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Synthesis and Characterization of Some Novel Tranexamic Acid Derivatives and Their Copper (II) Complexes

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The objective of this investigation was to synthesize and characterize some substitutive derivatives of Tranexamic Acid (TA) and their copper (II) complexes. N-Phthaloyltranexamic Acid (A-1), N-Acetyltranexamic Acid (A-2), Di-Tranexamate Diaquo Copper (II) (C-1), Di-N-Phthaloyltranexamate Diaquo Copper (II) (C-2) and Di-N-Acetyltranexamate Diaquo Copper (II) (C-3) were synthesized, using novel and reproducible procedures. These compounds were characterized by exploiting the techniques such as Fourier Transform Infra Red (FTIR) Spectroscopy, Mass Spectroscopy (MS) and FT ^{1}H NMR. Different methods, reported in the literature, have successfully been applied for qualitative and structural characterization of these compounds. C-1 showed unidentate bonding of carboxylic group to copper (II) while C-2 and C-3 indicated bidentate bonding of carboxylate group to copper (II).

Key words: Tranexamic acid, derivatives, copper complexes, spectroscopic techniques

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Introduction

Tranexamic acid (Trans-4-aminomethylcyclohexane carboxylic acid-C_8 H_{15} NO_2) is the derivative of amino acid lysine. This drug inhibits the proteolytic activity of plasmin and the conversion of plasminogen to plasmin by plasminogen activators. It is used for its antiplasminic, hemostatic, antiallergic and anti-inflammatory activities (Anthony *et al*., 1988; Nakanishi, 1999)

A review of the literature revealed that the substitutive derivatives of some drugs show different and in most of the cases, more effective activities than their parent compounds (Daidone *et al*., 1989; Ahmad *et al*., 2000; Pang *et al*., 1998). In another investigation it was found that the O-heterocyclic substituted salicylamide are more effective than salicylamide and in some cases less toxic (Fahmy and El-Eraki, 2001). As a part of our continuing research we have found that copper complexes of non-steroidal anti-inflammatory drugs (NSAID) are more effective than their parent drugs (Khan *et al*., 1997; 1997a).

Therefore, interest was emphasized in this investigation to synthesize some new and novel phthaloyl and acetyl derivatives of Tranexamic acid. Moreover, copper (II) complexes of the drug and their derivatives were also synthesized. In order to ascertain if the newly synthesized derivatives and/or the copper complexes would offer some better and different activities than their parent compound, comparative evaluation of their biological and pharmacological activities were performed.

Materials and Methods

Materials

Phthalic anhydride, sodium hydroxide (NaOH) and copper sulfate ($CuSO_4$) (Merck-Germany) were used as such without further purification. Tranexamic acid (TA) was a gratis supply from Tabros & Organon Pharmaceuticals (Pvt.) Ltd. Methanol (CH_3OH), ethanol (CH_3CH_2OH), chloroform ($CHCl_3$), acetone (CH_3COCH_3), methyl acetate ($CH_3COOC_2H_5$) and acetic anhydride (CH_3CO-O-CO-CH_3), etc. were of analytical grade.

All the experimental work i.e. synthesis of the derivatives and complexes including melting point and solubility determination were carried out in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gomal University, D. I. Khan.

Infrared absorption spectra were recorded on Fourier Transform IR (FTIR) spectrophotometer (Medic Corporation prospect-IR U.S.A.). Mini Press KBr (Potassium bromide) disc was used to prepare the transparent disc of samples. The major and important peaks are reported in cm^{-1}. FTIR measurements were carried out at Ferozsons Laboratories LTD, Nowshera.

^{1}HNMR spectra were recorded on Multinuclear FT NMR 400 MHZ (Brucker Co, model AM-400). All the chemical shifts are given in ppm relative to Trimetylsulfoxide (TMS) as an internal standard.

Mass spectra were recorded using model MAT 112 and 113, Doubled Focusing Mass Spectrophotometer (Finnigan) connected to IBM B at compatible PC based system. FT^{1}H NMR and Mass spectrophotometric measurements were carried out at H. E. J. International Research Institute of Chemistry, University of Karachi, Karachi.

Melting points were determined on Reichert Thermo-var (F.G. Bode Co., Austria) by taking crystals of the samples on a cover slip.

Digital pH/MV meter, model Nova B 210° C (Nova Scientific Co. Ltd., Korea) was used for pH measurement of the samples.

In vitro bactericidal, fungicidal, anti-yeast bioassay, effects of the derivatives on the blood pressure of anesthetized rats, analgesic activity, *in vitro* phytotoxic (anti-tumor) activity (potato disc assay) of the samples were made at H. E. J. International Research Institute of Chemistry, University of Karachi, Karachi.

Synthesis of N-phthaloyltranexamic Acid (A-1): Tranexamic acid (TA) was made to react with phthalic anhydride and the derivative was synthesized according to the reported procedure of N-phthaloyl amino acids (Daniel *et al*., 1975).

Equimolar quantities of TA and phthalic anhydride reagents were uniformly mixed and the mixture was transferred into a test tube. The test tube was immersed in paraffin oil bath which was pre-heated at 200°C. The reaction mixture started melting in the test tube. When the reaction was completed, the test tube was left undisturbed for 10 minutes in the oil bath at a constant temperature of 200°C. The test tube was then removed from the oil bath and allowed it to cool until the liquid mass became solidified. The solid mass, which was actually, N-phthaloyltranexamic acid (A-1), was dissolved in CH_3OH. This solution was concentrated in a flask on a heating plate with continuous stirring and was left undisturbed to crystallize out. The white crystals of the product were recovered on the next day by filtration process.

Synthesis of N-acetyltranexamic Acid (A-2): 1.00g of TA (6.3661 mM) was added into 10ml of CH_3-CO-O-CO-CH_3 and refluxed for two hours. A clear solution was obtained which was then cooled at room temperature. The 30ml of distilled water was added into this solution. Two layers were formed. The upper aqueous layer was separated and then concentrated by evaporation. The concentrated solution was left undisturbed for crystallization. After a period of 2 days white crystals of the required product were recovered by filtration, which were then re-crystallized from CH_3OH.

Synthesis of Di-tranexamate Diaquo Copper (II) (C-1): 0.500g (3.18 mM) of TA was dissolved in 50 ml of distilled water. The pH of the solution was 7.10. Then 0.1272g (3.18 mM) of NaOH was added to this solution, with constant stirring. The pH of the solution was raised to 9.50. It was a sodium salt of TA. In a separate beaker, 0.3971g (1.591 mM) of $CuSO_4.5H_2O$ was dissolved in 50 ml of distilled water. The pH of the solution was 5.03. It was poured into the burette and was added dropwise to the sodium salt of TA, while stirring constantly, for about half an hour. A blue precipitated product was obtained with a pH value of 6.95, which was filtered off, washed with distilled water and dried.

Synthesis of Di-N-phthaloyltranexamate Diaquo Copper (II) (C-2): 0.5g (1.7413 mM) of A-1 was taken. 50mL of distilled water and 5mL of 1N NaOH solutions were added into it with constant stirring, resulting in the formation of a clear solution having a pH value of 9.53.

0.2174 g (0.871 mM) of $CuSO_4.5H_2O$ was dissolved in 50mL of distilled water; the pH of the solution was 5.03. This $CuSO_4$ solution was added dropwise into the drug solution while stirring constantly for about half an hour. A blue precipitated product with a pH value of 6.60 was obtained which was filtered off, washed with distilled water and dried.

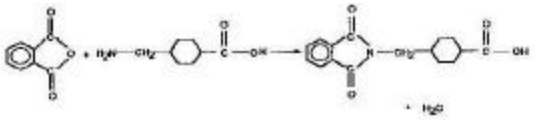
Synthesis of Di-N-acetyltranexamate Diaquo Copper (II) (C-3): 1.00 g (5.022 mM) of **A-2** was taken in a conical flask; 50mL of distilled water and 5mL of 1N NaOH solution was added into it with constant stirring. A clear solution was obtained having a pH value of 9.55.

By dissolving 0.6269g (2.51; 1mM) of $CuSO_4.5H_2O$ in 50ml of distilled water, a solution with pH 5.06 was obtained. This copper sulfate solution was added dropwise into the drug solution with constant stirring. The mixture was stirred well for about half an hour. A light green precipitated product was obtained, with a pH value of 6.17, which was filtered off, washed with distilled water and dried.

Qualitative Metal Analysis of Complexes : Whether, the metal cations are free or complexed in nature, elemental identification tests were carried out on the metal complexes with or without decomposition. Without decomposition tests failed, indicating the complexed nature of metal cations. To make it free metal complexes were decomposed. For this purpose, small amount of complex was taken in a china dish. A few drops of conc. HNO_3 and HCl were added into it and the mixture was heated up to dryness by evaporation. Into the dry residue, a few more drops of conc. HCl were added and evaporated again to dryness. The dry residue was dissolved in distilled water and the resultant aqueous solution was used to perform the Cu identification tests (Rao *et al*., 1998). The biological and pharmacological evaluation of the derivatives and the complexes will be published separately.

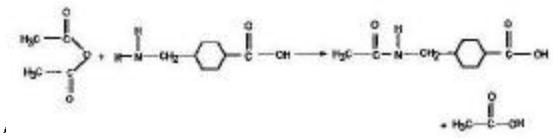
Results and Discussions

Synthesis: The synthesis of A-1 took place through a chemical reaction between TA and phthalic anhydride in 1:1 molar ratios. The yield was 81.6 % and the chemical reaction is as follows:

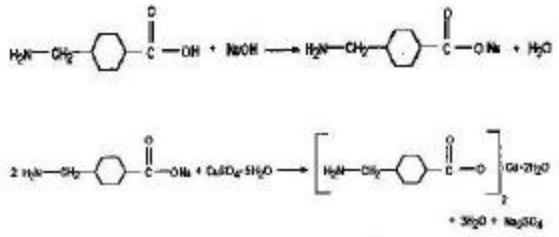


Phthalic anhydride TA A-1 (81.6%)

The synthesis of A-2 took place through a chemical reaction between TA and acetic anhydride in 1:1 molar ratios. The yield was 83.2 % and the chemical reaction is as follows:



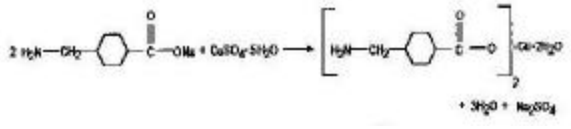
It is reported (Rao *et al*., 1998) that Cu reacts with Carboxylic acid to form copper (II) complexes with a molar ratio of 2:1. The synthesis of C-1 took place through the chemical reaction between TA and $CuSO_4$ in 2:1 molar ratio of the two constituents. The yield was 82% and the chemical equation is as follows:



Sodium salt of TA *C.1* (82 %)

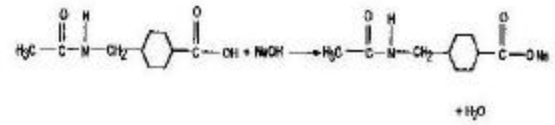
C-2 was synthesized through a chemical reaction carried out in 2:1 molar ratio of A-1 and copper sulfate. The yield was 79% and the chemical equation is as follows:

A-1 Sodium salt of A-1

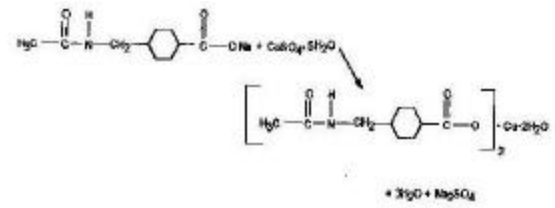


Sodium salt of T. A. C-1 (79 %)

C-3 was synthesized through a chemical reaction carried out in 2:1 molar ratio of A-2 and $CuSO_4$. The yield was 78%. The chemical equation is as follows:



A-2 Sodium salt of A-2



Sodium salt of A-2 C-3 (78 %)

Qualitative Metal Analysis of Complexes:

Tests for copper: A dark-blue color was developed upon addition of excess of NH_4OH to the aqueous solution obtained through the qualitative metal analysis of the complexes, this confirms the presence of copper in the complexes.

Addition of a few drops of potassium ferrocyanide to the aqueous solution gave red color, which further confirms the presence of copper.

Characterization Techniques

Physical Nature: Physical properties such as melting points and solubility of the TA derivatives and the copper (II) complexes are given in Table 1. As shown, the melting points of A-1, A-2, C-1, C-2 and C-3 are totally different than that of the TA (286-290 °C). This may indicate the confirmation about the synthesis of the respective derivatives and complexes.

2. Spectroscopic Studies: The I. R. data and vibrational behavior of the derivatives and the copper (II) complexes are given in Table 2. The IR spectra of A-1 showed bands for phthalimide group ($-C_2O_2N$) at 1770 cm^{-1} and for carbonyl of an acid (COO) at 1710-1670 cm^{-1} and 1320 cm^{-1}. The carboxylate (COOH) band was seen at 3550-3400 cm^{-1}. The NH_2 broad band at 3550-3300 cm^{-1} for TA had disappeared from the I.R. spectra of A-1, which indicates confirmation of the synthesis of the derivative.

Table 1: Physical properties of the Derivatives and Cu (II) Complexes

Comp. No.	Mol. formula	Odour, colour and Physical state	Melting points	Solubilities					
				H_2O	CH_3OH	C_2H_5OH	$CHCl_3$	0.1 N NaOH	Methyl Acetate
A-1	$C_{16}H_{17}NO_4$	Odourless, white crystalline	193-195°C	Insol.	Sol.	Sol.	Insol.	-----	-----
A-2	$C_{16}H_{17}NO_3$	Odourless, white crystalline	247-250°C	P. Sol	F. Sol	F. Sol.	Insol.	-----	-----
C-1	$(C_8H_{14}NO_2)_2.Cu.2H_2O$	Odourless, blue Amorphos (solid)	220°C (completely back)	Insol.	Insol.	Insol.	Insol.	Sol.	Sparingly Soluble
C-2	$(C_{16}H_{16}NO_4)_2.Cu.2H_2O$	Odourless, light blue, Amorphos (solid)	300°C (completely back)	Insol.	Insol.	Insol.	Insol.	Sol.	Sparingly Soluble
C-3	$(C_{10}H_{16}NO_3)_2.Cu.2H_2O$	Odourless, light green, Amorphos (solid)	280°C (completely back)	Insol.	Insol.	Insol.	Insol.	Sol.	Sparingly Sooluble

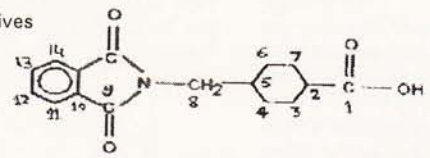
Key: A-1 = N-phthaloytranexamic acid, A-2 = N-Acetyltranexamic acid, C-1 = Copper complex of Tranexamic acid, C-2 = Copper complex of N-phthaloytranexamic acid, C-3 = Copper complex of N-Acetyltranexamic acid, Sol = soluble, Insol = Insoluble, F.Sol. = Freely soluble, P. Sol. Partially soluble. Comp. = Compound, Mol = Molecular

Table 2: I. R. Data of Derivatives and Copper (II) Complexes

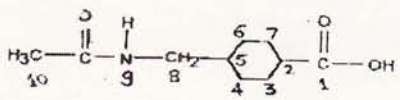
Comp. No.	Phthalamide (C_2O_2N) Asym (cm^{-1})	Phthalamide (C_2O_2N) Sym (cm^{-1})	Carbonyl of an acid (COO) Asym (cm^{-1})	Carbonyl of an acid (COO) Sym (cm^{-1})	Acetyl (CH_3-COO) (cm^{-1})	Carboxylic acid COOH (cm^{-1})	NH_2 (cm^{-1})	Cu-O (Copper Oxygen) (cm^{-1})
TA	-	-	1600-1500 (s.b)	1390 (s)	-	3100-2400 (b)	3550-3320 (b)	-
A-1	1770 (sp)	1730 (w)	1710-1670 (s.b)	1320 (w)	-	3550-3400 (s.b)	-	-
A-2	-	-	1630 (s.sp)	1430 (w)	1690 (s.sp)	3110 (m)	3300 (sp)	-
C-1	-	-	1625 (w)	1350 (sp)	-	2860 (w)	3550-3150 (s.b)	815 (w)
C-2	1630 (s)	1580 (w)	1550 (sp)	1360 (sp)	-	3520-3200 (s.b)	-	800 (w)
C-3	-	-	1530 (w)	1450 (w)	1640 (s.b)	-	-	815 (m)

Key: A-1 = N-phthaloytranexamic acid, A-2 = N-Acetyltranexamic acid, C-1 = Copper complex of Tranexamic acid, C-2 = Copper complex of N-pathaloytranexamic acid, C-3 = Copper complex of N-Acetyltranexamic acid, T.A = Tramnexamic acid, s = strong, b = broad, sp = sharp, w = weak, m = medium, Asym = Asymmetric, sym = symmetric

Table 3: ^{1}H NMR Data of the Derivatives



A-1



A-2

Proton No.	A-1 (δppm)	A-1 (δppm)
2	2.17-2.27 (m)	2.10-2.17 (m)
3,7	1.9-2.05 (m)	1.32 - 1.51 (m)
4,6	1.7-1.82 (m)	-
5	1.31 - 1.41 (m)	2.17 - 2.21 (m)
8	3.50 - 3.52 (d)	2.99 - 3.03 (m)
9	-	7.80 - 7.82 (b)
10	-	1.93 (s)
11,12	7.75-7.82 (m)	-

Key: A-1 = N-phthaloytranexamic acid, A-2 = N-Acetyltranexmic acid m = multiplet, d = dublet, s = singlet

The I.R spectra of A-2 showed stretching for NH at 3300 cm^{-1}, acetyl (CH_3-C= O) at 1690 cm^{-1} and for carbonyl of an acid (COO) at 1630 and 1430 cm^{-1}, indicating the confirmation of A-2 synthesis.

The I. R. spectra of C-1 showed that the broad peak between 3100 to 2400 cm^{-1} in the spectra for TA, which was due to the presence of carboxylic (COOH) group, has been disappeared. This showed the linkage of Cu with the ligand (TA) through the hydroxyl (OH) bond of COOH group. Furthermore, the bands associated with v_{asym} and v_{sym} (COO) mode were stretched at range 1625 and 1350 cm^{-1}, respectively. Comparing the v_{asym} and v_{sym} vibration values of the complex (C-1) with that of the ligand, it was found that v_{asym} values were raised and v_{sym} values were lowered for the complex. This indicates the unidentate or asymmetric bonding of carboxylate group to the Copper (II).

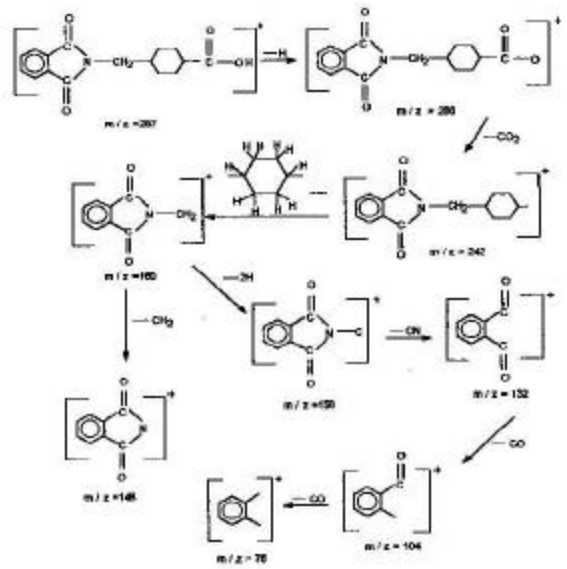
In case of C-2, the I. R spectra showed that the sharp broad peak of A-1 at 3550-3400 cm^{-1}, which was due the presence of COOH group, has been disappeared. This shows the linkage of copper with the phthaloyl-derivative of the ligand through the COOH group. Moreover, two bands of phthalimide (-C_2O_2N) were seen at range of 1630 and 1580 cm^{-1} for asymmetric and symmetric vibration, respectively. The v_{asym} and v_{sym} vibration values of the C-2 complex were compared with that of its ligand (A-1). The v_{asyn} values were lowered and the v_{asym} values were raised for the C-2, which indicates the bidentate or symmetric bonding of carboxylate group to the Cu (II).

While in case of C-3, the I.R spectra showed that the medium peak of A-2 at 3110 cm^{-1}, which was due to the presence of COOH group, has been totally disappeared. This shows the linkage of Copper (II) with its ligand (A-2) through the carboxylate (COO) group. Moreover, the acetyl (CH_3-C= O) band was seen at 1640 cm^{-1}. The bands associated with v_{asym} and v_{sym} (COO) modes were stretched at range 1530 and 1450 Cm^{-1}, respectively. The v_{asym} and v_{sym} vibration values of the C-3 were compared with the values of its ligand (A-2). The v_{asym} values were lowered and the v_{sym} values were raised for the complex, which indicated the bidentate or symmetric bonding of carboxylate group to the Copper (II).

^{1}H-NMR Spectroscopic Studies: The results regarding the chemical shift of protons of A-1 and A-2, as revealed from their ^{1}HNMR spectra, are given in Table 3. In case of A-1, the protons No. 11 and 12 of Phthalic ring were seen in range 7.82-7.75 ppm and it was a multiplet (Thorntonton and Collet, 1979). The proton No. 2 was seen in range 2.27-2.17 ppm values and was seen as multiplet peaks. The protons No. 3, 7 and 4, 6 were seen in range 2.05-1.9 and 1.82-1.7 ppm values. They were not properly resolute since they were overlapped with each other. The proton No. 5 was seen in range 1.41-1.31 ppm values and was seen as multiplet peaks. The proton No. 8 was seen in the range 3.52-3.50 ppm values and was seen as doublet peak.

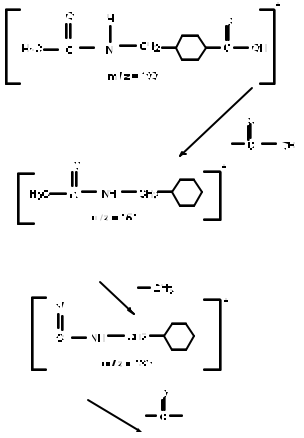
In the case of A-2 the proton No. 2 was seen in range 2.17-2.10 PPM values and it was not properly resolved. The protons No. 3 and 7 were seen in the range 1.51-1.32 ppm values. They were not properly resolute since they were overlapped with each other (Pavia *et al*., 1979). The proton No.8 was seen in range 3.03-2.99 ppm values and was seen as multiplet. The proton No.9 was seen in range 7.82-7.80 ppm values and was seen as broad doublet peak. The proton No. 10 was seen in range 1.93 ppm value and it appeared as a singlet peak.

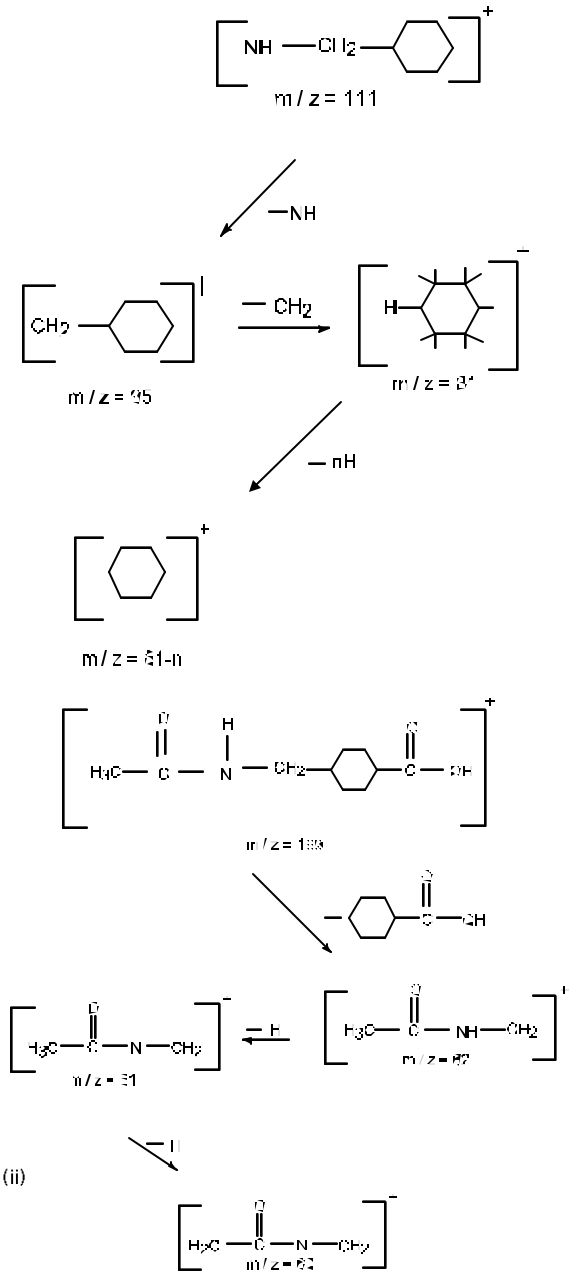
Mass Spectroscopic Studies: A-1 (C_{16} $H_{17}NO_4$) produced intense molecular ion peaks. As shown below, the most important fragmentation pathways involved the loss of H to form $C_{16}H_{16}$ NO_4^+ ion (m/z = 286), followed in sequence, by loss of CO_2 to form $C_{15}H_{16}NO_2^+$ ion (m/z = 242), loss of C_6H_{10} to form C_9H_6 NO_2^+ ion (m/z = 160), loss of two H to form C_9H_4 NO_2^+ ion (m/z = 158), loss of CN to form C_8H_4 O_2^+ ion (m/z = 132), loss of CO to form $C_7H_4O^+$ ion (m/z = 104), and loss of CO to form $C_6H_4^+$ ion (m/z = 76). The C_9H_6 NO_2^+ ion (m/z = 160) may also involved the loss of CH_2 to form $C_8H_4NO_2^+$ ion (m/z = 147–148).



peaks. The most important fragmentation pathways involved the loss of COOH to form $C_9H_{16}NO^+$ ion (m/z = 154), followed in sequence, by loss of CH_3 to form $C_8H_{13}NO^+$ ion (m/z 139), loss of CO to form C_7H_{13} NO^+ ion (m/z = 111), loss of NH to form $C_7H_{12}^+$ ion (m/z = 95), and loss of CH_2 to form $C_6H_{10}^+$ ion (m/z = 81). The fragmentation pathways are given as follows:

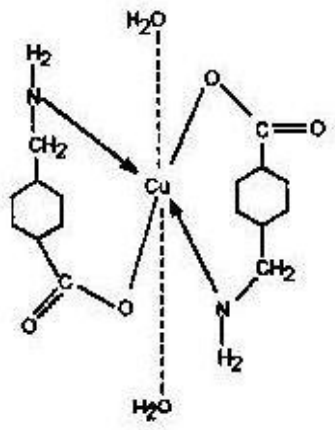
(I)



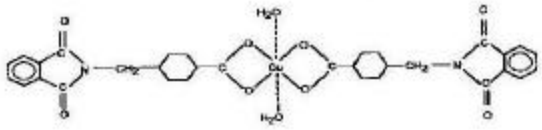


From the investigation, we concluded that, A-1 and A-2 were synthesized from TA Their synthesis was confirmed by physical methods and through various spectroscopic techniques.

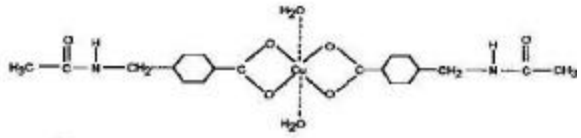
In the second stage, C-1, C-2 and C-3 were synthesized from their respective ligand i.e. T-A, A-1 and A-2, respectively and their synthesis was confirmed by physical method and through spectroscopic techniques. C-1 indicated unidentate bonding of carboxylate group to copper (II). While C-2 and C-3 indicated bidentate bonding of carboxylate group to copper (II). The structures of Copper (II) Complexes are as follows:



Structure of C-1



Structure of C-2



Structure of C-3

Acknowledgment

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