Contribution from the Department of Chemistry,
Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

^1H and ^{51}V NMR Investigations of the Complexes Formed between Vanadate and Nucleosides

Alan S. Tracey* and Chui Har Leon-Lai

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^1H and ^{51}V NMR spectroscopy as well as ultraviolet absorbance spectroscopy has been used to investigate the reactions between vanadate and methyl β-D-ribofuranoside, adenosine, and inosine. The major products are stereoisomers of a dimeric binuclear bis(ligand) complex. Variable ligand and vanadate concentration studies of the riboside system indicated that the monomeric precursor to the major product had a formation constant of 1.8 ± 1.5 M^{-1} at pH 7.5. This value cannot unequivocally be distinguished from zero, but if it is accepted, the dimerization constant has a value of 6.2×10^6 M^{-1}. 2-D correlation spectroscopy experiments allowed the ^1H chemical shifts and coupling constants to be determined for the ribose rings of the major inosine products from which conformational assignments were made. 2-D exchange spectroscopy showed that exchange on the 0.5-1.0-s time scale occurred only between specific products. On the basis of the NMR results, structures were assigned to the various vanadate complexes. The adenosine system was found to be very similar to that of inosine.

Introduction

The vanadate ion, $VO_4H_2^-$, undergoes facile condensation reactions with *cis*-1,2-diols to form binuclear bis(ligand) derivatives as the major products.[1-3] The spatial arrangement of the 2'- and 3'-hydroxyls of the common nucleosides is particularly suitable for such reactions and product formation is highly favored.[3] There have, however, been some problems with the interpretation of ^{51}V and ^1H nuclear magnetic resonance (NMR) studies of these systems, leading to a recent proposal that the major products in the nucleoside systems are mono- and bis(ligand) products that are mononuclear in vanadium.[4-6]

The question of product stoichiometry is not an unimportant one. Although it is of interest to delineate the chemistry properly, these materials also have utility as probes into the biochemistry of ribonucleases where they function as transition-state analogues of the 2',3'-cyclic phosphates.[7,8] Without having an understanding of the aqueous chemistry it is exceedingly difficult to properly interpret the results of studies of enzymic systems.

Overall, the preponderance of available evidence supports the conclusion that the major products are $V_2\ell_2$ derivatives.[3] On the basis of ^{51}V NMR studies, it has been proposed that each vanadium nucleus is pentacoordinate and possibly trigonal bipyramidal.[1,9] Evidently, the products form from $VO_4H_2^-$ without a requirement for additional protons although there is evidence for a pK_a of 6.7.[10] Further work on this aspect of the chemistry is necessary.

The formation of the monomeric pentacoordinate $V_1\ell_1$ precursor to the above dimer ($V_2\ell_2$) had not unequivocally been observed. Although formation of the $V_1\ell_1$ product with a formation constant of 130 M^{-1} has been reported,[4,5] those conclusions have been disputed[3] and are at variance with earlier work on these systems.[10]

The formation of a pentacoordinate $V_1\ell_2$ complex has also been reported as a major product along with $V_1\ell_1$.[4,5] Although this also has been disputed, it has been suggested that an $n(V\ell_2)$ product might form from $V_2\ell_2$ by incorporation of additional nucleosides into $V_2\ell_2$ at high ligand concentrations.[3] Unfortunately, the chemistry at high nucleoside concentrations is complicated by the well-known base-stacking interactions that nucleosides undergo.

Previous intensive study of the complexation of vanadate by a number of nucleosides, including both pyrimidines and purines, by ^{51}V NMR spectroscopy has indicated that there are no important differences in this aspect of the chemistry of the various nucleosides.[3]

The initial work on the formation of uridine/vanadate complexes explicitly assumed that a $V_1\ell_1$ derivative was the predominant product and utilized ultraviolet (UV) spectroscopy to measure the formation constant.[7] UV often is not as incisive as NMR spectroscopy but does offer an advantage in sensitivity.

In the present study, the formation of mono(ligand), $V_1\ell_1$, and bis(ligand), $V_2\ell_2$, products with β-methyl riboside, inosine, and adenosine have been investigated in detail by UV spectroscopy and by ^{51}V and ^1H NMR spectroscopy. In addition to this, the 2-dimensional NMR correlated and exchange spectroscopies have been utilized in an effort to gain information concerning the stereochemistry of the complexed ribose ring and the major pathways for chemical exchange.

Experimental Section

Materials. The details concerning the preparation of samples for the nuclear magnetic resonance studies do not differ significantly from those of ref 3. Unless specified otherwise, all samples were prepared at 1 M ionic strength by using KCl as the electrolyte. β-Methyl riboside was studied at pH 7.5 while the inosine and adenosine systems were studied at pH 7.3 in D_2O solution. The pH meter (Corning 150 ion analyzer) was calibrated immediately before the samples were prepared, using freshly opened pH standards.

Solutions for the UV studies were prepared by mixing appropriate amounts of stock solutions to yield final concentrations of 5.0 mM Tris (tris(hydroxymethyl)aminomethane) buffer, 0.35 M KCl, 0.096 mM vanadate, and varying proportions of β-methyl riboside. To avoid the use of HCl to adjust the pH and thus eliminate the problem of decavanadate formation, the vanadate stock solution was added to the Tris buffer-KCl mixture of pH 6.8. The pH was then adjusted to 7.0 using small aliquots of NaOH.

Spectroscopy. ^{51}V NMR spectra were obtained at 105 MHz by using a Bruker AMX-400 NMR spectrometer. Spectral widths of 80 KHz, 0.05-s acquisition times, and 60° pulse angles were used for all spectra. The number of transients obtained for the spectra varied between 5000 transients for vanadate concentrations above 3 mM to 100 000 transients for the 0.1 mM solutions.

^1H NMR spectra were obtained at 400 MHz by using 60° pulse angles and 6-s acquisition times with water nulling to remove the residual HOD signal.

The conditions for the 2-D COSY experiment were as follows: spectral width, 1450 Hz; data set size, 4K; pulse width, 90°; nulling pulse 3 s; number of scans, 16; number of spectra in F_1 domain, 512, zero filled to 1K. For the 2-D exchange spectrum, conditions were similar except that a 0.5-s mixing time for magnetization transfer was utilized.

All proton spectra were obtained at 275 K from D_2O solutions. Additionally, the conditions for the 2-D spectra were as follows: vanadate, 20 mM; adenosine or inosine, 30 mM; HEPES (N-(2-hydroxyethyl)-piperazine-N'-ethanesulfonic acid) buffer, 30 mM; ionic strength, 1.0 M (KCl); pH, 7.3.

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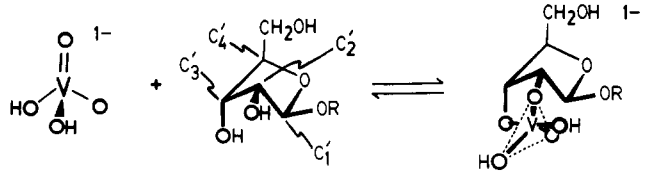
Scheme I



Ultraviolet spectra were recorded from 210 to 820 nm on a Hewlett Packard 8452A diode-array spectrophotometer. The signal averaging time was 25.5 s for each spectrum; i.e., each final spectrum was the average of 255 spectra. An internal reference procedure which zeroed the absorbance from 624 to 634 nm was applied to each spectrum for baseline correction.

A 2.0-mL aliquot of the 0.096 mM vanadate mixture was placed into a 3-mL UV cell containing a small magnetic stir bar. The sample was temperature equilibrated at 25 °C for 5 min before the first spectrum was recorded. A Hamilton syringe was used to add aliquots of a buffered solution of methyl β-D-ribofuranoside, pH 7.0. After each addition, the sample was temperature equilibrated and a spectrum was recorded.

The extinction coefficient for vandate was measured under the conditions described above except that no β-methyl riboside was added. Concentrations of 0.03, 0.05, 0.08, and 0.10 mM vanadate were used for the UV measurements. The extinction coefficient obtained was 1700 ± 18 M^{-1} cm^{-1} and was indistinguishable from that calculated from the first point of the titration curve.

In order to correct for the small absorbance of methyl β-D-ribofuranoside, a spectrum of the stock solution (1.01 M) was recorded and the extinction coefficient was calculated. The ϵ_{270} was 0.145 M^{-1} cm^{-1}. From this value, the absorbance at 270 nm, resulting from the presence of methyl β-D-ribofuranoside, was calculated and subtracted from the observed absorbance.

Methods. Analysis of the NMR data proceeded as outlined in the text. The relevant equations were put into their linear form and the best fit slope and intercept obtained. The errors quoted are standard deviations. The UV absorbance curve was fit to eq 8 using the Biomedical Data Processing program, BMDP. The reported errors are standard deviations. Each point in the experimental curve was corrected for the dilution factor before the results were analyzed.

Results and Discussion

In the ^{51}V NMR and the UV studies the buffer used was 1,1,1-tris(hydroxymethyl)aminomethane (Tris) because of concurrent investigations into the function of ribonuclease. It should be recognized that Tris undergoes reactions with riboside/vanadate complexes[3] and similarly with other diols.[11] At the concentrations of reactants used in this study, the formation of such products can be ignored. As a general rule, however, care must be taken in buffer selection as many undergo strong interactions with vanadate.[12] The buffer *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES) was used for the ^1H NMR studies. This buffer has not yet been observed to undergo significant interactions with vanadate complexes.[11]

The vanadate ion, $VO_4H_2^-$, forms a variety of products with the hydroxyl groups of nucleosides and of β-methyl riboside. Included among them are the simple tetrahedrally coordinated vanadate esters formed individually with the various hydroxyls of the ribose ring and apparently a 2′,3′-cyclic tetrahedral ester. The overall formation constant for these products is about 4 M^{-1} for the nucleosides and 3.3 ± 1.3 M^{-1} for β-methyl riboside itself.[3]

A primary objective of this work is to determine whether a riboside moiety, free of the complications resulting from the presence of nucleoside bases, behaves in a manner similar to that suggested for the nucleosides with respect to their condensation with vanadate. Furthermore, there is a question as to whether a ^{51}V NMR signal that could be assignable to a monomeric pentacoordinated 2′,3′-cyclic product (Vℓ) can be identified. This possibility has been studied only in a cursory fashion for ribose derivatives. The source of the problem in identifying such a product is that the monomer has a very large dimerization constant. As a consequence, except at very low ligand or very low total vanadate concentrations, the dominent product is the dimer. The formation of the monomer is depicted in Scheme I, which also shows the numbering scheme for the ribose ring.

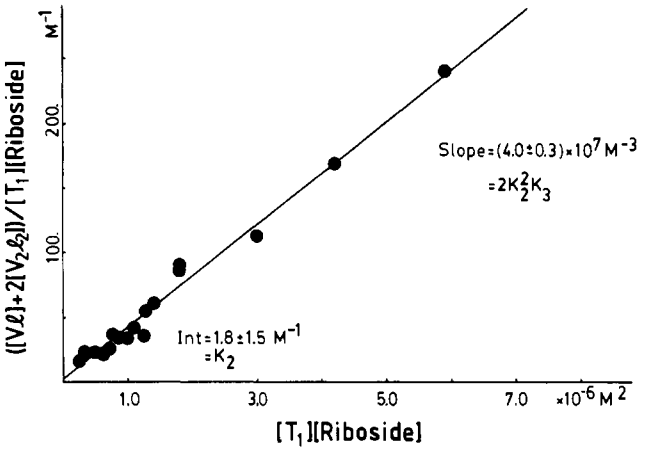


Figure 1. Graphical representation of the formation of monomeric (Vℓ) and dimeric ($V_2\ell_2$) pentacoordinate vanadate products. The conditions for the experiments were as follows: pH, 7.5; ionic strength, 1.0 M with KCl; Tris buffer, 5.0 mM; varying concentrations of vanadate (0.01–2 mM) at 25 mM β-methyl riboside; varying concentrations of β-methyl riboside (4–80 mM) at 0.10 mM vanadate.

Product formation in these systems can be written as in eqs 1–3.[3,10] In these equations Tℓ is used to represent the total of

$$T_1 + \ell \rightleftharpoons T\ell \qquad [T_1][\ell]K_1 = [T\ell] \tag{1}$$

$$T_1 + \ell \rightleftharpoons V\ell \qquad [T_1][\ell]K_2 = [V\ell] \tag{2}$$

$$2V\ell \rightleftharpoons V_2\ell_2 \qquad [V\ell]^2K_3 = [V_2\ell_2] \tag{3}$$

tetrahedral products while Vℓ is used to indicate the possible pentacoordinate monomers and $V_2\ell_2$ is used to indicate the binuclear products.

In the case of β-methyl riboside (and all nucleosides so far studied) separate ^{51}V NMR signals for vanadate, T_1, and its esters, Tℓ, were not observed. The value of K_1 obtained from a previous study of the riboside system[3] was therefore used to calculate the proportion of T_1 in the signal corresponding to [T_1] + [Tℓ]. Addition of eq 2 to eq 3 provided eq 4. A combination of vanadate and ligand concentration studies then provided the results displayed in Figure 1.

$$\frac{[V\ell] + 2[V_2\ell_2]}{[T_1][\ell]} = K_2 + 2K_2^2K_3[T_1][\ell] \tag{4}$$

The value obtained for the intercept of this graph (K_2 = 1.8 ± 1.5 M^{-1}) indicates that there is a monomeric pentacoordinate product formed. The slope ($2K_2^2K_3$ = (4.0 ± 0.3) × 10^7 M^{-1}) gives a value of 6.2 × 10^6 M^{-1} for the dimerization constant, K_3, if the value of 1.8 M^{-1} for K_2 is accepted. Only when [T_1][ℓ] is smaller than about 10^{-7} M^2 will there be significant proportions of Vℓ relative to $V_2\ell_2$. This is why it is difficult to obtain an accurate measure of K_2. In fact, as is evident from the standard deviation, the value of K_2 cannot really be distinguished from zero. Certainly Vℓ is not a major product except, perhaps, in very dilute solution, a result similar to that obtained from the studies of nucleoside/vanadate complex formation.[3,10]

The possibility that the $V_2\ell_2$ product can incorporate additional ligands to give products such as $V_2\ell_3$ or $V\ell_2$ has previously been raised.[3] In that work the graphs obtained apparently had a small amount of curvature. In Figure 1, such curvature is not observed where methyl riboside instead of the nucleosides of the previous study has been investigated. Therefore, such products if they are formed must be in small proportions, and this result indicates that any curvature that may have been observed for the nucleosides probably had the major portion of its origin in base-stacking interactions.

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Table I. ^{1}H Chemical Shifts and Coupling Constants for the Ribose Ring of the Complexes Formed between Inosine and Vanadate[a]

	free inosine	complexes[b]								
		A	B	C	D	E	F	G	H	I
δ_1	5.880	6.219 (0.34)	6.204 (0.32)	6.162 (0.28)	6.067 (0.19)	6.063 (0.18)	6.053 (0.17)	5.480 (0.14)	5.929 (0.05)	5.87 (−0.01)
δ_2	4.567	4.056 (0.49)	5.263 (0.70)	4.057 (0.48)	4.90 (0.33)	4.962 (0.40)	5.04 (0.47)		4.87 (0.30)	
δ_3	4.243	4.664 (0.42)	4.916 (0.67)	4.867 (0.62)						
δ_4	3.095	4.485 (0.39)	4.179 (0.08)	4.217 (0.12)						
δ_5	3.721	3.854 (0.13)	3.66 (−.06)	3.659 (−0.06)						
δ_5'	3.644	3.783 (0.14)	3.597 (−.05)	3.565 (−0.08)						
J_{12}	5.7	6.4	5.5	3.8		6.3	5.4	6.3	3.8	
J_{23}	5.1	5.7	5.3	5.5		5.6	>5			
J_{34}	3.8	1.8	3.9	5.5						
J_{45}	2.8	2.4	2.9	2.7						
J_{45}'	4.0	3.6	4.6	5.0						
J_{55}'	13.0	12.8	13.0	12.6						

[a] Conditions of the experiments were as follows: vanadate, 20 mM; inosine, 30 mM; HEPES buffer, 30 mM; pH, 7.3; ionic sternth, 1.0 M (KCl); temperature, 275 K. [b] Numbers in parentheses give the difference between the chemical shift for the proton in the complex and the corresponding proton in the free ligand.

In principle, it should be possible to utilize the sensitivity of UV spectroscopy to obtain a better measure of K_2. Unfortunately, there are several problems associated with this approach. Both the $V\ell$ and $V_2\ell_2$ products have stereoisomers, each with its own extinction coefficient. However, the proportions between the stereoisomers within each group are a constant factor so an averaged coefficient is suitable. A much more serious problem is the formation of acyclic vanadate esters at the various hydroxyl groups. It has also been proposed that a cyclic ester is formed between the 2',3'-hydroxyl groups.[3] These esters have tetrahedral coordination as does vanadate. There is evidence that the ester extinction coefficients at 270 nm are significantly larger than that of vanadate.[13] Fortunately, ester formation constants are small enough, approximately 0.2 M^{-1},[14] that the change in extinction coefficient can be tolerated. However, the extinction coefficient for the hypothesized 2,3-tetrahedral cyclic ester is not known and it has formation constant of about 3.0 M^{-1}.[3] The UV analysis can then not distinguish between formation of a pentacoordinate or tetrahedral cyclic product. In the following equations, the preceding two products are referred to collectively as $V\ell$ and the formation of acyclic esters is neglected.

The above conditions allow the conservation equations, eqs 5–7, to be written where A_t is the measured absorbance and V_t and L_t are the total concentrations of vandate and ligand, respectively

$$A_t = \epsilon_{T_1}[T_1] + \epsilon_{V\ell}[V\ell] + \epsilon_{V_2\ell_2}[V_2\ell_2] \quad (5)$$

$$V_t = [T_1] + [V\ell] + 2[V_2\ell_2] \quad (6)$$

$$L_t = [\ell] + [V\ell] + 2[V_2\ell_2] \quad (7)$$

and ϵ are the extinction coefficients. With the low total vanadate concentration of this study, L_t is never significantly different from $[\ell]$ so that conservation eq 7 is not required and eqs 5 and 6 in combination with eq 2 and 3 provide eq 8.

$$A_t = C(\epsilon_{T_1} + \epsilon_{V\ell}K_2[\ell]) + C^2\epsilon_{V_2l_2}K_3[\ell]^2 \quad (8)$$

$$C = (-(1 + K_2[\ell]) + ((1 + K_2[\ell])^2 + 8V_t[\ell]^2K_3)^{1/2})/4[\ell]^2K_3$$

Figure 2 shows the results of fitting eq 8 to the measured absorbances at 270 nm. The agreement is excellent. The parameters describing the calculated curve are as follows: K_2 = 1.1 ± 0.9 M^{-1}, K_3 = (2.1 ± 0.1) × 10^7 M^{-3}; ϵ_{T_1} = 1700 M^{-1} cm^{-1}; $\epsilon_{V\ell}$ = 2550 ± 220 M^{-1} cm^{-1}; $\epsilon_{V_2\ell_2}$ = 5350 ± 35 M^{-1} cm^{-1}. The values of K_2 and K_3 are very close to those obtained from the NMR study and within the limits of the approximations leading to eq 8 lend support to a nonzero value for K_2. Unfortunately, neither of the values obtained here can unequivocally be differentiated from zero. It is clear however that if K_2 is not zero it must be small, probably less than 2 M^{-1}.

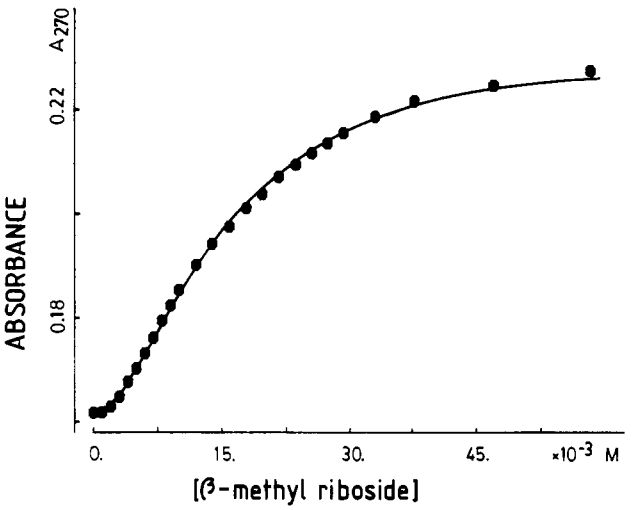

Figure 2. UV absorbance at 270 nm as a function of β-methyl riboside concentration is shown. The solid line was calculated as discussed in the text. The conditions for the experiments were as follows: Vanadate, 0.096 mM; Tris buffer, 5.0 mM; ionic strength 0.35 M (KCl); pH, 7.0.

In an effort to characterize these systems more completely, ^{1}H NMR spectroscopic investigations of the inosine/vanadate and adenosine/vanadate systems have been undertaken. Figure 3 shows ^{1}H NMR spectra of the anomeric protons of the nucleoside and its various products at pH 7.3 and 2 °C. The excess line broadening in certain resonances derives from exchange broadening. Under the conditions of these experiments, the major products are $V_2\ell_2$ derivatives; stereoisomers give rise to the different NMR signals.

In view of the possibility of products other than $V_2\ell_2$ being formed at high ligand concentrations, an inosine concentration study was done. Both ^{1}H and ^{51}V NMR spectra were obtained for a constant total concentration of vanadate (20 mM) and varying amounts of inosine (20, 40, 60, and 100 mM). No signals ascribable to other than $V_2\ell_2$ products were observed.

Detailed examination of the spectra of Figure 3 indicates that at least nine signals from various $V_2\ell_2$ complexes can be assigned to separate anomeric resonances. In an effort to obtain structural information concerning these products, correlated spectroscopy experiments were undertaken in order to assign the signals for the individual ribose rings. The J couplings for the various derivatives were then determined. Not surprisingly, it did not prove possible to trace out the complete coupling pathway for the minor products since there was considerable overlap of resonances with those of the free ligand and the major products. Table I provides the chemical shifts and coupling constants that were obtained.

Although the ribose ring of the nucleosides undergoes facile reorientation,[15-17] it is evident from the coupling constants of Table

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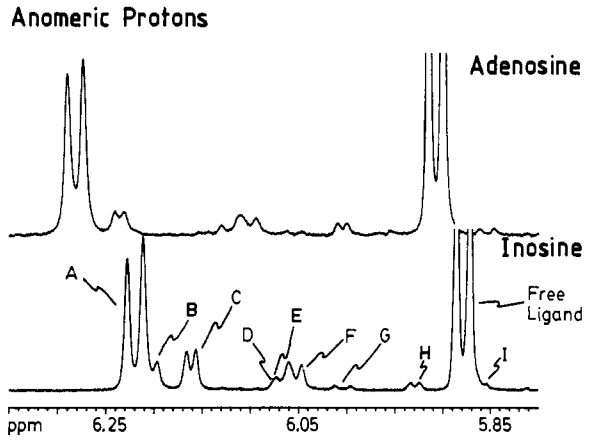


Figure 3. NMR spectra of the anomeric protons of the complexes of vanadate with inosine and adenosine. The products are labeled in alphabetical order and correspond to the listing in Tables I and II. The conditions for the experiments were as follows: nucleoside, 30 mM; vanadate, 20 mM; HEPES buffer, 30 mM; ionic strength, 1.0 M (KCl); temperature 275 K; pH, 7.3 in D_2O solvent.

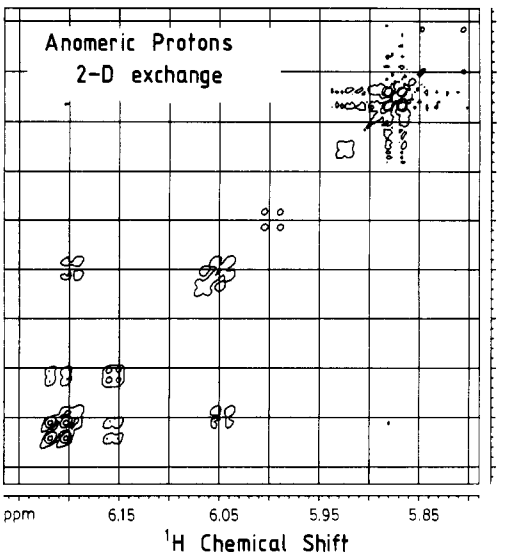


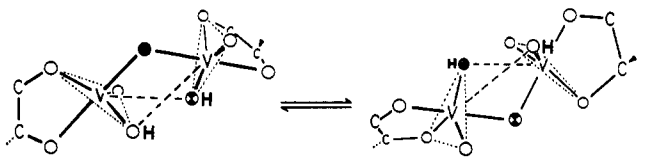
Figure 4. 2-D NMR spectrum demonstrating selective exchange pathways between the anomeric protons of the various riboside moieties of the vanadate complexes. The region and conditions of the spectrum corresponds to that of Figure 3. The mixing time for exchange to occur was 0.5 s.

I that there are distinct conformational preferences and that these can be different for the various inosine derivatives. The coupling constants for the free inosine ligand are consistent with a preferred ^2E–$^2T_1$–E_1 conformation. It is of interest that the J values for the major product, A of Table I, indicate a similar conformation. In this case, however, the relative increase in size of J_{12} and decrease in J_{34} indicate a deepening preference for this conformation. Of course, the tying of the 2′- and 3′-hydroxyl groups to the vanadate as the complex is formed probably restricts internal motion. Product formation does not require a significant change in conformation of the ribose ring so this probably accounts for the preferential formation of product A. Since this dimeric product has only one set of ^1H signals, it is a symmetrical compound (C_2 symmetry). If it is not symmetrical then rapid equilibration must be occurring. Such equilibration is unlikely to involve V–O bond scission as then rapid scrambling between all the isomers might be expected. This leaves the possibility of a correlated internal rotation that interconverts two different conformations. Exchange spectroscopy indicates that such interconversions occur on the $^1/_2$-s time scale.

The results of 2-D NMR exchange experiments (mixing time, 0.5 s) clearly show that product A is in chemical exchange with product C (Figure 4). The J couplings of Table I are consistent with an internal rotation within the ribose ring to the $^3T_4$–^3E–$^3T_2$ conformation. This also is a low-energy conformation for ribose rings. Thus the observed conformations of A and of C are the same as the two conformations normally observed for nucleosides.[15,17] Their interconversion involves a process that leads to a rotation about the C_2'–C_3' carbon–carbon bond. The required rotation transports the O_2' and O_3' oxygens from one gauche formation through an eclipsed intermediate to a second gauche conformation. The results obtained here are consistent with both the A and C products being symmetric conformational isomers. No exchange to a product of mixed conformation or, indeed, any other species was observed.

Efficient chemical exchange between product B and product F was also observed. Unfortunately, under the conditions of these experiments, it did not prove possible to trace out the complete J-coupling pattern for product F; possibly the signal from proton 4′ occurred under the HOD signal and was eliminated when the solvent signal was nulled. It is interesting to note that the signal intensity from the anomeric proton of both of these products is the same. This suggests that the NMR signals of products B and F derive from the same complex. If so, then as near as can be

Scheme II



judged from the coupling constants available, the two ribose rings have a similar conformation. This means that the two nucleosides cannot be interconverted by a conformational change within the ribose ring as suggested for products A and C. An alternate possibility is that the two nucleoside residues are interconverted by rotations about the V–O–V bonds. In this case, exchange into products A and C or other products would be expected but is not observed. This suggests the mechanism of exchange for both sets (A/C and B/F) of products is not a simple one.

It has previously been proposed[3,10] that the $V_2\ell_2$ products have trigonal-bipyramidal geometry about each vanadium and that the vanadium nuclei are linked through an oxygen. Apical and equatorial positions in a trigonal-bipyramidal coordination can be interconverted by pseudorotation about the central nucleus, in this case vanadium. In the general case, pseudorotation will convert symmetrical to asymmetrical products, which is contrary to observations here, where two distinct types of products are observed. The 2-D exchange results can be explained if concerted interactions are involved that allow transformation from one type of symmetrical product to a second type and similarly for interconversion of the two types of nucleosides in an asymmetrical product.

A clue to the process involved is obtained from the dimerization constant, K_3. At room temperature the energy of dimerization cannot be significantly less than 50 kJ mol^{-1}, from $\Delta G = -RT \ln K_3$. This value is comparable to those of chelation and suggests that the $V\ell$ monomers are linked by multiple bonds in the product $V_2\ell_2$. Inspection of molecular models suggests that bridging ligands do not allow the required interconversions.

If it is assumed that water requirements for the formation of the binuclear products are the same for the nucleosides as for ethylene glycol, then a structure that satisfies the water[1] and proton[10] stoichiometry for product formation is one in which the two vanadiums are coupled by a bridging oxygen and undergo longer range interactions to an equatorial hydroxo group of each vanadium, as depicted in Scheme II, to give a trigonal-bipyramidal geometry distorted toward octahedral coordination. A somewhat

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Table II. ^1H Chemical Shifts and Coupling Constants Determined for the Ribose Ring of the Complexes Formed between Adenosine and Vanadate[a]

	free adenosine	complexes								
		A	B	C	D	E	F	G	H	I
δ_1	5.908	5.282	6.28	6.24	6.15	6.12	6.10	6.06	6.00	5.85
δ_2	4.655	5.124	5.37	5.12						
δ_3	4.325	4.75								
δ_4	3.193	4.620								
δ_5	3.823	3.978								
δ_5'	3.731	3.882								
J_{12}	6.0	6.5	6	3.8	3.8	6	6	6.5	3.7	6.0
J_{23}	5.0	5	4							
J_{34}	3.7	1.5								
J_{45}	2.5	1.8								
J_{45}'	3.5	2.6								
J_{55}'	12.8	12.5								

[a] Conditions of the experiments were as follows: vanadate, 20 mM; adenosine, 30 mM; HEPES buffer, 30 mM; pH, 7.3; ionic strength, 1.0 M (KCl); temperature, 275 K.

similar type of interaction has been observed in X-ray studies of $VO(OCH_2CH_2CL)_3$, which is dimeric in the crystal and has each vanadium trigonal bipyramidal.[18] $VO(OCH_3)_3$ behaves somewhat similarly, although here an octahedral structure is obtained.[19] If pseudorotation occurs at one of the vanadium centers, an accompanying interchange of the bridging oxygen for a hydroxo group will cause pseudorotation about the second vanadium (see Scheme II). The effect of this on the ribose rings is to convert the apical C–O bonds to equatorial positions and vice versa. The conformational changes observed will be correlated with these interconversions. This mechanism provides a facile route to a concerted rearrangement of the products that does not give rise to an interchange of symmetrical for asymmetrical products and readily accounts for the processes observed. Other products could be formed by cogwheeling through the remaining possibilities of Scheme II, and this provides for all the products observed. The NMR spectra indicate that one bridging situation is highly preferred, possibly, though certainly not necessarily, the arrangement depicted in Scheme II. This bridging structure could give rise to both the symmetrical and asymmetrical products and account for the four major signals in the NMR spectrum. Of the minor products formed, exchange between product H and either D or E (probably E as near as could be judged from the overlapping signals) was observed.

It is difficult to arrive at an alternative scheme that can account both for the NMR observations and for the water stoichiometry unless some products have a differing coordination geometry from others. Since ^{51}V NMR signals are observed only at −523 ppm, this seems unlikely. Alternate coordination schemes such as square-pyramidal geometry do not readily explain the observations.

The ^1H chemical shifts observed for the free inosine ligand and its complexes are given in Table I which also includes the complexation shifts (the difference between the shift observed for the protons of the complex and those of the free ligand). The largest complexation shifts, averaging about 0.5 ppm, occur at H_2' and H_3'. This is consistent with the 2′- and 3′-hydroxyls being the sites for complex formation. Only small changes in chemical shift were observed for the C_5' methylene protons. There are differential shifts between positions 1′ and 4′ that give some hints to the conformation of the various complexes.

The conformation of the ribose ring, as previously discussed, is specified by the coupling constants. If this conformation and the distorted-trigonal-bipyramidal geometry of Scheme I about vanadium are assumed, then inspection of molecular models provides some useful insights. When the C_2'–O occupies an apical position in the complex and the C_3'–O occupies an equatorial position, then H_3' is approximately 1,3-diaxial to one of the equatorial VO oxygens while H_2' is more distant. Alternatively, when the C_2'-O is in the apical position, then H_2' and H_3' are both directed away from the equatorial vanadium-bonded oxygen. Both H_1' and H_4' are in a 1,3-arrangement with such oxygens but H_1' is significantly closer than H_4' to them. Because of the flexibility inherent in these systems it is difficult to unambiguously assign stereoisomers on the basis of chemical shifts; however, the former situation seems to correspond most closely to product A while the second corresponds to product C.

A major problem with the stereochemistry proposed here is that there is no ready explanation for the large complexation shifts for H_2' and H_3' of product B, 0.1–0.3 ppm larger than for products A and C. However, this may simply be a result of flexing within the five-membered $-VO_2'C_2'C_3'O_3'-$ ring or changes in the arrangement of the hydrating water.

As can be seen from the spectra of Figure 3, the adenosine system of complexes is in slightly more rapid chemical exchange than the inosine system. As a consequence, it did not prove possible to obtain details comparable to those for the inosine complexes. The results that were obtained indicated there are no significant differences between the two systems. Nine anomeric proton resonances were detected, as observed for inosine. Only with complex A did it prove possible to trace out the complete coupling pathway. The results that were obtained are summarized in Table II. Because the results that were obtained for the adenosine system were so similar to those determined for the inosine system, no intensive effort to study the adenosine system was made.

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