STUDY OF THERMAL STABILITY AND ANTIMICROBIAL ACTIVITIES OF N, N’-DIMETHYLBENZYLAMINE DERIVATIVES

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ABSTRACT: *Ortho Nitro N, N’-dimethylbenzylamine and its cmoplexes with Zn(II), Cd(II) and Hg(II) are reported. Physical properties of the ligand such as boiling point, miscibility, relative density, viscosity, surface tension and refractive index have been determined. The compounds were characterized on the basis of chemical characteristics and spectroscopic evidence. Relative thermal decomposition was noted by differential thermal analysis (DTA). Antibacterial activity of the ligand, metal salts and their complexes were investigated against Bacillus subtilis (B.s), Escherichia coli (E.c), Micrococcus luteus (M.l) and Staphylococcus aureus (S.a) by diffusion plate method.*

Key Words: Antimicrobial activities, N,N-dimethylbenzylamine derivatives

INTRODUCTION

Scientists [1] gave the method of tertiary amine synthesis by reaction of dimethylamine e.g., R_1NHR_2 with organometallic compounds like reaction of $LiCuR_2$ at -78^oC to $+25^oC$ which gave respective RNR R1.2N’-methylation of Me $(CH_2)_6NHBu$ and PhNHMe by CuMe gave $Me(CH_2)_6NBuMe$ and $PhN(Me)_2$. Korostylew and Litvineko [2] have reported the most versatile method of tertiary amine synthesis by alkyl halides and variety of benzyl substituted amines to give respective substituted tertiary benzylamines.

It has been reported that the reactions between copper (II), silver (I) and $o\text{-}LiC_6H_4CH_2NMe$ reagents give polynuclear complexes which contain metalated ligands bridging I-B metals [3].

On further reaction with I-B metal chlorides hexanuclear stable complexes are formed. These compounds are capable of forming five or six membered ring systems which would enhance the stabilities of the complexes formed with metals in favourable oxidation states. The reaction of N, N’-dimethylbenzylamine with platinum (II) and palladium (II) chloride has been reported and is found to occur by an initial rapid co-ordination of nitrogen to the metal [4].

MATERIALS AND METHODS

Chemicals used were mostly from E. Merck, British Drug House and Riedel de Haen. Solvents were of reagent quality and redistilled before use. Nitro derivative of n, N’-dimethylbenzylamine was prepared by treating o-nitrotoluene with N-bromosuccinimide in CCl_4 using benzoylperoxide as catalyst under reflux followed by the addition of dimethylamine. The ligand was separated by passing through column packed with silica gel No. 60 (70-230 mesh ASTM). Purity of the ligand was checked on T.L.C., coated with silica gel. λmax. of the ligand was taken on Hitachi UV-Vis Spectrophotometer, model U-2000 in absolute alcohol. The derivative of the ligand with II-B metals were synthesized in ethanol-water mixture (4:1), separated, washed and dried through vacuum line. Infrared absorption spectra of the ligand and metal complexes were recorded in thin film and nujol respectively on Hitachi Infrared Spectrophotometer, model 270-30 in the spectral range of 4000-400 cm^{-1}.

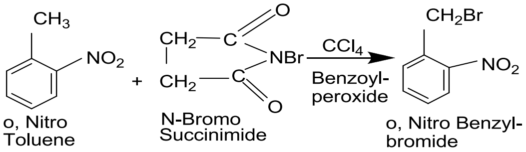
Differential thermal analysis of the complexes was performed on Shimadzu Thermal Analyzer, model DT-40 in the temperature range of 20-600^oC using air as atmosphere and calcined alumina as reference material.

Antibacterial activities of metal ions, ligand and their complexes were determined by agar diffusion method [5] against Bacillus subtilis (B.s), Escherichia coli (E.c), Micrococcus luteus (M.l) and Staphylococcus aureus (S.a). Assay medium was adjusted before the addition of agar. The nutrient agar was also used for the bioassay. The bio-assay medium consisted of (g/L):- beef extract 1.0, yeast extract 2.0, peptone 5.0, NaCl 5.0, agar 15.0, and water 1000mL. pH of the medium was adjusted before the addition of agar. The medium was then dispensed in 250mL Erlenmeyer flask and sterilized at 15 psi pressure for 30 minutes. The petri-plates were washed and sterilized in the oven at 120^oC overnight. 20mL of the molten bioassay medium poured in the sterile plates was allowed to set. Then 4.0 mL of the molten assay medium was incubated with predetermined concentrations of the microorganisms and spread uniformly over the first layer and was allowed to set. Then four wells of 0.8 cm were made in the plates, aseptically using stainless borer. An emulsion of the sample (0.1% salt, complex) in Gumacacia was used for testing its antibacterial activity. 0.12mL of the emulsion was added to a hole incubated at 37^oC for 24 hours. Clear zones of inhibition were developed and the diameter of the zones of inhibition were measured in mm.

SYNTHESIS OF O, NITRO N, N’-DIMETHYL-BENZYLAMINE

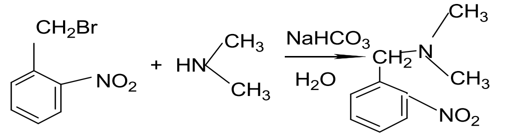
Step-I: Preparation of o-nitrobenzylbromide

o-Nitrotoluene (58.8 mL, 0.5 mol), N-bromosuccinimide (88.5 g, 0.5 mole) in 250 mL and benzoylperoxide (1.0 g) in CCl_4 (250 mL) were charged in a quickfit flask equipped with water condenser and reaction mixture was heated under reflux for 6-8h, until all the solid started floating on the liquid surface. The mixture was allowed to cool down to room temperature and regenerated succinimide was filtered off. The filtrate was evaporated to give o-nitrobenzylbromide.



Step-II: Preparation of o, Nitro N, N’-dimethylbenzylamine

o-Nitrobenzylbromide, $NaHCO_3$ (42.0 g, 0.5 mole) and distilled water (100 mL) were taken in quickfit flask equipped with water condenser. Dimethylamine (25.0 mL, 0.5 mole) was added to the reaction mixture through condenser with the help of separating funnel drop wise. The mixture was again refluxed for four hours. Then the mixture was allowed to cool and filtered off. The filtrate was transferred to a separating funnel where two layers were formed. The lower organic layer was collected and passed through a column packed with silica gel No. 60 (70-230 mesh ASTM). The ether **:** n-hexane (3:1) fraction was collected which on evaporation gave orange red oily liquid (compound-I).



o, Nitro benzylbromide

Dimethyl-amine

N,N-Dicyclohexyl-benzylamine

SYNTHESIS OF METAL DERIVATIVES:

Reactions of o-Nitro N, N'-dimethyl-benzylamine with:

a).ZnCl$_2$: A white solid settled when o-Nitro N, N'-dimethylbenzylamine (0.5 mL, 4.0 mmol) in 20.0 mL ethanol was mixed with a clear solution of $ZnCl_2$ (0.2725g, 2.0 mmol) in 10 mL distilled water. The product was filtered, washed thrice with distilled water, ethanol and n-hexane and dried to get the compound (II).

b) CdCl$_2$: An off-white solid settled when o,Nitro N, N'-dimethylbenzylamine (0.5 mL, 4.0 mmol) in 20.0 mL ethanol was mixed with a clear solution of $CdCl_2$ (0.402 g, 2.0 mmol) in 10 mL distilled water. The product was filtered, washed thrice with distilled water, ethanol and n-hexane and dried to get the compound (III).

c) HgCl$_2$: A pale yellow solid settled when o,Nitro N, N'-dimethylbenzylamine (0.5 mL, 4.0 mmol) in 20.0 mL ethanol was mixed with a clear solution of $HgCl_2$ (0.543 g, 2.0 mmol) in 10 mL distilled water. The product was filtered, washed thrice with distilled water, ethanol and n-hexane and dried to get the compound (IV).

RESULTS AND DISCUSSION

An indirect route was adopted for the preparation of o-nitro N, N'-dimethylbenzylamine. Nitrotoluene was refluxed with N-bromosuccinimide in CCl_4 in the presence of benzoylperoxide to get o-nitrobenzylbromide which on further treatment with dimethylamine in the presence of $NaHCO_3$, produced o, nitro N, N'-dimethylbenzylamine. The reaction products were passed through a column packed with silica gel. Ether: n-hexane mixture (3:1) eluted an orange red band. The orange solution was evaporation in a rotary evaporator to get red oily liquid which was dried under vacuum. The liquid was found soluble in almost all organic solvents and was fairly stable with highly pungent smell.

The IR spectra (Table III) of the ligand indicate C-H stretching frequency of unsaturated bonds at 3100-3025 cm^{-1}, C-H stretching frequency for CH_3, CH_2 and the benzene ring are observed at 2900 cm^{-1}, 2825 and 1460 cm^{-1}, respectively. Aromatic ring deformation and bending vibrations are indicated at 1100-1040 cm^{-1}. Characteristic stretching frequency of C-N bond of $(CH_3)_2N\text{-}CH_2$ type is indicated at 1260-1180 cm^{-1} and that of nitro-aryl at 1580-1460 cm^{-1}. The IR spectra of the complexes show lowering in frequencies in the region 1600-1300 cm^{-1} indicating the coordinate bond formation. The new bands appeared at 500 cm^{-1} in case of Zn(II) complex, 450 cm^{-1} in case of Cd(II) complex and 510 cm^{-1} in case of Hg(II) complex are due to M-N frequencies [6,7]. The compounds were found insoluble in organic solvents indicating their dimeric or polymeric nature bonded through halogen bridges as already reported [8]

Estimation of metal in the complex was carried out by atomic absorption spectrophotometer and nitrogen by Kjeldhal's method. The results of metal analysis indicate 1:1 ratio of metal atom to be the ligand molecule.

Table I: Physical properties of o-Nitro N, N'-dimethylbenzylamine

No.	Property	Value
1.	Boiling Point	196 ± 2^{o}C
2.	Colour	Red Oily Liquid
3.	Odour	Highly Pungent
4.	Relative Density	1.3553 g/mL at 20^{o}C
5.	Viscosity	26.0953 g/cm^{-1} sec^{-1}
6.	Surface Tension	31.849 Dynes cm^{-1} at 20^{o}C
7.	Refractive Index	1.533 at 25^{o}C
8.	λmax	250 mm (in absolute alcohol)
9.	Miscibility	Miscible in almost all organic solvents

Table II % yield, melting / decomposition point, nitrogen and metal estimation

No.	% yield	m.p/d.p ± 2^{o}C	%N Exp. (Theor.)	%Metal Exp. (Theor.)
I	29.11	170	9.85 (10.00)	22.09 (22.36)
II	40.29	230	8.40 (8.56)	33.99 (34.39)
II	35.49	280	6.49 (6.74)	47.89 (48.32)

Differential thermal analysis was carried out in order to find out the thermal stability of the complexes. o-Nitro N, N'-dimethylbenzylamine zinc (II) chloride was ignited up to 600^{o}C. It was found that complex was fairly stable up to 160^{o}C and then started decomposing DTA showed one endothermic peak at 175^{o}C due to melting of the complex. DTA showed continuous endothermic trend in the temperature range of 220 to 600^{o}C. o,Nitro N, N'-dimethylbenzylamine cadmium (II) chloride was completely anhydrous as no DTA peak was observed up to 220 C due to loss of water. It started decomposing at 230^{o}C and DTA showed two endotherms at 240.4^{o}C and 318.3^{o}C. DTA curve showed endothermic trend up to 600^{o}C which indicated slow degradation of the compound.

o-Nitro N, N'-dimethylbenzylamine mercury (II) chloride was also found to be anhydrous and fairly stable up to 210^{o}C. DTA showed two endothermic peaks at 240.6^{o}C and 317.6^{o}C due to decomposition of the complex. In the temperature range of 350 to 600^{o}C DTA curve showed continuous endothermic trend indicating slow degradation. The order of thermal stability observed from thermograms is:

Hg(II)complex > Cd(II) complex > Zn(II) complex

Antimicrobial activities of the ligand, metal salts and their complexes were investigated against some species of bacteria such as Bacillus subtilis (B.s), Escherichia coli (E.c), Micrococcus luteus (M.l) and Staphylococcus aureus (S.a) [5] by diffusion plate method. In case of II-B metal salts, $HgCl_2$ exhibited maximum activity followed by $CdCl_2$ and $ZnCl_2$ respectively. This trend was upset in the complexes. o-Nitro N, N'-dimethylbenzylamine cadmium (II) complex exhibited maximum activity followed by Zn(II) and Hg(II) complexes against Micrococcus luteus (M.l), Staphylococcus aureus (S.a) and Escherichia coli (E.c) but this trend was changed against Bacillus subtilis (B.s) as:

Cd(II)complex > Hg(II) complex > Zn(II) complex.

Table III: IR absorption spectra of ligand & II-B metal complexes

No	Infrared absorption bands(cm^{-1})
I.	3100w, 3025w, 2925vw, 2900m, 2825vw, 2725w, 1700s, 1625s, 1580m, 1530vs, 1460m, 1350vs, 1310vs, 1100m, 1040w, 830vw, 810vs, 755s, 730vw, 690w.
II.	3450s, 3400m, 3100m, 2350w, 1560w, 1310m, 1015, 930vw, 750w, 500w.
III.	3450m, 3400m, 3125m, 2325w, 1515m, 1325s, 970w, 740s, 450w.
IV.	3420m, 3080m, 2340m, 1530s, 1320w, 1165vw, 100m, 750s, 510w.

Table IV: Antibacterial activities exhibited by metal ions, ligand & II-B metal derivatives

Compound	**Inhibition Zone Diameter (mm)**			
	E.c	***B.s***	***S.a***	***M.l***
$ZnCl_2$	17.0	13.0	16.0	21.0
$CdCl_2$	18.0	26.0	27.0	30.0
$HgCl_2$	29.0	27.0	30.0	31.0
Compound I	9.0	8.0	9.0	8.0
Compound II	25.0	16.0	20.0	21.0
Compound III	34.0	28.0	34.0	34.0
Compound IV	23.0	26.0	19.0	14.0

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