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AMAVADINE, AN OXOVANADIUM (IV) COMPLEX OF N-HYDROXY-IMINO-α,α'-DIPROPIONIC ACID

by

G. Bemski[1], J. Felcman[2], J.J.R. Frausto da Silva[3], I. Moura[3], J.J.G. Moura[3], M.C. Vaz[3] and L.F. Vilas-Boas[3]

[1]Centro Brasileiro de Pesquisas Físicas - CNPq/CBPF
Rua Dr. Xavier Sigaud, 150
22290 - Rio de Janeiro, RJ - Brasil

[2]Departamento de Química
Pontifícia Universidade Católica
22452 - Rio de Janeiro, RJ - Brasil

[3]Centro de Química Estrutural
Complexo I, Instituto Superior Técnico
1000 - Lisboa - Portugal

Although the presence of vanadium in vegetal ashes has been referred to for the first time more than one century ago, it wasn't until 1931 that Ter Meulen reported a definite determination of the contents of this element in a plant, namely in the toadstool *Amanita muscaria* [1].

Results obtained since then have shown that *Amanita muscaria* is indeed unusual in these respects, concentrating comparatively high amounts of vanadium, up to 120 ppm dry weight.

More recently it has been reported that high vanadium content is not restricted to *Amanita muscaria* and that other *Amanita* species also contain this metal, e.g. *Amanita regalis* (169 ppm) and, particularly, *Amanita velatipes* (397 ppm), an american variety of *Amanita pantherina* [2]. Still, the ability to concentrate vanadium seems to be a unique property of just a few probably primitive species of the genus *Amanita*.

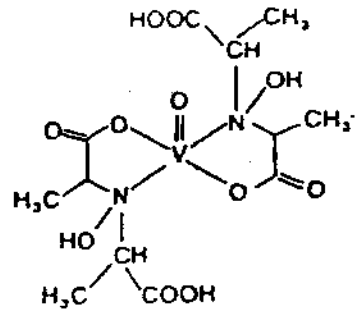
In 1972, Bayer and Kneifel isolated a vanadium containing compound from a german variety of *Amanita muscaria* (Black Forest and Schonbuch) which they named "Amavadine" [3]. About 40mg of the compound were obtained per kg of the fresh mushrooms by an elaborated procedure which included extraction with methanol of a thawed mixture of frozen mushrooms, followed by isolation through a series of chromatographic processes using celulose, sephadex and cation exchange resins.

Table 1 summarizes the properties of Amavadine, as reported by Bayer and Kneifel.

Following hydrolysis by 6N HCl, which gives mainly alanine, or by 1N NaOH, which affords sodium pyruvate and acetaldehyde as well as alanine, and after reduction by zinc and acetic acid which yields αα'-iminodipropionic acid, a first model of amavadine as a 2:1 com-

plex of this last ligand was assumed. Additional information came from EPR spectroscopic detection of a nitroxyl radical on oxidation of amavadine in alkaline media. Finally, a dimethylesther $C_8H_{15}NO_5$ was isolated after methanolysis of amavadine in methanol/H_2SO_4; this was identified as dimethyl N-hydroxy-imino-αα'--dipropionate and the corresponding acid was postulated as the natural ligand in the complex [4].

The structure proposed by these authors for amavadine, taking into account the various data obtained, is represented below as (I):



I

Structure proposed for Amavadine

To confirm this structure Kneifel and Bayer refer the preparation of the ligand N-hydroxy-imino-αα'-dipropionic acid from hydroxoammonium chloride and α-bromopropionic acid and state, without details, that a 2:1 complex with VO^{2+} is identical with natural amavadine in chromatographic behaviour, EPR, electronic spectra and IR absorption, differing only on chirality from the natural complex whose two optically active carbon atoms exist in the L--configuration. They also refer that it could not be excluded that in the fungal mycelium of the toadstool, the amavadine may be fixed as a metal cofactor to a macromolecular component by a loose

bond destroyed during the process of isolation [4].

No further papers have been published by these or other authors since this preliminary findings to which all reviews on biological vanadium are referred to, but, recently, Gillard and Lancashire compared the EPR spectra of segments of frozen mushrooms to vanadyl complexes of various amino-acids, as models for amavadine, and discussed the results in a short note [5]. According to these authors the 2:1 complexes of simple amino-acids are not good models, the type of spectrum observed for amavadine being closer to that found for the 2:1 complexes of L-cysteine or L-serine [5]. Since the original observations of Bayer and Kneifel had not been confirmed and N-hydroxy-imino-αα'-dipropionic acid seemed a rather unusual selection for a biological ligand, we have decided to synthesize this and other related compounds to see how the introduction of the N-hydroxyl and the two methyl groups in the more common iminodiacetic acid skeleton affected its metal complexation properties.

The study of the VO^{2+} complexes of these ligands would also allow a more direct comparison with the amavadine also present in specimens of *Amanita muscaria* collected in Portugal (Melides).

The synthesis of N-hydroxy-imino-αα'-dipropionic acid (HIDPA) is not easy due to the high solubility of the ligand in water and alcohol; this may be the reason for the absence of definite or further studies since the work of Bayer and Kneifel and failures to synthesize it have indeed been reported [6].

After various attempts we managed to obtain pure samples of $NaHL.LH_2$ and of NaHL (L being the completely ionised ligand), confirmed by elemental analysis, titration and NMR spectra. The re-

lated ligands imino-αα'-dipropionic acid (IDPA) and the closely similar N-hydroxyiminodiacetic acid (HIDA) were easier to synthesize [7].

The most striking effect observed was the pronounced lowering of the basicity of the imino nitrogen of HIDA and HIDPA compared with that of IDPA or of iminodiacetic acid (IDA); the practical consequence of this fact is that formation of ML_2 complexes of VO^{2+} with HIDPA is possible, whereas with IDPA and the normal IDA derivatives the introduction of the second molecule of the ligand occurs at a pH in which the hydroxide ion competes more favourably for VOL, yielding not VOL_2 but VOL.OH and the dimer $(VO)_2L_2(OH)_2$.

Table 2 and Figs. 1 and 2 illustrate the results obtained [7].

The hypothesis of Bayer and Kneifel is therefore supported by our results but the availability of the ligands allowed more direct confirmations.

In Fig. 3 and 4 the UV and visible electronic spectra of the vanadyl complexes of IDPA, HIDA and HIDPA are presented and the data are summarized in Table 3.

Comparing these results with those obtained by Bayer and Kneifel for amavadine, the vanadyl complex extracted from the toadstool, the close similarity between this complex and $VO^{2+}(HIDPA)_2$ is apparent. The absorption peaks are practically identical and the differences in molar absorptivities indicate just that the extracted amavadine is more dissociated at the ligand to metal ratio 2:1.

The EPR spectra of the 2:1 VO^{2+} complexes of the three novel ligands and that of frozen pieces of specimens of *Amanita muscaria* were also recorded and the g and A parameters obtained by su

perimposing these with spectra simulated with an adequate computer program [8] shown in Table 4, together with the corresponding data obtained by Gillard and Lancashire for 2:1 VO^{2+} complexes of some amino-acids and by Pilbrow et al. for 1:1 complexes of polyamino-carboxylic acids [9].

In Figs. 5 and 6 the EPR spectra obtained from pieces of *Amanita muscaria* and for the 2:1 complex of VO^{2+} with N-hydroxy--imino-αα'-dipropionic acid are presented together with the simulated spectra.

The data presented in Table 4 again show the striking similarity of amavadine and $VO(HIDPA)_2$ giving further and definite support to the structure proposed by Bayer and Kneifel for the product isolated from *Amanita muscaria*.

N-hydroxyiminodiacetic acid behaves in very much the same manner as N-hydroxyimino-αα'-dipropionic acid but its VO^{2+} complexes are not so closely similar to amavadine.

It can be shown that $g_{\|}$ and $A_{\|}$ are approximate functions of the last ionization constants of the ligands (different for 2:1 and 1:1 complexes) and that $A_{\perp}$ is in the range 45-46 for 2:1 complexes and 60-63 for 1:1 complexes.

The obvious question for which no answer has been found is why is a VOL_2 complex necessary for the toadstool and which function does it perform.

A speculative suggestion is offered [7], taking into account the characteristics that make VO^{2+} unique among the common metal ions.

Firstly, VO^{2+} behaves as a transition metal ion forming complexes as stable as those of nickel (II) [10] with the donor atoms

occupying the remaining octahedral sites around the V(IV) ion, i.e., complexes with a square pyramidal structure relative to VO^{2+}. However, unlike all common metal ions, these coordination sites are not all equivalent: the apical site *trans* to the oxo ligand on vanadium (IV) is far more labile towards substitution reactions than the *cis* equatorial sites [11]-typical rate constants are $k > 10^7 s^{-1}$ for the first case and $k \approx 10^{-1} s^{-1}$ for the second. Furthermore, oxovanadium (VI) complexes are oxidised by outer sphere oxidants provided that an aqua-ligand is present in an equatorial site, but the conjugate base, the hydroxo complex is oxidised much more rapidly to give *cis*-oxo species [11]. Other metal ions of sub-groups IV, V and VI of the Periodic Table also form oxocations, e.g. Ti, Cr and Mo, but solubility reasons exclude titanium complexes, redox properties and inertness of Cr(III) exclude chromium, and molybdenum (V) complexes with common ligands are frequently binuclear with $Mo_2O_4^{2+}$ cores.

Hence a VO^{2+} complex is particularly advantageous if a reaction center ensuring high substitution rates is necessary, provided that the equatorial coordination positions are blocked to avoid the formation of hydroxocomplexes or their dimers and to prevent oxidation; such a complex must expose the apical site *trans* to the oxo ligand to the reaction medium. The selection of a ligand such as N-hydroxy-imino-αα'-dipropionic acid satisfies the required conditions: a 2:1 square pyramidal complex of VO^{2+} can be formed, avoiding the formation of hydroxocomplexes and their dimers, which might prevent coordination to the apical sites besides being more easily oxidisable.

The choice of a tridentate ligand may also be of some signi

ficance; note that in the VO^{2+} complexes of tetradentate nitrilo triacetic acid or pyridinemethylimino-diacetic acid the apical site *trans* to oxygen is blocked by the nitrogen atom of the iminodiacetic moiety and substitution rates of reaction are much smaller [11]. In these conditions it is likely that "amavadine" is indeed "unique" for its function, but it is still not clear what kind of function it performs.

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FIGURE CAPTIONS

Fig. 1: Distribution of the species as function of pH for VO^{2+} complexes with IDPA, in the ligand to metal ratio 5:1. Total vanadium concentration=7.69×10^{-4} M. T=25°C; μ=0.10 M. KNO_3. A K_{ML_2} constant of the order of that found for the VO^{2+} complex of glycine was tentatively adopted ($K_{ML_2}=5.4 \times 10^4$).

Fig. 2: Distribution of the species as function of pH for VO^{2+} complexes with HIDPA, in the ligand to metal ratio 5:1. Total vanadium concentration=7.69×10^{-4} M; T=25°C; μ=0.10 M KNO_3

Fig. 3: UV electronic spectra of VO^{2+} complexes of HIDPA, HIDA and IDPA in the ligand to metal ratio 5:1. Total vanadium concentration=1.82×10^{-4} M.

Fig. 4: Visible spectra of VO^{2+} complexes of HIDPA, HIDA and IDPA in the ligand to metal ratio 5:1. Total vanadium concentration=4.54×10^{-3} M.

Fig. 5: EPR spectrum of "amavadine". Experimental conditions: temperature 20 K, microwave power 2 mW, modulation amplitude 0.5 mT, microwave frequency 9.451 GHz, scan time 500 s. The superimposed dotted spectrum was simulated using the spectrum parameters of Table 4.

Fig. 6: EPR spectrum of 2:1 VO^{2+} complex of HIDPA. Experimental conditions: temperature 77 K, microwave power 2 mW, modulation amplitude 0.5 mT, microwave frequency 9.261 GHz, scan time 500 s. The superimposed dotted spectrum was simulated using the spectrum parameters of Table 4.

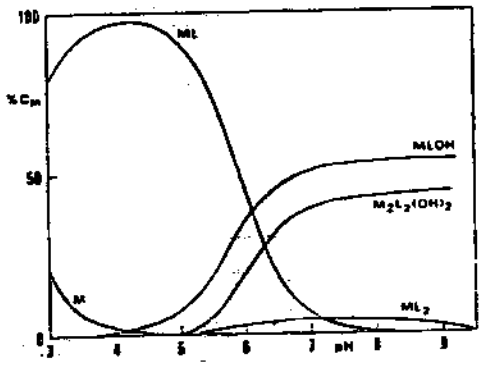
100
%C_M
50
0
ML
MLOH
M2L2(OH)2
ML2
M
3
4
5
6
7
pH
8
9

Fig. 1

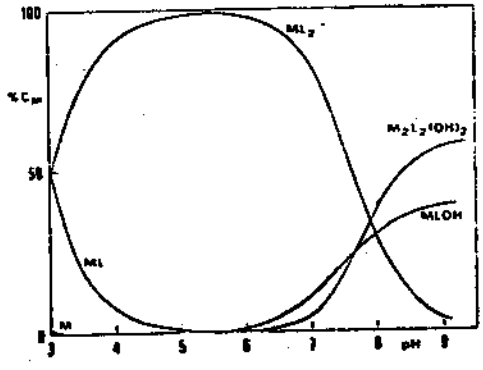
100
%C_M
50
0
ML2
M2L2(OH)2
MLOH
ML
M
3
4
5
6
7
8
pH
9

Fig. 2

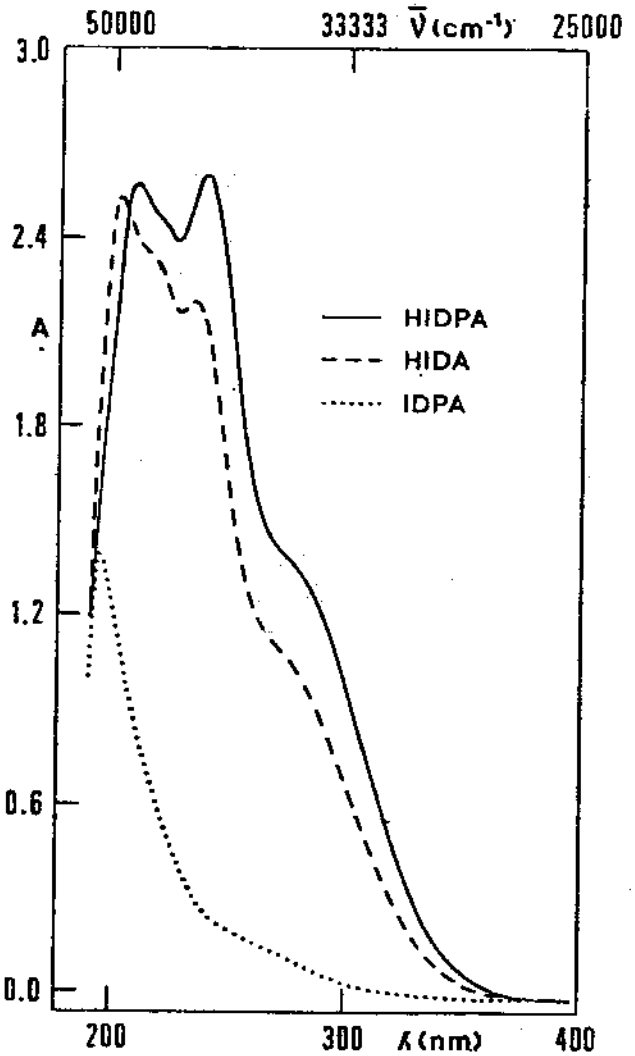
50000
33333 $\bar{\nu}$(cm^{-1})
25000
3.0
2.4
A
1.8
1.2
0.6
0.0
HIDPA
HIDA
IDPA
200
300
λ(nm)
400

Fig. 3

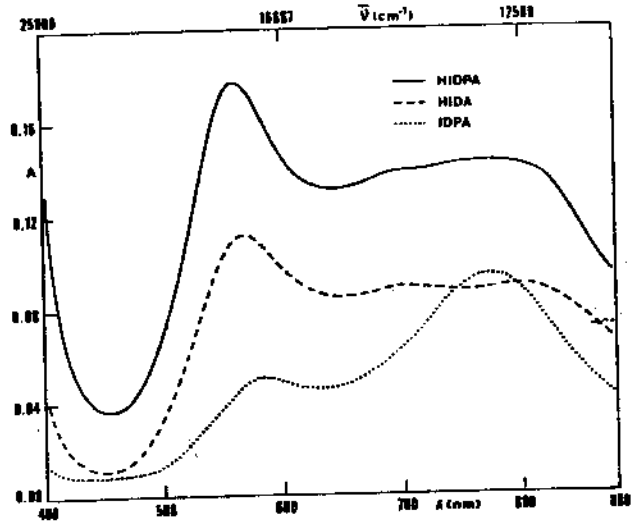

Fig. 4

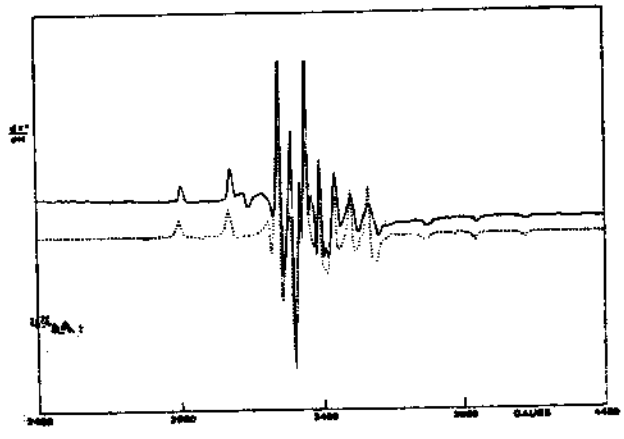

Fig. 5

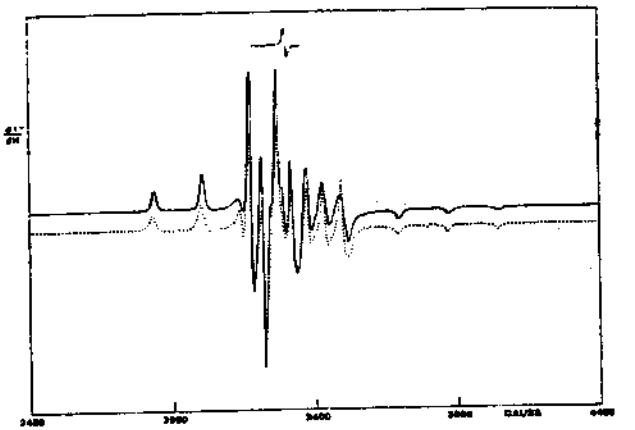

Fig. 6

TABLE 1

Some properties of Amavadine

Colour	pale blue
Melting point	no melting point; colour disappears at 170°C, turns to yellow at 185°C and to brown at 220°C.
UV, vis, spectra	bands at 755 nm (ε=19.3), 715 nm (ε=18.9), 565 nm (ε=23.5), 270 nm (sh., ε=6800), 235 nm.
(ε/mol^{-1}lcm^{-1})	(sh., ε=12300), 218 nm (sh., ε=12600).
IR	strong CO band at 1600-1650 cm^{-1} and a V=0 band at 985 cm^{-1}.
EPR	indicative of VO^{2}.
M.W. (osmometry)	415.
Composition (analysis)	$C_{12}H_{22}N_2VO_{12}$ (with two free acid groups).

TABLE 2

Proton ionization constants (pKa_1 and pKa_2), stability constants of VO^{2+} complexes ($\log K_{ML}$ and $\log K_{ML_2}$) and proton ionization constants of VO^{2+} aqua-aminopolycarboxylates. T=25.0 ± 0.1°C, μ=0.10 M (KNO_3).

Ligand (acid)	H^+		VO^{2+}			
	pKa_1	pKa_2	$\log K_{ML}$	$\log K_{ML_2}$	$-\log K_1$	$-\log \beta_{22}$
Iminodiacetic	2.61 ± 0.02	9.34 ± 0.01	9.00 ± 0.02		5.8 ± 0.1	9.1 ± 0.1
Imino-αα'-dipropionic	2.43 ± 0.01	9.38 ± 0.01	9.54 ± 0.01		6.1 ± 0.1	9.2 ± 0.1
N-hydroxyiminodiacetic	2.82 ± 0.01	5.48 ± 0.03	7.16 ± 0.03	6.10 ± 0.05	5.0 ± 0.1	6.4 ± 0.1
N-hydroxyimino-αα'-dipropionic	2.74 ± 0.02	5.77 ± 0.02	7.34 ± 0.02	5.51 ± 0.05	5.0 ± 0.1	6.6 ± 0.1

$K_1 = [VO(OH)L][H^+]/[VO(H_2O)L]$; $\beta_{22} = [(VO)_2(OH)_2L_2][H^+]^2/[VO(H_2O)L]^2$

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TABLE 3

Spectral parameters for VO^{2+} complexes of IDPA, HIDA and HIDPA (concentration of the complexes for UV=1.82×10^{-4}M; for vis. 4.54×10^{-3}M). T=25°C

VO(IDPA) (pH=4.6)		$VO(HIDA)_2$ (pH=6.3)		$VO(HIDPA)_2$ (pH=5.8)	
λ/nm	$\varepsilon/mol^{-1}lcm^{-1}$	λ/nm	$\varepsilon/mol^{-1}lcm^{-1}$	λ/nm	$\varepsilon/mol^{-1}lcm^{-1}$
260	990	214(sh)	12400	220(sh)	13900
580	10.6	232	11870	236	14800
776	20.5	270(sh)	5500	272(sh)	7750
		565	24.4	560	29.0
		706	19.6	700	23.1
		790	20.0	790	23.8

TABLE 4

EPR parameters for "amavadine" and for various oxovanadium (IV) complexes of amino acids [5] and aminopolycarboxylic acids (T=77 K)

	Conditions	$g_{\parallel}$	$g_{\perp}$	$10^4 A_{\parallel}/cm^{-1}$	$10^4 A_{\perp}/cm^{-1}$
A. *muscaria* (England)	direct in the mushroom	1.920	1.982	153	45
A. *muscaria* (Portugal)	"	1.919	1.982	157	46
$VO(L\text{-ala})_2$	pH 6.6	1.943	1.976	163	55
$VO(serine)_2$	pH 11.0	1.955	1.976	150	45
$VO(cysteine)_2$	pH 7.8	1.962	1.976	143	45
EDTA	pH 5.8	1.943	1.980	168	60
EGTA	pH 5.5	1.941	1.975	173	63
DTPA	pH 5.5	1.943	1.980	167	63
TTHA	pH 5.5	1.943	1.980	168	60
VO(IDPA)	pH 5.3	1.939	1.980	170	60
$VO(HIDA)_2$	pH 5.4	1.913	1.983	157	45
VO(HIDPA	pH 5.4	1.919	1.982	157	46

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