −570 ppm respectively (Figure 2). Precise determination of the concentrations of t-butyl esters were obtained at 0.1 mM vanadate concentration, where the vanadate tetramer did not obscure the observations of the *tert*-butyl mono- and diesters. Corresponding conditions yielded the same $K_{eq\,1,EtOH}$ and $K_{eq\,2,EtOH}$ for the ethyl esters as determined above using 10 mM vanadate. $K_{eq\,1,t\text{-}BuOH}$ was determined to be 3.4 and $K_{eq\,2,t\text{-}BuOH}$ was 1.4. We observe a small but steady decrease in the ester stability constants when substituting primary with secondary and tertiary alcohols. These changes in the formation constants in 0.10 M imidazole and at approximately 0.4 ionic strength are contrary to the previous observations at low ionic strength.[15,16] Our results suggest that formation constants for vanadate esters in solutions containing imidazole at 0.4 ionic strength show significant sensitivity to the sterics of the alcohol moiety.

Diols. The reaction of vanadate with ethylene glycol is more complicated because cyclic vanadates form in addition to the mono- and diesters. The cyclic vanadates can be either mono- or dinuclear in vanadium. In solution at pH 7.00 (±0.05) containing 0.10 M imidazole at 0.4 ionic strength, the vanadate monoester of ethylene glycol could not be distinguished from the vanadate monomer (Figure 2). This observation suggests that either the ethylene glycol monoester is in fast exchange with the vanadate monomer or that the chemical shifts of the ethylene glycol vanadate ester and the monomer are identical. Previous studies of ethylene glycol with vanadate in Tris buffer showed a 4.5 ppm separation between the chemical shift of the monomer and the ethylene glycol monoester.[6] Therefore, it is likely that the ethylene glycol monoester of vanadate and the vanadate monomer are in rapid exchange in the presence of imidazole. The 0.10 M imidazole and 0.35 M KCl (compared to 20 mM Hepes or 20 mM Tris) seem to affect the kinetics of the vanadate ester exchange reactions.

The concentration of ethylene glycol monoester could be calculated by using the analysis described above for the isopropyl vanadate ester and the 2-chloroethanol esters (eq 10). In a solution of 0.5 M ethylene glycol, the concentration of vanadate monomer was calcualted as 0.65 mM, whereas 0.66 mM was observed at the −557 ppm resonance. In a solution of 4 M ethylene glycol, the concentration of vanadate monomer was calculated to 0.46 mM, whereas 0.70 mM was observed at the −557 ppm resonance. The $K_{\mathrm{eq\,1,diol}}$ was determined to be 1.7. The $K_{\mathrm{eq\,1,diol}}$ is of particular interest because it is much smaller than the formation constants of ethanol and *n*-propanol previously determined at low ionic strength in Hepes. We therefore conclude that the buffer and/or salt concentrations significantly affect the stability of the vanadate ester.

Relationship between $K_{\mathrm{eq}\,1}$ and $\mathrm{p}K_a^{\mathrm{ROH}}$. A linear relationship was previously observed between log $(1/K_{\mathrm{eq}\,1})$ and $\mathrm{p}K_a^{\mathrm{ROH}}$ (the $\mathrm{p}K_a$ of the alcohol) for $K_{\mathrm{eq}\,1}$s determined at low ionic strength.[16] Seven out of ten alcohols showed a very good fit to such a relationship ($\rho = -0.09$), one alcohol showed an acceptable fit whereas two alcohols showed a poor correlation with this linear relationship. One of these alcohols, *t*-BuOH, was completely opposite to the observed relationship, suggesting that other effects are contributing to this particular formation constant. In general it appears that there is a linear relationship between the formation constant $\mathrm{p}K_a^{\mathrm{ROH}}$ although ρ is only −0.09.[16] In view of such a small ρ, changes in the $\mathrm{p}K_a$ for the ROH in each reaction series do not appear to affect the ester formation constant significantly. It is possible that steric hindrance or interactions facilitate the unexpectedly low formation constant for the vanadate monoester formed from *t*-BuOH. Such a conclusion is supported by our determinations of $K_{\mathrm{eq}\,1}$s in 0.10 M imidazole and 0.35 M KCl. We found the formation constant of vanadate monoester decreased in the order 1° > 2° > 3° > (1° with electronegative substitutents). A plot of log $(1/K_{\mathrm{eq}\,1})$ against $\mathrm{p}K_a^{\mathrm{ROH}}$ of our formation constants would therefore result in a small positive slope contrary to the negative slope observed at low ionic strength (data not shown).

In both cases the ρ is very small ($-0.1 < \rho < 0.1$). The change in the sign of the slope may be indicative of the changes in the conditions under which the vanadate monoester is observed in the two experiments. There are several reasonable explanations for a small ρ. The experiments may have been carried out at or near the isoequilibrium temperature where the effects of substituents on the stability of the product are small. The $\mathrm{p}K_a^{\mathrm{ROH}}$ has only little effect on the K_{eq} and it may not be the only factor affecting the stabilization of vanadate monoesters. An additional factor may be the steric bulk of the alcohol, and this effect may only be present in very bulky alcohols or at higher ionic strengths with buffers such as imidazole. Vanadate may require small alkyl ligands around the vanadium to obtain maximum bonding stabilization or, expressed somewhat differently, the more bulky alkyl ligands interfere with solvation of the ester; however, the effect is small. In either case the formation constants of monoesters in 0.10 M imidazole and 0.35 M KCl decrease when the steric bulk of the alcohol increases.

^{51}V NMR Chemical Shifts of Various Organic Vanadate Monoesters. The ^{51}V NMR resonance of monoesters shifts toward higher frequency as an increasingly substituted alcohol forms the vanadate ester (Figure 2). The chemical shift at highest frequency is exhibited by the *tert*-butyl complex, which is also the most hindered ester (−572 ppm). These results correspond to previous observations under different conditions.[15] Replacing an H in ethanol with a methyl group (*n*-propanol) does not affect the chemical shift of the ester. Substituting an H in ethanol with Cl or other electronegative group yields a ^{51}V NMR spectrum in which the monoester is superimposed on the vanadate monomer signal due to coincidental chemical shifts and/or rapid exchange. The substitution of H with an OH group yields a vanadate monoester that is in rapid equilibrium with the vanadate monomer when imidazole is used as buffer at high ionic strength. Rapid exchange is not observed when this reaction is studied in Tris at low ionic strength.[6] It appears that the chemical shifts for the ^{51}V NMR resonances are fairly constant although the kinetics may change significantly and possibly affect whether the derivatives will be in fast or slow exchange on the NMR timescale. The assignments given in imidazole at high ionic strength are in general agreement with the assignments previously reported in Hepes at low ionic strength. These assignments should therefore be appropriate for a variety of conditions. However, if unusual line broadening of the monomeric vanadate resonance is observed, this may indicate a case of fast exchange on the NMR time-scale.

Vanadate and Ethanol with and without Imidazole. The changes in equilibrium constants and chemical shifts induced by the media could be caused by changes in the ionic strength and/or the buffer. We have examined the effects of imidazole in ethanol and vanadate solutions at low ionic strength. In the case of ethanol, little change was expected for $K_{\mathrm{eq\,1,EtOH}}$ as the concentration of imidazole changed, since the $K_{\mathrm{eq\,1,EtOH}}$ determined in this study corresponded to that observed previously. Correspondingly little change was observed in $K_{\mathrm{eq\,1,EtOH}}$ at varius imidazole concentrations. However, a drastic variation in the $[V_4]/[V_1]^4$ ratio was observed. A change in this ratio demonstrates that the added imidazole affects the formation constant of tetramer [see eqs 4 and 7]. It is therefore possible that the imidazole contributes to the observed changes in formation constants and that changes in ionic strength may not be completely responsible for these effects.

The 1D ^{51}V NMR spectrum of 10 mM vanadate and 4.9 M ethanol at pH 7.8 solution showed signals for a vanadate diester at −547 ppm, a monoester at −552 ppm, a monomer at −557 ppm, two presumed dimer derived esters at −564, −566, and −572 ppm, a dimer at −571 ppm, and a tetramer at −576 ppm (Figure 4b). In order to appropriately investigate this spectrum, we used Glinfit to simulate the spectrum (Figure 4a). The 1D ^{51}V NMR spectrum of 10 mM vanadate, 5.2 M ethanol, and 5.0 mM imidazole at pH 7.5 was assigned as follows: vanadate diester at −547 ppm, monoester at −552 ppm, vanadate monomer at −557 ppm, two presumed dimer derived esters at −564, −566, and −572 ppm, dimer at −571 ppm, and tetramer at −576 ppm (Figure 4c). Glinfit was also used to simulate this spectrum (spectrum not shown). The presence of the 5 mM imidazole in the vanadate solution significantly affects the line

Table II. T_1s (in ms) for Vanadate Derivatives under Various Conditions (131.5 MHz)[a]

	vanadate derivative					
conditions	R_2V	RV	V_1	presumed V_2 derivatives	V_2	V_4
10 mM vanadate, pH 8.6, 23 °C			15		13	10
10 mM vanadate, 4.9 M ethanol, pH 7.8	14	13	14	8.3/8.8	11	5.1
10 mM vanadate, 5.2 M ethanol, 5 mM imidazole, pH 7.5	14	11	10	8.8	9.9	5.4
10 mM vanadate, 5.1 M ethanol, 50 mM imidazole, pH 7.5	~9[b]	4.9	3.9	6.2	3.5	5.0

[a] T_1s were obtained from plots of ln $(M_0 - M_t)$ as a function of t measured from modified Freeman–Hill inversion recovery experiments (Freeman, R.; Hill, H. D. W. *J. Chem. Phys.* **1974**, *54*, 3367–77). We estimate that the uncertainties on the T_1s were not above 15%, although we expect that the real uncertainty is much less than this. [b] This value was determined with much greater uncertainty.

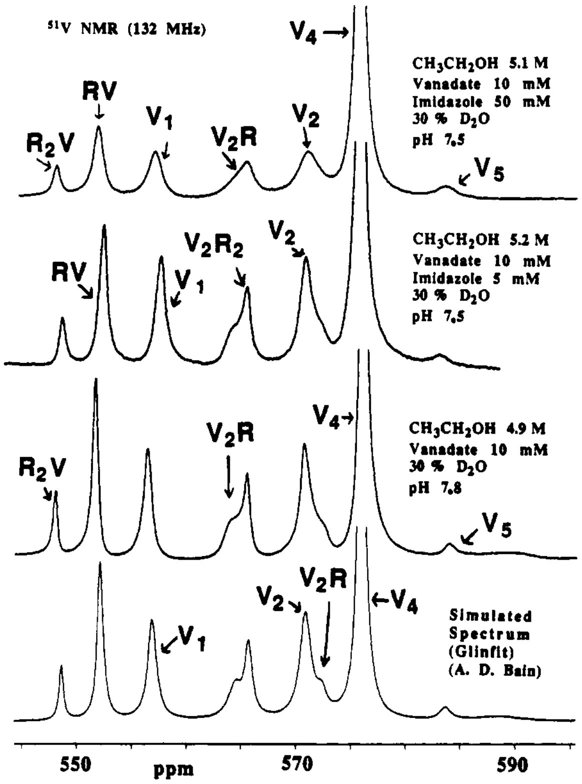


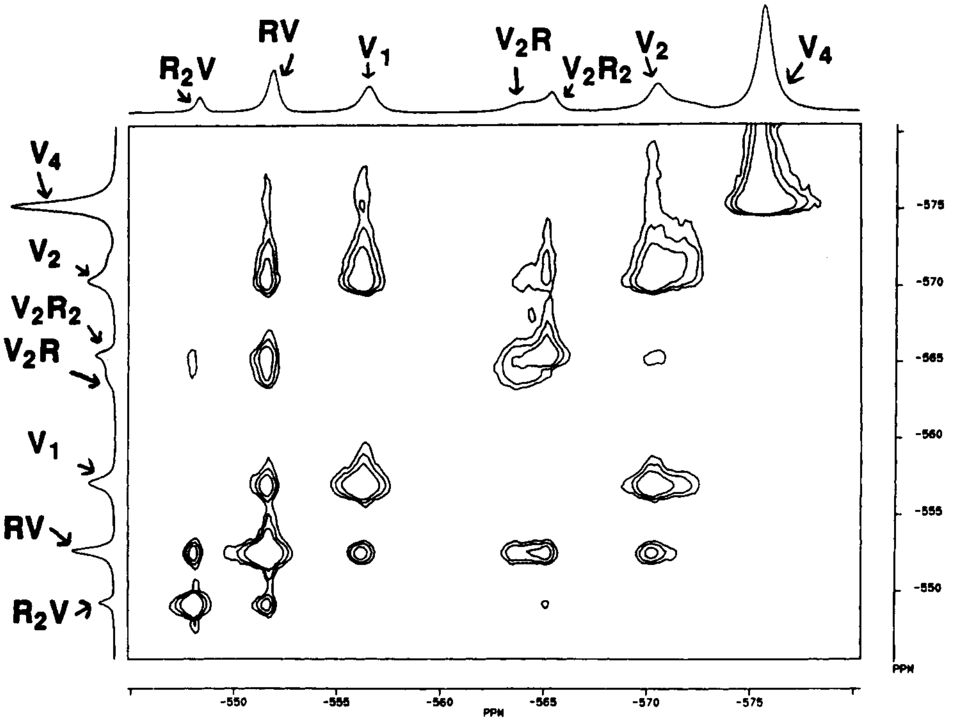
Figure 4. ^{51}V NMR spectra at 131.5 MHz (11.7 T) are shown of (a) the corresponding Glinfit simulated spectrum (within a 3% fit) of (b), (b) 4.9 M ethanol and 10 mM vanadate at pH 7.8, (c) 5.2 M ethanol, 10 mM vanadate, and 5 mM imidazole at pH 7.5, and (d) 5.1 M ethanol, 10 mM vanadate, and 50 mM imidazole at pH 7.5.

shape of the ^{51}V NMR resonances at −564, −566, and −572 ppm.

The 1D ^{51}V NMR spectrum of 10 mM vanadate, 5.1 M ethanol, and 50 mM imidazole at pH 7.5 showed a spectrum with signals for a diester at −549 ppm, a monoester at −552 ppm, a monomer at −557 ppm, one dimer derived ester at −566 ppm, a dimer at 571 ppm, and a tetramer at −576 ppm (Figure 4d). We again used Glinfit to simulate the spectrum (spectrum not shown). The presence of 50 mM imidazole in the solution has altered the spectrum to show six resolved resonances in place of eight resonances. Two of the six resonances in the spectrum recorded with imidazole are asymmetric and broad, suggesting that these signals may each be the result of two resonances near coalescence merging to one resonance. The two resonances that are no longer observed as separated signals are the presumed dimer-derived resonances at −564 and −571 ppm. On the basis of previous assignments, these resonances are assigned to a R–V–V species that is likely to give one resonance for each vanadium.[7,10] The addition of 50 mM imidazole changed the ^{51}V NMR spectrum significantly with respect to the relative concentrations of monomer, tetramer, and pentamer. Since a catalyst does not change the thermodynamics of reactions, the changes in the ^{51}V NMR spectra can be explained by loosely coordinated imidazoles to the vanadate derivatives. The changes in the line broadening for all signals suggest imidazole affects the exchange rates and can in some cases generate a situation of rapid exchange on the NMR timescale.

The observed changes in the formation constants obtained from the ^{51}V NMR spectra could possibly be due to direct interaction of imidazole with the vanadate derivatives. This direct interaction is further supported by the changes in T_1s without imidazole, with 5 mM imidazole, and with 50 mM imidazole (Table II). The T_1s for the vanadate esters and oligomers significantly decrease when imidazole is added to the solutions. The decrease in T_1s suggests that significant changes occur in the environment of the vanadium. As seen from Table II, significant changes are observed for all vanadium derivatives, and the higher the concentration of imidazole, the larger the effect. Although it is possible that imidazole forms a loose complex with the vanadate derivatives, we present no structural proposal for such complexes before further studies have been carried out to characterize these complexes. The decrease in the T_1s in the presence of 50 mM imidazole causes technical problems because it is so large that accurate exchange rate determinations can no longer be obtained under these conditions. The small T_1s of several resonances results in rapid decay of the magnetization transfer of these signals, whereas the long T_1s for other resonances requires long mixing times for observation of the magnetization transfer. Consequently, we have determined the exchange rates in ethanol–vanadate solutions with lower concentrations of imidazole (5 mM) and compare these exchange rates with those of ethanol–vanadate solutions containing no imidazole. While this approach may only yield small changes in the exchange rate, one should keep in mind that 5 mM is a factor of 10 or 20 smaller than the imidazole concentration commonly used in enzyme reactions. The rate changes in 50 mM imidazole are expected to be 10 or 20 times larger than those observed in 5 mM imidazole.

Kinetics. Phase-sensitive ^{51}V homonuclear 2D EXSY experiments[17] were used to measure microscopic exchange rates between various vanadate oligomers and vanadate esters. The 2D ^{51}V NMR spectra were recorded of solutions containing 10 mM vanadate and 4.9 M ethanol at pH 7.8 (Figure 5), 10 mM vanadate, 5.2 M ethanol, and 5 mM imidazole at pH 7.5 (Figure 6), and 10 mM vanadate, 5.1 M ethanol, and 50 mM imidazole at pH 7.5 (Figure 7).[19] The 2D ^{51}V NMR spectra showed exchange cross-peaks between selected diagonal signals in both Figures 5 and 6. The integration of the peak volumes shown in the contour plots was carried out by using the standard Bruker Software, DISNMR for process controller, and the resulting rate terms are given in Tables III and IV. The program produces an exchange rate constant matrix; we convert the microscopic rate constants to rate terms because this simplifies the following kinetic analysis. Details



CH$_3$CH$_2$OH + Vanadate

Figure 5. 2D ^{51}V NMR EXSY experiment (132 MHz) of a solution containing 4.9 M ethanol and 10 mM vanadate at pH 7.8 (30% D_2O). Integration of exchange cross-peaks leads to the microscopic rates shown in Table III.

Table III. Microscopic Exchange Rates for 4.9 M Ethanol and 10 mM Vanadate Solutions at pH 7.8[a]

x	$Y_{R_2V_x}$	Y_{RV_x}	Y_{1x}	$Y_{RV_{2x}}$	Y_{2x}	Y_{4x}
	k					
R_2V		20	0	15	11	0
RV	20		38	54	22	0
1	0	38		0	180	12
RV_2	15	54	0		37	1
2	11	22	180	37		0
4	0	0	12	1	0	
concn	0.324	1.27	1.21	1.36	1.89	4.23

[a] A rate term labeled Y_{2x} is the exchange rate that determines the rate by which the vanadate dimer forms the x derivative (rate of x formation = $k_{2x}[V_2]$. The units on the rates are mM s^{-1}. The concentrations of vanadate derivatives are in mM vanadium atoms.

Table IV. Microscopic Exchange Rates for Solution Containing 5.2 M Ethanol, 10 mM Vanadate, and 5 mM Imidazole at pH 7.5[a]

x	$Y_{R_2V_x}$	Y_{RV_x}	Y_{1x}	$Y_{RV_{2x}}$	Y_{2x}	Y_{4x}
	k					
R_2V		41	0	9	3	0
RV	41		63	45	21	0
1	0	63		0	170	0
RV_2	9	45	0		65	0
2	3	21	170	65		0
4	0	0	0	0	0	

[a] A rate term labeled Y_{2x} is the exchange rate that determines the rate by which the vanadate dimer forms the x derivative (rate of x formation = $k_{2x}[V_2]$ = Y_{2x}). The units on the rates are mM s^{-1}. The concentrations of vanadate derivatives are in mM vanadium atoms.

concerning the 2D ^{51}V EXSY NMR method have been described elsewhere.[17]

Qualitatively the 2D ^{51}V NMR spectrum of the sample containing 10 mM vanadate and 4.9 M ethanol at pH 7.8 shows that the vanadate diester forms mainly from the monoester as well as from V_2R and V_2R_2 (Figure 5). Vanadate monoester forms through several pathways, including decomposition of V_2R_2, V_2R, and R_2V and the reactions of both vanadate monomer and dimer with ethanol. The formation of the dimer derived esters of −564 and −566 ppm occurs mainly from the vanadate monoester and the dimer. Minor pathways involving these esters include reactions from vanadate diester and vanadate tetramer. The quantitative results from the 2D ^{51}V NMR EXSY experiment of the pathways under these conditions are shown in Table III.

Table IV and Figure 6 show the exchange rates and 2D ^{51}V NMR spectrum of 5.2 M ethanol and 10 mM vanadate at pH 7.5 in the presence of 5 mM imidazole. The vanadate diester is generated from the vanadate monoester, dimer-derived esters, and dimer. The vandate monoester forms from all other vanadate derivatives except the tetramer, which shows little exchange on the NMR timescale. This exchange pattern varies from the exchange pattern observed in a sample containing no imidazole. Although the conditions are not identical for the two samples with respect to alcohol concentration and pH, the conditions are sufficiently similar such that a qualitative comparison can be made. It appears that the imidazole has increased the exchange rates between vanadate monoester with vanadate monomer and diester. The imidazole has also increased the exchange rate between vanadate dimer and the dimer derived esters. On the other hand, the presence of imidazole has decreased the exchange rates between the diester and dimer or the dimer-derived ester. Imidazole furthermore decreased the exchange between monomer and tetramer and the exchange between monoester and the dimer-derived ester.

Based on the present evidence, it is possible that imidazole interacts with the vanadate oligomers and monomer anions to generate a complex that reacts more rapidly with ligand or other vanadate ions. No detectable change in

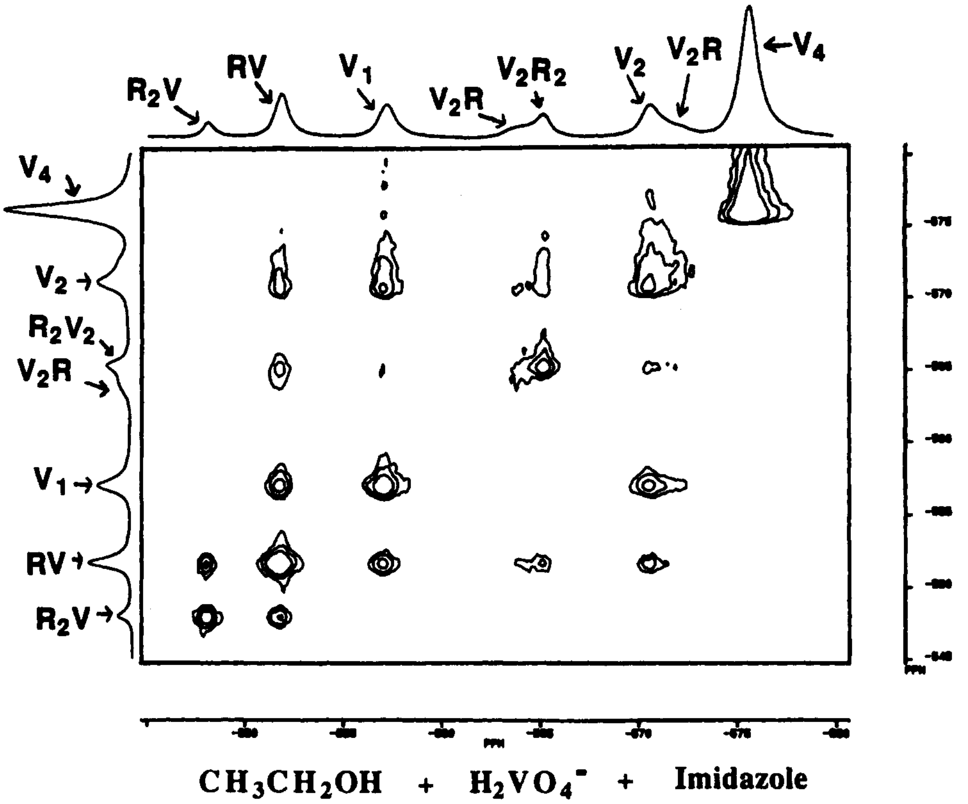


Figure 6. 2D ^{51}V NMR EXSY experiment (132 MHz) on a solution containing 5.2 M ethanol, 10 mM vanadate, and 5 mM imidazole at pH 7.5 (30% D_2O). Integration of exchange cross-peaks leads to the microscopic rate constants shown in Table IV.

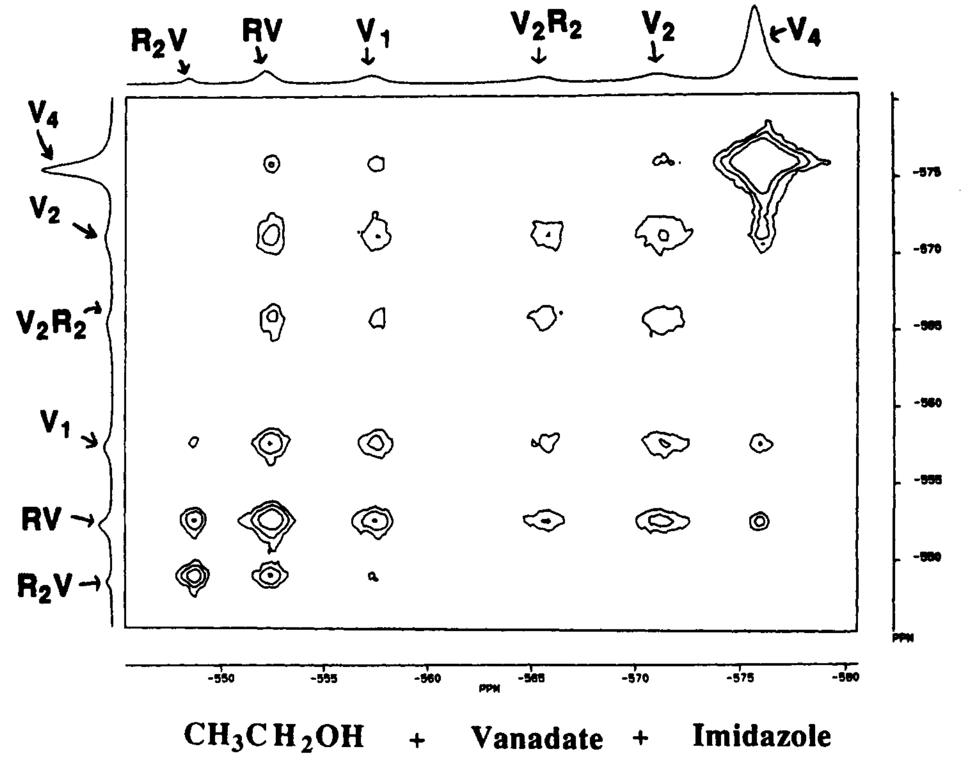


Figure 7. 2D ^{51}V NMR EXSY experiment (132 MHz) of a solution containing 5.1 M ethanol, 10 mM vanadate, and 50 mM imidazole at pH 7.5 (30% D_2O).

chemical shifts is observed on adding increasing concentrations of imidazole to the ethanol–vanadate solution, suggesting that the interaction of imidazole with vanadate is not likely to involve a change in the coordination number around the vanadium. An alternative explanation involves the possibility that imidazole acts as a catalyst (general acid–base catalyst) and affects specific reactions by (for example) facilitating loss of alcohol. The variation in exchange rates may suggest how imidazole affects the exchange reactions. The fastest reaction in solutions without any imidazole is the monomer–dimer exchange, whereas the exchange between vanadate monoester and dimer derived esters is almost four times slower (Table III). Adding imidazole to the solution does not affect the mo-

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nomer–dimer exchange rates. However, the monoester–monomer exchange and the dimer-derived ester–dimer exchange increase significantly. The exchange rate between monoester and dimer-derived ester decreased and became comparable with the diester and monoester exchange rate (Table IV). In summary, imidazole seems to increase some exchange rates, it affects the concentrations of monomer, tetramer, and pentamer, and it prevents reduction of vanadium(V) in solutions containing complex organic ligands. The two most likely possibilities explaining these observations include direct interaction between the vanadate derivatives and imidazole or that the imidazole acts as a general base catalyst. Although the presented results favor the former, studies appropriately testing the latter possibility are underway. Conclusive evidence for whether imidazole acts as a general base or via complex formation must therefore await further detailed studies of the vanadate ester system.

Conclusion

Formation of organic vanadate esters in solutions containing vanadate, alcohol, imidazole, and KCl were studied in order to determine how the nature of the alcohol affects the ^{51}V NMR chemical shift and the stability of the vanadate monoesters and diesters. Primary, secondary, tertiary, and substituted alcohols generate vanadate monoesters with a chemical shift defined mainly by the alcohol and possibly modified by the conditions under which the reaction is being observed. The ^{51}V NMR chemical shift of the vanadate monoester can, in general, be used to assign the organic vanadate monoester when several organic vanadate derivatives are present in solution. The formation constants of vanadate monoesters were found to decrease as follows: primary > secondary > tertiary > (primary with electronegative substituents). This stability order of equilibrium constants for vanadate monoesters differs from the results reported previously by Tracey et al.[15,16] and suggests that not only do the stability constants vary with respect to ionic strength and buffers but the relative order of the stability constants changes with conditions. It seems reasonable to expect that if steric bulk and hydrophobicity of the esters were to be important in the stability of mono- and diesters, such effects will be largest at high ionic strengths. Using relaxation times, 1D and 2D ^{51}V NMR, we show that imidazole affects both the equilibria and the kinetics of the vanadate reaction. Specifically, the presence of imidazole in the solution resulted in larger exchange rates between vanadate esters and other vanadate derivatives, whereas vanadate oligomer exchange decreased. It is possible that vanadate esters form a complex with imidazole that facilitates these exchange reactions and that such a complex contains coordinated imidazole. The studies presented in this paper presently support such a mechanism. It is also possible that imidazole acts as a general base catalyst, which facilitates the loss of alcohols as leaving groups. Detailed understanding of the ester formation reaction without imidazole is necessary before the role of imidazole can conclusively be determined. The work presented in this paper shows qualitative and quantitative results suggesting that apparently innocuous molecules in solutions containing vanadate have the potential to affect both equilibria and kinetics of vanadate reactions. It is possible the catalytic and biochemical properties of vanadates are closely linked to the variability in the reactivity and stability of vanadate derivatives.

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Thermodynamically Controlled 1,3-Asymmetric Induction in an Acyclic System: Equilibration of α-Amino Nitriles Derived from α-Alkylbenzylamines and Aldehydes

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Acyclic α-amino nitriles **1**, derived from α-alkylbenzylamines **3** and aldehydes **4** via a Strecker-type reaction, readily epimerize in MeOH at the newly emerged asymmetric center α to the cyano group. The equilibrium diastereomeric ratio [(R^*,R^*)-**1**]:[(R^*,S^*)-**1**] varies from 73:27 for **1e** to 90:10 for **1h**. The latter ratio is unprecedentedly high for thermodynamically controlled 1,3-asymmetric induction. The aromatic group and a bulky alkyl group (R^1) of the parent amine **3** are required to obtain high concentrations of (R^*,R^*)-**1**, whereas the bulkiness of the alkyl group (R^2) of the parent aldehyde **4** has little effect. The reasons for the thermodynamic predominance of (R^*,R^*)-**1** over (R^*,S^*)-**1** were addressed by MM2 calculations, which predicted the most stable conformers of (R^*,R^*)-**1** and (R^*,S^*)-**1**. The structures of these conformers were verified by ^{1}H NMR spectroscopy and X-ray crystallography.

Introduction

There have been few reports on the highly efficient thermodynamic control of 1,3-asymmetric induction in an acyclic system, probably because thermodynamic control does not achieve satsifactory selectivity in comparison with kinetic control. To study thermodynamically controlled 1,3-asymmetric induction, our attention was focused on the equilibrium between the two diastereomers of an α-amino nitrile (**1**) (eq 1). At equilibrium, (R^*,R^*)-**1** is known to be the major component of the mixture, with a diastereomeric excess of about 50% when R^1 is Me and

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