# Vanadate–Nuceloside Interactions

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Complex formation between the vanadium(V) oxoanion, vanadate and the nucleosides inosine and adenosine in aqueous solution has been studied. $^1$H NMR spectroscopy clearly revealed four major product signals and an additional four minor signals. Variation of the concentration of KCl added to the aqueous solution showed that the formation of the major products was enhanced by decreasing the electrolyte concentration, while the formation of the minor products was less favoured. Replacement of KCl by $KNO_3$ or $K_2SO_4$ had only a small effect on the formation of all products. Elimination of the pH regulation buffer, HEPES, from the solution had no observable effect on product formation. Two-dimensional $^1$H–$^{13}$C correlation spectroscopy was used in order to assign the carbon signals of the major product, which is a binuclear bisligand product. The results of this and other studies were shown to be consistent with a $V_2O_9$ chelation skeleton with ligands in a distorted trigonal bipyramidal geometry about each vanadium. A number of alternative structures were examined and shown not to be consistent with the known properties of these complexes.

The past few years have seen a rapid development of interest in the chemistry and biochemistry of vanadium(V). Much of this interest has its origins in the unique ability of vanadate to act as a phosphate analogue in a variety of biochemical reactions. This ability allows vanadate to activate the function of a number of enzymes while inhibiting that of others. Vanadium also is found in the haloperoxidases of some marine algae and in nitrogen-fixing enzymes of some bacteria. Extensive reviews of these and a number of other biochemical functions of vanadium have recently appeared.[1-3]

A topic that has generated a considerable amount of interest, and some controversy, is the chemistry of the reactions between vanadate and 1,2-diol functionalities. This interest has centred on the nucleosides, to a large extent because vanadate in the presence of uridine is an exceedingly effective inhibitor of ribonuclease.[4] Neutron and X-ray diffraction studies of the ribonuclease/vanadate/uridine complex clearly have shown a pentacoordinate structure about the vanadium nucleus.[5] The structure proposed in that study is similar to the one expected for the ribonuclease 2′,3′-uridine cyclic phosphate transition state complex, an intermediate in the hydrolysis of uridine 2′,3′-cyclic phosphate.

In the absence of ribonuclease the major products of the reaction between vanadate and nucleosides are binuclear bisligand complexes[6-8] similar to those formed with glycols.[9] The structure of these binuclear products is of interest. In the case of ethylene glycol, only one $^{51}$V nuclear magnetic resonance (NMR) signal is observed at −523 ppm. However, if the ligands are chiral, multiple signals are obtained, and this has been attributed to the formation of stereoisomers as, for instance, in the cases of: 1,2-propane diol,[9] the cyclohexane diols,[10] monosaccharides[10] and nucleosides.[11] Proton NMR spectroscopy has also shown the presence of several products[6,7,12] attributed to stereoisomers.[7,12] Recently, however, on the basis of $^{13}$C NMR spectra, it has been stated that, for conditions comparable to the proton work that indicated the presence of several stereoisomers,[6,7] only one product complex is formed.[8] These incompatible results raised a number of questions concerning the two sets of studies, including whether the buffer was giving rise to incorrectly assigned products, whether the ionic strength of the medium was having an unexpectedly large effect on the equilibria or whether the carbon spectra were being incorrectly interpreted.

In an effort to resolve this disagreement between the results of different workers, and ultimately to understand more thoroughly vanadate/nucleoside interactions, a study of the effects of the presence of the buffer used in the proton work, of the effects of ionic strength and of the replacement of the KCl electrolyte with $Na_2SO_4$ or $KNO_3$ was initiated. The effects were monitored by $^{51}$V and $^1$H NMR spectroscopy and also by $^{13}$C NMR spectroscopy for selected solutions.

## Experimental

*Materials.* Inosine, adenosine and HEPES [*N*-2-(hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid] were purchased from SIGMA Chemical Corp. Vanadium(V) oxide (gold label, 99.99 %) was obtained from Aldrich Chemical Co.

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*Table 1.* The compositions of samples for NMR studies.[a]

| No. | Vanadate / mM | Ligand / mM | HEPES / mM | Ionic strength / M |
|---|---|---|---|---|
| $^1$H NMR (Inosine) | | | | |
| 1 | 20 | 30 | 5 | 0.0 |
| $KNO_3$ electrolyte | | | | |
| 2 | 20 | 30 | 5 | 1.5 |
| 3 | 20 | 30 | 5 | 1.0 |
| 4 | 20 | 30 | 5 | 0.5 |
| $K_2SO_4$ electrolyte | | | | |
| 5 | 20 | 30 | 5 | 1.5 |
| 6 | 20 | 30 | 5 | 1.0 |
| 7 | 20 | 30 | 5 | 0.5 |
| KCl electrolyte | | | | |
| 8 | 20 | 30 | 5 | 1.5 |
| 9 | 20 | 30 | 5 | 1.0 |
| 10 | 20 | 30 | 5 | 0.5 |
| 11 | 20 | 30 | 0 | 1.0 |
| 12 | 20 | 30 | 0 | 1.0 |
| 13 | 20 | 30 | 30 | 1.0 |
| 14 | 20 | 30 | 30 | 1.0 |
| $^1$H–$^{13}$C NMR (Adenosine) | | | | |
| 15[b] | 15 | 25 | 0 | 0.0 |
| 16 | 60 | 60 | 0 | 0.0 |

[a] $^{51}$V NMR spectra were obtained for selected samples. [b] This sample was prepared according to the conditions of Ref. 8 and spectra obtained at 294 and 275 K.

Deuterium oxide ($D_2O$, 99.9%) was from ISOTEC Inc. All chemicals were used without further purification.

*Solutions.* Stock solutions of 1.0 M HEPES buffer and stock solutions of KCl, $KNO_3$ and $K_2SO_4$ of 2.0 M ionic strength were prepared in $D_2O$. The stock solution of sodium vanadate was prepared by the addition of 0.5 molar equivalent of vanadium pentoxide to a 1.0 M solution of NaOH; this was then stirred overnight until the orange solution became colourless. This solution was then diluted to 0.1 M with $D_2O$.

Solutions for $^1$H NMR studies were prepared by combining appropriate amounts of stock solutions and solid inosine ligand for the desired final concentrations of vanadate, buffer, ligand and salt (ionic strength) after diluting to the final volume. It was important that the pH of the intermediate solution had been initially adjusted to an adequate value with DCl solution before the vanadate stock solution was added. The pH value of the final solution was then adjusted only by the addition of NaOD. This procedure avoided exposure of the vanadate solution to acidic conditions and the subsequent formation of decavanadate. The volume was then adjusted by addition of $D_2O$, the pH was checked, and small adjustments were made when necessary by adding NaOD solution. All solutions were prepared at pH 7.1; the pH measurements were made with a pH meter calibrated with freshly opened pH standard solutions. The solutions for $^{13}$C NMR studies were prepared using similar procedures except that adenosine was used as ligand, which required warming (ca. 40 °C) for dissolution, and the pH was adjusted to 7.0. Table 1 lists the compositions of the samples (in $D_2O$ solvent) used for NMR studies.

*Spectroscopy.* All $^1$H NMR spectra were obtained from a Bruker AMX-400 NMR spectrometer operating at 275 K. The $^{13}$C NMR and the $^1$H–$^{13}$C 2-D correlation spectra were also obtained at 275 K. The 2-D correlation spectra were obtained using proton detection for the 60 mM sample. The $^{13}$C spectrum of this sample did not vary significantly from that of the 25 mM adenosine sample. The conditions for the inverse correlation spectrum were as follows: $^1$H spectral width, 3kHz; $^{13}$C spectral width, 10 kHz; frequency domain data points, 2 K; number of scans per spectrum, 24; number of spectra, 4 K; incremental delay between spectra, 2.5 μs; $^{13}$C decoupling; real time domain data points in $f_2$, 2 K; real time domain data points in $f_1$, 4 K; phase sensitive with time proportional phase incrementation (TPPI).

## Results

Under the conditions of this and similar studies[6–8,11,12] vanadate undergoes a facile complexation reaction with nucleosides to form, as the major products, a mixture of binuclear

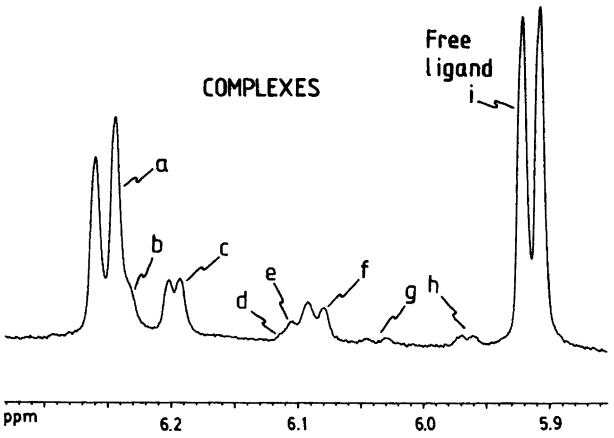



*Fig. 1.* $^1$H NMR spectrum of the anomeric protons of inosine and its complexes formed in the presence of vanadate and KCl. The conditions of the experiment were: inosine, 30 mM; vanadate, 20 mM; KCl, 1.0 M; no buffer; pH 7.1 (at 294 K); temperature, 275 K.

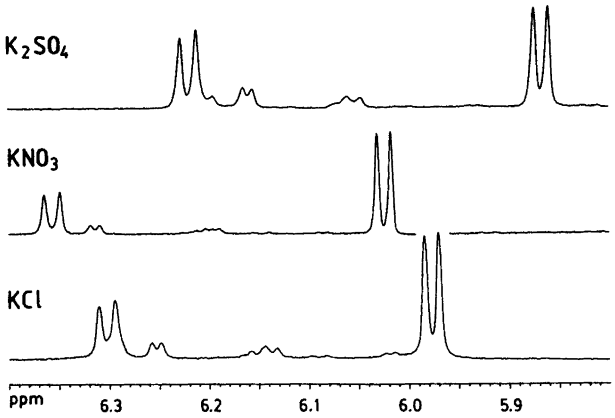



*Fig. 3.* Comparison of the proton NMR spectra of the anomeric protons of inosine and its vanadate complexes obtained in the presence of different electrolytes. Conditions of the experiments were: vanadate, 20 mM; inosine, 30 mM; HEPES buffer, 5 mM; pH 7.1 (at 294 K); temperature, 275 K; $\mu = 1.5$ molar with the indicated electrolyte.

bisligand complexes referred to collectively as $V_2L_2$. The formation of the various different complexes is most readily observed using $^1$H NMR spectroscopy and concentrating on the signals from the anomeric proton of the riboside moiety. Fig. 1 shows the proton spectrum of this region of the NMR spectrum obtained at 275 K for a solution containing inosine, vanadate and KCl. Eight sets of product signals can be counted directly from this spectrum as indicated in the figure. None of these signals can be attributed to products arising from buffer interactions, since no buffer was present in the solution. This spectrum does not vary substantially from those of Fig. 2, where 5 mM HEPES buffer was incorporated into the solution in order to regulate the pH more easily.

In order to ensure that the formation of none of the products was dependent on the presence of KCl in the reaction medium, the concentration of KCl was varied in a systematic manner. Some of the results are displayed in Fig. 2. A decrease in electrolyte concentration from 1.5 M KCl to no added KCl favours product formation by about a factor of three. It seems from these spectra that the minor products are less favoured relative to the major products as the ionic strength of the medium was lowered. The signals, however, did not appear to be completely lost, but were broadened significantly by chemical exchange.

In order to verify this interpretation of the spectra and to ensure that there were no specific effects arising from the interactions of chloride ion with the vanadium nucleus, KCl was replaced by $K_2SO_4$ and $KNO_3$ in two independent ionic strength studies. The results obtained from these studies

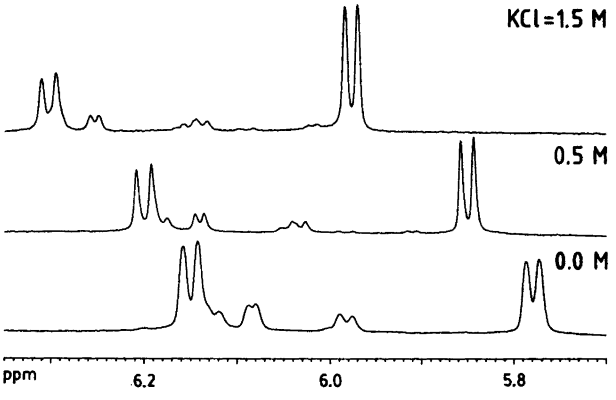



*Fig. 2.* $^1$H NMR spectra of the anomeric protons of inosine and its vanadate complexes shown as a function of the concentration of added KCl. The conditions of the experiments were: vanadate, 20 mM; inosine, 30 mM; HEPES buffer, 5 mM; pH 7.1 (at 294 K); temperature, 275 K and the indicated proportions of KCl.

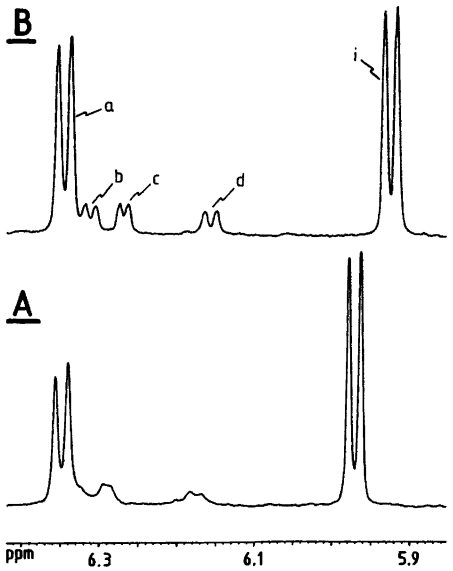



*Fig. 4.* Effect of temperature on the proton NMR spectra of the anomeric protons of adenosine and its vanadate complexes. The conditions of the experiments were: vanadate, 15 mM; adenosine, 25 mM; no buffer; pH 7.1 (at 294 K); temperature, 294 K, spectrum (A), 275 K, spectrum (B).

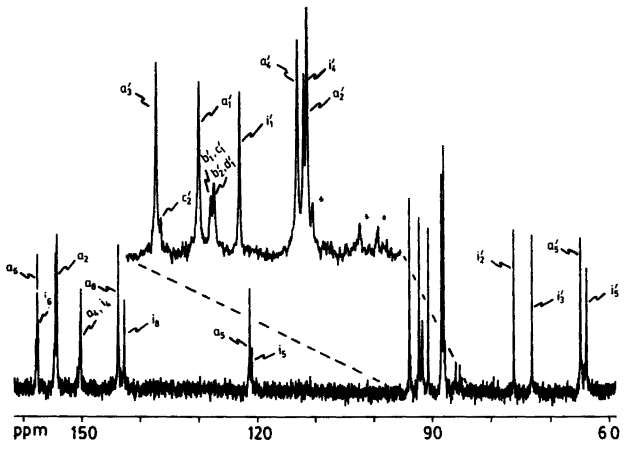



*Fig. 5.* $^{13}C$ NMR spectrum of the equilibrium solution of 60 mM vanadate in the presence of 60 mM adenosine at 275 K and pH 7.0, with no buffer. No correlations to the signals marked with an asterisk were obtained from the 2-D correlation experiment.

were similar to those of the KCl concentration study of Fig. 2. Fig. 3 shows NMR spectra obtained with $\mu = 1.5$ M for the three different electrolytes. Product formation clearly is not very sensitive to these types of electrolytes. Interestingly, it was observed that the product distribution in the presence of 0.5 M $K_2SO_4$ ($\mu = 1.5$ M) was more similar to that of 0.5 M KCl or 0.5 M $KNO_3$ ($\mu = 0.5$ M) than to 1.5 M KCl or $KNO_3$.

It seems clear from these experiments that the formation of multiple products is not a result of the interactions of an extraneous material. It is then necessary to ascertain why, as has been reported,[8] the $^{13}C$ spectrum of adenosine in the presence of vanadate did not show more than one product. Fig. 4 shows proton spectra of a vanadate–adenosine solution prepared under conditions similar to those previously reported.[8] Spectrum (A) was obtained at 294 K while spectrum (B) was obtained at 275 K. At the lower temperature, chemical exchange is slowed significantly and the spectrum was much better resolved. As found for the inosine case, the least favoured of the minor products are not readily observed at low ionic strength, although they are evident in the presence of 1 M KCl.[7] The other three minor products signals are clearly evident.

The $^{13}C$ NMR spectrum of the same sample used to obtain the spectra of Fig. 4 was obtained at 275 K. The signals of the major product were readily identified. The minor products tended to give signals under or very close to those of the major product. At higher temperature these signals are more highly exchange-broadened, and since, individually, they represent at most only about 15 % of the intensity of the major signals, they are easily overlooked. As a whole, however, they do represent about 30 % of the total product formed.

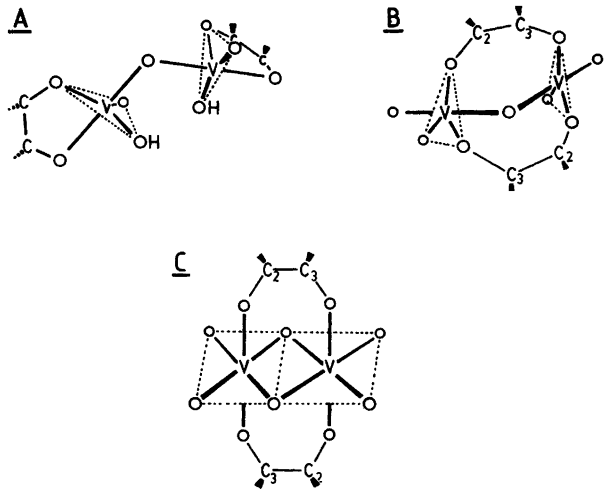
In order to confirm the origin of the carbon signals a $^1H$–$^{13}C$ two-dimensional correlation experiment was carried out on a 60 mM vanadate, 60 mM adenosine sample.

*Table 2.* Chemical and complexation shifts for inosine and adenosine complexes with vanadate.[a]

| | $^1H$ Chemical shifts | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $H_2$ | $H_8$ | $H_1'$ | $H_2'$ | $H_3'$ | $H_4'$ | $H_5'$ | $H_5''$ |
| Adenosine | | | | | | | | |
| Free ligand | 7.69 | 7.92 | 5.68 | 4.45 | 4.12 | 4.00 | 3.64 | 3.53 |
| Complex | 7.73 | 7.90 | 6.12 | 4.90 | 4.57 | 4.48 | 3.81 | 3.72 |
| Complexation shift | 0.04 | −0.02 | 0.44 | 0.45 | 0.45 | 0.48 | 0.17 | 0.19 |
| Inosine | | | | | | | | |
| Free ligand | 8.17 | 8.03 | 5.88 | 4.57 | 4.24 | 4.10 | 3.72 | 3.64 |
| Complex | 8.15 | 8.01 | 6.22 | 5.06 | 4.66 | 4.49 | 3.85 | 3.78 |
| Complexation shift | −0.02 | −0.02 | 0.34 | 0.49 | 0.42 | 0.39 | 0.13 | 0.14 |

| | $^{13}C$ Chemical shifts | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $C_2$ | $C_4$ | $C_5$ | $C_6$ | $C_8$ | $C_1'$ | $C_2'$ | $C_3'$ | $C_4'$ | $C_5'$ |
| Adenosine | | | | | | | | | | |
| Free ligand | 154.6 | 150.5 | 121.2 | 157.7 | 142.8 | 90.7 | 76.1 | 73.0 | 88.2 | 63.8 |
| Major complex[b] | 154.4 | 150.5 | 121.6 | 157.8 | 143.6 | 91.9 | 87.9 | 93.7 | 88.5 | 64.7 |
| Complexation shift | −0.2 | 0.0 | 0.4 | 0.1 | 0.8 | 1.2 | 11.8 | 20.7 | 0.3 | 0.9 |

[a]The $^{13}C$ chemical shifts of the free ligand were assigned in accordance with the information in Ref. 13. The riboside ring assignments were confirmed by the $^1H$–$^{13}C$ correlation study. The chemical shifts of the carbons of the product complex were assigned from the correlation experiment, except for the quaternary carbons of the base. These quaternary carbons were assumed not to shift much with complexation, since the protiated carbons of the base did not shift significantly. [b]The assignments of carbons $C_1'$, $C_2'$ and $C_3'$ of the product by other workers[8] are not in agreement with this work.



*Scheme 1.*

The $^{13}C$ spectrum of this sample was not significantly different from that of the lower-concentration sample but it did allow the lower intensity signals to be more easily observed. The spectrum is shown in Fig. 5. Carbon assignments for the uncomplexed adenosine are from Ref. 13 and have been confirmed here. The 2-D correlation experiment also allowed unambiguous assignment of all $^{13}C$ resonances for the major product and some signals of the minor products. The signal positions and the effects of complexation on those positions are given in Table 2. Although the $^{13}C$ NMR signals of the product complexes have previously been assigned,[8,12] no experimental basis for those assignments has been reported. Some of those previous assignments for the major product are not consistent with this work.

## Discussion

The condensation of vanadate with nucleosides proceeds favourably to form a major product with the stoichiometry of two vanadiums and two ligands.[6,7] Proton NMR spectroscopy revealed the presence of at least eight different types of nucleosides in the complexes, as judged by the observation of different signals for the anomeric proton of the riboside ring (Ref. 7 and this work). These observations were made for inosine and adenosine ligands under the experimental conditions of 30 mM total nucleoside, 20 mM total vanadate, 20 mM HEPES buffer, 1.0 M KCl and pH 7.1 in $D_2O$ at 275 K. At room temperature (294 K) exchange broadening in the $^1H$ spectrum was significant but, as was evident form Fig. 4A, a number of products were clearly observable. A similar result has been reported for the inosine nucleoside.[6] This work contrasts with a recent $^{13}C$ study in which the formation of only one product was reported for conditions not substantially different from those of these proton studies.[8] This is a serious discrepancy that calls into question the results of a number of studies of diol/vanadate systems.[6,7,9–12,14]

At 1.5 M KCl the $^1H$ spectrum of the inosine anomeric protons consisted of four major signals corresponding to about 90 % of total product. The remaining signals derived from several minor products. Of the major products, one accounted for about 60 % of the total, while the remaining three signals were in roughly equal proportions. This proportion between major products stayed roughly constant on changing to no added electrolyte, while overall, formation of the major products was favoured. Replacing KCl by either $K_2SO_4$ or $KNO_3$ gave rise to no significant changes in the NMR spectrum. This makes it very unlikely that specific electrolyte interactions are giving rise to the multiple products. Similarly, removal of the HEPES buffer from the equilibrium solution had no observable effect on the signals deriving from the anomeric protons. It has previously been reported from studies with the cyclohexane diols that HEPES buffer had no significant effects on the equilibria of that system, although tris(hydroxymethyl)-aminomethane clearly did.[15] Similarly, the imidazole buffer enters into the equilibrium reactions.[8]

In the adenosine system the major equilibrium product is slightly more favoured over the remaining products than in the inosine system, but only by about 0.7:0.3 compared to 0.6:0.4. It is therefore surprising that the $^{13}C$ NMR spectrum did not clearly indicate the formation of more than one major adenosine product. A detailed look at a carbon spectrum obtained at a temperature of 294 K, however, did reveal some signals deriving from the additional products. At 275 K the signals were much more clearly resolved.

It is evident from these studies that in fact vanadate in the presence of nucleosides, at near neutral pH, gives rise to a number of major products and that these do not arise from the presence of buffer or from interactions with the electrolyte. Furthermore, it has been shown that these products have the same $V_2L_2$ stoichiometry.[7] Unlike the major products, a decrease in electrolyte concentration tends to disfavour the formation of the minor products.

The coordination about vanadium and the nature of the linkage between the two subunits of the dimeric product has been the subject of some degree of speculation. The initial work with glycol systems proposed that the product was pentacoordinate about each vanadium, with the two subunits joined by a bridging oxygen as shown in Scheme 1, structure **A**.[9] An alternative arrangement, with pentacoordinate vanadium and a bridging oxygen and two bridging glycol units, was also proposed (structure **B**, Scheme 1), but considered to be less likely than the previous structure.[9] Recently, on the basis of $^{13}C$ NMR, magnetic circular dichroism studies, it was proposed that the vanadium nuclei each have an edge-shared octahedral coordination with two bridging ligands as depicted in structure **C** of Scheme 1.[8] The formation of such a product requires no overall uptake of water molecules when the product is formed from two tetrahedral vanadate esters. However, there is evidence that eqn. (1) represents the equilibrium that occurs. In the case of ethylene glycol, one water was observed to be

$$2HOCHCH\text{–}OVO_3H^- \rightleftharpoons V_2L_2 + H_2O \quad (1)$$

released as product formation proceeded from two vanadate esters. There seems little reason to suspect problems with the determination of water stoichiometry in the ethylene glycol study, since the release of water as the esters themselves were formed was accurately quantitated.[9] It also seems unlikely that the complexes formed with nucleosides or other glycols differ much in their coordination from that of ethylene glycol. The $^{51}V$ chemical shifts, for instance, are virtually identical for all systems so far studied, generally occurring within 2 or 3 ppm of −523 ppm.[6–12,14–16] Additionally, all such products have the $V_2L_2$ stoichiometry.[6–11,14,15] The water production observed is consistent with the pentacoordinate structures **A** and **B** of Scheme 1 but, as previously noted, only if vanadate and its esters themselves are tetracoordinated because only a change in water stoichiometry is observable. It seems unlikely that $VO_4H_2$ does not have a tetrahedral coordination.[17,18]

If the vanadate complexes have the $V_2O_{10}$ fused octahedral skeleton, then with a chiral ligand such as adenosine or inosine there is a possibility for formation of only two chemically distinct product complexes when the ligands are bridging from one vanadate to the other. In each complex the two ligands are chemically indistinguishable because each complex has $C_2$ symmetry. As a consequence, only two sets of proton or carbon NMR signals will be observed in the spectrum of the ligand. (Note, however, that three $^{51}V$ signals are possible, two for one product, one for the other.) It seems evident, both on the basis of the observation of four major sets of product NMR signals, and on the water stoichiometry observed for ethylene glycol, that a coordination such as structure **C** of Scheme 1 is very unlikely. Structure **A** and **B** both satisfy the water stoichiometry, but there is evidence that neither of these represents the correct coordination.

It has been shown that, at 275 K, chemical exchange occurs on the 0.5 s timescale between the signals labelled (a) and (c) and between those labelled (b) and (d) of Fig. 1. A concerted change in conformation of the riboside ring was found to accompany each exchange. Exchange of (a) and (c) with (b) and (d) or the free ligand was not observed, nor did (b) or (d) exchange with the free ligand.[7] It is of interest to examine the observed exchange processes in terms of the possible structures of Scheme 1.

It seems reasonable to expect that rotation about the V–O–V bonds in a structure such as that of **A** of Scheme 1 would be fast on the NMR timescale. This means that only three distinguishable products could be formed, two symmetrical products and one with no symmetry. In order for interconversion between these three products to occur it would be necessary for pseudorotation within the bipyramidal subunits to proceed. In such an event, exchange between all products should be observed, since concerted interconversions in a structure such as this would not be expected.

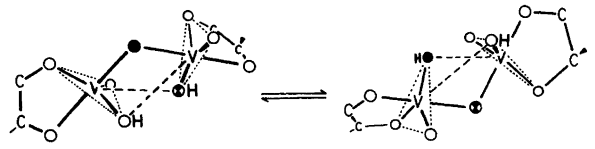
If the V–O–V linkage of structure **A** were via an equatorial, rather than an apical oxygen, then six chemically distinct products are possible that are not rotational isomers. In this case pseudorotation would give rise to two sets of three products, with exchange within each set. One facet of such an exchange mechanism would be the interconversion of asymmetric complexes with those of $C_2$ symmetry. This type of exchange was not observed, but rather symmetric to symmetric and asymmetric to asymmetric exchange was observed.[7] An alternative scheme involving pentacoordinate products is one involving a single bridging oxygen and two bridging ligands (structure **B**, Scheme 1). Such a structure would be in accord with the water stoichiometry, but it is difficult to propose a viable mechanism that would account for the exchange processes that were reported. On the basis of the kinetics it therefore seems very unlikely that either structure **A** or **B** is an accurate representation of the structure of the product.

The results of the studies reported here have clearly shown that $^1H$ NMR signals assigned to a number of $V_2L_2$ complexes of different stereochemistries in a previous study[7] did not derive from artifacts arising from the presence of the HEPES buffer. They also did not occur because of specific interactions deriving from the presence of the electrolyte. Electrolytes, however, do shift the equilibria away from product formation with an increase in electrolyte concentration. Four major sets of ligand NMR signals were observed. Of these signals, two sets are assigned to products with $C_2$ symmetry, so that each individual set of signals derives from two nucleoside residues. The remaining two sets of proton NMR signals were assigned to the individual nucleosides of an asymmetric product.

For the adenosine ligand the $^1H$ complexation shifts of Table 2 are interesting and somewhat difficult to interpret. The ribose ring protons all show large shifts of about 0.45 ppm. Similar complexation shifts were observed for the inosine complex and have been previously discussed.[7] The corresponding changes in chemical shift of the $H_2$ and $H_8$ protons of the nucleoside bases of the two complexes were very small.

The $^{13}C$ chemical shifts of the adenosine complex show a significantly different pattern. Only $C_2'$ and $C_3'$ show large complexation shifts, being 11.8 and 20.7 ppm, respectively. The next largest shift is 1.2 ppm for $C_1'$. This is fully consistent with carbons $C_2'$ and $C_3'$ being the site of complexation.

In a bridged octahedral coordination, as in Scheme 1, structure **C**, it might be expected that the complexation shifts for $C_2'$ and $C_3'$ would be similar, since the $C_2'$ and $C_3'$ oxygens are similarly situated in the complex. The situation is quite different for pentacoordinate structure, where one oxygen would occupy an apical site in the complex, the other oxygen an equatorial position. In this case the complexation shifts of the appropriate carbons would be expected to be quite different, and this is what is observed, one carbon being shifted 9 ppm more than the other. Un-



*Scheme 2.*

fortunately, because of problems arising from the residual HOD NMR signal, it did not prove possible to determine the $^{13}C$ resonance positions for both the 2′ and 3′ carbons of the second symmetrical product which would have provided a test for the above arguments.

In order to account for the water stoichiometry observed for the ethylene glycol product and assumed to be the same for the nucleosides, for the NMR spectra, and for the exchange kinetics, the structures depicted in Scheme 2 have previously been proposed.[7] It may well be that there are alternative structures that equally well explain the experimental results. However, the proposed structures do provide a ready explanation for both the major and the minor products observed, since non- or slowly-exchanging rotomers are possible. Although this proposed geometry is in accord with the experimental results for the three major products, there is no firm basis for the assignment of a similar coordination to the minor products, and alternatives such as bridging structures seem quite likely.

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