Electrochemical characterization of a new vanadium penicillamine–ester complex

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Abstract

The synthesis and isolation of $VO(Pen\text{-}OCH_3)_2$ ($Pen\text{-}OCH_3$ = the anion of the carboxylate ester of penicillamine) is reported. This is the first report of the isolation of a vanadyl complex with penicillamine ligands. Based on IR data, the structure is proposed to be similar to that of $VO(Cys\text{-}OCH_3)_2$ ($Cys\text{-}OCH_3$ = the anion of the carboxylate ester of cysteine) which is known to be square pyramidal with bonding of *trans* sulfur atoms and *trans* nitrogen atoms to vanadium. The oxidation of $VO(Pen\text{-}OCH_3)_2$ in DMSO is a reversible process at 0.3 V versus SCE followed by a rapid chemical reaction of the initial product. Controlled potential coulometric oxidation did not give reproducible results until a ruthenium mediator was added. Under these conditions the coulometric data indicated a one electron oxidation, as previously reported for $VO(Cys\text{-}OCH_3)_2$. Thus, these two vanadyl compounds exhibit similar one electron oxidations giving vanadium(V) products.

Introduction

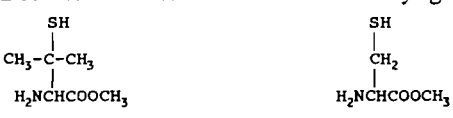
We have recently been involved in the study of model complexes for the inhibition of Na^+,K^+(ATPase) by vanadium. It has been demonstrated that naturally occurring tissue levels of vanadium are sufficient for inhibition of the enzyme when the vanadium is oxidized to the V oxidation state [1]. Although the lifetime of the vanadium(V) species may be short [2], the redox cycling of species between the IV and V oxidation states may supply a high enough steady state concentration of vanadium(V) to inhibit the enzyme and be a factor in hypertension. Thus, we have focused on the electrochemistry of vanadium(IV) and (V) model complexes.

Glutathione has been suggested as the primary reducing agent for vanadium(V) in this system [3]. As a reducing agent, glutathione undergoes a one electron oxidation and dimerizes by means of a disulfide bridge. Other thiols such as cysteine exhibit a similar electrochemistry. Because of the varied functional groups in glutathione (a tripeptide of glutamic acid, cysteine, and glycine) we have chosen individual amino acids and derivatives as model ligands. Amino acid complexes of vanadium are generally soluble and difficult to isolate because of the acid (–COOH) group. As first shown by Sakurai and coworkers [4] esterifying cysteine (–COOR) leads to the straightforward isolation of complexes with vanadium(IV). The structure of the vanadium(IV) cysteine ester complex has recently been reported [5], and it is believed that the bonding of the acid (–COOH) form of the ligand is similar [6]. we previously reported an improved synthesis of the cysteine ester ligand complex, bis(methoxycysteinato)oxovanadium(IV), $[VO(Cys\text{-}OCH_3)_2]$ and characterized its electrochemistry [7].

As an additional model compound, we have synthesized and studied the electrochemistry of the analogous penicillamine ester complex. Penicillamine differs from cysteine by two methyl groups on the carbon adjacent to the sulfur:

Penicillamine Cysteine

Both can be esterified at the carboxyl group giving:



Penicillamine methyl ester Cysteine methyl ester

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In our preliminary work with the acid form of penicillamine and VO^{2+}, no solid compounds could be isolated, in agreement with earlier reports [8–10]. Using the ester form of the ligand a new complex of VO^{2+} was isolated and characterized. We report here the synthesis and electrochemistry of the first known vanadium penicillamine carboxylic-ester complex.

Experimental

Instrumentation

Electrochemical experiments were made using two systems. Some cyclic voltammetry and bulk electrolysis experiments were carried out using a Bioanalytical Systems model 100A electrochemical analyzer. The data was transferred to an IBM-XT computer and stored on floppy disks. Final hard copies were output to a Houston Instruments Hiplot DMP-40 Series digital plotter. Other cyclic voltammetric measurements were made with a Princeton Applied Research model 173 three electrode potentiostat and model 175 universal programmer. The voltammograms were recorded on a Houston Instruments model 2000 omnigraphic X-Y recorder. Controlled-potential electrolysis was carried out with the above potentiostat and a Princeton Applied Research model 179 digital coulometer.

All-glass electrochemical cells with joints for a three electrode system were used. The non-aqueous electrochemistry was studied with a Bioanalytical Systems model ME-2013 platinum electrode. The electrode was held in the cell via a thermometer adapter so that height adjustments could be made. A platinum mesh electrode was used for coulometric studies. The reference electrode consisted of a Ag/AgCl electrode in aqueous tetramethylammonium chloride (Aldrich) with the concentration adjusted to make the electrode potential 0.000 V versus SCE. The reference junction was a platinum wire sealed in the tip of a pyrex tube. The electrode was positioned in a Luggin capillary in the cell assembly. The auxiliary electrode was either platinum foil or folded platinum mesh. It was separated from the cell solution by a fine porosity frit. The studies were conducted under an inert atmosphere of prepurified nitrogen.

A Varian model DMS 90 UV–Vis spectrometer was used for spectrophotometric studies. Infrared spectra were obtained using a Perkin-Elmer model 683 spectrometer with data station.

Reagents

Non-aqueous electrochemistry was done in high purity dimethyl sulfoxide (DMSO) (0.07% water) from Burdick and Jackson Laboratories. It was used as obtained. Tetraethylammonium perchlorate (TEAP) was the supporting electrolyte. It was prepared from tetraethylammonium bromide (Aldrich) and perchloric acid (Fisher) as previously described [11]. Tetraethylammonium hydroxide (TEAOH) was obtained from Aldrich as a 40% solution in water. D-(−)penicillamine was obtained from Sigma and D-penicillamine methyl ester was from Fluka. Both were used as obtained. Vanadyl chloride was obtained from ICN Biomedical or Custom Chemicals Lab, Livermore, CA, U.S.A.

The ruthenium compound, bis(bipyridyl)chloro(tri-n-butylphosphine)ruthenium(II) hexafluorophosphate, $\{[(bpy)_2Ru(tri\text{-}n\text{-}Bu)P]Cl\}PF_6$, was made by the procedure of Sullivan *et al.* [12]. This compound was used as a mediator in some experiments as demonstrated by the work of Thackrey and Riechel [13].

Synthesis

Bis(methoxypenicillaminato)oxovanadium(IV), [VO(Pen-OCH$_3$)$_2$]

The synthesis of this compound was based on that previously reported for the cysteine methyl ester analogue [7]. First, vanadyl acetate was prepared by the dropwise addition of excess sodium acetate (1.23 g, 0.023 mol, dissolved in 50.0 ml of reagent grade methanol) to 1.23 g $VOCl_2 \cdot 6H_2O$ (0.005 mol) chilled in an ice bath. The mixture was stirred for 15 min in the ice bath, producing a blue–green solution accompanied by precipitation of NaCl. After chilling for an additional 30 min, the solution was filtered on a fine porosity fritted glass funnel to remove the salt.

A three-neck round-bottom flask, with gas inlet and outlet, was used for the reaction of vanadyl acetate with the ligand. The central neck held a separatory funnel for vanadyl acetate. First, 50.0 ml 0.1 M borate buffer (pH 11.0) was placed in the flask and deoxygenated with prepurified nitrogen for 15 min. Then 2.50 g penicillamine methyl ester (0.0125 mol) was dissolved in the buffer with stirring and deoxygenation, lowering the pH by about 2 units. (The borate buffer concentration was near its limit of solubility and thus could not be increased to give a true buffer. The final pH of 9.0–9.5 was sufficiently high to deprotonate the –SH group and promote complex formation with vanadyl ion.) The ligand solution was stirred and deoxygenated for an additional 10 min. Deoxygenated vanadyl acetate was then added dropwise from the separatory funnel to the stirred ligand solution. Halfway through the addition, a purple solid precipitated, at which point addition was stopped. (Vanadyl acetate added was

approximately 0.00250 mol.) This insured that the ligand was in excess. The solid was filtered from the solution on a fine porosity fritted funnel, washed with cold methanol, air-dried for 2 h on the funnel, then sealed in a 50 ml round bottom flask and dried under vacuum overnight. The solid was stored under vacuum. Yield was about 60% for the monohydrate. *Anal.* Calc. for $C_{12}H_{24}N_2O_5S_2V \cdot H_2O$: C, 35.20; H, 6.40; S, 15.66. Found: C, 35.84; H, 6.38; S, 15.88%.

Results and discussion

Synthesis

The synthesis of the penicillamine methyl ester compound, $VO(Pen\text{-}OCH_3)_2$ is as follows.

$$2CH_3COONa + VOCl_2 \xrightarrow{CH_3OH} VO(CH_3COO)_2 + 2NaCl_{(ppt)}$$

$$VO(CH_3COO)_2 + 2NH_2CH(COOCH_3)C(CH_3)_2SH \xrightarrow[N_2]{\text{pH 9.3, borate buffer}} VO[NH_2CH(COOCH_3)C(CH_3)_2S]_2 + 2CH_3COOH$$

This is the first example of an isolated vanadyl complex with penicillamine methyl ester.

The IR spectrum of $VO(Pen\text{-}OCH_3)_2$ was recorded and compared to those for penicillamine and penicillamine methyl ester. The data are tabulated in Table 1. Both ligands exhibit an S–H stretch near 2600 cm^{-1} while the spectrum of the complex is flat in this region. This verifies S–H bonding in the ligands and a deprotonated sulfur group in $VO(Pen\text{-}OCH_3)_2$, as expected for bonding to the vanadyl ion. The C=O stretch for penicillamine is very low perhaps because of Zwitterion formation. The C=O stretches for the ester ligand and the complex (1739 and 1726 cm^{-1} respectively) are in the region expected for esters. The spectrum of the complex also exhibits a V=O stretch at 974 cm^{-1} as is characteristic of vanadyl complexes. The data for the complex also compare well with the cysteine methyl ester complex described earlier [4, 7].

TABLE 1. Infrared data (cm^{-1})[a]

Compound	Functional group		
	S–H	C=O	V=O
Penicillamine	2601	1594	
Penicillamine methyl ester	2601	1739	
$VO(Pen\text{-}OCH_3)_2$		1726	974

[a]All spectra were recorded on KBr pellets.

Based on these comparisons we propose that the structure of the penicillamine ester complex is similar to that for the cysteine ester complex as reported by Sakurai *et al.* [5]. Our proposed structure is given in Fig. 1. The coordination geometry around the central vanadium atom is essentially octahedral. The pairs of sulfur and nitrogen atoms form a square plane around the vanadium with the two sulfurs and two nitrogens both *trans* to one another. The terminal oxygen atom and a weakly bound water molecule are expected to reside above and below the plane, *trans* to one another. This geometry is common for five- or six-coordinate vanadyl compounds.

Electrochemistry

Although the polarography of penicillamine has been studied [14] no reports on the electrochemistry of penicillamine methyl ester have been published. We carried out cyclic voltammetry on penicillamine methyl ester to aid in our study of the complex containing this ligand. (All cyclic voltammetry experiments were carried out in DMSO at a platinum working electrode with tetraethylammonium perchlorate (TEAP) as supporting electrolyte.) Voltammograms of 0.60 mM penicillamine methyl ester are shown in Fig. 2. Scan A, scanning negatively first, exhibits a reduction peak at −1.7 V versus SCE, a small oxidation peak at −0.4 V, and a major oxidation peak at 1.1 V. The reduction process at −1.7 V appears to produce the species oxidized at −0.4 V. The oxidation at 1.1 V is assigned to the oxidation of the thiol group, based on comparison to other thiol containing compounds. Scan B shows that this oxidation process leads to two new reduction peaks at −0.5 and −0.85 V. The peak at −0.5 V is due to protons, since it can be eliminated by the addition of TEAOH. The protons come from the dissociation of the thiol hydrogen (–SH) which accompanies the oxidation of the thiol group.

Figure 3 shows cyclic voltammograms of the penicillamine ester complex, $VO(Pen\text{-}OCH_3)_2$. The initial cathodic scan (scan A) shows a reduction peak at −1.7 V and a broad oxidation wave near −0.5 V

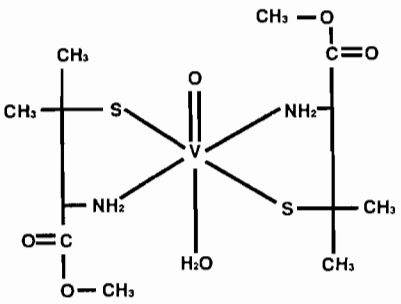

Fig. 1. The proposed structure of $VO(Pen\text{-}OCH_3)_2 \cdot H_2O$.

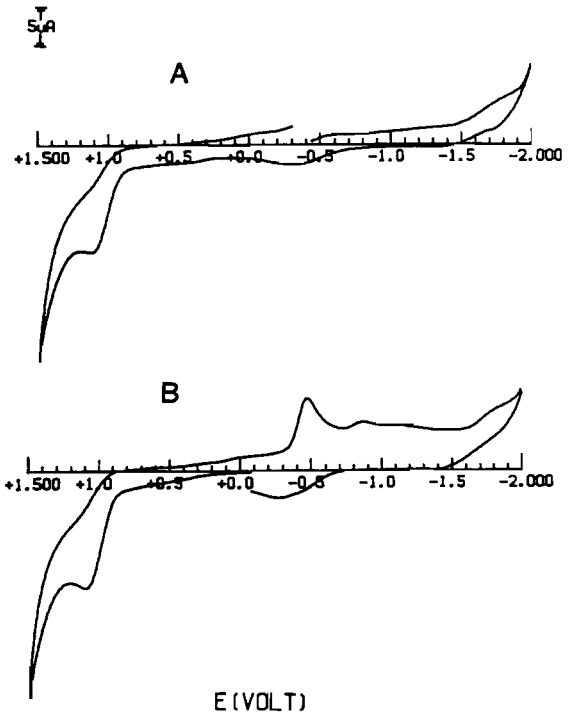

Fig. 2. Cyclic voltammograms of 0.60 mM D-penicillamine methyl ester in 0.1 M TEAP/DMSO, at a platinum electrode. Scan rate 200 mV/s.

which coincide with processes observed for the ligand. The initial anodic scan (scan B) shows a major oxidation wave at 0.35 V, perhaps coupled to a small reduction wave at 0.25 V. The two reduction waves at −0.70 and −1.15 V are a result of the oxidation at 0.35 V.

A scan rate study was carried out on the couple with peaks at 0.35 and 0.25 V (Fig. 4). For scan rates of 0.10, 0.20, 0.50, 1.00 and 2.00 V/s, the corresponding peak height ratios (i_{pc}/i_{pa}) were 0.16, 0.19, 0.25, 0.48 and 0.77. Thus, as the scan rate increases, the peak height ratio approaches unity. This suggests an EC mechanism in which a chemical reaction competes with the reduction step of a reversible redox process. This agrees with the electrochemistry of the cysteine methyl ester complex, $VO(Cys\text{-}OCH_3)_2$, reported earlier [7]. Also, this suggests that the reduction peaks at −0.70 and −1.15 V (Fig. 3) are due to the product of the chemical step in this mechanism.

Controlled potential electrolysis was carried out at 0.5 V in order to exhaustively oxidize the complex and determine the number of electrons in the process. Cyclic voltammograms of an initial solution, the solution following oxidation, and the solution following subsequent reduction are shown in Fig. 5. Scan B exhibits reduction peaks at −0.7 and −1.15

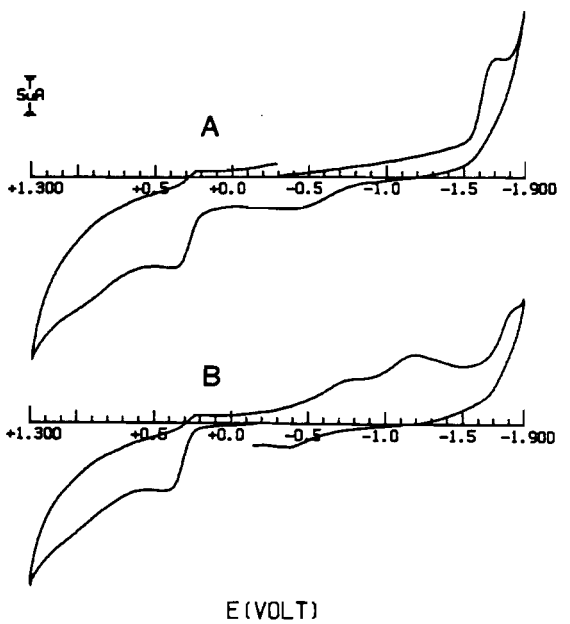

Fig. 3. Cyclic voltammograms of 3.8 mM $VO(Pen\text{-}OCH_3)_2$ in 0.1 M TEAP/DMSO, at a platinum electrode. Scan rate 200 mV/s.

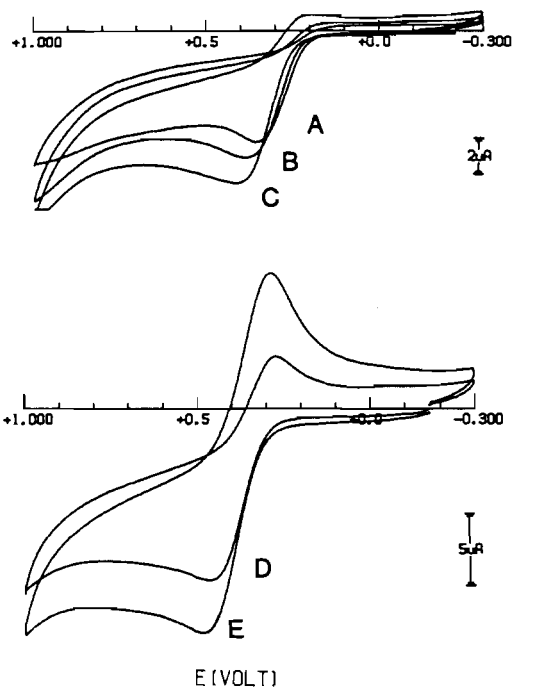

Fig. 4. Scan rate study of the 0.3 V couple of 3.8 mM $VO(Pen\text{-}OCH_3)_2$, 0.1 M TEAP/DMSO, at a platinum electrode. A, 100, B, 200; C, 500; D, 1000; E, 2000 mV/s.

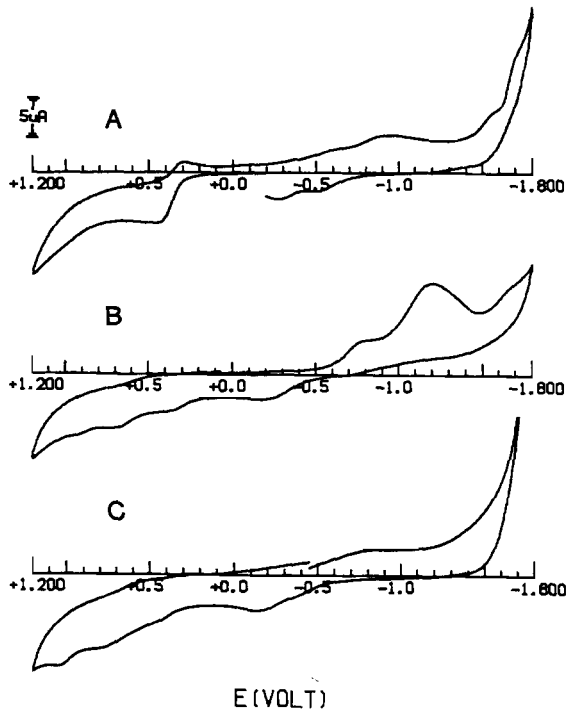

Fig. 5. Cyclic voltammograms resulting from oxidation and the subsequent reduction of 3.8 mM VO(Pen-$OCH_3)_2$ in 0.1 M TEAP/DMSO, at a platinum electrode. Scan rate 200 mV/s. A, Original solution; B, after complete oxidation at 0.5 V; C, after reduction of solution B at −1.6 V.

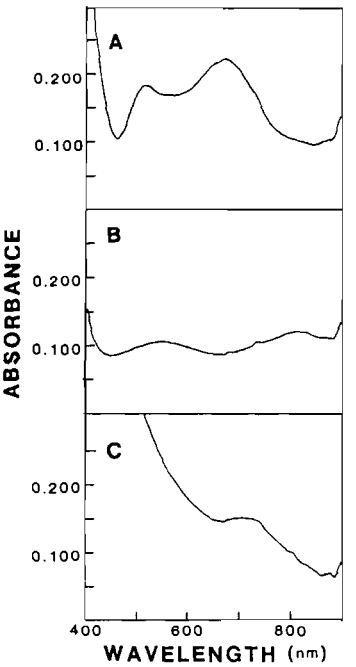

Fig. 6. Visible spectra (under nitrogen) resulting from oxidation and subsequent reduction of 3.8 mM VO(Pen-$OCH_3)_2$ in 0.1 M TEAP/DMSO, at a platinum electrode. A, Original solution; B, after complete oxidation at 0.5 V; C, after reduction of solution B at −1.6 V.

V indicative of the final products of the EC process. Scan C demonstrates that the reduction processes of scan B have gone to completion, but there is no evidence of a dominant vanadium complex remaining. These data likewise, compare well with those for VO(Cys-$OCH_3)_2$ [7]. Spectra corresponding to each of these solutions are shown in Fig. 6. Once again, these are analogous to those of VO(Cys-$OCH_3)_2$ undergoing the same electrolysis steps.

It was concluded earlier [7] that VO(Cys-$OCH_3)_2$ undergoes a one electron reversible oxidation to vanadium(V), followed by a chemical reaction to another vanadium(V) species reduced at −1.30 V. Based on the cyclic voltammetry and spectra, the electrochemistry of VO(Pen-$OCH_3)_2$ appears to be similar, with the final reduction occurring at −1.15 V. (In each case a proton reduction peak is also observed at −0.7 or −0.9 V.) In contrast, the coulometric results do not agree. The reduction of VO(Cys-$OCH_3)_2$ is a one electron process [7] while the experiments reported here for VO(Pen-$OCH_3)_2$ gave a variable number of electrons for the process, averaging about 2.

Due to the irreproducibility of the coulometric results and the extreme times required for electrolysis (~6 h), we concluded that other processes may be involved leading to high, variable results. To facilitate the electrolysis, we carried out further experiments in the presence of a ruthenium mediator {[$(bpy)_2$Ru(tri-n-Bu)P]Cl}PF_6 [12, 13].

Figure 7, scan A shows a cyclic voltammogram of 1.0 mM VO(Pen-$OCH_3)_2$ and 0.1 mM ruthenium mediator. The voltammogram exhibits the vanadium oxidation couple near 0.3 V and the product reduction peaks near −0.7 and −1.15 V. The oxidation peak at 0.6 V and two couples near −1.3 and −1.5 V are due to the ruthenium, as shown by voltammograms of the mediator alone.

Several coulometric experiments were carried out at a fixed potential of 0.6 V on solutions of VO(Pen-$OCH_3)_2$ plus the mediator. Electrolysis proceeded much more quickly than without the mediator, and residual current levels were reached within 45 min. The resulting voltammogram (Fig. 7, scan B) shows that oxidation of the complex is complete and the same final product is formed (reduction peak near −1.2 V). The coulomb values were corrected for the amount of ruthenium oxidized and gave values of 0.79, 1.10 and 1.00 electrons per vanadium for three experiments. The average value of 0.96 electrons suggests a one electron process. The ease of oxidation, the number of electrons involved, and the final yellow color of the solution all indicate a vanadium(V) product.

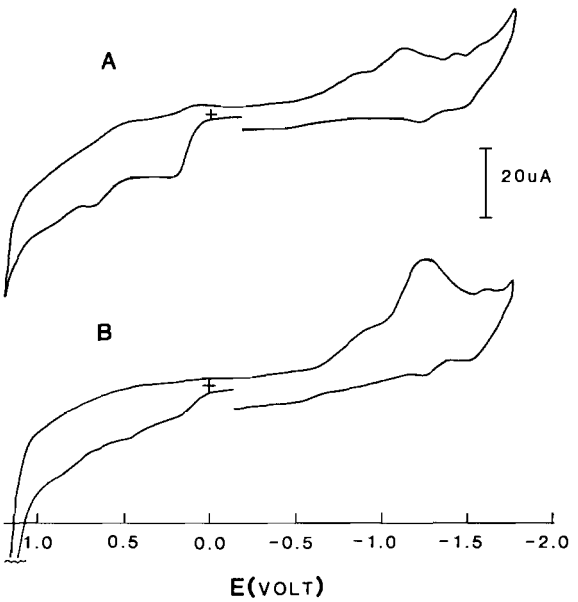

Fig. 7. Cyclic voltammograms of 1.0 mM VO(Pen-$OCH_3)_2$ + 0.1 mM $\{[(bpy)_2Ru(tri\text{-}n\text{-}Bu)P]Cl\}PF_6$ in 0.1 M TEAP/DMSO, at a platinum electrode. Scan rate 200 mV/s. A, Original solution; B, after complete oxidation at 0.6 V.

Conclusions

Evidence from the synthesis, IR spectra, UV–Vis spectra and electrochemistry all demonstrate great similarity between VO(Pen-$OCH_3)_2$ and VO(Cys-$OCH_3)_2$. The penicillamine ester ligand causes VO(Pen-$OCH_3)_2$ to be difficult to oxidize, but this can be accomplished cleanly by use of a mediator.

This work represents the first report of an isolated vanadium–penicillamine complex. This compound and the cysteine analogue should both be useful as models for vanadium–glutathione complexes in biological systems.

Acknowledgements

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