

Synthesis, Physico-Chemical and Antimicrobial Studies of Ionic Liquid

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A novel ammonium salt, an ionic liquid (ILs) was synthesized at room temperature. Its structure was elucidated by elemental analyses and various spectroscopic techniques like FT-IR, ¹H and ¹³C NMR and ESI-MS spectrometry. Thermal studies were also carried out to get thermal stability of the ionic compound. X-ray crystallographic study was carried out to ascertain the structure of ammonium cation which is bonded to three anion via O-H...Cl intermolecular hydrogen bonds. Synthesized compound was also investigated for *in vitro* antimicrobial study against number microbes and results showed excellent results.

Keywords: Ionic liquid, Spectroscopic studies, Antimicrobial studies.

INTRODUCTION

Ionic liquids are a novel class of compounds composed of an organic cations and organic or inorganic anions¹⁻⁴ and are liquid up to 200 °C⁵⁻⁷. Ionic liquids generally constitute of a pair of cation like ammonium, phosphonium, pyridinium, imidazolium or chlorinium cation and an anion like a fluorinated anion. These cation-anion combinations have been extensively studied for physical properties of ionic liquids and recent studies have proven that the interaction between cations and anions depend on the type of solvent, particularly on its polarity⁸⁻¹¹. Ionic liquids are thermally stable, non-explosive and flammable and don't evaporate since they have very low vapour pressure. Due to low vapor pressure and lack of flammability, ionic liquids are good candidate for replacement of toxic and volatile organic compounds¹²⁻¹⁵. Moreover, the low volatility of ionic liquids results in an increase in process safety, chemical reactivity and thus led to more effective processes⁸⁻¹¹. Ionic liquids have been considered as environment-friendly media as highly biodegradable and exceptionally harmless materials and are more attractive and ecologically acceptable green solvents^{12,13,15,16}. They have been regarded as powerful alternatives to the volatile organic compounds in the field of organic synthesis¹⁶ and find extensive applications in electrochemistry, chromatography, transition metal catalysis, biocatalytic transformations asymmetric synthesis, polymers and biomaterials and material science¹⁷⁻²². The effect of ionic liquids on micro-

organisms has been also studied^{23,24}. The antimicrobial activities of five new groups of choline-like quaternary ammonium chloride ionic liquids were evaluated against a number of Gram positive and Gram negative bacteria²⁵ and showed good antimicrobial activity and confirmed that lipophilicity was the main factor in determining antimicrobial activity²⁶. Due to these facts that ionic liquids related research has become one of the most exciting topics nowadays. Hereby, we synthesize quaternary ammonium cation, an ionic liquid and characterize it by various spectroscopic studies like IR and ¹H and ¹³C NMR and ESI-MS spectrometry. Single crystal X-ray crystallography has also been performed to confirm the structure of the ionic liquid.

EXPERIMENTAL

All the reagents used were of anal AR grade and were purchased from E. Merck and used as received. Elemental analyses were recorded on a ElementarVario EL analyzer. ¹H and ¹³C NMR spectra were recorded in DMSO-*d*₆ using JEOL 400 spectrometer. FT-IR (4000-400 cm⁻¹) spectra were recorded as KBr pellet on a Perkin Elmer 621 spectrophotometer. Mass spectrometry was performed with a Micromass Quattro Premier tandem MS fitted with an ESI interface and controlled by MassLynx 4.1 software. MS/MS detection was performed with electrospray positive ionization mode.

Synthesis of quaternary ammonium cation: A solution of Zn(II) chloride (0.50 mmol) dissolved in 15 mL methanol was added dropwise into 10 mL methanol solution of 2-[(3-

pyridinylimino)methyl]phenol. The resultant reaction mixture was stirred for 0.5 h resulting into a clear yellow colored solution. Small quantity of triethanolamine was added dropwise in the reaction mixture to make the solution alkaline. White precipitate appears which removed by filtration and recrystallized in water-methanol mixture. After few days, colorless crystal suitable for single crystal X-ray diffraction appeared.

Yield: 68 %, Color: colorless, m.f.: $C_6H_{16}NO_3Cl$; 1H NMR (DMSO- d_6): δ (ppm) 4.48 (NH), 3.35 (-CH₂-NH), 3.43 (-CH₂-OH), ^{13}C NMR (DMSO- d_6): δ (ppm) 57.5, 59.5 Anal: calc.d, C, 38.89; H, 8.77; N, 7.67; Cl, 19.18; found, C, 38.81; H, 8.68; N, 7.54; Cl, 19.09; IR, 3421 cm^{-1} ν (OH), 3050 cm^{-1} ν (NH).

Single crystal X-ray diffraction analysis: The colourless needle crystal of the ionic compound was sealed in glass capillary filled with helium and it was mounted on a KM-4-CCD automatic diffractometer equipped with CCD detector and used for data collection. X-ray intensity data were collected with graphite monochromated CuK_{α} ($\lambda = 1.54178$ Å) radiation at temperature 100(1) K, with ω scan mode. The 7 seconds exposure time was used and reflections inside Ewald sphere were collected up to $\theta = 72.31^\circ$. The unit cell parameters were determined from 972 strongest reflections. Details concerning crystal data and refinement are given in Table-1. Examination of reflections on two reference frames monitored after each 20 frames measured showed no loss of the intensity during measurement. During the data reduction Lorentz, polarization and numerical absorption²⁷ corrections were applied. The structure was solved by partial structure expansion procedure. All the non-hydrogen atoms were refined anisotropically using full-matrix, least-squares technique on F^2 . All the hydrogen atoms were found from difference fourier synthesis after four cycles of anisotropic refinement and refined as "riding" on the adjacent atom with geometric idealization after each cycle of refinement and individual isotropic displacement factors equal 1.2 times the value of equivalent displacement factor of the parent carbon and nitrogen atoms and 1.5 times of parent oxygen atoms. The flack parameter was refined as full matrix parameter. The SHELXS97, SHELXL97 and SHELXTL²⁸ programs were used for all the calculations. Atomic scattering factors were those incorporated in the computer programs. The intermolecular interactions are listed in Table-2.

Determination of antimicrobial activity: Antimicrobial activities of the synthesized novel ammonium salt were performed against six bacterial isolates (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Pantoea dispersa* and *Acromobacter xylosoxidans*) and six fungal isolates (*Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus oryza*, *Fusarium oxysporum* and *Alternaria* sp.). The antimicrobial activity of synthesized compounds was done by modified Kirby Bauer agar diffusion method²⁹. The pure cultures of organisms were subculture on Müller-Hinton broth for bacteria at $30 \pm 2^\circ C$ and Potato dextrose broth for fungus at $35 \pm 2^\circ C$ on a rotary shaker at 140 rpm. Each strain was spread uniformly onto the individual plates using sterile glass rod spreader. A lawn of culture was prepared by spreading the 100 mL culture broth having 10^6 CFU/mL of each test organism on nutrient agar plates. Plates were left standing for 10 min to let the culture get absorbed. The 8 mm size wells

TABLE-1
CRYSTAL AND STRUCTURE REFINEMENT
DATA OF COMPOUND

Empirical formula	$C_6H_{16}NO_3Cl$
Formula weight	185.65
Crystal system, space group	hexagonal, R3c (No. 161)
Unit cell dimensions [Å, °]	$a = b = 8.2308(3)$ $c = 22.7412(15)$
Volume [Å ³]	1334.22(15)
Z, Calculated density [Mg/m ³]	6, 1.386
Absorption coefficient [mm ⁻¹]	3.541
F(000)	600
Crystal size [mm]	0.210, 0.068, 0.061
θ range for data collection [°]	7.33 to 72.31
Index ranges	$-10 \leq h \leq 10$, $-10 \leq k \leq 10$, $-26 \leq l \leq 27$
Reflections collected / unique	4467 / 559 [$R_{int} = 0.0215$]
Completeness [%]	100.0 (to $\theta = 67^\circ$)
Min. and max. transmission	0.698 and 0.943
Data/restraints / parameters	559 / 1 ^s / 35
Goodness-of-fit on F^2	1.138
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0191$, $wR2 = 0.0497$
R indices (all data)	$R1 = 0.0191$, $wR2 = 0.0497$
Largest diff. peak and hole [$e \cdot \text{\AA}^{-3}$]	0.141, -0.155
^s Floating origin restrain	

TABLE-2
HYDROGEN BONDS GEOMETRY OF COMPOUND 1 [Å, °]

D—H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N1—H1N...O1	0.90	2.34	2.8013(16)	111.7
O1—H1O...Cl1	0.91	2.23	3.1390(10)	178.1

were punched into the agar with the head of sterile micropipette tips. Wells were sealed with one drop of melt agar (0.8 % agar) to avoid leakage from the bottom of the plate. Using a micropipette, 100 μ L (100 μ g) of the sample of drug solution was poured onto each of wells on all plates. After incubation at optimum temperature for 24 h, the different levels of zone of inhibition were measured. Solvent blank was used as negative control and standard drug used as a positive control.

RESULTS AND DISCUSSION

The ionic compound was synthesized by the reaction of $ZnCl_2$ with 2-[(3-pyridinylimino)methyl]phenol in methanol in the alkaline medium of triethanolamine. The analytical data agree well with the proposed composition of the synthesized ionic liquid. Its formation was confirmed on the basis of results of elemental analyses, molecular ion peak in mass spectra, characteristic bands in FT-IR and resonance signals in the 1H and ^{13}C NMR spectra and single crystal XRD. So formed ionic liquid is thermally stable and soluble in water.

Single crystal X-ray diffraction studies: A perspective view of the synthesized ionic liquid structure is shown in Fig. 1. The compound is composed from quaternary ammonium cation $C_6H_{16}NO_3^+$ and charge balancing chloride anion. The Cl1, N1 and H1 atoms occupy the special position a at 0, 0, z, with the site symmetry 3, thus the asymmetric unit contains one third of organic cation and one third of chloride anion. The bond distances within the cation are normal and the N1-C1-C2-O1 torsion angle of $57.73(12)^\circ$ is close to one of preferred angles (60 and 180°) in such systems.

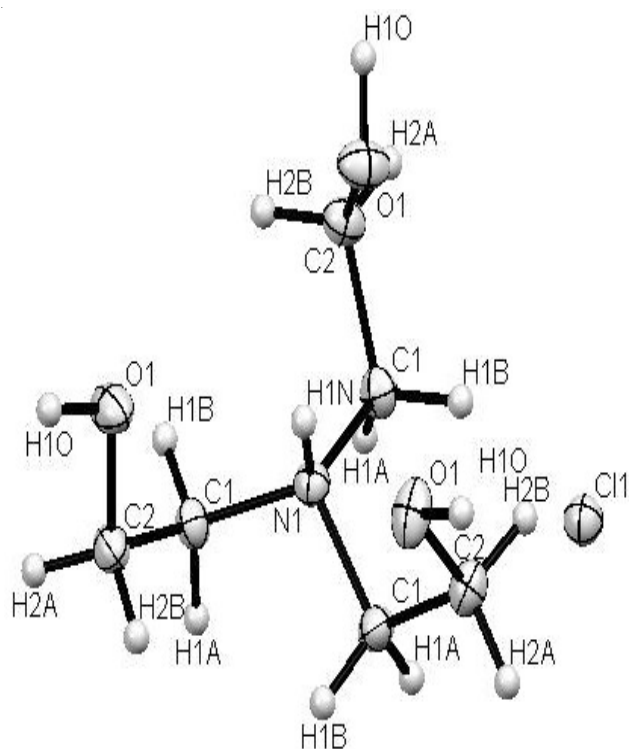


Fig. 1. Molecular structure of ionic liquid plotted with 50 % probability of displacement ellipsoids. The hydrogen atoms were omitted for clarity. The symmetry equivalent atoms generated by $-y + 1, x - y, z$ and $-x + y + 1, -x + 1, z$ transformations were labelled by additional A and B letter respectively

The each cation is bonded to three anions *via* O-H...Cl intermolecular hydrogen bonds and *vice versa*, each anion is bonded to the three cations (Table-2). These interactions form the $R_6^2(30)[C(10)]$ motifs of basic unitary graph. Additionally the intramolecular short N-H...O contacts can be classified as the hydrogen bonds³⁰ (Table-2).

Spectroscopic studies: The IR studies have been undertaken to obtain information of the organic cations. The IR spectrum shows a broad band observed at 3050 cm^{-1} assigned to $\nu(\text{NH})$ stretching mode. The symmetric bending vibration is observed at 2905 cm^{-1} . The absorption band observed at 1058 cm^{-1} is ascribed to $\nu(\text{C-C})$ stretching mode. The $\nu(\text{OH})$ absorption band appears at 3421 cm^{-1} , while the other bands were found at their expected positions. The ^1H NMR spectrum of the ammonium cation provides its important structural information and the results obtained are in close agreement with those obtained by X-ray and IR studies. The ^1H NMR spectrum of the cation shows two broad singlets at 3.35 ppm and 3.43 ppm assigned to $-\text{CH}_2-\text{OH}$ group and $-\text{CH}_2-\text{NH}_2$, respectively. The $-\text{OH}$ and $-\text{NH}$ protons appear at 3.53 ppm and 4.48 ppm, respectively. The ^{13}C NMR spectrum shows two signals at 57.5 ppm and 59.5 ppm attributed to $-\text{CH}_2-\text{OH}$ and $\text{NH}-\text{CH}_2$, respectively. Mass spectra of the ammonium cation exhibited molecular ion peak $[\text{M} + \text{H}]^+$, m/z at 186.65, corresponding to molecular formulae, $[\text{C}_6\text{H}_{16}\text{ClNO}_3]$ as its calculated m/z being 185.65 for its corresponding compound.

Antimicrobial activity: *in vitro* antibacterial and antifungal activity of the ammonium salt was tested in relation to considerable number of microorganisms, including human

pathogenic bacteria and fungal isolates in order to evaluate broad-spectrum antimicrobial activity. The tested compounds showed low antimicrobial activity or no activity at tested concentrations (Tables 3 and 4). There was no significant difference in activity between corresponding complexes and its precursors. Detectable MIC values were in range from $300\text{ }\mu\text{g/mL}$ to $500\text{ }\mu\text{g/mL}$ for bacterial isolates, while MIC for fungal isolates were at $500\text{ }\mu\text{g/mL}$ and $800\text{ }\mu\text{g/mL}$. This could be explaining the low antibacterial activity of tested compounds.

TABLE-3
ANTIBACTERIAL ACTIVITY OF NOVEL AMMONIUM SALT AGAINST SELECTIVE BACTERIAL SPECIES

Bacterial organisms	Novel ammonium salt		Tetracyclin	
	MIC	MBC	MIC	MBC
<i>Bacillus subtilis</i>	300	>350	5	>5
<i>Escherichia coli</i>	400	>400	5	>5
<i>Pseudomonas aeruginosa</i>	500	>500	10	>10
<i>Staphylococcus aureus</i>	500	>500	10	>10
<i>Pantoea dispersa</i>	300	>300	6	>6
<i>Acromobacter xylosoxidans</i>	300	>350	5	>5

Concentration used in $\mu\text{g/mL}$

TABLE-4
ANTIFUNGAL ACTIVITY OF NOVEL AMMONIUM SALT AGAINST SELECTIVE FUNGUS SPECIES

Fungal organisms	Novel Ammonium salt		Nystatin	
	MIC	MFC	MIC	MFC
<i>Candida albicans</i>	500	>550	4	>4
<i>Aspergillus niger</i>	600	>600	4	>5
<i>Aspergillus flavus</i>	800	>850	5	>5
<i>Rhizopus oryza</i>	700	>750	5	>5
<i>Fusarium oxysporum</i>	600	>700	4	>4
<i>Alternaria sp.</i>	500	>500	5	>5

Concentration used in $\mu\text{g/mL}$

Thermal stability: Thermal stabilities of the tertiary ammonium cation was determined by thermal analyses (TGA and DTA) in N_2 atmosphere at a heating rate of $20\text{ }^\circ\text{C min}^{-1}$ in the temperature range $20\text{--}600\text{ }^\circ\text{C}$. These compounds undergo two distinct steps of weight loss (Fig. 2). The first step shows a weight loss of 10 % corresponding to moisture in the temperature range of $20\text{--}232\text{ }^\circ\text{C}$. Further elevation in temperature from $252\text{--}363\text{ }^\circ\text{C}$ leads to a rapid weight loss of 21 % attributed to the loss of NH and two methyl groups. The TGA data is further supported by DTA, which shows two main steps at $20\text{--}232, 232\text{--}363$. The results of TGA and DTA clearly indicated that the second stage is the main degradation stage.

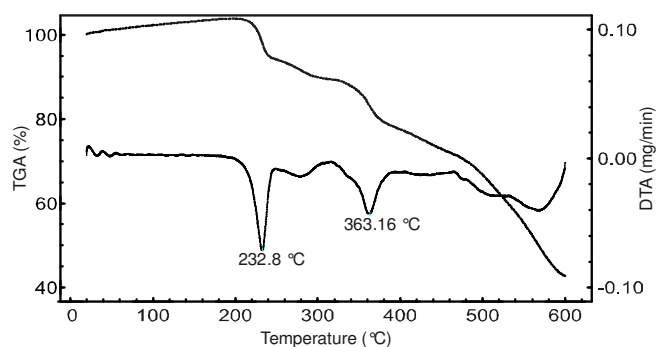


Fig. 2. TGA/DTA of ionic liquid

Supplementary material available: CCDC925982 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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