The Electrochemistry of Amavadine, a Vanadium Natural Product

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Abstract

Amavadine, a vanadium natural product of the mushroom *Amanita muscaria*, has been synthesized and its electrochemistry characterized in DMSO, DMF, and water. In each solvent a one electron reversible oxidation couple is observed and the vanadium(V) product is stable. In DMSO the couple is at 0.03 V *versus* SCE and in water it occurs at 0.53 V. Several model complexes show irreversible oxidations at much higher potentials. The unique electrochemistry of Amavadine, as compared to the model complexes, suggests that it may function as an electron-transfer catalyst in the mushroom.

Introduction

Although the natural occurrence of vanadium in biological systems has been known for many years, only one natural product has been isolated. Bayer and Kneifel reported a vanadium content of 120 ppm (dry weight) for the mushroom *Amanita muscaria* [1]. They characterized a vanadium extract from the mushroom as a vanadyl (VO^{2+}) complex with two *N*-hydroxy-α,α-iminodipropionic acid ligands and named the product Amavadine [2].

Two EPR studies have recently appeared [3, 4] which indicate that Amavadine is indeed an oxovanadium(IV) compound and probably does not change form upon isolation. Felcman and coworkers [5] synthesized the ligand present in Amavadine, *N*-hydroxy-α,α-iminodipropionic acid, and studied the formation of complexes with various metal cations, including VO^{2+}. No vanadium compound was isolated. Nawi and Riechel [6] isolated and reported the electrochemistry of models for Amavadine, such as bis(iminodiacetato)oxovanadium(IV) and bis(α,α-iminodipropionato)oxovanadium(IV). Most recently Kneifel and Bayer [7] have reported a total synthesis of Amavadine and verified its structure and stereochemistry. The structure of the compound (Fig. 1) is as those authors originally proposed.

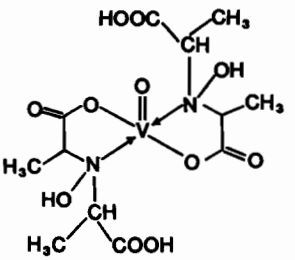

Fig. 1. The structure of Amavadine as determined by Kneifel and Bayer.

We present here an alternate synthesis of Amavadine, spectral characterizations, and extensive electrochemical data. We believe that the electrochemical results shed light on the probable role of Amavadine in the mushroom *Amanita muscaria*.

Experimental

Instrumentation

Cyclic voltammetry experiments were carried out with a Princeton Applied Research Model 173, three electrode potentiostat and a Model 175 Universal Programmer. The voltammograms were recorded on a Houston Instrument model 2000 Omnigraphic X–Y recorder.

The working electrode for the aqueous studies was a Bioanalytical Systems glassy carbon electrode. A Beckman platinum-inlay electrode was used for both aqueous as well as non-aqueous studies. The reference electrode consisted of a Ag/AgCl electrode in aqueous tetramethyl ammonium chloride (Aldrich) with the concentration adjusted to make the reference electrode potential 0.000 V *versus* SCE. The reference electrode junction was a soft-glass cracked bead sealed into a pyrex tube. The electrode was positioned in a luggin capillary in the cell assembly. All studies utilized an auxiliary electrode made from a small piece of platinum foil separated from the cell solution by a fine porosity frit.

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All cyclic voltammetry experiments were done under an inert atmosphere by either bubbling nitrogen through an electrochemical cell or by using a Vacuum Atmospheres Co. model HE-43-2 glove box with an HE 493 Dri-Train under dry nitrogen atmosphere.

Spectrophotometric measurements were made on a Hewlett Packard 8450-A UV–Vis spectrophotometer and a Beckman DU 40 spectrophotometer. IR spectroscopy was done using a Perkin-Elmer Model 683 spectrometer with data station while the NMR was done by using a Varian EM 360A NMR spectrometer. Atomic absorption for vanadium elemental analysis was done by using a Perkin-Elmer 560 spectrometer.

Reagents

High purity dimethylsulfoxide (DMSO) and dimethylformamide (DMF) were obtained from Burdick and Jackson Laboratories. These were used as received. Tetraethylammonium perchlorate (TEAP) was prepared from tetraethylammonium bromide (Aldrich) and perchloric acid as previously described [8] and was used as supporting electrolyte. Potassium chloride or potassium nitrate for the supporting electrolyte in aqueous solvent were obtained from MCB. Tetraethylammonium hydroxide (TEAOH) was obtained from Aldrich as a 25% solution in water.

Synthesis

Bis(N-hydroxy-α,α-iminodipropionato)oxovanadium(IV) monohydrate, Amavadine

The synthesis of Amavadine involved the addition of vanadyl acetate to the ligand, *N*-hydroxy-α,α-iminodipropionic acid. Vanadyl acetate was prepared in methanol from $VOCl_2$ and sodium acetate, as described previously [6]. The ligand was prepared according to the method of Felcman and coworkers [5]. As indicated by those authors, the final product is sticky and is a mixture of both the diacid and the monosodium salt of the diacid. The NMR spectrum in D_2O of our product was similar to that of Felcman, but a pure product could not be isolated. Hence, the ligand was used in aqueous solution.

An excess of ligand (about 0.10 mol) in aqueous solution was treated with 6 M HCl until a pH of 2.5 was achieved. The solvent was stripped off leaving a viscous liquid. This liquid was treated with concentrated HCl to precipitate NaCl. The NaCl was filtered off, the solvent was removed, and the HCl treatment was repeated until no more salt precipitated. Excess HCl was then removed by repeatedly adding water and stripping off the solvent. The final pH was approximately 2.

A vanadyl acetate solution in methanol (0.02 mol) was added dropwise to the solution of ligand and a purplish blue solution resulted. Since no crystals formed when left in a refrigerator overnight, the solvent was stripped off completely. A viscous purplish blue liquid was obtained. This was treated repeatedly with methylene chloride until no more greyish methylene chloride solution was obtained. The remaining residue was dried on a vacuum line, yielding a purplish blue solid. This impure product was contaminated with excess ligand and was hygroscopic.

Purification of Amavadine was attained by dissolving the solid in methanol. The solution was chilled and the insoluble residue was filtered off. To the filtrate was added acetone whereby a pale blue precipitate formed immediately. Reprecipitation was repeated several times. The final purification was done by dissolving the compound in ethanol and precipitating with acetone. The solid was then washed with acetone and dried under vacuum. The overall yield including synthesis of the ligand was about 10%. *Anal.*: Calc. for $C_{12}H_{20}N_2O_{11}V \cdot H_2O$: C, 32.95; H, 5.03; V, 11.66. Found: C, 32.51; H, 4.84; V, 11.51%.

Results and Discussion

Synthesis

The synthesis of Amavadine is not straightforward, due mainly to complexities with the ligand synthesis. Kneifel and Bayer [2] were first to suggest the reaction of hydroxylamine hydrochloride with α-bromopropionic acid to form *N*-hydroxy-α,α-iminodipropionic acid, but details of the synthesis were not given. Their later work [7] uses this approach and requires the isolation of a zinc complex followed by ion exchange chromatography to obtain a pure product. We followed the synthesis of Felcman and coworkers [5] which gave the protonated form of the diacid directly, but was always contaminated by the monosodium salt. The product was also extremely hygroscopic. Since the ligand can be used in solution for the synthesis of Amavadine and the sodium can be removed by precipitation as NaCl, this is a practical and convenient alternative approach to obtaining *N*-hydroxy-α,α-iminodipropionic acid†.

Kneifel and Bayer [7] prepared Amavadine from $VOSO_4$ and the ligand. $BaCO_3$ was added to precipitate the sulfate as $BaSO_4$, and ion-exchange was used, probably to replace excess barium with protons. Our procedure was based on the addition of vanadyl acetate to the ligand. The only by-product was acetic acid, which was easily removed. Once the ligand is prepared, the actual synthesis of Amavadine gives high yields in both cases, but our method seems better suited for larger scale preparations. (The

† Kneifel and Bayer (ref. 7) also give a procedure for separating the stereoisomers of the ligand.

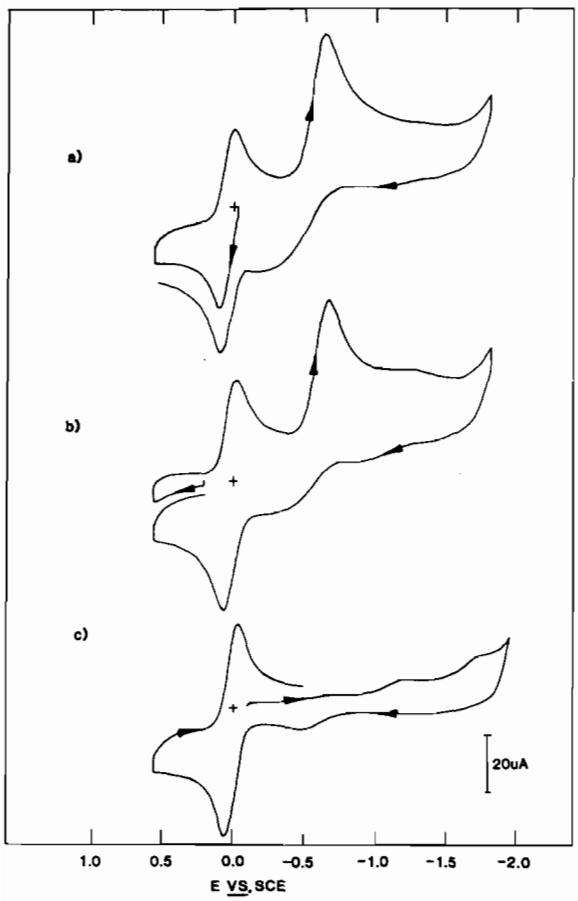

Fig. 2. Cyclic voltammograms of Amavadine in 0.1 M TEAP/DMSO. (a) 1.23 mM Amavadine; (b) solution [a] after oxidation at +0.45 V *vs.* SCE; (c) Amavadine after treatment with TEAOH. Scan rate, 0.2 V/s. Pt working electrode.

infrared and UV–Vis spectra of our product compared very well with those of Kneifel and Bayer [1, 7].)

Electrochemistry

A cyclic voltammogram of Amavadine in 0.1 M TEAP/DMSO using a Pt working electrode is shown in Fig. 2a. The anodic scan indicated an oxidation peak at 0.06 V *versus* SCE. The reverse scan produced a reduction peak at 0.0 V. Scans done at various scan rates did not alter the peak potentials. The ratio of cathodic peak current to anodic peak current was close to unity and the peak separation was about 60 mV. Thus, these processes form a reversible couple. The redox potential of this couple is taken as the average between the anodic and cathodic peaks, 0.03 V. Figure 2b shows the result of controlled potential coulometry done at 0.45 V. When the solution of Amavadine was exhaustively oxidized at this potential using a platinum mesh-electrode, a pink solution was obtained and a one electron transfer was observed. The CV in Fig. 2b shows that on an initial anodic scan there is no oxidation peak at 0.06 V. This indicates that the oxidation process is complete. On the reverse scan, the reduction peak at 0.0 V was obtained. Since cyclic voltammograms of the ligand alone (not shown) exhibit no peaks in the positive potential range, this means that the pink solution corresponds to the vanadium(V) analogue of Amavadine. That is, the processes at 0.03 V represent the reversible one electron oxidation of Amavadine to a similar vanadium(V) species. A subsequent reduction of the resulting solution at −1.0 V resulted in a CV similar to Fig. 2c. This means that Amavadine is reformed by the reduction.

Figure 2a also shows a large reduction peak at −0.65 V and a broad oxidation peak at −0.20 V. The controlled potential reduction of an Amavadine solution at −1.0 volt gave a two electron transfer. No effect on the CV was observed except for the disappearance of the reduction peak at −0.65 V and the diminishing of the oxidation peak at −0.20 V. Therefore, the reduction peak at −0.65 V and the redox couple at 0.03 V are independent of each other. The identity of the reduction peak is revealed by treating a solution of Amavadine with tetraethylammonium hydroxide (TEAOH). As shown in Fig. 2c, when two equivalents of TEAOH were added, the reduction peak at −0.65 V disappeared. There was no effect on the redox couple at 0.03 V. A solution of $HClO_4$ in DMSO produced a reduction peak identical to that observed at −0.65 V for Amavadine. A similar CV was obtained for HCl in DMSO. Thus, the −0.65 V peak is due to the reduction of protons. Apparently, these protons originate from the free carboxylic moieties of Amavadine. The fact that a two electron transfer was observed when this peak was reduced supports this suggestion.

Figure 2c also exemplifies the highly simplistic electrochemistry of Amavadine. It involves simply two oxidation states, vanadium(IV) and vanadium(V), which form a reversible couple. Both of these oxidation states are quite stable. Oxidized Amavadine was in fact so stable that it could be left open to the air for several days without producing significant changes in its CV.

The preceding processes were also followed by spectroscopy. Figure 3 shows visible spectra of the initial solution of Amavadine, the oxidized species, and the reduced solution of the oxidized species. The visible spectrum of Amavadine (spectrum (a)) is essentially identical with the spectrum of Amavadine in water. Oxidized Amavadine (spectrum (b)) is pink (peak maximum at 500 nm) and has a much stronger molar absorptivity than Amavadine. (Spectrum (b) corresponds to the absorbance scale on the left). When oxidized Amavadine was reduced, the pink color changed back to purplish-blue (spectrum (c)) with a one-electron transfer. Comparing spectrum (c)

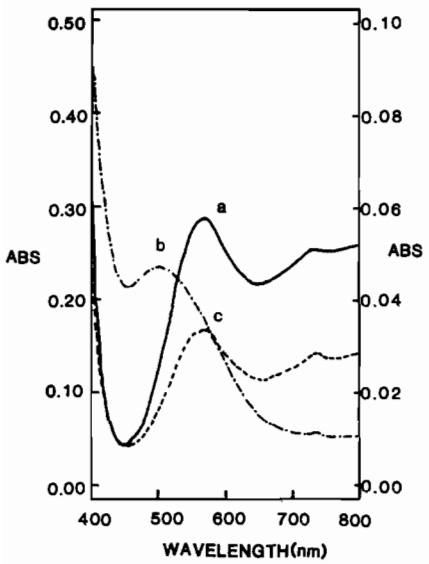

Fig. 3. Visible spectra in DMSO of (a) the initial solution of Amavadine, (b) the oxidized Amavadine and (c) the reduced solution of oxidized Amavadine. The absorbance scale for (b) is on the left.

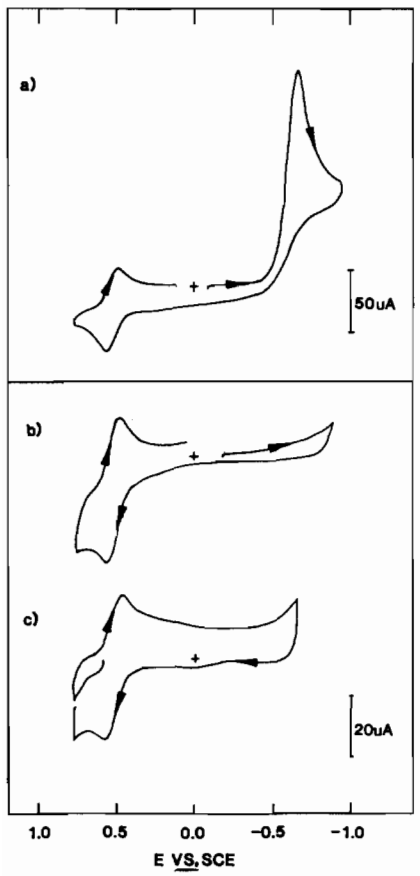

Fig. 4. Cyclic voltammograms of Amavadine in 0.1 M KNO_3/H_2O using a platinum working electrode. (a) 1 mM Amavadine; (b) solution [a] treated with 0.1 M NaOH and (c) solution [b] after oxidation at + 0.65 V. Scan rate, 0.2 V/s.

with spectrum (a) shows that they are qualitatively identical, although the final spectrum has lower absorbances.

A 1 mM solution of Amavadine in water with 0.1 M KNO_3 supporting electrolyte and a Pt working electrode has a CV as shown in Fig. 4a. The electrochemistry of Amavadine in water is similar to that in DMSO and is also quite simple. Curve (a) consists of only a reversible couple with an oxidation peak at 0.580 V and a reduction peak at 0.480 V, and a large reduction peak at −0.650 V. Once again, the couple (0.530 V) belongs to the oxidation of Amavadine at 0.580 V to vanadium(V) and subsequent reduction at 0.480 V back to Amavadine. This couple was also observed on the initial anodic scan. There is a significant shift in the redox potential of the couple from 0.030 V in DMSO to 0.530 V in water.

The large reduction peak at −0.650 V is independent of the redox couple. Figure 4b is solution (a) after being treated with 0.1 N NaOH. The reduction peak at −0.650 V is totally eliminated. Once again, this reduction peak is assigned to the reduction of protons.

Figure 4c shows the product of exhaustive coulometry done at 0.65 V. One electron was transferred. The solution obtained was identical in color to oxidized Amavadine in DMSO (pink). The oxidized Amavadine is very stable in water. As shown, an initial anodic scan indicates the absence of the anodic peak at 0.580 V meaning that the oxidation process was complete. The reverse scan gives the reduction peak at 0.480 V.

Figure 5 shows a similar study of Amavadine in water but using a glassy carbon electrode. The advantage of using a glassy carbon electrode in water is the expanded working potential range. When platinum was used, the potential working range was limited to +0.75 V to −1.1 V due to reduction of water at negative potentials and oxide formation at positive potentials. The carbon electrode extended the potential working range to between +1.4 volt and −1.5 volts. Figure 5a shows an oxidation peak at 0.54 V and a reduction peak at 0.43 V upon reversal of the initial anodic scan. This is similar to what was observed with the platinum electrode. However, in the case of the carbon electrode, the oxidation peak is not as sharp. The CV of oxidized Amavadine observed with the carbon electrode is shown in Fig. 5b. It also demonstrates complete oxidation to vanadium(V).

As expected, the glassy carbon electrode provides similar voltammograms of Amavadine as the platinum electrode. However, no reduction of protons was observed. This is probably due to the higher hydrogen overpotential for this electrode as compared to platinum [9]. The peak of the redox couple is also broadened indicating a sluggish electron transfer occurring between the electrode and the complex.

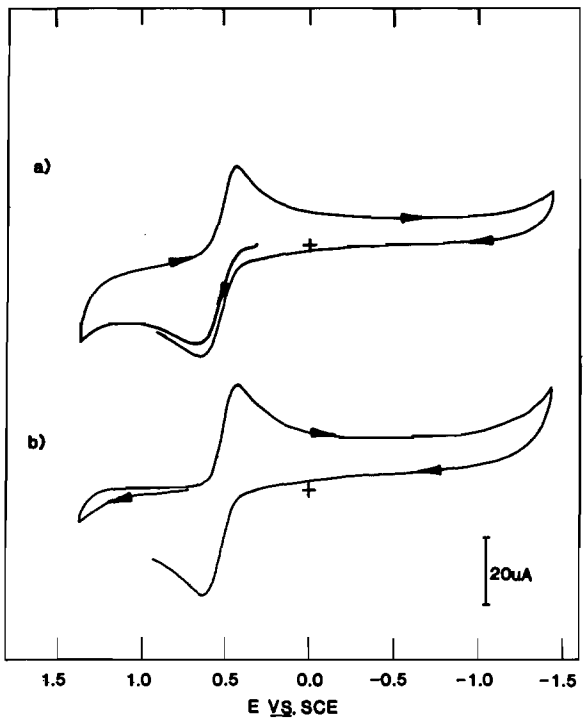

Fig. 5. Cyclic voltammograms of Amavadine in 0.1 M KNO_3/H_2O. (a) 3.5 mM Amavadine; (b) solution [a] after it has been oxidized at +0.65 V. Scan rate, 0.2 V/s. Glassy carbon working electrode.

The electrochemical experiments in water were accompanied by spectral measurements. The spectra for Amavadine, oxidized Amavadine, and the final reduced product were identical to those shown in Fig. 3. Thus, the vanadium(V) species formed by the oxidation of Amavadine is the same in both DMSO and water.

The effect of pH on the redox potential of the Amavadine couple was studied. This was done by preparing a solution of Amavadine in water with 0.1 M KNO_3. The pH was measured directly in the electrochemical cell. After degassing, a cyclic voltammogram was recorded on the solution. The pH was then varied by adding 0.1 M NaOH. When a platinum electrode was used, no effect was observed on the redox couple of Amavadine. However, pH affects the redox couple of Amavadine when a carbon electrode is used. The results are shown in Table I. As the pH was increased, the peak potential separation increased. The average redox potential of the couple also increased with pH. Thus, at pH 9.4 the redox potential was 0.59 V which was an increase of greater than 0.1 V when compared to the initial potential of Amavadine a pH 2.4. Both effects were reversed when the pH was lowered.

The increase in peak potential separation means that the kinetics of electron transport across the electrode/solution interface are less favorable at

TABLE I. Effects of pH on the Redox Couple of Amavadine as Observed with the Glassy Carbon Electrode

pH	E_{anodic} (V)	$E_{cathodic}$ (V)	ΔE_p (V)	E' (V)
2.4	0.54	0.43	0.11	0.48
3.0	0.56	0.42	0.14	0.49
3.6	0.58	0.42	0.16	0.50
5.2	0.60	0.41	0.19	0.51
6.7	0.70	0.38	0.32	0.54
7.0	0.76	0.37	0.39	0.56
7.4	0.80	0.37	0.43	0.58
9.0	0.80	0.36	0.44	0.58
9.4	0.84	0.34	0.50	0.59

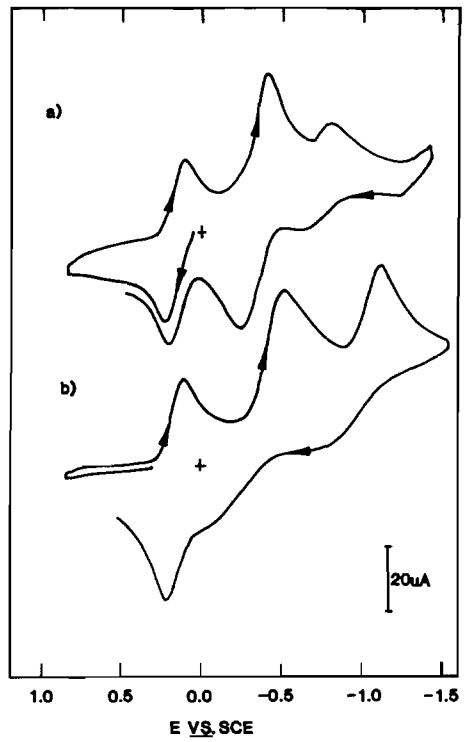

Fig. 6. Cyclic voltammograms of Amavadine in 0.1 M TEAP/DMF. (a) 1 mM Amavadine; (b) solution [a] after oxidation at 0.8 V *vs.* SCE. Scan rate 0.2 V/s. Pt working electrode.

higher pH, but the products are the same. The fact that the major features of the voltammogram remain the same qualitatively, shows that Amavadine does not decompose at high pH. Furthermore, these pH changes do not affect the visible spectrum.

A solution of 1 mV Amavadine 0.1 M TEAP/DMF (Pt electrode) has a CV as shown in Fig. 6a. On the initial anodic scan, an oxidation peak at 0.23 V was obtained, while on the reverse scan, a reduction peak at 0.13 V was obtained. These peaks represent a reversible couple with an average potential of 0.18 V. There was also a reduction peak at –0.43 V. This reduction peak disappeared when TEAOH was added

to the solution. Also, another reduction at −0.83 V occurs. This reduction peak was not observed in solutions of Amavadine in DMSO or water. On the reverse scan, an oxidation peak at −0.66 V was obtained. This oxidation peak is dependent on the reduction peak at −0.83 V and they could be a redox couple, although the potential separation is very large. Another oxidation peak at −0.27 V was also observed. This oxidation peak is a direct result of the reduction at −0.43 V. Addition of base also eliminated this oxidation peak.

Oxidation of Amavadine in DMF consumed one electron. The resultant solution has a CV as shown in Fig. 6b. Clearly, the CV indicates that the oxidation process is complete since no oxidation peak at 0.23 V was observed. The solution was pink and has a visible spectrum identical to the oxidized Amavadine in the other solvents. (Air oxidation of Amavadine in DMF gives the same result.) Besides the reduction peak at −0.83 V which was observed in the initial solution of Amavadine, the oxidized Amavadine also produced a large reduction peak at −1.20 V. These processes have not been identified.

The redox potentials for the reversible oxidation couple of Amavadine in various solvent/electrode systems are summarized in Table II. The potentials in water are significantly higher than those in the non-aqueous solvents, and nearly independent of the type of electrode used. The potentials may correlate approximately to the dielectric constants of the solvents.

TABLE II. Redox Potentials of Amavadine in Various Solvent/Electrode Systems

Solvent/Electrode	Dielectric constant of solvent	E' *vs.* SCE (V)
H_2O/Pt	78.4	0.53
H_2O/C	78.4	0.49
DMSO/Pt	46.68	0.03
DMF/Pt	36.71	0.18

Conclusion

The electrochemistry of Amavadine is quite simple involving only two oxidation states. Amavadine is reversibly oxidized to a vanadium(V) version near 0.03 V in DMSO, and both species are stable. The proton reduction peak present on the cyclic voltammograms indicates that dissociation of protons from the carboxylic acid groups occurs before oxidation. These reactions of Amavadine are given in Table III. Although the potentials are different, a single redox couple for Amavadine is also observed in water and DMF.

TABLE III. The Electrochemistry of Amavadine in DMSO

Reaction[a]	E' *vs.* SCE (V)
$VO(LH)_2 \rightleftharpoons VO(L)_2^{2-} + 2H^+$	
$VO(L)_2^{2-} \rightleftharpoons VO(L)_2^{-} + e^-$	0.03
$H^+ + e^- \rightleftharpoons \frac{1}{2}H_2(g)$	−0.65

[a] LH_2 = *N*-hydroxy-α,α-iminodipropionic acid.

TABLE IV. Oxidation Couples for Amavadine and its Analogs

Complex[a]	E', DMSO V *vs.* SCE	E', Water V *vs.* SCE	Reference
Amavadine $VO(NHIDP)_2$	0.03	0.53	This work
$VO(NHIDA)_2$	0.14	0.65	f
$VO(\alpha,\alpha\text{-IDP})_2$	1.10[b]	c	6
$VO(\beta,\beta\text{-IDP})_2$	0.75[b]	d	6
$VO(IDA)_2$	e	c	6

[a] Abbreviations: NHIDP = *N*-hydroxy-α,α-iminodipropionic acid; NHIDA = *N*-hydroxyiminodiacetic acid; α,α-IDP = α,α-iminodipropionic acid; β,β-IDP = β,β-iminodipropionic acid; IDA = iminodiacetic acid. [b] Irreversible. [c] Positive potential range not accessible with Hg electrode. [d] Not formed in water. [e] Not soluble in DMSO. [f] The *N*-hydroxyiminodiacetic acid complex was synthesized as part of this study, but was contaminated by the mono-sodium salt of the ligand. Its spectroscopic and physical properties were similar to that of Amavadine. The electrochemistry exhibited reversible oxidation couples in both DMSO and water as indicated in the Table.

A comparison of the redox potentials of Amavadine and several model complexes is given in Table IV. Of major significance is that only Amavadine and the *N*-hydroxyiminodiacetic acid complexes exhibit reversible oxidations. That is, it appears that reversibility (and stability of the vanadium(V) products) is related to the hydroxyl group bound to the nitrogen atom. Comparing potentials in DMSO, Amavadine also has a potential 1 V more negative than $VO(\alpha,\alpha\text{-IDP})_2$, which is its analog without the N–OH group. The fact that the hydroxyl group lowers the potential of a system was also observed by Masui and coworkers [10] in a study of alkylamines and alkanolamines.

The data presented here suggest that Amavadine may have a role in the mushroom *Amanita muscaria* as an electron-transfer catalyst or mediator titrant [11]. The redox potential for Amavadine (in either water or DMSO) is within the typical range for biological redox agents. Its electrochemistry is simple and provides a reversible couple as is required for a

mediator titrant. We are currently pursuing applications of Amavadine as a mediator titrant to demonstrate the feasibility of this role in the mushroom.

Acknowledgements

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