**PREPARATION, CHARACTERIZATION AND ANTI MICROBIAL STUDIES OF SOME HYDRAZINUM CARBOXYLATES**

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**ABSTRACT**

Some new hydrazinium salt of aromatic carboxylic acids have been prepared by neutralization of acid with hydrazine hydrate in aqueous medium and characterized by Analytical, IR spectral and TG-DTA analysis. All compounds undergo two, three or four step decomposition yielding carbon residue as the final product. The in vitro antibacterial screening of 2,4-dichlorophenoxyacetic acid and its hydrazinium salt against *Escherichia coli* have been investigated. The antibacterial activity of the prepared hydrazinium salt shows more promising activity than the free acid.

**Keywords:** Hydrazinium salt, Aromatic carboxylic acids, IR spectral.

1. **INTRODUCTION**

Hydrazine is the simplest diamine and forms salt with mineral and carboxylic acids (Vogel, 1962; Yasodhai and Govindarajan, 1999). The preparation of hydrazinium salts has become a subject of recent interest due to their wide use as additives in propellants, drugs to treat cancer and Hodgkin's diseases and explosives (Vogel, 1962). They have also been used as ligands for the preparation of metal hydrazinium/hydrazine complexes (Patil et al., 1980, Govindarajan et al., 1986 a and b; Chandra and Singh, 1983). Some of these salts are used as flame– retardants (Schmidt, 1984; Balague et al., 2001) and proton conductors (Patil et al., 1979). Only few of these salts show antibacterial activity (Govindarajan, et al., 1980). Preparation and thermal behaviour of some salts from few aliphatic acid (Patil et al., 1981) and aromatic carboxylic acids (Allan, et al., 1998; Vairam and Govindarajan, 2004) have been reported. There is no literature citations about hydrazinium salt of aromatic substituted acetic acids, aromatic unsaturated acids and hetero acids except hydrazinium salt of pyrazine mono and di-carboxylic acids (Premkumar and Govindarajan, 2006). It is therefore, considered interesting to prepare hydrazinium salt of aromatic substituted acetic acids namely 2,4-dichlorophenylacetic acid, phenoxy acetic acid, 2,4-dichlorophenoxyacetic acid and Diphenylacetic acid, aromatic unsaturated acid namely cinnamic acid, hetero acids namely picolinic acid and nicotinic acid.

Generally all phenolic derivatives show antibacterial property. Particularly 2,4-dichlorophenoxyacetic acid altered envelope properties of the bacteria *Escherichia coli*, such as hydrophobic index Unsubstituted phenoxyacetic acid is also a phenolic derivative. But, it has no potent substitutes to have antibacterial property. 2,4-dichlorophenoxyacetic acid contain two potent chloro substituents. It influences it to have antibacterial property like chloroxylenol (4-chloro-2,5-Xylenol) which acts as antiseptic as well as disinfectants. This prompted us to make antibacterial study of hydrazinium salt of 2,4-dichlorophenoxyacetic acid against *Escherichia coli*.

The structures of acids and their designations used are shown below for clarity

![Structures of Acids](image)

2. **EXPERIMENTAL**

All the salts reported have been prepared by the neutralization of the respective carboxylic acids in aqueous medium with hydrazine hydrate (99 - 100 %) in appropriate molar ratios.
2.1. Preparation

2.2.1. Hydrazinium 2,4-dichlorophenyl acetate

\[
\text{[CH-COO}^+ \text{Cl}]_2 \text{(N}_2\text{H}_3\text{)}_2\text{N}_2\text{H}_6^{2+}
\]

This is prepared by mixing hydrazine hydrate and 2,4-dichlorophenylacetic acid in 2:3 molar ratio in 50 mL of distilled water. The resulting turbid solution is heated over water bath to obtain clear solution and concentrated to nearly 20 mL. Then it is allowed to crystallize at room temperature. Light yellow coloured hydrazinium salt is crystallized out after 24 hours. The crystals are filtered off and washed by using benzene and air dried.

2.2.2. Hydrazinium phenoxyacetate

\[
\text{[O-CH}_{2}-\text{COO}^+ \text{Cl}]_2 \text{(N}_2\text{H}_3\text{)}_2\text{N}_2\text{H}_6^{2+}
\]

This is also prepared by the same procedure as above by mixing the acid with the base in the molar ratio of 2:3. Spongy white coloured salt is crystallized within 20 minutes. The product is washed by using benzene and air dried.

2.2.3. Hydrazinium 2,4-dichlorophenoxy acetate hydrate

\[
\text{[O-CH}_{2}-\text{COO}^+ \text{Cl}]_2 \text{(N}_2\text{H}_3\text{)}_2\text{N}_2\text{H}_6^{2+} \text{H}_2\text{O}
\]

The hydrated salt is also prepared by the same procedure by mixing hydrazine hydrate and 2,4-dichlorophenoxyacetic acid with molar ratio 1:1. White coloured salt is crystallized out immediately. This is washed by using alcohol and air dried.

2.2.4. Hydrazinium diphenyl acetate hydrate and hydrazinium cinnamate hydrate

\[
\text{[CH-CH}_{2}-\text{COO}^+ \text{H}_2\text{O}]	ext{ and [CH=CH-CH}_{2}-\text{COO}^+ \text{H}_2\text{O}}
\]

These are prepared by mixing hydrazine hydrate and Diphenyl acetic acid or cinnamic acid in 1:1 molar ratio in 50 mL of water. The contents of the beaker are heated on water bath. Then the undissolved acid is removed by filtration. The resulting clear solution is concentrated on water bath to 20 mL. Then it is allowed to crystallize at room temperature. While the white coloured monohydrated hydrazinium(+1) salt of cinnamic acid separated out after 24 hours, whereas light yellow coloured monohydrated hydrazinium (+1) salt of Diphenylacetic acid separated out after 48 hours, they are filtered off and washed by using alcohol and air dried.

2.2.5. Hydrazinium picolinate and Hydrazinium nicolinate

\[
\text{[N}_2\text{H}_6^{2+} \text{COO}^- \text{H}_2\text{O}]	ext{ and [N}_2\text{H}_6^{2+} \text{COO}^- \text{H}_2\text{O}}
\]

These are prepared by mixing hydrazine hydrate and picolinic acid or nicotinic acid with 1:1 molar ratio in 50 mL of distilled water. The resulting clear solution is concentrated on water bath to 20 mL. Then it is allowed to crystallize in a vacuum desiccator over calcium chloride. Light yellow coloured hydrazinium(+2) salt of picolinic acid separated out after 2 days, filtered and washed by using alcohol, whereas white coloured hydrazinium(+2) salt of nicotinic acid is also separated out after 2 days, filtered and washed by using ether.

2.2.6. Physico-Chemical techniques

The hydrazine content of these salts are determined volumetrically using a standard (0.025 m) KIO₃ solution under Andrew’s condition [17]. IR spectra are recorded as KBr pellets with a Shimadzu spectrophotometer in the range 4000-400 cm⁻¹. Elemental analyses are performed on a Perkin-Elmer 240 B CHN analyzer. Simultaneous TG-DTA measurements are carried out using STA 1500 thermal analyzer. All thermal analyses are carried out in air at a heating rate of 10°C per minute. Platinum cups are used as sample holders and alumina as reference.

The microorganism used to test the biological potential of 2,4-dichlorophenoxyacetic acid and its hydrazinium(+1) salt is Escherichia coli, obtained from the stock cultures of the Biotechnology Laboratory of the Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore, India.

2.2. Antibacterial activity

The antibacterial activity of 2,4-dichlorophenoxyacetic acid and its hydrazinium salt are determined by the disc diffusion method.
(Cruickshank, 1968). The bacteria are cultured in nutrient agar medium and used as inoculum for the study. Bacterial cells are swabbed on to nutrient agar medium (prepared from NaCl (5.0g), peptone (5.0g), beef extract powder (3.0g), yeast extract powder (3.0 g), agar (20.0 g) in 100 mL distilled water, pH = 7.5 ± 0.2) in Petri dishes. The test solutions are prepared in distilled water to a final concentration of 2% and 4% and then applied to filter paper discs (Whatmann/No. 4.5 mm dia). These discs were placed on the already seeded plates and incubated at 35±2°C for 24hr. the zone of inhibition around the discs are measured after 24hr. Co-trimoxazole is used as a standard positive control.

3. RESULTS AND DISCUSSION

The analytical data of the salts (Table 1) are consistent with the proposed formulae for them.

3.1. Infrared spectra

The important IR bands of the salts are listed in Table 2. The IR spectra of the hydrated salts display absorption bands in the region of 3346 - 3330 cm⁻¹ due to O-H stretching of water molecule. The bands in the region of 1390-1323 cm⁻¹ and 1598-1521 cm⁻¹ for these salts are corresponds to symmetric and asymmetric stretching frequencies of the carboxylate ions. The N-N stretching frequencies of N₂H₅+ ion appear in the range of 963-951 cm⁻¹ and the N-N stretching frequencies of N₂H₆²⁺ ion shows bands in the region of 1047 - 1026 cm⁻¹.

3.2. Thermal analysis

3.2.1. Hydrazinium 2,4-dichlorophenyl acetate

\[
\text{CH} = \text{CH-CH} = \text{CH} \quad (\text{N}_2\text{H}_5)^+ \text{N}_2\text{H}_6 \quad 2+ \\
\text{Cl} \quad \text{Cl} \\
\text{Cl} \quad \text{Cl}
\]

The thermogram of this salt indicates that the decomposition of the salt takes place in multi steps. In the first step, dehydration and dehydrozination occur simultaneously showing a sharp endotherm at 170°C. In the second step the acid intermediate decomposes exothermally at 285°C and 488°C to carbon residue.

3.2.2. Hydrazinium phenoxy acetate

\[
\text{O-CH} = \text{COO}^- (\text{N}_2\text{H}_5)^+ \text{N}_2\text{H}_6 \quad 2+ \\
\text{Cl} \quad \text{Cl} \\
\text{Cl} \quad \text{Cl}
\]

This undergoes decomposition in three steps. In the first step, compound undergoes melt with endothermic peak at 99°C. Then the compound decomposes to diphenyl glycol with exothermic peak at 149°C and 265°C. Finally at 327°C it decomposes to carbon residue.

3.2.3. Hydrazinium 2,4-dichlorophenoxy acetate hydrate

\[
\text{CH} = \text{CH-CH} = \text{CH} \quad (\text{N}_2\text{H}_5)^+ \text{H}_2\text{O}
\]

The thermogram of this salt indicates that the decomposition of the salt takes place in two steps. In the first step, both dehydration and dehydrozination occur simultaneously showing a sharp endotherm at 170°C. In the second step the acid intermediate decomposes exothermally at 285°C and 488°C to carbon residue.

3.2.4. Hydrazinium diphenyl acetate hydrate

\[
\text{CH} = \text{CH} \quad (\text{N}_2\text{H}_5)^+ \text{H}_2\text{O}
\]

This undergoes decomposition in three steps. First step shows an endothermic peak at 106°C corresponding to the removal of moisture. In the second step, both dehydration and dehydrozination occur simultaneously showing two exothermic peaks at 186°C and 233°C to form formic acid. In the final step, formic acid completely decomposes to carbon residue.

3.2.5. Hydrazinium cinnamate hydrate

\[
\text{CH}=\text{CH-CH} = \text{COO}^- (\text{N}_2\text{H}_5)^+ \text{H}_2\text{O}
\]

This compound also undergoes three steps decomposition. First step shows an endothermic peak at 92°C corresponding to liberation of water molecule. Second step shows on endothermic peak at 150°C due to the elimination of one molecule of hydrazine. In the third step complete decomposition to carbon residue takes place.

3.2.6. Hydrazinium picolinate and hydrazinium nicolate

\[
\text{N} \quad \text{COO}^- \quad \text{NH}^+ \quad 2+ \\
\text{N} \quad \text{COO}^- \\
\text{N} \quad \text{H}^+ \quad 2+ \\
\text{N} \quad \text{H}^+ \quad 2+
\]
Both compounds undergo two step decomposition. First step is the removal of moisture with endothermic peaks at 121°C and 117°C respectively. In the second step, the compound completely decomposes to carbon residue.

TG – DTA curves of some compounds are given (Fig 1 - 4) as representative examples.

3.3. Antibacterial activity

The antibacterial activity of the 2,4-dichlorophenoxyacetic acid and its salt are determined by disc diffusion method. From the result (Table 4) it has been observed that hydrazinium salt of 2,4-dichlorophenoxyacetic acid shows more activity than the free acid.

4. CONCLUSION

2,4-dichlorophenylacetic acid and Phenoxyacetic acid form peculiar type of hydrazinium salts similar to double salts. These type of salts are not been reported so far in the literature. They contain both hydrazinium(+1) and hydrazinium(+2) ions. Their compositions are confirmed by analytical, IR spectral and Thermal studies. In these salts ‘CH₂’ group loses H⁺ ion because of the presence of more electronegative groups on both sides and becomes carbanion and their charges are compensated by the extra groups on both sides and becomes carbanion and nicotinic acid formed as hydrazinium(+2) salts.

All compounds undergo two, three or four step exothermic or endothermic decomposition through various intermediates. The double salts have more lattice energy than the other salts. Therefore they undergo melting before decomposition as observed in TG – DTA analysis, whereas the other simple salts decomposes before melting. All the salts decompose completely to give carbon residue as the final product.

The antibacterial activity of 2,4-dichlorophenoxyacetic acid and its hydrazinium salt against Escherichia coli has been studied. The antibacterial activity of the hydrazinium salt show more promising activity than the free acid.

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### Table 1. Analytical data

| S.No | Compound                  | Colour       | Found(calculated) % | Hydrazine | Carbon | Hydrogen | Nitrogen |
|------|---------------------------|--------------|---------------------|-----------|--------|----------|----------|
| 1    | (N\textsubscript{2}H\textsubscript{5})\textsuperscript{2+}N\textsubscript{2}H\textsubscript{6}\textsuperscript{2+}O\textsubscript{2+}Cl\textsubscript{2}ClCH\textsubscript{-}COO\textsuperscript{-} | Light yellow | 19.20(19.47)       | 37.40(37.70) | 4.50(4.91) | 16.30(16.49) |
| 2    | (N\textsubscript{2}H\textsubscript{5})\textsuperscript{2+}N\textsubscript{2}H\textsubscript{6}\textsuperscript{2+}O\textsubscript{2+}Cl\textsubscript{2}ClO\textsubscript{-}CH\textsubscript{-}COO\textsuperscript{-} | White       | 24.19(24.70)       | 47.20(47.82) | 7.11(7.20) | 20.14(20.92) |
| 3    | (N\textsubscript{2}H\textsubscript{5})\textsuperscript{2+}N\textsubscript{2}H\textsubscript{6}\textsuperscript{2+}O\textsubscript{2+}Cl\textsubscript{2}ClCH\textsubscript{-}COO\textsuperscript{-} | White       | 11.21(12.14)       | 47.10(47.04) | 4.10(4.41) | 8.80(8.82) |
| 4    | (N\textsubscript{2}H\textsubscript{5})\textsuperscript{2+}N\textsubscript{2}H\textsubscript{6}\textsuperscript{2+}O\textsubscript{2+}Cl\textsubscript{2}ClCH=CH\textsubscript{-}COO\textsuperscript{-} | Light yellow | 12.00(12.55)       | 63.20(63.80) | 6.20(6.83) | 10.59(10.63) |
| 5    | (N\textsubscript{2}H\textsubscript{5})\textsuperscript{2+}N\textsubscript{2}H\textsubscript{6}\textsuperscript{2+}O\textsubscript{2+}Cl\textsubscript{2}ClCH=CH\textsubscript{-}COO\textsuperscript{-} | White       | 17.40(17.19)       | 51.00(51.19) | 7.04(7.02) | 14.85(14.05) |
| 6    | (N\textsubscript{2}H\textsubscript{5})\textsuperscript{2+}N\textsubscript{2}H\textsubscript{6}\textsuperscript{2+}O\textsubscript{2+}Cl\textsubscript{2}ClCH=CH\textsubscript{-}COO\textsuperscript{-} | Light yellow | 12.71(12.10)       | 51.29(51.38) | 2.89(2.85) | 9.96(9.99) |
| 7    | (N\textsubscript{2}H\textsubscript{5})\textsuperscript{2+}N\textsubscript{2}H\textsubscript{6}\textsuperscript{2+}O\textsubscript{2+}Cl\textsubscript{2}ClCH=CH\textsubscript{-}COO\textsuperscript{-} | White       | 12.83(12.10)       | 51.20(51.38) | 2.80(2.85) | 9.95(9.99) |

### Table 2. IR spectral data (cm\textsuperscript{-1})

| S.No | Compound                  | \(\nu\text{max}\) of water/acid | \(\nu\text{max}\) of H\textsubscript{2}O | \(\nu\text{max}\) of \(\nu\text{sym}\) (OCO) | \(\nu\text{max}\) of \(\nu\text{asym}\) (OCO) | \(\nu\text{max}\) of \(\nu\text{N-N}\) |
|------|---------------------------|-----------------------------------|------------------------------------------|---------------------------------------------|---------------------------------------------|----------------------------------------|
| 2    | (N\textsubscript{2}H\textsubscript{5})\textsuperscript{2+}N\textsubscript{2}H\textsubscript{6}\textsuperscript{2+}O\textsubscript{2+}Cl\textsubscript{2}ClCH\textsubscript{-}COO\textsuperscript{-} | - | 3276 | 1590 | 1380 | 958 |
| 4    | (N\textsubscript{2}H\textsubscript{5})\textsuperscript{2+}N\textsubscript{2}H\textsubscript{6}\textsuperscript{2+}O\textsubscript{2+}Cl\textsubscript{2}ClCH=CH\textsubscript{-}COO\textsuperscript{-} | - | 3443(b) | 1589 | 1375 | 959 | 1026 | 1047 |
Table 3. TG - DTA data

| S.No | Compound | DTA peak temp (°C) | Thermogravimetry(TG) | Decomposition product |
|------|----------|-------------------|----------------------|----------------------|
|      |          |                   | Temp range °C | Mass loss (%) | Observed | Calculated |
| 1    | \[
\text{Cl} \text{Cl} \]
\[
\text{O-CH}_2\text{COO}^+ \text{(N}_2\text{H}_3^+ \text{H}_2\text{O})
\] | 3330(s) 3072 | 25-133 5.18 | 6.49 | Melting and dehydrazination |
|      |          |                   | 1390 1328     | 951    |          |
|      |          |                   | 3172 1577     |        |          |
|      |          |                   | 1569 1339     |        |          |
|      |          |                   | 1328 951      |        |          |
| 6    | \[
\text{Cl} \text{Cl} \]
\[
\text{CH-COO}^- \text{(N}_2\text{H}_3^+ \text{H}_2\text{O})
\] | 3346(s) 3276 | 3251 1558 | 1386 | Phenol decomposition to formic acid |
|      |          |                   | 1375 953      | 1043   |          |
|      |          |                   | 3236 1521     |        |          |
|      |          |                   | 1340 1043     |        |          |
| 8    | \[
\text{CH}\equiv\text{CH-COO}
\] | 3331(s) 3245 | 3213 1560 | 1331 | Phenol decomposition to carbon residue |
|      |          |                   | 1598 1029     | 1029   |          |
|      |          |                   | 1386 1029     |        |          |
| 10   | \[
\text{CH}\equiv\text{CH-COO}
\] | 3332(b) 3251 | 3276 1583 | 1385 | Phenol decomposition to formic acid |
|      |          |                   | 1386 1043     | 1043   |          |
|      |          |                   | 3236 1521     |        |          |
|      |          |                   | 1331 1043     |        |          |
| 11   | \[
\text{N} \text{H}_2^+ \text{N} \text{H}_2^+ \text{N} \text{H}_2^+ \text{N} \text{H}_2^+
\] | - | 3265 1598 | 1386 | Phenol decomposition to carbon residue |
|      |          |                   | 1323 1029     | 1029   |          |
|      |          |                   | 3246 1544     |        |          |
| 12   | \[
\text{N} \text{H}_2^+ \text{N} \text{H}_2^+ \text{N} \text{H}_2^+ \text{N} \text{H}_2^+
\] | - | 3265 1598 | 1386 | Phenol decomposition to carbon residue |
|      |          |                   | 1323 1029     | 1029   |          |
|      |          |                   | 3246 1544     |        |          |
| No | Molecular Structure | Chemical Formula | Melting Point | Decomposition Temperature | Decomposition Products |
|----|---------------------|------------------|---------------|----------------------------|------------------------|
| 2  | ![Molecular Structure](image1) | \((N_2H_5)_2^+N_2H_6^{2+}\) | 99(+) 25-90  | 149(-) 90-234 50.41 | 47.00 | Decomposition leads to diphenyl glycol Decomposition to carbon residue |
|    |                     |                  | 149(-) 90-234  | 265(-) 234-344 97.6  | - | |
|    |                     |                  | 327(-) 234-344  | 97.6 - | - | |
| 3  | ![Molecular Structure](image2) | \((N_2H_5)_3^+H_2O\) | 170(+) 103-225 | 285(-) 103-225 18.21 | 18.39 | Dehydration and dehydrazination Complete decomposition to carbon residue |
|    |                     |                  | 285(-) 103-225  | 18.21 - | - | |
|    |                     |                  | 225-528 96 - | 96 - | - | |
| 4  | ![Molecular Structure](image3) | \((N_2H_5)_3^+H_2O\) | 106(+) 25-86 1 | 186(-) 86-354 81.51 | 82.53 | Removal of moisture Dehydration and dehydrazination leads to formic acid Complete decomposition to carbon residue |
|    |                     |                  | 233(-) 86-354 81.51 | 82.53 - | - | |
|    |                     |                  | 484(-) 354-483  | 97 - | - | |
| 5  | ![Molecular Structure](image4) | \((N_2H_5)_3^+H_2O\) | 92(+) 25-102  | 150(+) 150-481 98.41 | 9.03 | Dehydration dehydrazination Decomposition to carbon residue |
|    |                     |                  | 150(+) 150-481  | 98.41 - | - | |
|    |                     |                  | 289(-) 150-481  | 26.13 - | - | |
|    |                     |                  | 458(-) 150-481  | - | - | |
| 6  | ![Molecular Structure](image5) | \(N_2^2+\) | 121(+) 25-99 | 155(-) 99-218 87.70 | 3.69 | Removal of moisture Decomposition to carbon residue |
|    |                     |                  | 121(+) 25-99 | 87.70 - | - | |
|    |                     |                  | 191(+) 99-218  | - | - | |
| 7  | ![Molecular Structure](image6) | \(N_2^2+\) | 117(+) 25-127 | 183(-) 127 | 90.20 | Removal of moisture Decomposition to carbon residue |
|    |                     |                  | 117(+) 25-127 | 90.20 - | - | |
|    |                     |                  | 284(+) 90.20  | 90.20 - | - | |

Dehydration and dehydrazination leads to formic acid.
### Table 4. Antibacterial activity of 2,4-dichlorophenylacetic acid and its hydrazinium salt (The test solution is prepared in distilled water)

| S.No | Compound | Diameter of inhibition zone (mm) 2% | Diameter of inhibition zone (mm) 4% |
|------|----------|----------------------------------|----------------------------------|
| 1.   | ![Chemical Structure](image1) | 7                               | 10                               |
| 2.   | ![Chemical Structure](image2) | 19                              | 26                               |

Diameter of Zone of inhibition is a mean of triplicates

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**Fig 1** TG – DTA of ![Graph](image1)

**Fig 2** TG – DTA of ![Graph](image2)

**Fig 3** TG – DTA of ![Graph](image3)

**Fig 4** TG – DTA of ![Graph](image4)