Synthesis, Characterization and Antibacterial Activity of Mononuclear Complexes of Chiral Schiff Base 2,6-Bis (1*S*,2*R*-2- Hydroxy-1-Methyl-2-Phenyl-Ethylimino)4-Heptanone

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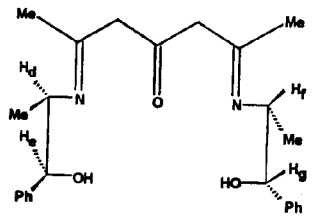
(Received 20th September, 2000, revised 22nd Feburary, 2001)

Summary: Mononuclear complexes of a new chiral ligand 2,6 bis(1*S*,2*R*-2 hydroxy-1-methyl-2-phenylethylimino) 4-heptanone with VO(IV), Co(II), Ni(II) and UO_2(VI) are reported. The ligand and its complexes are characterized by elemental analysis and infra-red spectra. The ligand has very weak inhibitory effects upon a number of gram positive and gram negative bacteria. However, some of these complexes show greater inhibitory effects upon various bacteria.

Introduction

Compartmental ligands synthesized by the condensation of diamines with triketones provide dissimilar bonding sites for the accomodation of metal ions. Encapsulation of metal ions in different environments may lead to study the behavior of metal ions. Further problems like magnetic exchange, structural patterns and electrochemical nature of the metal ligand bonding in such systems have been investigated [1,2]. These compounds act as models for metalloenzymes in the biological systems. Some of the enzymes require the presence of metal ions e.g. carbonic anhydrase is a zinc-based enzyme which catalyse reversible hydration of carbon dioxide [3]. Chirality plays an important role in biochemical reactions. A rapid process of aging due to decrease in the concentration of certain optically active compounds in the body has been observed. A number of chiral compounds like (+)-tartaric acid, (+)-ephedrine, (-)-nicotine, (+)-cinchonine etc. are synthesized by plants and extracted from these sources. Enzymes are generally very good at chiral recognition, and much of the work in this area has been an attempt to mimic the action of enzymes [4]. Metal ions are involved in a number of enzymatic reactions. The essential process of replication, transcription and translation involving nucleic acids are also dependant upon metal ions. Recently antibacterial studies on mononuclear complexes of a new chiral Schiff base have been reported from our laboratory [5]. In this paper, synthesis of a number of mononuclear complexes of a new Schiff base (I) which has two similar compartments for metal bonding is reported. These complexes have been screened for their antibacterial effects against a number of gram positive and gram negative bacteria. The ligand and its complexes have weak inhibitory effects (MIC 160-1280 μg/ml) against the tested bacteria.



(I)- (1S, 2R-Nore)$_2$DAA

Results and Discussion

A new chiral ligand, 2,6-bis(1*S*,2*R*-2-hydorxy-1-methyl-2-phenylethylimino)4- heptanone ((1*S*,2*R*-Nore)$_2$DAA) was synthesized by condensing two moles of (+)-norephedrine (1*S*,2*R*-2-hydroxy-1-methyl-2-phenylethylamine) with one mole of 2,4,6-heptanetrione. The ligand was characterized by its elemental and thermal analysis, infra-red and ^{1}H NMR spectral studies. Mononuclear complexes of this ligand with metal ions VO(IV), Co(II), Ni(II), Cu(II) and UO_2(VI) were synthesised by reacting metal acetates with the ligand in 1:1 molar ratio from an acetone:water mixed solvent.

The elemental analysis of the ligand suggests that it is associated with 1.5 H_2O molecules which is

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confirmed by thermal analysis. The IR spectrum of the ligand consists of a broad band at 1308 cm^{-1} which may be assigned to -OH bending and C-O stretching vibrations. The other prominent bands are present at 3392 cm^{-1} due to NH stretching vibration, carbonyl stretching at 1700 cm^{-1} , C=N at 1564 cm^{-1} and C—N—C at 1132 cm^{-1}. The ^1H NMR spectrum of the ligand consists of two multiplets of 12 protons between 1.32 - 2.2 ppm arising from four methyl groups present in different environments. The methyl protons labelled as H_d and H_f resonate as doublets in the region 3.3 - 3.6 ppm. A doublet of two protons is observed at 3.85 ppm due to H_e and H_f. Different methylene protons resonate in the region 3.92, 3.98 and 4.01 ppm. Vinylic protons of the different tautomeric forms resonate between 4.8 - 5.8 ppm. The signals due to aromatic protons of two phenyl rings are observed at 7.3 - 8.2 ppm.

Thermal analysis of the ligand shows a 6.0% loss of weight between 100-190 °C which indicates the loss of 1.5 water molecules (calc. 6.2% loss). The degradation is completed at 360 °C and overall 70% weight loss is observed. In this temperature range a major portion of the molecular structure breaks down. In the 180 - 280 °C range, degradation is slow and probably loss in weight is accompanied by a cyclization reaction. The residue seems to be a polymeric compound of formula $C_7H_{10}N_2O$ which accounts for 31.68%. DTA shows two endothermic peaks at 380 °C and 450 °C which may be due to loss in weight with rearrangement and cyclization respectively.

The mononuclear complexes of this ligand are synthesized by reacting equimolar quantities of the ligand with VO(IV), Co(II), Ni(II) and UO_2(VI) acetates in acetone-water solution and refluxing. The reactions were carried out under basic conditions. The Co(II), Ni(II) and Cu(II) complexes are brown in colour while VO(IV) complex is green and UO_2(VI) complex is orange red. These complexes decompose between 92-130 °C (Table-1). These complexes are insoluble in water, however, easily dissolve in Py, DMSO, DMF, CH_3OH, CH_3CN, $CHCl_3$, CH_3NO_2, dioxane and $C_6H_5NO_2$. The elemental analysis of these complexes fit in the formulae [VO(1*S*,2*R*-Nore)$_2$DAA(OAc)], [Co(1*S*,2*R*-Nore)$_2$DAA(OAc)] $0.5H_2O$, [Ni(1*S*,2*R*-Nore)$_2$DAA(OAc)]$7H_2O$, [Cu(1*S*,2*R*-Nore)$_2$DAA(OAc)]$1.5H_2O$ and [UO_2 (1*S*,2*R* Nore)$_2$DAA(OAc)]$5H_2O$. A comparison of IR spectra of these complexes with the ligand (Table-1) reveals that vibrational bands due to secondary alcoholic group (OH) at 1048 cm^{-1}, C = O at 1708 cm^{-1}, C = N at 1492 cm^{-1} and C—N—C at 1132 cm^{-1} have been shifted upon coordination. The IR spectrum of the ligand exhibits a strong band due to carbonyl stretching at 1708 cm^{-1} which has been split up into 2-3 weak bands and shifted to 1624 cm^{-1} in VO(IV), Co(II) and UO_2(VI) while to 1665 cm^{-1} and 1674 cm^{-1} in Cu(II) and Ni(II) complexes. A medium intensity band at 1048 cm^{-1} attributed to alcoholic (OH) bending vibration has been slightly shifted upon complexation and observed at 1023-1046 cm^{-1} in IR of these complexes indicating the presence of uncoordinated OH moiety. Another medium intensity multiplet band due to C=N stretching vibration at 1492 cm^{-1} has been shifted to 1448-1472 cm^{-1} in VO(IV), Co(II), Cu(II) and UO_2(VI) complexes. A sharp band at 1132 cm^{-1} due to C—N—C stretching vibration in the ligand is observed between 1104--1128 cm^{-1} in these complexes. The acetate vibrations are usually observed between 1578-1414 cm^{-1} and 646-460 cm^{-1}. The first band is observed between 1408-1348 cm^{-1} in these complexes, while a second band between 652-676 cm^{-1} is observed in Ni(II) Cu(II) and UO_2(VI) complexes. Therefore it is proposed that in these complexes, the ligand (1*S*,2*R* Nore)$_2$DAA) acts as tridentate and binds metal ions through imine nitrogen, carbonyl oxygen and alcoholic oxygen after deprotonation while an acetate satisfies the fourth coordination site of the metal ion.

Table-1: Physical Constants and Prominent Infra Red Bands of the Ligand and its Metal Complexes.

Compound	Color	M.P/Dec. Point °C	Prominent infrared bands (cm^{-1})												
			ν_{H_2O}	ν_{N-H}	ν_{C-H}	$\nu_{C=O}$	$\nu_{C=O}$ +$\nu_{C=C}$	$\nu_{C=N}$ +C=C	ν_{OAc} vibrations	ν_{C-N-C}	ν_{CH} out of plane bending	OH bending	ν_{M-O}	ν_{pheny} ring	ν_{M-O}	ν_{M-N}
(1*S*,2*R*-Nore)$_2$DAA(L)	Light yellow	72	3400	3084	2976	1708	1564	1492	--	1132	1080	1048	--	753	--	--
[VOL(OAc)]	Green	92	--	3128	2978	1624	1542	1468	1380	1104	1080	1044	980	690	616	568
[CoL(OAc)]$0.5H_2O$	Brown	110	3392	3160	2978	1624	1566	1448	1389		1080	1046	--	700	--	564
[NiL(OAc)$(H_2O)_2$]$5H_2O$	Brown	116	3420	3064	2980	1674	1563	1482	1408 1384 652	1104	1080	1024	--	744	616	444 512
[CuL(OAc)$(H_2O)_{1.5}$]	Brown	120	3392	3088	2980	1665	1554	1448	1384 666	1107	1080	1023	--	700	618	596
[UO_2L(OAc)(H_2O)]$4H_2O$	Red	82	3412	3060	2980	1624	1540	1472	1408 1384 676	1128	--	1024	932	744	608	440 536

A number of other bands due to C—H stretching, ring bending and out of plane bending observed in the ligand at 3084 cm^{-1}, 1084 cm^{-1} and 700 cm^{-1} remain unshifted in the IR. spectra of these complexes (Table-1).

The V=O and U=O stretching vibrations are observed at 972 cm^{-1} and 932 cm^{-1} in these complexes respectively. The M—N stretching vibrations are identified between 608-618 cm^{-1} while M—O stretching bands have been identified at 440-596 cm^{-1} for these complexes.

The elemental analysis shows that different number of water molecules are associated with these complexes. Some of which may be present in coordination sphere of metal ions. It may be assumed that [VO(1*S*,2*R*-Nore)$_2$DAA(OAc)] and [Co(1*S*,2*R* Nore)$_2$DAA(OAc)(H_2O)] have square pyramidal environment of donor atoms around metal ions. The metal ions in [Ni(1*S*,2*R*-Nore)$_2$DAA(OAc)(H_2O)$_2$] $5H_2O$ and [Cu(1*S*,2*R* Nore)$_2$DAA(H_2O)$_2$] are six coordinated octahedral species while UO_2(1*S*,2*R* Nore)$_2$DAA(H_2O)].$4H_2O$ may be assumed as a seven coordinated pentagonal bipyramidal complex.

Antibacterial Studies

The ligand and its monometallic complexes were screened for their antibacterial effects against a number of gram positive bacteria like *Staphylococcus* AFIP-P-5283, *Staphcocgulase* (-ive) AFIP-5381, *Staphaureus* AFIP-P-53f69, *Staphylococcus* (control), *E.*coli (control) and gram negative as *Enterobacter*, *Salmonella* (resistant), *Typhimurium*, *Pseudomonas*, *Salmonella* (sensitive) *S. Paratyphium*, *P. Vulgaris*, *Pseudomonas* (control) and *K. Pneumoniae*.

The ligand and complexes were dissolved in DMSO and minimum inhibitory concentration (MIC) was determined for each compound against these bacteria. The MIC study was also conducted using blank solvent in order to ascertain that the solvent has no inhibitory effects upon bacteria. The inhibitory effects of complexes upon bacteria are reported in Tables 2-3.

The ligand (1*S*,2*R*-Nore)$_2$DAA has very weak inhibitory effects(MIC > 1280 μg/mL) against most of the bacteria studied. The monometallic VO(IV), Co(II) and Cu(II) complexes of this ligand are found to be more active against a variety of bacteria in a much lower concentration range as compared to the ligand itself. The Cu(II) and Co(II) complexes of this ligand are active against *P.Vulgaris*, *Pseudomonas*, *K. Pneumoniae*, *Staphcoagulase(-ve)*, *S. Paratyphium*, *Salmonella* (sensitive) *Pseudomonas* and *Enterobacter* in the concentration range of 160

Table 2: Determination of minimum inhibitory concentration of (1*S*,2*R*-Nore)$_2$DAA and its complexes against gram negative bacteria (μg/mL)

COMPOUND	ORGANISM NO.									
	1	2	3	4	5	6	7	8	9	10
(1*S*,2*R*-Nore)$_2$DAA (L)	>	1280	>	>	1280	>	>	>	>	>
[VOL(OAc)]	1280	640	>	320	320	320	>	>	>	>
[CoL(OAc)]0.5H_2O	160	>	160	160	320	160	160	320	320	160
[NiL(OAc)(H_2O)$_2$].5H_2O	>	>	>	>	>	>	>	>	>	>
[CuL(OAc)(H_2O)$_{1.5}$]	320	640	640	640	640	640	640	320	640	320
[UO_2L(OAc)(H_2O)].$4H_2O$	>	>	>	>	>	>	>	>	>	>

1. *Entrobactor* 2. *Salmonela* (resistant) 3. *Escherichia coli* 4. *Skalmonella thphimurium* 5. *Pseudomonas* 6. *Salmonella* (sensitive) 7. *Salmonella paratyphium* 8. *Protius vulgarus* 9. *Pseudomonas* (control) 10. *Klepsiela Pneumoniae*

Table 3: Determination of minimum inhibitory concentration of (1*S*,2*R*-Nore)$_2$DAA and its complexes against gram positive bacteria (μg/mL)

COMPOUND	ORGANISM NO.						
	1	2	3	4	5	6	7
(1*S*,2*R*-Nore)$_2$DAA (L)	>	>	>	>	>	>	>
[VOL(OAc)]	>	>	>	>	>	>	>
[CoL(OAc)]0.5H_2O	160	160	160	160	320	160	160
[NiL(OAc)(H_2O)$_2$].5H_2O	1280	1280	>	>	>	>	>
[CuL(OAc)(H_2O)$_{1.5}$]	640	640	160	640	640	160	1280
[UO_2L(OAc)(H_2O)].$4H_2O$	>	>	>	>	>	>	>

1. *Staphylococcus* AFIP-P-5283 2. *Staphcogulase* (-ve) 3. *Staphylococcus* AFIP-P-5381 4. *Streptococcus Group-D* 5. *Staphaureus AFIP-P-5369* 6. *Staphylococcus (control)* 7. *Escherichia coli (control)*

μg/mL. The VO(IV) complex of this ligand is found to be active against *Salmonella* (resistant) and *Staphcoagulose* in the concentration range 320 μg/mL. The UO_2 (VI) complex is not found active against any bacteria in the concentration range 1280 μg/mL. The Co(II) complex of this ligand is active against *Pseudomonas* in lowest concentration (MIC 80 μg/mL) which is slightly higher than that of tetracycline (MIC 64 μg/mL).

It may be concluded from preceeding discussion that the new ligand [(1*S*,2*R* Nore)$_2$DAA] forms mononuclear complexes with VO(IV), Co(II), Ni(II), Cu(II) and UO_2(VI), some of which have relatively stronger inhibitory effects upon a number of gram positive and gram negative bacteria.

Experimental

(a) Materials

All metal salts and other reagents used were of highest purity analytical reagent grade and obtained from commercial sources. These were used without further purification.

The triketone 2,4,6-heptanetrione (diacetylacetone) was prepared by a literature method [6]. The amino alcohol (+)-1*S*, 2*R* norephedrine was obtained from Fluka, Switzerland.

*(b)Synthesis of 2,6-bis(1*S*,2*R*-2-hydroxy-1-methyl-2-phenylethylimino)-4-heptanone1.5 hydrate, (1*S*,2*R*-Nore)$_2$DAA)1.5 H_2O.*

To a well stirred methanol(100 mL) solution of 7.14 g (0.049 mol) of diacetylacetone at 60 °C was dropwise added 15.138 g (0.1 mol) of norephedrine in 50 mL methanol over a period of 1h. The reaction mixture was further stirred for 1h and the solution was then concentrated to 20 mL. The yellow condensation product was precipitated on keeping the concentrated solution in refrigerator overnight. The product was filtered, washed several times with acetone and finally with ether. It was recrystallized from $CHCl_3$. Yield 86% M.Pt. 68 °C, $[\alpha]^{25}$ -285.8°. Anal.Calcd for $C_{25}H_{32}N_2O_3$ 1.5 H_2O C 68.94 H 8.10 N 6.43% Found: C 68.62 H 7.58 N 6.27%, ^{1}H NMR ($CDCl_3$) δ 1.3-2.2 (m 12H) 3.3 - 3.6 (d 2H) 3.85 (d, 2H) 3.92 (s, CH_2), 3.98[m, 4H], 4.01, 4.8 - 5.8 (m, vinyl) 7.3 - 8.2 (m, 10H).

(c)Synthesis of complexes

(i) Acetato-2,6-bis(1S,2R-2-hydroxy-1-methyl 2-phenylethylimino)-4-heptanato oxovanadium (IV), [VO(1S,2R-Nore)$_2$DAA (OAc)]

To a boiling solution of 4.35 g (0.01 mol) of the ligand in 50 mL of acetone was slowly added an aqueous solution containing 0.01 mol VO^{2+} ions. The solution turned green instantaneously indicating complex formation. The solution was refluxed for 15-20 min. The product precipitated upon reducing volume of the solution. The complex was filtered, washed with acetone, ether and dried. Yield 86%. Anal. Calcd. for VO $C_{25}H_3N_2O_3(C_2H_3O_2)$ C 60.78, H 6.42 N 5.25 V 9.56% Found: C 60.60 H 6.20 N 4.91 V 9.37%

*(ii) Hemiaquo acetato-2,6-bis(1*S*,2*R*-2-hydroxy-1-methyl-2-phenylethylimino)-4-heptanatocobalt (II), [Co(1S,2R-Nore)$_2$ DAA(OAc)(H_2O)$_{0.5}$]*

An acetone solution of 4.35g (0.01 mol) of the ligand in 100 mL was brought to boiling. A solution of 2.49 g (0.01 mol) of cobalt acetate tetrahydrate in minimum volume of water was added to the boiling ligand solution. The complex precipitated upon refluxing the solution for 1h. It was filtered, washed several times with acetone, ether and dried. Yield 72% Anal. Calcd.for $CoC_{25}H_{31}N_2O_3$ ($C_2H_3O_2$)0.5 H_2O. C 60.67, H 6.67, N 5.24, Co 9.85, Found: C 60.71, H 6.55, N 4.87, Co 9.64%.

*(iii)Diaquo acetato-2,6-bis(1*S*,2*R*-2-hydroxy-1-methyl-2-phenylethylimino)-4-heptanatonickel (II) pentahydrate.*
[Ni(1S,2R Nore)$_2$ DAA (OAc)(H_2O)$_2$]0.5 H_2O.

A 50 mL acetone solution of 4.35 g (0.01 mol) of the ligand was brought to boiling. An aqueous solution of 2.18 g (0.01 mol) of nickel acetate dihydrate was dropwise added to the boiling ligand solution with constant stirring followed by 0.2 mL of triethylamine. The reaction mixture was stirred for 15-20 min. during which the complex precipitated in good yield. The product was filtered, washed three times with acetone, ether and dried. Yield 76% Anal. Calcd. For Ni $C_{25}H_{31}N_2O_3$ ($C_2H_3O_2$).7H_2O. C 49.78, H 7.43, N 4.30, Ni 9.0% Found: C 49.53, H 6.52, N 4.24, Ni 9.2%.

(iv)Acetato-2,6-bis(1S,2R-2-hydroxy-1-methyl-2-phenylethylimino)-4-heptanato-copper (II) 1.5 hydrate.
[Cu(1S,2R-Nore)$_2$ DAA (OAc)]1.5 H_2O

A 50 mL acetone solution of the ligand (4.35 g, 0.01 mol) was brought to boiling. An aqueous solution of 1.99 g (0.01 mol) of copper acetate in minimum volume of water was dropwise added to the boiling ligand solution with constant stirring followed by 0.2 mL of triethylamine. The reaction mixture was refluxed for 15-20 min. during which green complex precipitated. The product was filtered, washed with acetone, ether and dried. Yield 84% Anal Calcd. For Cu $C_{25}H_{31}N_2O_3$ ($C_2H_3O_2$) 1.5 H_2O. C 58.20, H 6.69, N 5.03, Cu 11.4% Found: C 58.31, H 6.34, N 5.38, Cu 11.87 %

(v)Aquo acetato-2,6-bis(1S,2R-2-hydroxy-1-methyl-2-phenylethylimino)-4-heptanatodioxouranium (VI) tetrahydrate,
[UO_2(1S,2R Nore)$_2$DAA(OAc) (H_2O)].4H_2O.

To a boiling 25 mL solution of 2.18g (0.005 mol) of the ligand in acetone was added an aqueous solution of uranyl acetate (2.12 g, 0.005 mol) followed by 4 mL of triethylamine. The reaction mixture was refluxed for 3h. The semisolid orange red product separated upon cooling was removed and dissolved in hot ethanol. The solution was cooled to room temperature and kept for slow evaporation. The orange red precipitates formed were separated, washed several times with ethanol, finally with ether and dried. Yield 81% Anal. Calcd. For $UO_2C_{25}H_{31}N_2O_3$ ($C_2H_3O_2$).5H_2O C 39.23, H 5.36, N 3.39 found: C 39.11, H 4.47, N 3.03 %.

Physical Measurements

Elemental analyses were obtained from Midwest microlabs, Indianapolis, Indiana, USA. The metal contents were determined by reported spectrophotometric methods [7] on Shimadzu model 120-2 spectrophotometer using 1 cm quartz cells. The melting points were determined on MP-D metlting point apparatus. The infra red spectra of complexes in KBr disc were recorded on Perkin Elmer 1710 FT IR spectrophotometer. The proton NMR spectrum of the ligand in $CDCl_3$ was recorded on Braker WP-200 spectrometer. For the determination of minimum inhibitory concentration of the ligand and its complexes, Danley's multipoint inoculator was used for application of inoculum.

Antibacterial Activity Studies

The ligand and its complexes were dissolved in DMSO to test their minimum inhibitory concentration against a variety of gram positive and gram negative bacteria. The method applied the preparation of agar plates with double dilution of antibiotics.

Procedure

The ligand and metal complexes (1.280 g each) were dissolved separately in minimum quantity of DMSO and diluted to 5 mL. A series of test tubes was prepared containing 1280 μg, 640 μg, 320 μg, 160 μg, 80 μg, 40 μg, 20 μg and 10 μg per mL of the sample in agar solution. Sterilized pipettes and test tubes were used. The nutrient agar solution was prepared in distilled water and autoclaved. One mL of the ligand/metal complex solution was transferred to sterilized 20 mL flask and diluted to 20 mL with agar solution. This was then transferred to a labelled petri dish and covered. These plates were refrigerated.

The test organisms were subcultured on blood agar, MacConkey agar and nutrient agar plates. The plates were incubated at 37 °C aerobically and anaerobically for 24 h. These were reidentified by screening methods before subjecting to MIC study. From nutrient agar plates (numbered for every individual organism) a loop full of growth from the single or more colonies were inoculated in 2 mL of Mueller Hinton broth in Bijon bottles and incubated at 37 °C aerobically for 18-24 h. Just prior to the test, the turbidity was adjusted to 10^5 - 10^6 CFU/mL with that of Kirby-Bauer standard and diluting the suspension with sterilized Mueller-Hinton broth. The thin broth culture (0.1 mL) of each test strain was transferred into each well of the multipoint inoculator. All the strains inoculated in the study were given definite number and the same number was maintained throughout the study. Danley's multipoint inoculator was used for the application of inoculum. The strains of bacteria were inoculated on prepared Mueller-Hinton agar plates containing predried different compounds. The plates were allowed to dry at room temperature for 10-15 min after inoculation and incubated aerobically for 10-24 h. at 37 °C. The MIC was the lowest concentration of compounds in agar plates at which no growth of the organism was visible to the naked eye. The results are reproduced in Tables 2 and 3.

Acknowledgement:

We would like to acknowledge the help extended by Lt. Col. Tariq Mahmood and Maj. Zia Ahmed of virology section, Armed Forces Institute of Pathology, Rawalpindi for antibacterial activity studies.

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