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Synthesis and Characterization of Valero and Isovalero Hydroxamic Acids and their Complexes with Zn(II) And Al(III)

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Abstract. Valerohydroxamic acid (VAH) and isovalerohydroxamic acid (IVAH) were synthesized and characterized by m.p. and pK_a determination, IR and ^1H NMR studies. The ligands were complexed with Zn^{2+} and Al^{3+} and the complexes were characterized by metal analysis, IR and conductance studies. Antimicrobial studies of all the compounds were carried out. The pK_a of the ligands are 9.50 ± 0.01 (VAH) and 9.51± 0.01 (IVAH) at 25 °C and ionic strength is 0.1 mol/dm^3, while their melting points are 77.8 °C and 76.8 °C, respectively. IR and ^1H NMR data are consistent with the proposed formula. The complexes are non-electrolytes in EtOH. Coordination mode (0,0) is consistent with the IR data of the complexes. The compounds exhibited no significant antimicrobial activity.

Keywords: hydroxamic acid, matrix metalloproteases, zinc, aluminum, valerohydroxamic acid

Introduction

Hydroxamic acids are compounds that have one or more CON(H)OH groups. These compounds play important biological role due to their metal complexing ability (Kurzak *et al.*, 1992). They are also important due to their pharmacological, toxicological and pathological properties (Nielands, 1968). They are intimately associated with iron transport phenomena in microbes (Kehl, 1982) and are also able to chelate other metal ions. In fact, the only drug currently available for the treatment of aluminum overload is desferrioxamine B (a tris-hydroxamate) (Kaim and Schwederski, 1994).

The matrix metalloproteases (MMPs) are a class of zinc containing hydrolytic enzymes necessary for tissue remodeling and healing cascade (Levy *et al.*, 1998). Malfunction in MMP activity can contribute to many diseased conditions (Holleran *et al.*, 1997; Weckroth *et al.*, 1996). Wounds possessing too much MMP activity may become ulcerated rather than heal properly (Chiu *et al.*, 2005). Consequently, the inhibition of MMPs has received great attention. Prominent among the successful MMP inhibitors is the hydroxamate functional group as the zinc binding group (ZBG). The mode of coordination of hydroxamates to metal ions is also important. While most reports reveal coordination *via* the hydroxamate O atoms (Nwabueze, 1996; Brown and Roche, 1983; Brown *et al.*, 1979), coordination *via* N atom is also known (Brown et al, 1982). Besides many reports available on metal complexes of some hydroxamic acids, a few reports are on those of VAH and IVAH (Porcheddu and Giacomelli, 2006: Katritzky *et al.*, 2003).

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This work reports the synthesis of VAH and IVAH, their characterization and complexation with Zn^{2+}. An understanding of their mode of coordination will give more insight into the interaction of hydroxamates with Zn^{2+} and Al^{3+} in biological systems.

Materials and Methods

Preparation of hydroxamic acids. Hydroxamic acids were prepared by the adaptation of the method described in the literature (Nwabueze, 1996; Brown and Roche, 1983).

***Valerohydroxamic acid (VAH)*:** Sodium metal 11.5 g (0.5 mol) was dissolved in 250 ml of distilled MeOH and added to a solution of hydroxylamine hydrochloride 34.5 g (0.5 mol) in 250 ml of MeOH. The mixture was cooled to room temperature, 75 ml (65.6 g; 0.5 mol) of ethylvalerate was added and stirred for one h. A solution of 11.5 g (0.5 mol) of sodium metal in 250 ml MeOH was further added and stirred for 20 min. The mixture was filtered to remove the precipitated NaCl and the filtrate was acidified with conc. HCl. The precipitated NaCl was removed by filtration and the filtrate was left in a deep freezer for two weeks. The resulting crystals were filtered and recrystalized in ethyl acetate (yield 72%).

Isovalerohydroxamic acid (IVAH) was prepared similarly using 75 ml (65.6 g, 0.5 mol) of ethyl isovalerate (yield 68%).

Preparation of complexes. *Zn (II) Complexes:* A solution of $ZnCl_2$ 1.36 g (0.01 mol) in 25 ml of ethanol was added to a solution of valerohydroxamic acid 2.34 g (0.02 mol) in 10 ml

of ethanol. The mixture was well stirred and the pH was raised to 6 by the addition of sodium ethoxide. The mixture was filtered, the filtrate was concentrated and left in a deep freezer for 48 hs. Cream coloured crystals were obtained which were filtered and dried over $CaCl_2$ in a vacuum desiccator (yield 53%). The same procedure was used for the preparation of Zn-IVAH complex.

Al (III) complexes: A solution of $AlCl_3$. $6H_2O$ 2.41 g (0.01 mol) in 20 ml of ethanol was added to a solution of valerohydroxamic acid 3.51 g (0.03 mol) in 10 ml of ethanol. The mixture was stirred and the pH was adjusted to about 6 using sodium ethoxide. The mixture was filtered and the filtrate was left in a deep freezer for 48 hs to crystallize. The crystals were filtered and dried over $CaCl_2$ in vacuum desiccator (yield 47%). Al-IVAH complex was similarly prepared. The metal content of each complex was determined complexometrically according to the standard methods (Vogel, 1963).

Spectrophotometric determination of the pK_a of the ligands. The pK_a values for the ligands were determined spectrophotometrically by the method of Albert and Serjeant (1971) using phosphoric acid and tris (hydroxymethyl) methyl amine buffer. In each case, the ligand stock solution was $5.0x10^{-4}$ M, which was diluted five fold in the buffer solution. The optical densities of the solutions were measured at the analytical wave length of 210 nm. Seven buffer solutions were used for each ligand.

Instrumental measurements. The IR spectra of the ligands and complexes were run in CCl_4 using a Ferrin Elmer 1310R IR spectrophotometer. ^1H NMR spectra of the ligands in $CDCl_3$ were recorded using a Varian Mercury 200BB NMR Spectrometer, while conductivity measurements in EtOH were taken using LF340/SET conductivity meter.

Antimicrobial studies. Antimicrobial screening of the ligands and complexes in aqueous EtOH was carried out using nutrient agar. Petri dishes containing already jelled nutrient agar were inoculated with microorganisms *viz - Staphylococcus aureous* (Sa), *Bacillus subtilis* (Bs), *Eschericia coli* (Ec), *Pseudomonas aeruginosa* (Ps) and *Candida albicans* (Ca). Petri dishes were then impregnated with the discs containing solutions of the ligands and the complexes and incubated for 24 hs at 37 °C and tests were carried out in duplicate.

Results and Discussion

The ligands were prepared as reported in the literature (Nwabueze, 1996). The ligands reacted with Zn^{2+} and Al^{3+} according to the equation:

$$M^{n+} + {}_nRCON(H)OH \rightarrow [MRCON(H)O]_n \times H_2O$$

where n = 2 or 3 and × = 1 or 2

Crystallization of the ligands was achieved after concentration under laboratory conditions for 2 weeks and afterwards refrigeration in deep freezer for further two weeks. The ligands were recrystalized in 10 cc of dry ethanol after filtration. Their relatively low melting points (Table 1) are an indication of weak intermolecular force between the molecules. Literature search confirms that these ligands are being reported for the first time. Both ligands show similar basicity indicated by their pK_a of 9.50 ± 0.01 and 9.51 ± 0.01 for VAH and IVAH, respectively, at 25 °C and ionic strength of 0.10 mol/dm^3. These values imply that they can act as good Lewis bases.

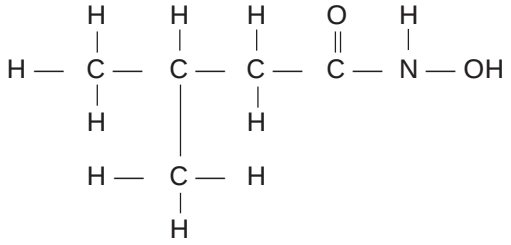
^1H NMR data for the ligands are shown in Table 2. The signals due to the N-H protons are located as a singlet around $\partial_{7.5.}$ The signal around $\partial_{2.4}$ is due to the O-H protons; broadening of the signal suggests some form of hydrogen bonding between the molecules.

Table 1. Analytical data and some physical constants

Compound	Formula	Formula wt	Yield (%)	M.P. (°C)	M (%)	Ω/cm^2/mol	H	C	N	Colour	Solubility
VAH	$C_5H_{11}NO_2$	117	58	77.78			0.854 (0.855)	10.270 (10.26)	11.99 (11.96)	White	MeOH,$CHCl_3$,H_2O
IVAH	$C_5H_{11}NO_2$	117	62	76.77			0.856 (0.855)	10.28 (10.26)	11.98 (11.96)	White Crystal	MeOH,$CHCl_3$,H_2O
A1$(IVA)_3$2H_2O	$C_{15}H_{34}N_3O_8A1$	411	32.50	194	6.61 (6.57)	0.051	0.245 (0.243)	2.93 (2.92)	3.42 (3.41)	White	MeOH,$CHCl_3$,H_2O
A1$(IVA)_3$2H_2O	$C_{15}H_{34}N_3O_8A1$	411	47.20	197	6.56 (6.57)	0.020	0.246 (0.243)	2.94 (2.92)	3.44 (3.41)	White	MeOH,$CHCl_3$,H_2O
Zn$(AV)_2H_2O$	$C_{10}H_{24}N_2O_6Zn$	315	53.20	145	19.48 (19.52)	0.0125	0.322 (0.317)	3.84 (3.81)	4.46 (4.44)	Creamy	MeOH,$CHCl_3$,H_2O
Zn$(IAV)_2H_2O$	$C_{10}H_{24}N_2O_6Zn$	315	61.88	165	19.43 (19.52)	0.0150	0.320 (0.310)	3.86 (3.81)	4.45 (4.44)	Creamy	MeOH,$CHCl_3$,H_2O

In the spectrum of VAH, the signal due to the methyl protons appear as a triplet centered at $\partial_{0.8}$. A sextet centered at $\partial_{1.3}$ is due to the -CH_2 protons adjacent to the methyl group while a quintet at $\partial_{1.5}$ is due to the methylene group next to it. A triplet at $\partial_{2.5}$ is due to the -CH_2 group adjacent to the carbonyl group. The appearance of this signal down field is due to anisotropy of the neighbouring carbonyl group. The mass spectrum of VAH and IVAH recorded at 70 eV electron energy shows a well defined peak at m/z=116 (M^+) with relative intensities of 98% and 95%, respectively. This data is consistent with the formula of VAH i.e. $CH_3CH_2CH_2CH_2CON(H)OH$.

In the spectrum of IVAH, there are two chemically equivalent -CH_3 groups whose signals appear as a doublet centered at $\partial_{0.9}$. A doublet at $\partial_{2.1}$ is due to the methylene protons, while a nanonet centered at $\partial_{2.3}$ is due to the C-H proton. The spectrum is consistent with the structure (Katritzky, 2003).



Infrared. The diagnostic IR bands for the ligands and complexes are shown in Table 3. All the bands and nature of the spectra are consistent with the common features reported for hydroxamic acids (Nwabueze, 1996; Brown and Roche, 1983; Brown *et al.,* 1982). The υ(C=O) band, which is located in the spectra of the ligands at ca 1650 cm^{-1}, is lower in the spectra of the complexes by between 24-41 cm^{-1}. This observation together with an increase in the υ(C-N) band from about 1365 cm^{-1} in the ligands to above 1380 cm^{-1} in the complexes is consistent with the coordination via the carbonyl oxygen (Mandlik and Aswar, 2003; Nwabueze, 1997).

Bands located around 3200 cm^{-1} in the ligands are assigned to υ(N-H) vibrations. Bands located above 3400 cm^{-1} in the complexes are assigned to υ(OH) of water and υ(N-H) of the ligands. It is, therefore, difficult to assign these bands unambiguously and establish the involvement of amino N in bonding. Non-ligand bands below 600 cm^{-1} have been assigned to M-N and M-O vibrations (Mandlia and Aswar, 2003).

Table 4. Microbial screening of the compounds

Compound	Sa	Ec	Ps	Bs	Ca
VAH	-	+	+	+	-
$Al(VA)_3.2H_2O$	+	+	+	+	+
$Zn(VA)_2.H_2O$	+	+	+	+	+
IVAH	-	++	-	++	-
$Al(VA)_3.2H_2O$	+	++	+	++	+
$Zn(IVA)_2.H_2O$	+	++	+	++	+

+ = mild activity; ++ = partial activity; - = no activity; Sa = *Staphylococcus aureus;* Ec = *Echericia coli;* Ps = *Pseudomonas aeruginosa;* Bs = *Bacillus subtilis;* Ca = *Candida albicans.*

Table 2. ^1H NMR data for the ligands in $CDCl_3$ (δ).

Compound	NH	CH	CH_2^a	CH_2^b	CH_2^c	CH_3
VAH	7.2 (si.1H)		2.5 (t.J6.5Hz2H)	1.5 (q.J6Hz2H)	1.3 (s.J4Hz2H)	0.8 (t.J6Hz3H)
IVAH	7.6 (s.1H)	2.3 (n.J6Hz, 1H)	2.1 (d.J6.8Hz, 2H)			

a = adjacent to carbonyl and b and c, to alkyl chain; s = singlet; d = doublet; t = triplet; q = quintet; si = sextet; n = nanonet

Table 3. IR diagnostic bands (cm^{-1}) for the ligands and complexes

Compound	υ(OH,NH)	υ (C=O)	Δυ (C-O)	υ (C-N)	Δυ (C-N)	υ (M-O)
VAH	3300, 3187	1650		1365		
$Al(VA)_3.2H_2O$	3420	1626	-24	1382	+17	440
$Zn(VA)_2.H_2O$	3430	1613	-37	1390	+25	462
IVAH	3198	1650		1370		
$Al(VA)_3.2H_2O$	3420	1609	-41	1385	+15	409
$Zn(IVA)_2.H_2O$	3420	1612	-28	1385	+15	440

Biological activity. The degree of antibacterial activity exhibited by the ligands and the complexes is shown in Table 4. Mild activities of the ligands were enhanced on complexation as revealed by the partial activity for lsovalerohydroxamates against *Bacillus subtilis* and *Eschericia coli*, organisms. While in the case of the valerohydroxamates, there is no noticeable difference from that exhibited by the ligand against the tested organisms.

Conclusion

The IR spectra of the complexes are consistent with the observed trend in the reported cases of hydroxamates. Non-activity of the compounds is an indication of their lack of usefulness as antimicrobial agents.

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