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Ethylenediammonium-Dicarboxylate Salts and Co-Crystallization for Biological and Pharmaceutical Applications

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***Abstract*— Several ammonium type salts and co-crystals have been used in drug formulations to increase or decrease solubility, to improve stability, toxicity and also reducing the hygroscopicity of the products. Basically, a wide range of chemically diverse acids and bases with different pK_a, solubilities , molecular weight and other properties have been used for the purpose for a very long time in both drug and food formulations. In the present study, six ethylenediamminium salts containing thiocyanate and the anion derived from diprotic acids namely oxalic (I), adipic (2), fumaric(3), succinic (4), azelaic (5) and sebacic (6) respectively, have been synthesized and characterized including their molecular structures. The difference in the pK_a values of the acids unable the salt formation to be generalized. The molar conductivities of the salts are between 218 and 453 $ohm^{-1}cm^2mol^{-1}$ with maximum value of 453 for salt (I). All the salts showed low activity against some selected bacteria and fungi and low toxicity against vero cell. The molecular structure of the salts will be presented and the biological activity will be compared with other ammonium salts.**

***Keywords*— Ethylenediammine; Thiocyanate; Oxalic; Adipic; Fumaric; Succinic; Azelaic; Sebacic; Antibacterial; Toxicity.**

I. INTRODUCTION

Synthesis of crystallined charge transfer complexes have been known for a long time for their vital role in biological systems such as antimicrobial activity and DNA-binding, as well as in laser technology, optoelectronics, optical communications, photocatalyst and optical signal processing [1, 2]. Some of these compounds show interesting electrical conductivity properties and further applications have found in electronics and solar cells [3]. Moreover, many of organic salt crystals have been extensively studied due to their properties in non linear optical (NLO) applications and their NLO coefficients have been found larger than those of inorganic materials [4, 5]. There are a wide range of chemically diverse acids and bases, with different range of pK_a, which are useful for this type of neutralisation reaction. In this study six ethyelendiammonium with oxalic (I), adipic (2), fumaric(3), succinic (4), azelaic (5) and sebacic (6) with the presence or absence on thiocyanate anions were synthesized. The antibacterial and antifungal activities along with cytotoxicity were investigated.

II. MATERIALS AND METHODS

A. Chemicals and instrumentation

All solvents and chemicals were of analytical grade and were used without purification. Elemental analysis was carried out with Fison EA 1108 for hydrogen, carbon and nitrogen contain in the compound. Infrared spectra of the salts were recorded in the range 4000-200 cm^{-1} with the help of Perkin Elmer Spectrum GX as KBr pellets. NMR spectra were recorded in DMSO-d_6 using TMS as standard on JOEL FX-400 spectrometer. The single crystal X-ray study was conducted by using Bruker SMART Apex diffractometer.

B. Preparation of Ethylenediammon- ium-dicarboxylate Salts

Six Ethyelendiammonium-dicarboxylate salts were prepared by addition of 10 ml aqueous solution of ethylenediamine (2 mmol) to 1mmol aqueous solution of each dicarboxylic acid in the presence of ammonium thiocyanate. After about one week of evaporation at room temperature, colourless crystals were obtained.

C. Biological studies

Antibacterial activity test for all the salts against 8 types of bacteria and 2 fungi were carried out by the disc diffusion technique. 100 mg of the samples were dissolved in 1 ml of distilled water using a 5200 Bran sonicator for 30 minutes.

The filter paper discs (6 mm in diameter) were individually impregnated with 20 µl of each diammonium-diprotic acid salt. The results were compared to commercially available chloramphenicol. MTT method used to determine the cytotoxic activity of the samples in two concentrations of 1 mg/ml and 25 mg/ml.

III. Results And Discussion

IR spectrum of compounds (1), (2), (5) and (6) showed the sharp absorption band at about 2064 cm^{-1} indicating the presence of thiocyanate ion. On the other hand, no stretching frequency for thiocyanate anion was observed in compounds (3) and (4). No signal for protonated amines were observed in the ^{1}H NMR spectra due to the H/D exchange in both D_2O and DMSO solvents. The ^{13}C NMR confirmed the formation of ethylenediammonium-dicarboxylate-thiocyanate complex with the thiocyanate chemical shifts of 172.81, 183.86, 183.61 and 182.05 ppm for compounds (1), (2), (5) and (6) respectively. Ultra-violet visible spectrum shows a maximum absorption peak around 220nm (ε=1500) due to π → π* electronic transitions of carboxylate ions.

The spectroscopic data for compound (1), (2) and (3) was supported by X-ray investigation. The oxalate, adipate and fumarate salts crystallized in triclinic system with space group Pī [6]. The unit cell of adipate salt (2) is a=7.1228(17), b=8.0920(19), c=9.383(2)Å, β=81.179(5)°, V=479.62(19)$Å^3$ and Z= 1. The molecular structure is shown in Fig.1. The ethylenediammonium cation in (1) is not planar as observed in (2) and (3) but twisted with the torsion angle of 62.64 (15)°. The asymmetric unit of fumarate salt (3) consists of two independent molecules and both fumarate anions are not planar due to the carbonyl groups. The structure of these compounds is stabilized by inter and inter-molecular hydrogen bonds forming three dimensional networks (Table 1).

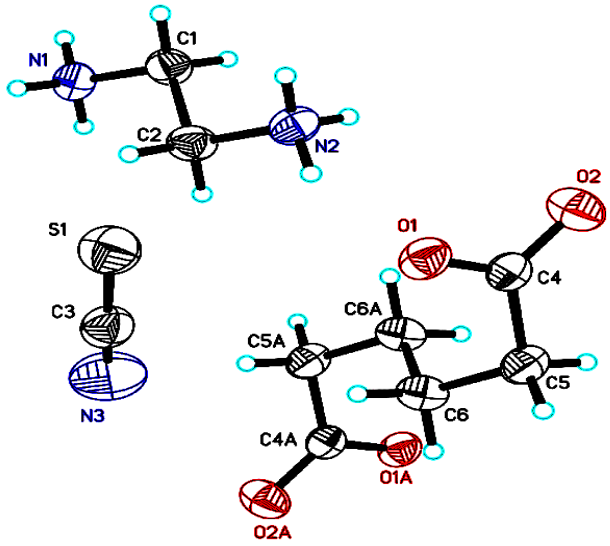

Fig. 1. The molecular structure of Ethyelenediammonium hemiadipate thiocyanate salt(2)

TABLE I
INTRA AND INTER MOLECULAR HYDROGEN BONDS FOR ETHYELENEDIAMMONIUM HEMIADIPATE THIOCYANATE SALT (2)

D-H…A	d(D—H)	d(H…A)	d(D…A)	(D-H…A)
N1—H10A…O1 [i]	0.89	2.03	2.7765 (19)	141
N1—H10B…O2 [ii]	0.89	1.90	2.760 (2)	161
N1—H10C…N3 [iii]	0.89	1.97	3.840 (2)	166
N2—H12A…O1	0.89	2.00	2.8138 (17)	152
N2—H12B…O2 [iv]	0.89	1.82	2.7095 (17)	175
N2—H12C…S1 [v]	0.89	2.56	2.4343 (17)	166

Symmetry codes: (i) 1+x, -1+y, z, (ii) x, -1+y, z, (iii) 1-x, -y, 1-z (v) 1-x, 1-y, -z., (iv) - 1+x, y, z

En-Oxalate-SCN(1): percentage yield = 92%; melting point = 457.1-458.3K; elemental analysis, expt C=30.65 H=6.40 N=25.17 S=18.90; cal C=29.26 H=6.14 N=25.59 S=19.5; infrared analysis, C=O = 1620 cm^{-1}, C=N = 2064 cm^{-1}, N-H = 2993 cm^{-1}, ^{1}H NMR $H(4,s)_{amine}$ = 3.14ppm, ^{13}C NMR C=N = 172.81ppm, C=O= 133.49ppm; $(C-H)_{amine}$=36.31ppm; UV λ_{max} = 225nm; Molar conductivity (Λ_m): 453 oh $m^{-1}cm^2mol^{-1}$

En-Adipate-SCN(2): percentage yield = 86%; melting point = 410.3-411K; elemental analysis, expt C=37.10 H=7.30 N=21.47 S=16.02; cal C=37.48 H=7.34 N=21.86 S=16.88; infrared analysis, C=O = 1648 cm^{-1}, C=N = 2074 cm^{-1}, N-H = 2925 cm^{-1}, ^{1}H NMR $H(4,s)_{amine}$ = 3.29ppm, $H(4,s)_{acid}$= 1.48ppm, $H(4,s)_{acid}$= 2.13ppm, ^{13}C NMR C=N = 183.86ppm, C=O= 135.64ppm; $(C-H)_{amine}$=36.78ppm, $(C-H)_{acid}$= 24.69, 35.8 ppm ;UV λ_{max} = 223nm; Molar conductivity (Λ_m): 433.29 oh $m^{-1}cm^2mol^{-1}$

En-Fumarate(3): percentage yield = 73%; melting point = 483-483.5K; elemental analysis, expt C=37.10 H=6.51 N=15.63; cal C=40.91 H=6.87 N=15.90; infrared analysis, C=O = 1659 cm^{-1}, N-H = 2721 cm^{-1}, ^{1}H NMR $H(4,s)_{amine}$ = 3.29ppm, $H(2,s)_{acid}$= 6.45ppm, ^{13}C NMR C=O= 133.49ppm; $(C-H)_{amine}$=36.65 ppm, $(C-H)_{acid}$= 135.43; UV λ_{max} = 218nm. Molar conductivity (Λ_m): 265.72 oh $m^{-1}cm^2mol^{-1}$

En-Succinate(4): percentage yield = 40%; melting point = 462-463K; elemental analysis, expt C=41.95 H=7.32 N=10.57; cal C=40.54 H=6.80 N=9.46; infrared analysis, C=O = 1665 cm^{-1}, N-H = 2932 cm^{-1}, ^{1}H NMR $H(4,s)_{amine}$ = 2.86ppm, $H(4,s)_{acid}$= 2.17ppm, ^{13}C NMR C=O= 131.64ppm; $(C-H)_{amine}$=34.17ppm, $(C-H)_{acid}$= 22.87; UV λ_{max} = 224nm. Molar conductivity (Λ_m): 254.31 oh $m^{-1}cm^2mol^{-1}$

En-Azelate-SCN(5): percentage yield = 55%; melting point = 420-421.5K; elemental analysis, expt C=43.12 H=8.40 N=18.50 S=14.92; cal C=42.23 H=8.03 N=19.70 S=15.00; infrared analysis, C=O = 1715 cm^{-1}, C=N = 2074 cm^{-1}, N-H = 2933 cm^{-1}, ^{1}H NMR $H(4,s)_{amine}$ = 3.35ppm, $H(8,s)_{acid}$= 1.27ppm, $H(4,s)_{acid}$= 1.53ppm, $H(2,s)_{acid}$= 2.23ppm, ^{13}C NMR C=N = 183.61ppm, C=O= 133.50ppm; $(C-H)_{amine}$=36.60ppm, $(C-H)_{acid}$= 25.67, 28.41, 28.65 and 37.1 ppm ; UV λ_{max} = 216nm; Molar conductivity (Λ_m): 391.70 oh $m^{-1}cm^2mol^{-1}$

En-Sebacate-SCN(6): percentage yield = 80%; melting point = 32.5K; elemental analysis, expt C=43.75 H=8.08 N=18.62 S=13.62; cal C=43.61 H=8.24 N=19.07 S=14.55; infrared analysis, C=O = 1720 cm^{-1}, C=N = 2079 cm^{-1}, N-H = 2929 cm^{-1}, ^{1}H NMR $H(4,s)_{amine}$ = 3.34ppm, $H(4,s)_{acid}$= 2.17ppm, $H(4,s)_{acid}$= 1.52ppm, $H(8,s)_{acid}$= 1.27ppm, ^{13}C

NMR C=N = 182.05ppm, C=O= 133.47ppm; $(C\text{-}H)_{amine}$=35.90ppm, $(C\text{-}H)_{acid}$= 25.17, 28.17, 28.52 and 36.6 ppm ;UV λ_{max} = 220nm; Molar conductivity (Λ_m): 384.35 oh $m^{-1}cm^2mol^{-1}$

Biological studies

Among the six chemically synthesized ethylenediammonium-dicarboxylate salts evaluated against the eight bacteria and two fungi, ethylenediammonium azelate thiocyanate salt (5) showed the highest activity against *Candida albicans (*inhibition zone =10± 0.57*)*, gram positive *Bacillus Subtilis (*inhibition zone =9.3 mm*)* and also gram negative *E. coli (*inhibition zone =9± 0.57 mm*)*, respectively. Cytotoxic study in Vero cell by MTT assay with CC59 values within 1 mg/ml to 25 mg/ml indicates the low toxicity of the ethylenediammonium-dicarboxylate salts compared to curcumin as positive control.

IV. Conclusion

A series of ethylenediammonium-dicarboxylate salts have been successfully synthesized. A multi-component structure of ethylenediammonium-dicarboxylate-thiocyanate was obtained except, in the case of fumaric and succinic acid. The molar conductivities of the salts were between 218 and 453 $ohm^{-1}cm^2mol^{-1}$ with maximum value of 453 for ethyelediammonium hemioxalate thiocyanate (1) salt. The salts showed low antibacterial activity against eight tested bacteria and two fungi. The synthesized products were not a cytotoxicity to Vero cells.

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