NMR studies on natural and synthetic Amavadin

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Abstract

The stereochemistry of isolated natural product Amavadin, which contains a 1:2 complex of V(IV) with N-hydroxyimino-2,2′-dipropionic acid (HIDPAH$_3$), and some synthetic complexes have been investigated. Amavadin was isolated from *Amanita muscaria* and oxidized with [NH$_4$]$_2$[Ce(NO$_3$)$_6$]. H$_2$[Δ-V(S,S-HIDPA)$_2$].3H$_2$O, H$_2$[Δ,Λ-V(S,S-HIDPA)$_2$].3H$_2$O and their equivalent oxidized species have been synthesized and characterized spectroscopically. A combination of COSY, NOE, ^{1}H, ^{13}C-NMR and CD spectroscopy have been used to prove that the isolated natural product Amavadin consists of an almost equal mixture of the Δ- and Λ-isomers of [V(S,S-HIDPA)$_2$]$^{2-}$. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

The fungal genus *Amanita* is known to concentrate vanadium which is isolated as a V(V)N-hydroxyiminodipropionic acid (HIDPAH$_3$) and named Amavadin [1]. Spectroscopic and X-ray crystallographic studies on the isolated natural product Amavadin and its chemical analogues showed that the vanadium center of Amavadin has an eight-coordinate non-oxo complex [2–6], contrary to the initial suggestion that it has a VO^{2+} center [7].

In Amavadin a 2:1 complex of HIDPAH$_3$ and vanadium is formed and each HIDPA^{3-} ligand possesses two chiral atoms and all the chiral carbon atoms have been reported as possessing the *S* configuration [7,8]. Characterization of [Δ-V(S,S-HIDPA)]$^{2-}$ by EPR and UV/VIS spectroscopy indicated that this complex was identical to the isolated natural product Amavadin. However, the CD spectrum was different from that of Amavadin. Oxidation of this complex enabled X-ray crystallographic studies (Fig. 1) and comparative NMR studies to be undertaken. Cycling between the oxidation states V(IV) and V(V) in these complexes was followed by CD and NMR experiments and the data showed that there was no change in the nature of the complex [9]. There are several possible distinct combinations of iso-

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mers with four chiral carbon atoms in the complex. The vanadium center itself is also a chiral center by bonding of an η^2-N-O moeity of the oxyimino group. This leads to two possible isomers Δ, and Λ at the vanadium center (Fig. 2).

In this work we report one and two dimensional NMR spectroscopy, and CD spectroscopy

data for the isolated natural product Amavadin and for synthetic Amavadin.

In order to understand the molecular and electronic structures and the reaction chemistry of Amavadin-like complexes, further investigations were also carried out. An interesting observation is isomerization at the vanadium center. NMR
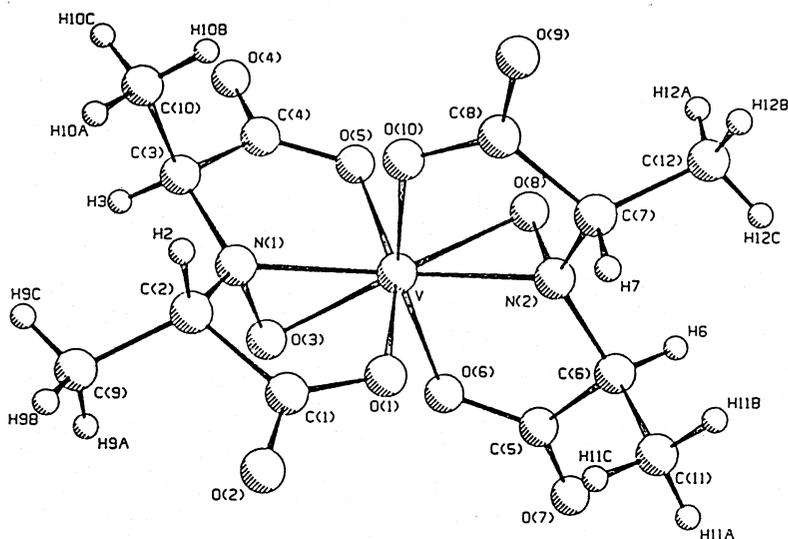

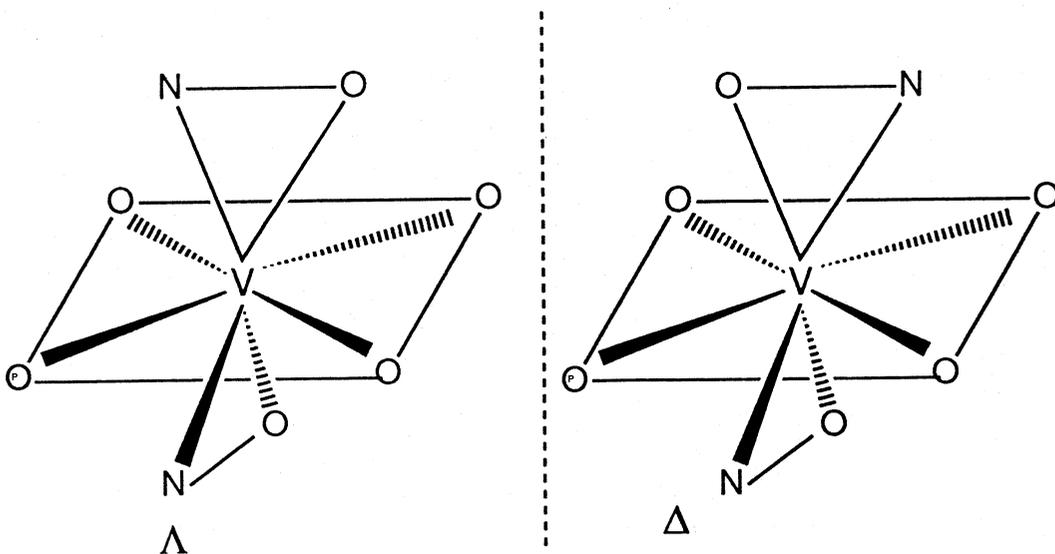Fig. 1. Structure of $[\Delta\text{-V}(S,S\text{-HIDPA})_2]^-$ in $[PPh_4]^+$ salt [9].

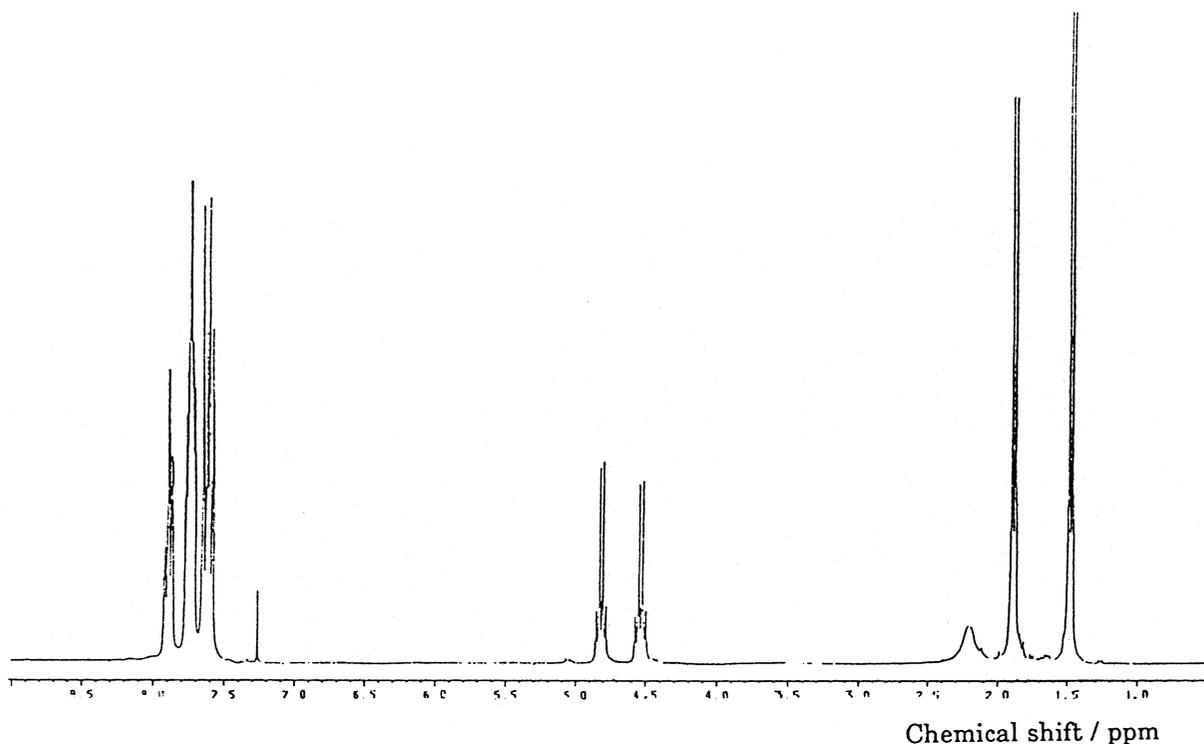Fig. 2. Illustration of chirality at the vanadium center in bis(HIDPA) complex.

Fig. 3. ^{1}H-NMR spectrum of $[PPh_4][\Delta\text{-}V(S,S\text{-HIDPA})_2].H_2O$.

studies together with CD and specific rotation measurements have shown that, when $[\Delta\text{-}V(S,S\text{-}HIDPA)]^{2-}$ allowed to stand for several hours in aqueous solution at room temperature isomerization occurred at the vanadium(IV) center.

2. Results

2.1. ^1H, ^{13}C, and COSY spectra

^{1}H (300 MHz), ^{13}C (75 MHz), and ^{1}H-^{1}H COSY NMR spectra have been obtained for the vanadium(V) complexes of S,S-HIDPA^{3-}, and the oxidized natural product Amavadin in CDCl$_3$. Samples for NMR spectroscopy were prepared either (i) by dissolving an isolated $[PPh_4]^+$ salt of a vanadium(V) complex in CDCl$_3$; or (ii) by oxidizing an aqueous solution of a vanadium(IV) complex which had been formed directly by mixing a $[VO(acac)_2]$ solution with a solution of the ligand, with $[NH_4]_2[Ce(NO_3)_6]$ and extracting into

CDCl$_3$. This latter method is termed an in situ complexation and oxidation. The individual complexes were obtained as described above. Chemical shifts for ^{1}H and ^{13}C spectra are given relative to TMS.

2.2. ^1H-NMR spectrum of $[PPh_4][\Delta\text{-}V(S,S\text{-}HIDPA)_2].H_2O$

The proton NMR spectrum (Fig. 3) of $[PPh_4][\Delta\text{-}V(S,S\text{-}HIDPA)_2].H_2O$ shows two quartets as a result of the methine protons at $\delta = 4.55$ and $\delta = 4.75$ ppm, two doublets as a result of methyl protons at $\delta = 1.43$ and $\delta = 1.85$ ppm. This material has been shown by single crystal X-ray diffraction [9] to contain a single isomer. The pairs of signals in the characteristic regions of the ^{1}H-NMR spectrum are consistent with a complex anion possessing C_2 point symmetry in solution. The ratio of the integrals of the methyl to methine protons is 3:1 and J_{H-H} is ca. 7.0 Hz.

2.3. ^{13}C NMR spectrum of $[PPh_4][\Delta$-$V(S,S$-HIDPA)$_2].H_2O$

The ^{13}C NMR spectrum (Fig. 4) of [PPh$_4$][Δ-V(S,S-HIDPA)$_2$].H$_2$O shows two signals for the carboxylate carbon atoms at $\delta = 174.4$ and $\delta = 172.8$ ppm, two signals for the methine carbon atoms at $\delta = 71.9$, $\delta = 66.8$ ppm and two signals for the methyl carbon atoms at $\delta = 15.8$, $\delta = 14.1$ ppm. These data are entirely consistent with the presence of a single isomer when [Δ-V(S,S-HIDPA)$_2$]$^-$ is dissolved in CDCl$_3$ and that this isomer possesses C$_2$ point symmetry.

2.4. ^{1}H-NMR spectrum of $[PPh_4][\Delta,\Lambda$-$V(S,S$-HIDPA)$_2].H_2O$ (oxidized synthetic Amavadin, generated by an in situ complexation and oxidation

This Δ,Λ-pair of isomers is designated 'synthetic Amavadin', since it will be shown that the predominant features in the various NMR and CD spectra of this complex are identical to those of the isolated, oxidized, natural product Amavadin. The ^{1}H-NMR spectrum of [PPh$_4$][Δ,Λ-V(S,S-HIDPA)$_2$].H$_2$O (Fig. 5) shows four doublets for the methyl protons at $\delta = 1.43$, $\delta = 1.48$, $\delta = 1.76$ and $\delta = 1.83$ ppm and four quartets for the methine protons at $\delta = 4.38$, $\delta = 4.50$, $\delta = 4.76$ and $\delta = 4.98$ ppm. $J_{H-H} = 7.0$ Hz. The integral ratio of Δ,Λ is ca. 1:1 indicating an approximately equimolar mixture of Δ- and Λ-isomers. Also, there is a weak apparent triplet (two overlapping doublets) at $\delta = 1.6$ ppm, which is indicative of the presence of some [Δ,Λ-V(R,S-HIDPA)$_2$]$^-$ or [Δ,Λ-V(S,S-HIDPA)(R,S-HIDPA)]$^-$.

2.5. ^{13}C-NMR spectrum of $[PPh_4][\Delta,\Lambda$-$V(S,S$-HIDPA)$_2].H_2O$ (oxidized synthetic Amavadin, generated by an in situ complexation and oxidation

In the ^{13}C-NMR spectrum (Fig. 6) of this compound there are seven signals for the methyl carbon atoms at $\delta = 14.0$, $\delta = 14.7$, $\delta = 15.8$ and $\delta = 16$, four signals for the methine carbon atoms
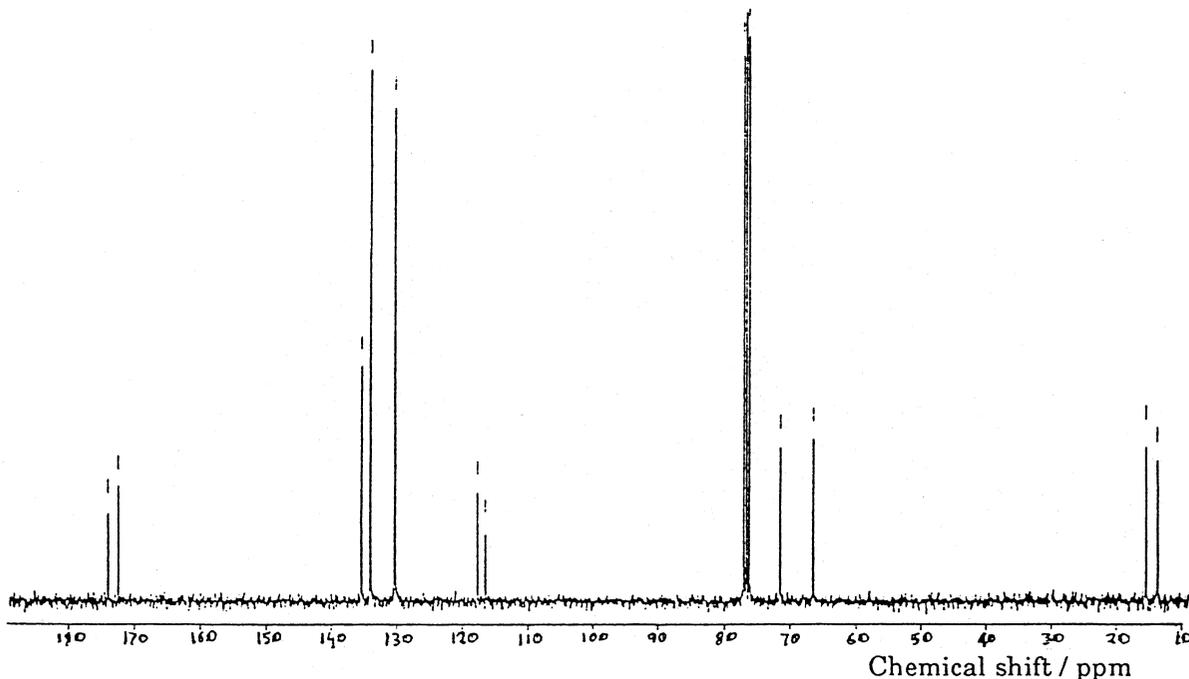

Fig. 4. ^{13}C NMR spectrum of [PPh$_4$][Δ-V(S,S-HIDPA)$_2$].H$_2$O.
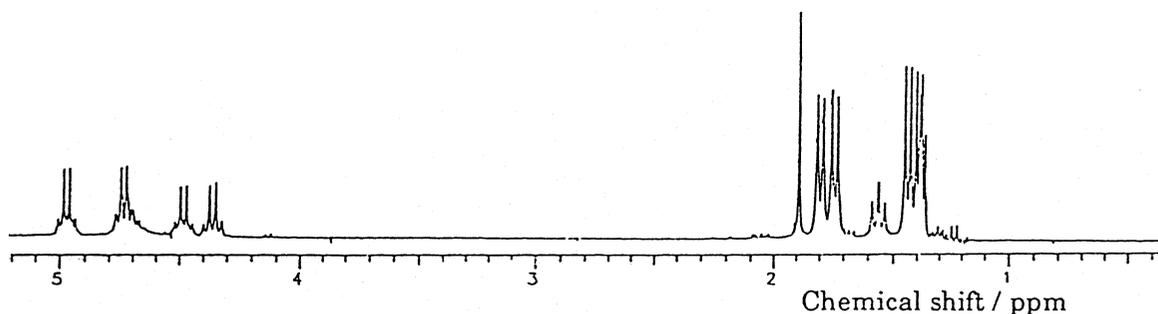
Fig. 5. ^{1}H-NMR spectrum of [PPh$_4$][Δ,Λ-V(S,S-HIDPA)$_2$].H$_2$O. Oxidized synthetic Amavadin, generated by an in situ complexation and oxidation.
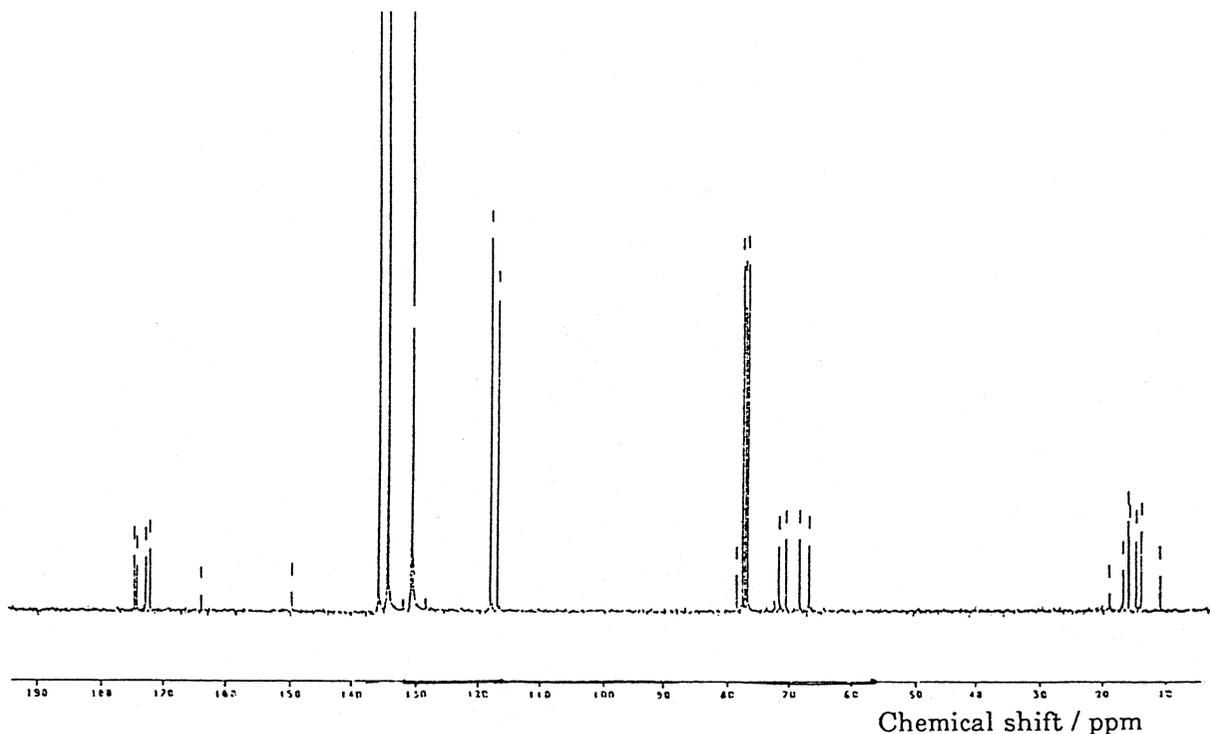
Fig. 6. ^{13}C-NMR spectrum of [PPh$_4$][Δ,Λ-V(S,S-HIDPA)$_2$].H$_2$O. Oxidized synthetic Amavadin, generated by an in situ complexation and oxidation.

at $\delta = 66.8$, $\delta = 68.3$, $\delta = 70.6$ and $\delta = 71.8$ ppm and four signals for the carboxylate carbon atoms at $\delta = 172.2$, $\delta = 173.0$, $\delta = 174.3$ and $\delta = 174.8$ ppm. These are consistent with the results of the ^{1}H-NMR studies. The extra weak signals in the methyl region (10–20 ppm) are as a result of small amounts of impurities of other isomeric forms present in the solution.

2.6. ^{1}H-COSY spectrum of [PPh$_4$][Δ,Λ-V(S,S-HIDPA)$_2$].H$_2$O (oxidized synthetic Amavadin, generated by an in situ complexation and oxidation

From the correlation spectrum [PPh$_4$][Δ,Λ-V(S,S-HIDPA)$_2$].H$_2$O the following major couplings are seen:

1. methine proton at $\delta = 4.38$ couples to methyl protons at $\delta = 1.76$ ppm;
2. methine proton at $\delta = 4.50$ couples to methyl protons at $\delta = 1.83$ ppm;
3. methine proton at $\delta = 4.76$ couples to methyl protons at $\delta = 1.43$ ppm;
4. methine proton at $\delta = 4.98$ couples to methyl protons at $\delta = 1.48$ ppm.

When the resonance positions in the ^{1}H, ^{13}C, and ^{1}H-^{1}H NMR spectra for $[\Delta,\Lambda$-V(*S,S*-HIDPA)$_2]^-$ are compared to those for $[\Delta$-V(*S,S*-HIDPA)$_2]^-$ it can be seen that the Δ-isomer does indeed account for half of the spectrum assigned as the Δ,Λ-isomeric mixture. When all four carbon atoms of the ligand have *S* chirality there is the possibility of two diastereoisomers which differ as a result of the relative orientation of the η^2-N, O groups (Fig. 2).

2.7. ^{1}H-NMR spectrum of the oxidised natural product Amavadin

In the ^{1}H spectrum (Fig. 7) of oxidised Amavadin four quartets for the methine protons and four doublets for the methyl protons are seen. There are no other quartets and doublets in these regions. Chemical shifts for the quartets are $\delta = 4.35$, 4.56, 4.75 and 5.00 ppm and for the doublets $\delta = 1.43$, 1.46, 1.78 and 1.85 ppm. $J_{\text{H-H}} = 7.0$ MHz. The stereochemistry at the carbon centres of Amavadin has previously been determined and the configuration at all the carbon atoms was found to be *S*. Therefore, the anion of the oxidised natural product can be formulated as $[\text{V}(S,S\text{-HIDPA})_2]^-$. Synthetically prepared $[\Delta$-V(*S,S*-HIDPA)$_2]^-$ displays only two sets of quartets in the methyl region. This synthetic anion has

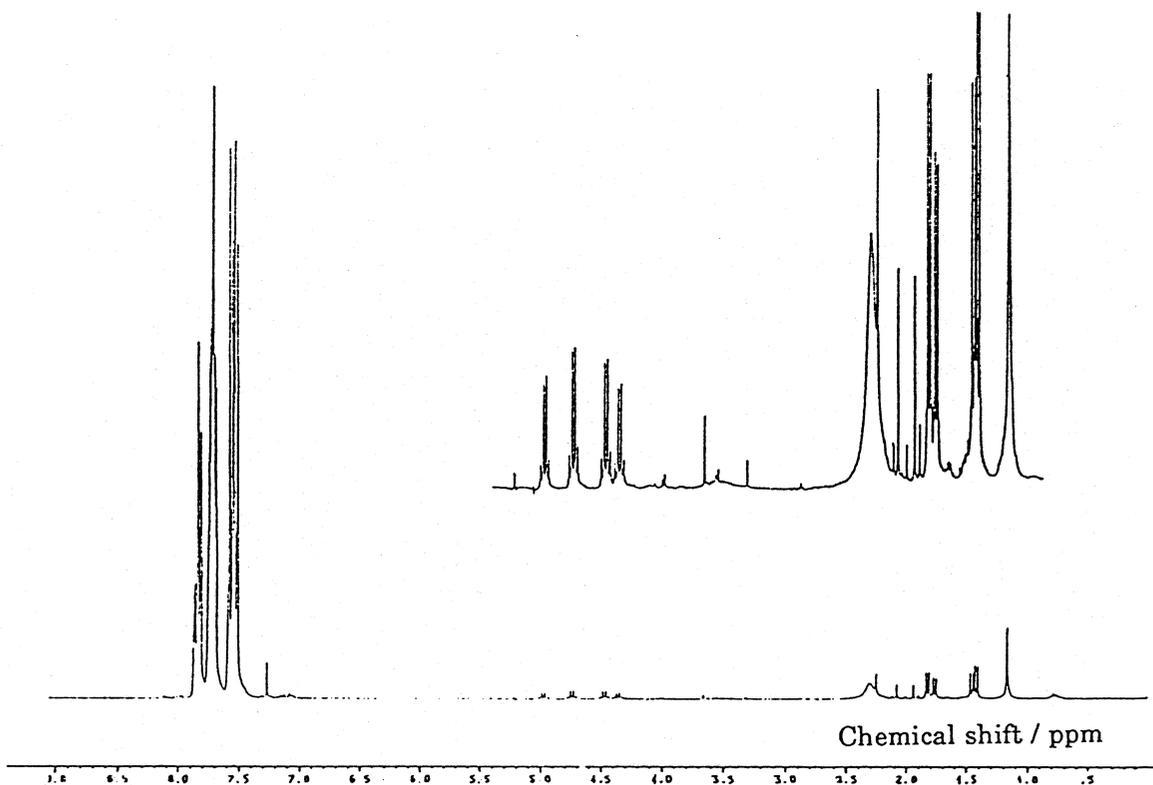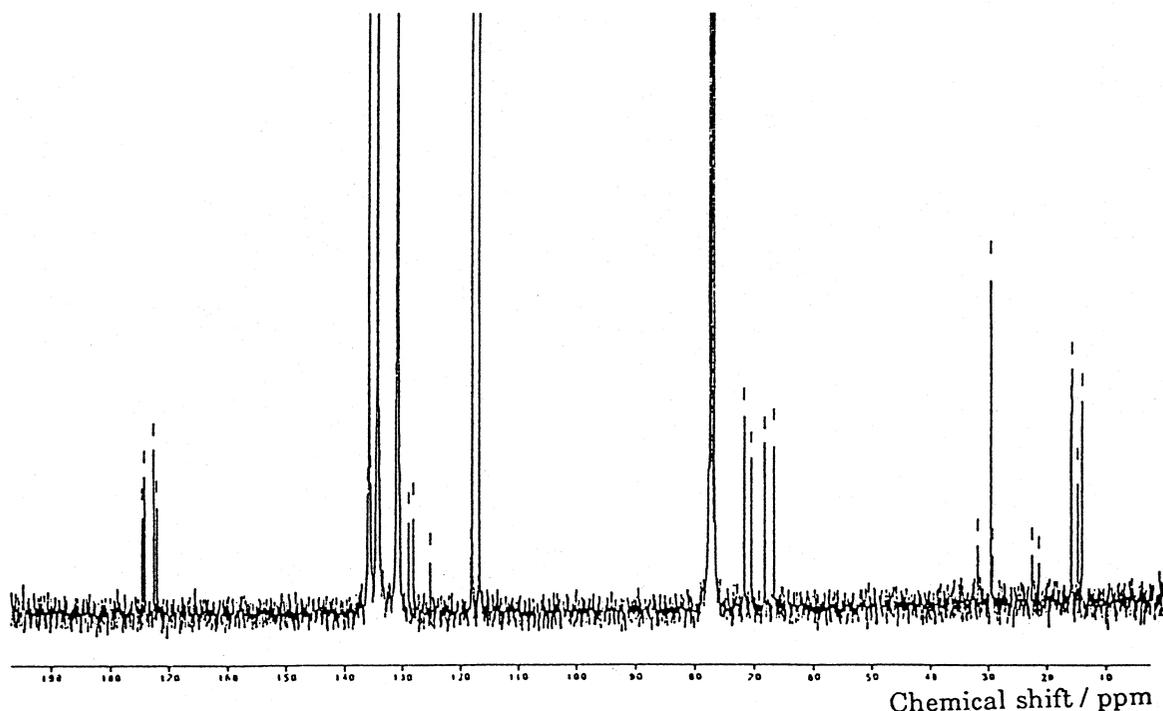Fig. 7. ^{1}H-NMR spectrum of the oxidised natural product Amavadin.

Fig. 8. ^{13}C-NMR spectrum of the oxidised natural product Amavadin.

been shown to possess Δ-stereochemistry at the vanadium center and the features in the methyl and methine regions of this spectrum correspond to half of the major features in the spectrum of synthetic Amavadin which involves an approximately equal mixture of the Δ- and Λ-isomers of $[V(S,S\text{-}HIDPA)_2]^-$. Thus, the chirality at the vanadium center of the natural product Amavadin may be explained by the presence of equal amounts of the Δ- and Λ-isomers, which are diastereomers.

2.8. ^{13}C-NMR spectrum of the oxidised natural product Amavadin

The ^{13}C-NMR spectrum (Fig. 8) of the oxidized natural product Amavadin shows four signals for the methyl carbon atoms at $\delta = 14.0, 14.8, 15.8$ and 16.0 ppm, four signals for the methine carbon atoms at $\delta = 66.8, 68.3, 70.6$ and 71.8 ppm, four signals for the carboxylate carbon atoms at $\delta = 172.1, 172.7, 174.2$ and 174.6 ppm. The other signals at $\delta = 116–136$ ppm are as a result of the aromatic carbon atoms from $[PPh_4]^+$, and those at $\delta = 76–77$ ppm are as a result of the methine carbon atoms from $CDCl_3$. The occurrence of four signals in each of the characteristic regions is again consistent with the presence of an approximately equal mixture of the Δ- and Λ-isomers of $[V(S,S\text{-}HIDPA)_2]^-$.

2.9. ^{1}H-COSY spectrum of the oxidised natural product Amavadin

The ^{1}H correlation spectrum (Fig. 9) of oxidised natural Amavadin involves the following couplings:
1. methyl protons at $\delta = 1.43$ couple to methine proton at $\delta = 4.75$ ppm;
2. methyl protons at $\delta = 1.85$ couple to methine proton at $\delta = 4.56$ ppm;
3. methyl protons at $\delta = 1.46$ couple to methine proton at $\delta = 4.35$ ppm;
4. methyl protons at $\delta = 1.78$ couple to methine proton at $\delta = 5.00$ ppm.

A combination of NMR and X-ray crystallographic data show that the couplings labelled (1) and (2) may be assigned to the isomer [Δ-V(*S,S*-HIDPA)$_2$]$^-$. Therefore, the couplings labelled (3) and (4) are assigned to the Λ-isomer of the anion.

All the NMR spectra obtained for synthetic oxidised Amavadin, and for the Δ-, Λ-isomeric mixture of [V(*S,S*-HIDPA)$_2$]$^-$, display the spectral features of natural, oxidised Amavadin. The additional weak features seen in the spectra of the synthetic material are taken to be indicative of the presence of other isomers of the anion which contain one (or more) carbon atoms with the *R*-configuration. The complete absence of such additional features in the spectra of the natural, oxidised Amavadin suggests that the biosynthesis

of Amavadin specifically produces a single stereochemistry at the carbon atoms. Whilst the NMR data alone cannot determine whether natural, oxidised Amavadin has entirely *S* or *R* stereochemistry at the carbon atoms, CD and NOE data are required. NOE spectra indicate that in both compounds the chirality of the four carbon atoms of the HIDPA ligand is the same [10].

Fig. 10 shows circular dichroism spectra of the natural product Amavadin, synthetic Amavadin H$_2$[Δ,Λ-V(*S,S*-HIDPA)$_2$].3H$_2$O, H$_2$[Δ-V(*S,S*-HIDPA)$_2$].3H$_2$O and H$_2$[Δ,Λ-V(*R,R*-HIDPA)$_2$].3H$_2$O. The CD spectra of natural Amavadin and synthetic Amavadin are almost the same and CD spectrum of H$_2$[Δ-V(*S,S*-HIDPA)$_2$].3H$_2$O differs from these. This is at-
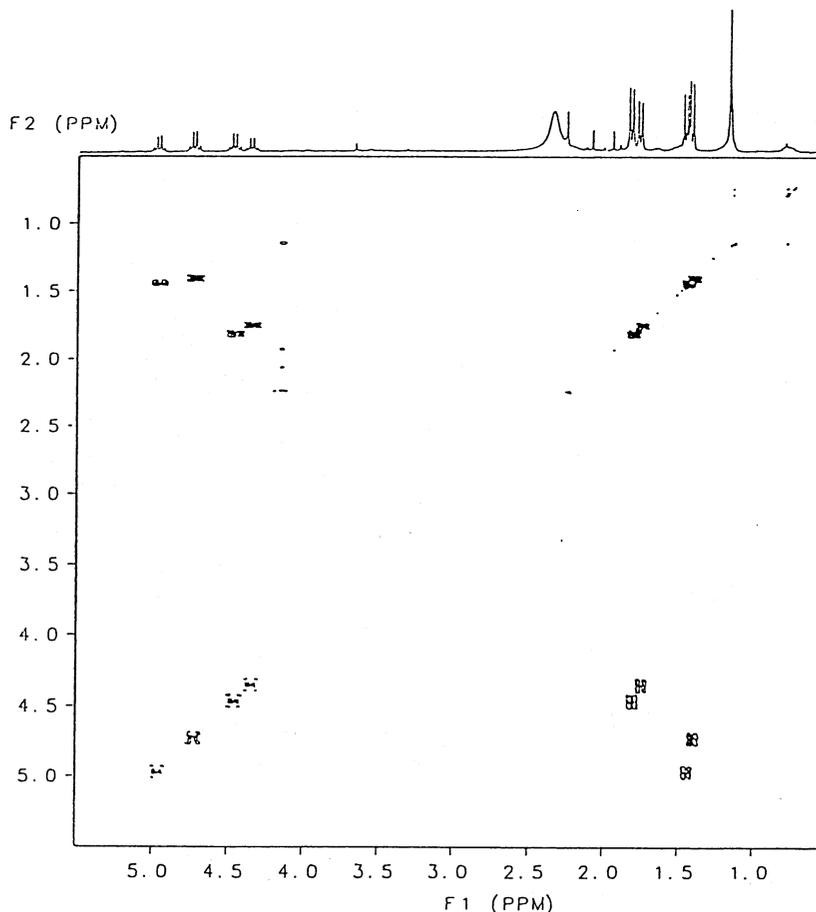

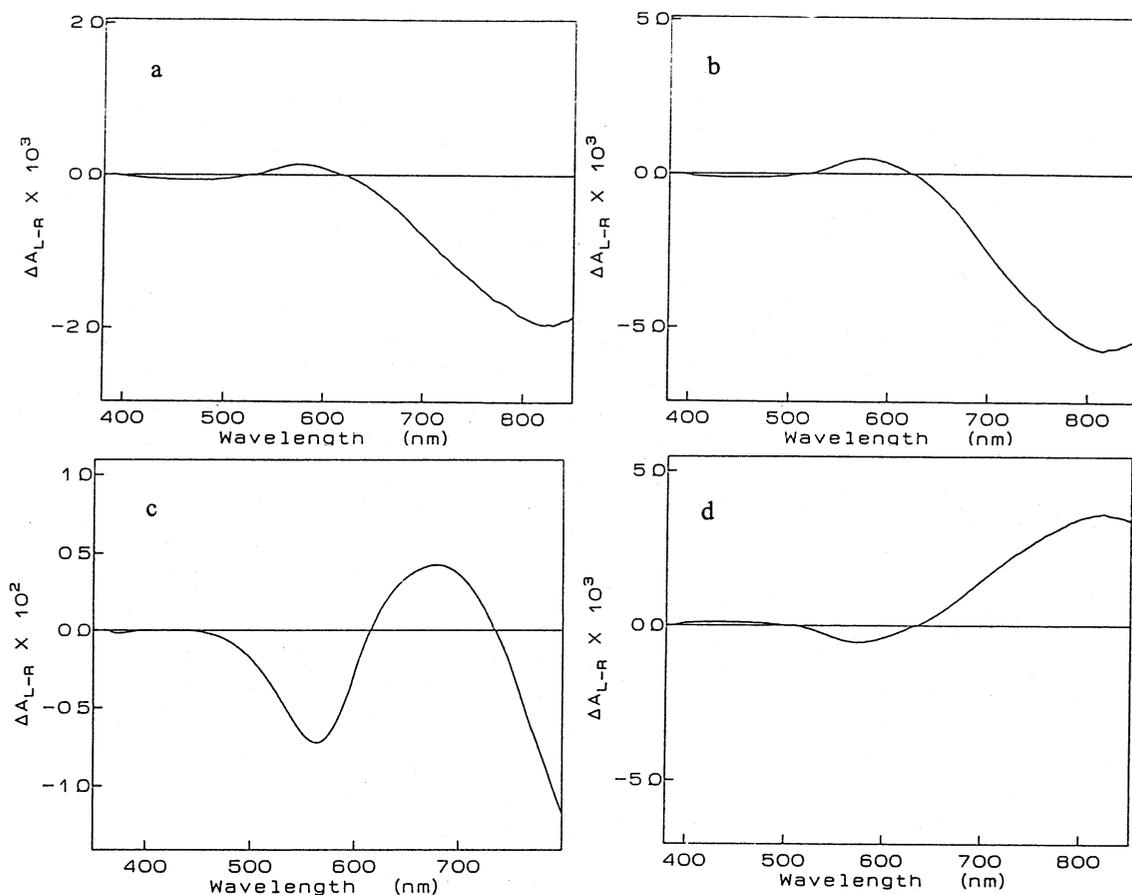Fig. 9. ^{1}H-COSY spectrum of the oxidised natural product Amavadin.

Fig. 10. CD spectra of (a) natural product Amavadin; (b) synthetic Amavadin $H_2[\Delta,\Lambda\text{-}V(S,S\text{-HIDPA})_2].3H_2O$; (c) $H_2[\Delta\text{-}V(S,S\text{-HIDPA})_2].3H_2O$; and (d) $H_2[\Delta,\Lambda\text{-}V(R,R\text{-HIDPA})_2].3H_2O$.

tributed to the effect of the chirality at the vanadium center. If all the asymmetric carbon atoms have the same configuration (in this case *S*), the isomers arise from the particular configuration adopted at the vanadium which could be either Δ- or Λ-, according to the relative positions of the two VON triangular units. The Δ- and Λ-isomers of $[\Delta\text{-}V(S,S\text{-HIDPA})_2]^{n-}$ ($n = 1$, 2) are diastereoisomers and Amavadin consists of ca. 1:1 mixture of them. This interpretation of the CD spectra is consistent with the ^{1}H-NMR spectra of $[\Delta\text{-}V(S,S\text{-HIDPA})_2]^-$ and the oxidized forms of the natural product Amavadin and synthetic Amavadin.

2.10. Isomerization at the vanadium center

Fig. 11 shows the CD spectra of $H_2[\Delta\text{-}V(S,S\text{-HIDPA})_2].3H_2O$ before and after isomerization. After standing for 3 h in aqueous solution at room temperature, the CD curve of the solution became like that of the isolated natural product Amavadin and synthetic Amavadin $H_2[\Delta,\Lambda\text{-}V(S,S\text{-HIDPA})_2].3H_2O$. In both natural Amavadin and $H_2[\Delta,\Lambda\text{-}V(S,S\text{-HIDPA})_2].3H_2O$ all carbon atoms of the HIDPA ligand have the *S* configuration. Therefore it appears that isomerization has occurred at the vanadium center. Thus the solution became a mixture of Δ- and Λ-iso-

mers. In the vanadium (V) state isomerization did not occur. Specific rotation measurement showed that isomerization of $H_2[\Delta-V(S,S-HIDPA)_2].3H_2O$ in aqueous solution at room temperature starts after 3 h and is complete in 2 days. ^{1}H-NMR spectra also demonstrated the isomerization of $H_2[\Delta-V(S,S-HIDPA)_2].3H_2O$ under these conditions. The initial spectrum of $[PPh_4][\Delta-V(S,S-HIDPA)_2]$ showed that there were two quartets in the methine region. The ^{1}H-NMR spectrum which was run after an aqueous solution of $[\Delta-V(S,S-HIDPA)_2]^{2-}$ was left for 4–5 days at room temperature and was then oxidized, showed four quartets in the methine region and four doublets in the methyl region (Fig. 12) as can be seen in the ^{1}H-NMR spectrum of isolated natural product Amavadin and synthetic Amavadin.

3. Discussion

The ^{1}H-NMR spectrum of the oxidized natural product Amavadin displays four sets of quartets in the methine region coupled to four sets of doublets in the methyl region, the relative integrals within each set are approximately equal and the ratio of the integrals from set to set is ca. 3(methyl):1(methine). Analogous sets of four resonances can be seen in the ^{13}C-NMR spectra correspond to four environments for the $CHCH_3$ moieties as well as for the carboxylate carbon

Fig. 11. CD Spectra of $H_2[\Delta-V(S,S-HIDPA)_2].3H_2O$ in water: (a) (– –) initial spectrum; and (b) (·–·–·–·) after isomerization.

atoms. The two sets of quartets and two doublets seen in the ^{1}H-NMR spectrum of $[\Delta-V(S,S-HIDPA)_2]^-$ and the sets of two resonances seen in the ^{13}C-NMR spectrum, can be overlaid with the half of the corresponding features of the oxidized natural product Amavadin. NOE spectra indicate that in both compounds the chirality of the four carbon atoms of the HIDPA ligands is the same. This is consistent with the CD spectra; for the vanadium (IV) and (V) forms of natural Amavadin and $[\Delta-V(S,S-HIDPA)_2]^{2/1-}$. The Cotton effects have the same sign, however the sign distribution is different. The combination of NMR and CD data confirm that the natural product Amavadin contains an almost equal mixture of the diastereoisomers of $[\Delta-V(S,S-HIDPA)_2]^-$ and of $[\Lambda-V(S,S-HIDPA)_2]^-$. The synthetic complex of $[\Delta,\Lambda-V(S,S-HIDPA)_2]^-$ displays very similar ^{1}H, ^{13}C, NOE and CD spectra to those of the natural product Amavadin and this also confirms the suggested stereochemistry of natural Amavadin. A further confirmation of this stereochemistry comes from the ^{1}H, ^{13}C, and COSY spectra of $[\Delta,\Lambda-V(R,R-HIDPA)_2]^-$ which are identical to those of $[\Delta,\Lambda-V(S,S-HIDPA)_2]^-$. However their CD spectra show opposite Cotton effects, as expected.

The prolonged isolation procedure necessary to produce a purified sample of Amavadin from the *Amanita muscaria* source means that there would be sufficient time for this isomerization reaction to occur. It is not possible to determine whether one or both of isomers are present in the mushroom. This will require solid state measurements, which are sensitive to the nature of the various diastereoisomers, to be made on lyophilized mushroom.

4. Experimental

4.1. Chemicals

All preparations were carried out in air. Solvents and chemicals were used as supplied, without further purification. Hydroxyl ammonium chloride, vanadyl chloride, ammonium ceric nitrate (AnalaR). Amberlite IR-120(H) from BDH, R,S-2-bromopropionic acid from Aldrich. [VO(a-

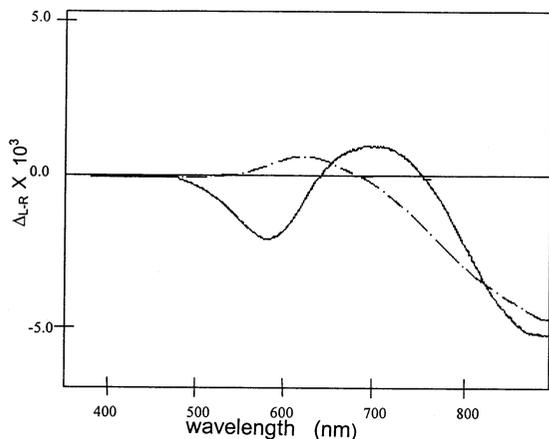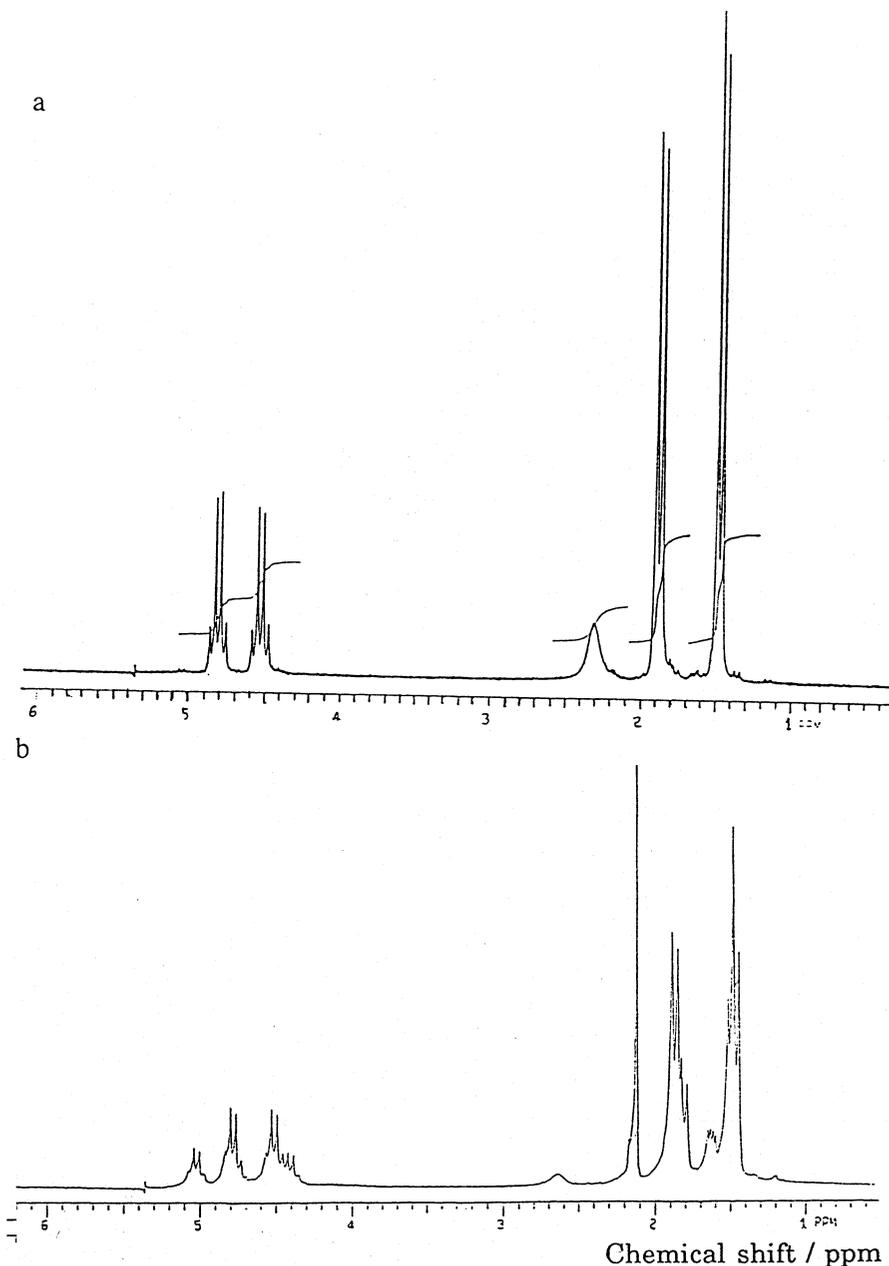Fig. 12. Two hundred mega-hertz ^{1}H-NMR spectrum of [PPh$_4$][Δ-V(S,S-HIDPA)$_2$].H$_2$O in CDCl$_3$: (a) initial spectrum; and (b) after isomerization.

cac)$_2$] was prepared as described Organic Synthesis, (5, 1957, 114). *R*-2-bromopropionic acid and *S*-2-bromopropionic acid were prepared by the method of Fu, Birnbaum and Greenstein [J. Am. Chem. Soc. 76 (1954) 6054].

4.2. Instruments

^{1}H-NMR spectra were recorded at 300 MHz and ^{13}C-NMR at 75 MHz on a Varian XL-300 MHz spectrometer. Shifts were quoted to an accu-

racy of ± 0.1 ppm. A total of 200 MHz NMR spectra were recorded on a Varian Gemini 200 Spectrometer.

Circular Dichroism spectra were recorded as solutions at room temperature on a Jobin-Yuon DC5 Spectrometer at 350–800 nm using 0.2 cm cells.

4.3. Preparation of R,S-N-hydroxyiminodipropionic acid (R,S-HIDPAH₃)

R,S-N-hydroxyiminodipropionic was prepared by the method of Felcman [11] with several modifications. Hydroxylamine hydrochloride (3.5 g, 0.05 mol), previously neutralised with 5 M NaOH (10 mol), was added to *R,S*-2-bromopropionic acid (8 cm³, 0.10 mol), also previously neutralised with 5 M NaOH (20 cm³). A total of 5 M NaOH (20 cm³) was then added keeping the temperature of the reaction mixture at about 10°C using an ice bath. The mixture was left to react for 3 days. After this period the clear solution was acidified to pH 1.0 with 5 M HCl and then concentrated under vacuum to a volume of ca. 10 cm³. The salts that had been precipitated (NaCl, NaBr) were removed by filtration. Concentration of the solution was then continued until the volume was ca. 3 cm³ and again the precipitated salts were removed by filtration. The remaining liquid was evaporated under vacuum to give white treacle which still contain a small amount of salt. Acetone (10 cm³) was then added, resulting in the formation of a sticky mass. Trituration with a glass rod aided dissolution of the acid, leaving the remaining as a precipitate, which was separated by centrifuging the mixture. The acetone was then removed by evaporation under vacuum to give a clear treacle of NaH₂L and H₃L, where L = HIDPA.

In order to remove any unwanted Na⁺ (the presence of which was found to a problem when trying to isolate pure vanadium(IV)-HIDPA complexes) the clear treacle was treated with an ion exchange resin. Amberlite IR-120(H) was used. To ensure that the ion exchange resin was in acid form before use it was washed with 1 M HCl and

then with distilled water. The ion exchange was then stirred with an aqueous solution of the hydroxyiminodipropionic acid mixture for an hour. The resin was then removed by filtration washed with water to ensure that no acid was still coating the resin. The filtrate and washing were combined and the solution was again concentrated under vacuum to give a clear colorless treacle.

4.4. Preparation of S,S-N-hydroxyiminodipropionic acid (S,S-HIDPAH₃)

Preparation of this compound is analogous to that of *R,S*-HIDPAH₃, here *R*-2-bromopropionic acid was used instead of *R* and *S* mixture. Using this method a single isomer of the *S,S* ligand could not be prepared, however this does not lead a problem in complexing it with vanadium (IV). Gas chromatography: (GC) analysis of the product using Chromotopac C-R6A indicated that the yield is about 63%. The retention time was 3.35 min. The measurements were carried out on a Carlo Erba Capillary Gas Chromatograph, with a 5 m × 0.22 mm column, H₂ carrier gas LV = 35 cm s⁻¹, temperature programme 50–280°C (10 min).

4.5. Preparation of R,R-N-hydroxyiminodipropionic acid (R,R-HIDPAH₃)

Preparation of this compound is analogous to that of *R,S*-HIDPAH₃, here *S*-2-bromopropionic acid was used instead of *R* and *S* mixture. GC analysis of the product using Chromotopac C-R6A indicated that the yield is about 75%. The retention time was 3.35 min.

4.6. Preparation of H₂[Δ,-V(S,S-HIDPA)₂].3H₂O

[VO(acac)₂] (0.469 g,1.8 mmol) was added slowly to an aqueous solution of *S,S*-HIDPAH₃ (3.6 mmol) with stirring. A brown color was obtained initially with the addition of each por-

tion of [VO(acac)$_2$], but soon this turned to the characteristic blue-purple solution of HIDPA-containing V(IV) complexes. To remove any unwanted cations (e.g. sodium), the blue solution was subjected to ion-exchange chromotography on a column, with DOWEX 50X8-400(H$^+$). The blue fraction of H$_2$[Δ(*S,S*-HIDPA)$_2$] was eluted with water and the water was removed by evaporation under reduced pressure The resulting oil was triturated with acetone to give a blue precipitate. Any unreacted blue-green [VO(acac)$_2$] was left unshifted at the top of the ion exchange column; it was subsequently removed by elution with 1 M HCl.

Analysis:

Found:	C: 32.6% H: 5.4% N: 6.2%	
	V: 11.3%	
Calculated for	C: 31.6% H: 5.3% N: 6.1%	
C$_{12}$H$_{24}$N$_2$O$_{13}$V:	V: 11.2%	

4.7. Preparation of H$_2$[*Δ,Λ-V(S,S*-HIDPA)$_2$].3H$_2$O

This compound was prepared by isomerization of H$_2$[Δ-V(*S,S*-HIDPA)$_2$].3H$_2$O. An aqueous solution of H$_2$[Δ-V(*S,S*-HIDPA)$_2$].3H$_2$O was left to stand overnight to isomerize and water was removed by evaporation under vacuum. The resulting oil was triturated with acetone to give a lilac precipitate. This was dried under vacuum.

Analysis:

Found:	C: 32.3% H: 5.2% N: 6.2%	
	V: 11.3%	
Calculated for	C: 31.6% H: 5.3% N: 6.1%	
C$_{12}$H$_{24}$N$_2$O$_{13}$V:	V: 11.2%	

4.8. Preparation of H$_2$[*Δ,Λ-V(R,R*-HIDPA)$_2$].3H$_2$O

This compound was prepared by isomerization of H$_2$[Λ-V(*R,R*-HIDPA)$_2$].3H$_2$O. An aqueous solution of H$_2$[Λ-V(*R,R*-HIDPA)$_2$].3H$_2$O was left to stand overnight to isomerize and water was removed by evaporation under vacuum. The resulting oil was triturated with acetone to give a lilac precipitate. This was dried under vacuum.

Analysis:

Found:	C: 32.1% H: 4.9% N: 6.6%	
	V: 11.0%	
Calculated for	C: 31.6% H: 5.3% N: 6.1%	
C$_{12}$H$_{24}$N$_2$O$_{13}$V:	V: 11.2%	

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