

## SYNTHESIS OF BIS-QUATERNARY SALTS OF AMMONIA FROM SUCCINIC ACID AND THEIR EVALUATION AS AGROCHEMICALS

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### ABSTRACT

Quaternary ammonium salts, also known as quats, are the nitrogen containing compounds with four alkyl groups bonded to nitrogen which are highly reactive having potential in various fields like agrochemicals, pharmaceuticals, organic and bio-organic synthesis. These are applied growth retardants to control the size and shape of plants. In the present investigation, two series of eight new bis-quaternary salts of ammonia have been synthesised from succinic acid. In the first series tertiary amines were prepared by subjecting different benzaldehydes to Leuckart's reaction. In the second series, tertiary amines were prepared from different cinnamic acids through the formation of epoxy compounds with epichlorohydrin. Structures of tertiary amines thus prepared were confirmed by IR and NMR spectroscopy. These amines were then converted into their corresponding bis- quaternary salts of ammonia by reacting with succinyl chloride. Plant growth retardant activity of these salts was conducted on rice seeds (*Oryza sativa*, PR-116). All the compounds showed plant growth retardant activity. Compounds containing nitro group were found to possess enhanced retardant activity.

**KEYWORDS:** *Oryza Sativa*, Bis-Quaternary Ammonium Salts, Succinic Acid, Leuckart's Reaction

### INTRODUCTION

The control of plant size can be of great importance in agriculture. For commercial benefit, it may be important to be able to reduce the overall size of the plant. This reduction in size of the plant is achieved by growth retardants. Eight new bis quaternary salts of ammonia from different aldehydes have been synthesized and tested as plant growth retardants using seed germination and seedling growth of rice (*Oryza sativa*, PR-116) seeds as bioassay. All the tested compounds (1a-d and 9e-h) inhibited seed germination and seedling growth. Compounds (1a, 1c, 9b,9h) with nitro and chloro group (meta and ortho) attached to benzene nucleus are found to possess pronounced inhibitory effect

CHART-1

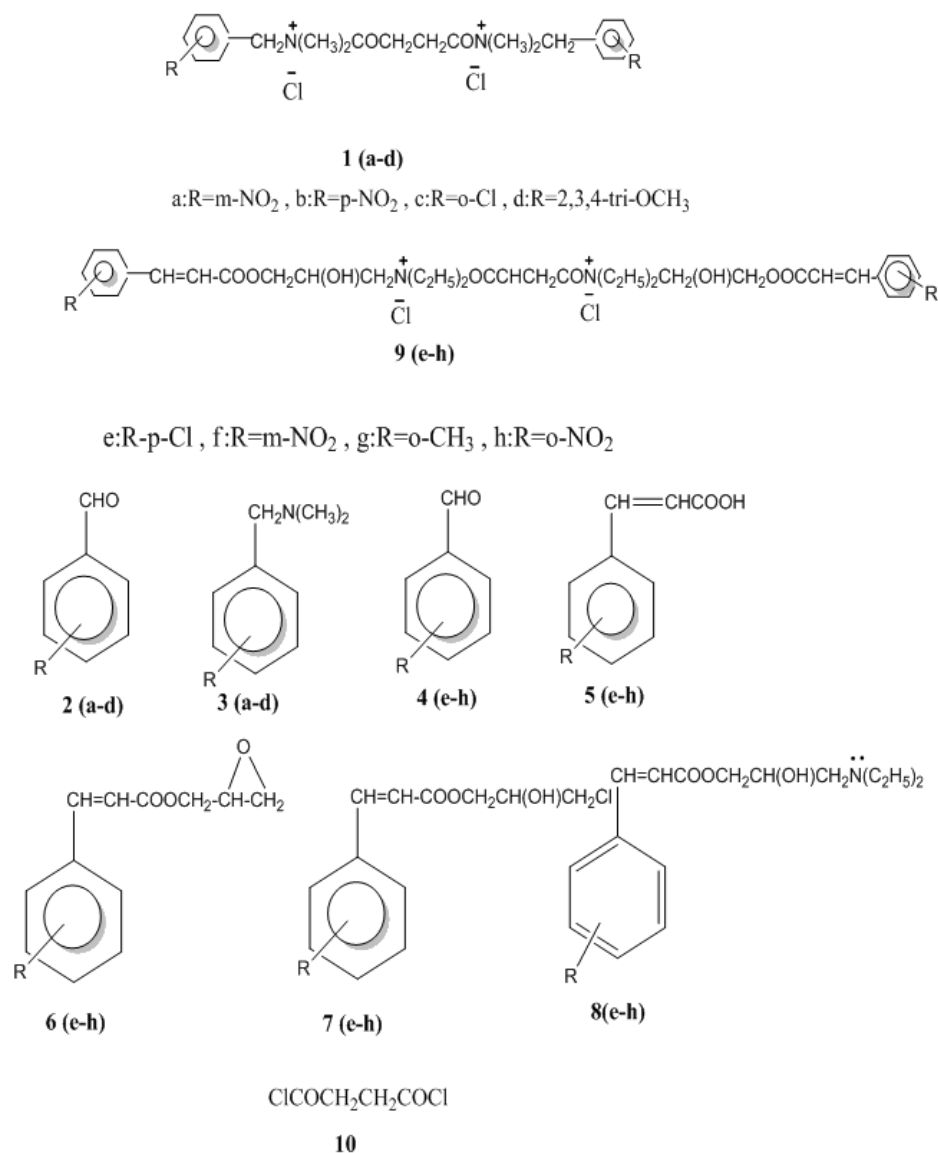


Figure 1

## MATERIAL AND METHODS

All organic extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Purity of samples was checked by TLC. IR spectra were recorded as neat liquid on Perkin Elmer-800 spectrometer. PMR spectra were taken in CDCl<sub>3</sub> on BURKER AVANCE II 400 NMR spectrometer SAIF, Panjab University, Chandigarh.

### Preparation of butan-1,4-dioylchloride(10)

Succinic acid 9g (0.05mol) was heated in a round bottom flask on water bath and thionyl chloride(22g) was added drop wise during 45 minutes. The reaction mixture was then refluxed for 30 minutes on water bath while shaking from time to time. The excess of thionyl chloride was distilled to give butan-1,4-dioylchloride (10).

**Preparation of N, N-dimethyl-3-nitrophenylmethanamine (3a)**

3-Nitrobenzaldehyde (3g, 0.02 mol), dimethylformamide (15.8g,0.21mol) and formic acid (7.2g,85%) were taken in a round bottom flask, fitted with a condenser and heated at 185-190<sup>0</sup> c for 6h on an oil bath. The homogeneous solution was added to a separating funnel containing 50ml of 10% HCl solution and aqueous aminehydrochloride was washed twice with ether. The aqueous phase was treated with 30% solution of sodium hydroxide and the tertiary amine was recovered by extracting with ether. After drying over Na<sub>2</sub>SO<sub>4</sub>, followed by distillation, tertiary amine (3a) as thick oil was recovered; yield 1.82g (50%)  $\nu_{\max}(\text{cm}^{-1})$ . 3075,2977, 2945,2822, 2778, 1614, 1529, 1458,1350,1258,1203, 1174,1148,1093,1036, 983,926, 898, 845,805.  $\delta$ . 2.26 [6H, s, -N(CH<sub>3</sub>)<sub>2</sub>], 3.50 [2H, s, -CH<sub>2</sub>-N(CH<sub>3</sub>)<sub>2</sub>], 7.5(1H, d, j=7Hz, Ar-H), 7.6(1H, d, j=7Hz, Ar-H), 8.1 (1H, d, j=9 Hz, Ar-H), 8.18 (1H, s, j=9Hz)

N, N-Dimethyl-4-nitrophenylmethanamine(3b) was prepared similarly by taking 4-nitrobenzaldehyde (4g, 0.03 mol), DMF(15.8g,0.21mol) and formic acid (85%,7.2g 0.15mol) and the yield obtained was 1.82g (45%).

N, N-Dimethyl-2-chlorophenylmethanamine (3c) was also prepared similarly by taking 2-chlorobenzaldehyde (2g,0.014mol), DMF (15.3g,0.21mol) and formic acid (85%,7.2g,0.15mol) and the yield obtained was 1.4g (58%).  $\nu_{\max}(\text{cm}^{-1})$  3066, 2974, 2943, 2856, 2818, 2771, 1596, 1444, 1362, 1256, 1174, 1150, 1122, 1097, 1033, 975, 944, 852, 752, 686.  $\delta$  2.32[6H, s, -N(CH<sub>3</sub>)<sub>2</sub>], 3.56[2H, s, -CH<sub>2</sub>N(CH<sub>3</sub>)<sub>2</sub>], 7.20-7.28 (2H, m, Ar-H), 7.36-7.38 (1H, m, Ar-H), 7.42-7.45(1H, m, Ar-H).

N,N-Dimethyl-3,4,5-trimethoxyphenylmethanamine (3d) was prepared by taking 3,4,5-Trimethoxybenzaldehyde (3.9g, 0.02mol), DMF (15.3g,0.21mol) and formic acid (85%,7.2g). yield obtained was 2.3g (51%)

**Preparation of 3-(4-Chlorophenyl)-prop-2-en-1-oic acid (5e)**

A mixture of 4-chlorobenzaldehyde (6.44g,0.03mol), malonic acid (6.2g,0.03 mol), pyridine(8ml), piperidine(2-3drops) as a catalyst were refluxed for 1h. The mixture was cooled and poured into excess of water containing enough HCl (20ml HCl+80 mlH<sub>2</sub>O) to react with pyridine. The precipitates thus formed were filtered, washed and dried to give unsaturated acid (5e), yield. 4.7g (85%), m.p.250-252<sup>0</sup>, (lit .m.p.249-251<sup>0</sup>, E isomer).

3-(3-Nitrophenyl)-prop-2-en-1-oic acid (5f) was prepared similarly by taking a mixture of 3-nitrobenzaldehyde (1.2g, 0.0083 mol), malonic acid (1.7g, 0.016 mol), pyridine (8ml) and piperidine (2-3 drops) as catalyst and the yield obtained of unsaturated acid (5f) was 1.2 g (80%), m.p.236-237<sup>0</sup> (lit m.p 239-240.5<sup>0</sup>, E isomer).

3-(2-Methoxyphenyl)-prop-2-en-1-oic acid (5g) was prepared similarly by taking a mixture of 2-methoxybenzaldehyde (4.2g, 0.03mol), malonic acid (6.2g,0.06mol), pyridine (12ml) and piperidine(2-3 drops) as catalyst and the yield obtained of acid (5g) was 7.2g (75%).

3-(2-Nitrophenyl) prop-2-en-1-oic acid (5h) was also prepared similarly by taking a mixture of 2-nitrobenzaldehyde(4.6g,0.03mol), malonic acid (3.4g,0.033mol), pyridine (8ml) and piperidine (2-3drops) as a catalyst and the yield obtained of acid (22) was 4.9g (82%) m.p 238-239<sup>0</sup> (lit m.p 249-241<sup>0</sup>), E isomer.

### Preparation of 3-N, N-Diethyl amino-2-hydroxypropyl-4-chlorophenylprop-2-en-1-oate(8e)

3-(4-Chlorophenyl)-prop-2-ene-1-oic acid(5e,3.1g), anhydrous  $K_2CO_3$  (5.4g) and epichlorohydrin (60ml) were refluxed under stirring for 13h . The reaction mixture was filtered, filtrate was concentrated and the residue was extracted with benzene. The organic phase was washed successively with 10% NaOH ,water, saturated aqueous NaCl ,dried and concentrated to afford mixture of (6e) and (7e) ,TLC (2spots) . The above mixture (4.5g) and diethyl amine (9ml) were refluxed in absolute alcohol (65ml) for 1h and alcohol was distilled off. The residue oil thus obtained was distilled under reduced pressure to give tertiary amine (8e) , yield 3.7g (40%) .

(3-N,N-Diethylamino-2-hydroxypropyl)-3-(3-nitrophenyl)-prop-2-en-1-oate(8f )was also prepared similarly by taking 3-(3-nitrophenyl)-prop-2-en-1-oic acid(5f,1.2g),anhydrous $K_2CO_3$  (7.62g) and epichlorohydrin (85ml) and the yield obtained was 1.2g (48%).

(3-N,N-Diethylamino-N-2-hydroxy propyl)-3-(2-methoxy phenyl)-prop-2-en-1-oate(8g) was also prepared similarly by taking 3-(2-methoxyphenyl)-prop-2-en-1-oic acid(5g,4.8g) anhydrous  $K_2CO_3$  (8.3g) and epichlorohydrin (90ml) and yield obtained of tertiary amine (8g) was 3.6g(40%) .

(3-N,N-Diethylamino-2-hydroxy propyl)-3-(2-nitrophenyl) prop -2 -en-1-oate (8h) was prepared similarly by taking 3-(2-nitrophenyl)-prop-2-en-1-oic acid(5h,4.9g),anhydrous $K_2CO_3$  (8.5g) and chemicals treated with 30% solution of sodium hydroxide and the tertiary amine was recovered by extracting with ether. After drying over  $Na_2SO_4$ , followed by distillation, tertiary amine (8h) as thick oil was recovered. Yield;1.82g (50%).

$\nu_{max}(cm^{-1})$  3075, 2977, 2945, 2822, 2778,1614,1529,1458, 1350,1258, 1203, 1174, 1148, 1093, 1036, 983,926,898,845,805.

General Procedure for the preparation of Quaternary Ammonium compounds of first series1(a-d) and second series 9(e-h)

A solution of butane-1,4-dioylchloride (1,0.7gm, 0.005mol) dissolved in chloroform (5ml) was added drop wise to tertiary amine (0.01mol) dissolved in chloroform (65ml) under anhydrous conditions at  $0-5^{\circ}C$  with continue stirring. After the addition was over, the mixture was stirred for 3 hrs. at the same temperature and then at room temperature for 1 hr. The removal of solvent afforded a thick oil. It was scratched with dry ether to remove, if any unreacted tertiary amine and dichloride) and decantation of the solvent afforded a semisolid bis quaternary salt of ammonia. Being hygroscopic in nature it was stored in a vacuum desiccator and its spectral studies could not be done. However it gave positive test with copper wire.

## RESULTS AND DISCUSSIONS

The present investigation includes the synthesis of eight new salts of ammonia and their biological studies as plant growth retardants. In these studies, two series of bis-quaternary salts of ammonia were prepared from different benzaldehydes. In the first series N,N-dimethyl tertiary amines 3(a-d) were prepared from differently substituted benzaldehyde (m- $NO_2$ , p- $NO_2$ , o-Cl and 2,3,4- trimethoxy) 2(a-d) by Leuckart's reaction with DMF and Formic acid. The products were characterized by a characteristic peak at  $2778\text{ cm}^{-1}$  in its IR spectrum and singlet at  $\delta 2.26$  for six protons in its PMR spectrum. Succinic acid dichloride was prepared by treating succinic acid with thionyl chloride under anhydrous

conditions. The quaternization of the tertiary amines 3(a-d) were then carried out by reacting two moles of these amines with one mole of butan-1,4-dioylchloride at 0-5°C to get the bis quaternary compounds. These products were scratched with anhydrous ether followed by decantation of ether to remove any unreacted tertiary amine or acid dichloride. The spectral studies of these compounds could not be done because of their hygroscopic nature and were preserved in vacuum desiccators.

In the preparation of compounds of second series, differently substituted benzaldehydes (p-Cl, m-NO<sub>2</sub>, o-OCH<sub>3</sub>, o-NO<sub>2</sub>) 4(e-h) were subjected to Knoevenagel reaction with malonic acid using pyridine as a base in the presence of catalytic amount of piperidine to get different cinnamic acids 5(e-h). Cinnamic acids obtained thus were found to have E geometry by the comparison with the melting points of acids reported in literature. The E configuration of the double bond was further confirmed by the doublet at  $\delta$  6.4 and 8.0 having coupling constant equal to 16 Hz, from the NMR spectra of tertiary amines (8e) and (8h) prepared from these acids. These acids were then reacted with epichlorohydrins in the presence of anhydrous K<sub>2</sub>CO<sub>3</sub> using absolute alcohol as a solvent to yield hydroxy tertiary amines 8(e-h). The tertiary amines were characterized by the presence of characteristic bands at 3393 cm<sup>-1</sup> (-OH) and 1713 cm<sup>-1</sup> (>C=O) in its IR spectra and a triplet at  $\delta$  1.2 for six protons and multiplet at  $\delta$  2.6 also for six protons in its NMR spectra. Bis quaternary salts of the tertiary amines 8(e-h) were prepared by reacting two moles of the amine with one mole of butan-1,4-dioylchloride under anhydrous conditions at 0-5°C. These compounds were purified by scratching with anhydrous ether followed by decantation of the ether, in order to remove any unreacted tertiary amine and acid dichloride. Being hygroscopic in nature, the spectral studies of these salts could not be done and stored in vacuum desiccator. However, these salts gave positive copper wire test

## BIOLOGICAL TESTING---EVALUATION OF PLANT GROWTH RETARDING ACTIVITIES

The biological activity of bis quaternary salts of ammonia having benzyl moiety 1(a-d) and cinnamic acid functionalities 9(e-h) was tested by using seeds of *Oryza sativa*(PR-116). The effects of different concentrations of the chemicals on the percent germination, seedling growth in terms of root length, shoot length, changes in fresh and dry weights of root and shoot were studied. Cycocel and ABA were used as standards for calibrating the potential tested compounds as retardant or inhibitor. Seeds germinated in water served as controls.

Seed germination studies of newly synthesised compounds resulted reduction in seed germination, but the magnitude of inhibition was less than when tested with CCC at all concentrations. Among the tested compounds, the compound having o-chloro substituent(1c) was found to possess comparatively more inhibitory effect. Synthesised compounds when tested for their effect on length of root and shoot showed significant effect as compared to control. Compound 1c and 1a having m- NO<sub>2</sub> and o-Cl substituent in benzene nucleus showed maximum inhibition of root length and shoot length. The magnitude of inhibition increased with increase in concentration of tested compound (Table 1 and 2). Other biological studies such as seedling mass and dry (fresh weights of root and shoot) as well as seedling vigour index were also carried out.

The biological studies of synthesised compounds revealed that nitro groups with negative inductive effect in general and o-nitro compounds in particular showed enhanced plant growth retardant activity. Chloro- substituents with negative inductive effect and methoxy group with positive mesomeric effect when substituted at ortho position, showed

improved plant growth retardant activity. The enhanced activity seems to be due to the ortho effect, which is both electronic as well as steric. Trimethoxy group activates the benzene nucleus due to mesomeric effect and quaternary salts prepared with 3,4,5-trimethoxy substitution in the benzene nucleus showed the lowest plant growth retardant activity amongst those compounds. Thus it may be concluded that negative inductive effect and ortho effect are responsible for the enhanced activity of some of the compounds.

**Table 1: Effect of Bis-Quaternary Salts of Ammonia Containing Benzyl/Cinnamic Acid Moiety on Germination (%) Of Oryza Sativa (PR-116)**

Compounds	Concentration ( $\mu\text{g MI}^{-1}$ )						
	0	25	50	100	200	400	500
H <sub>2</sub> O	98	–	–	–	–	–	–

**With Benzyl Moiety**

<b>1a</b>	–	96	95	89	86	82	79
<b>1b</b>	–	97	95	92	90	89	85
<b>1c</b>	–	92	90	89	85	79	75
<b>1d</b>	–	97	97	95	90	90	89

**With Cinnamic Acid Moiety**

<b>9e</b>	–	98	97	97	95	91	90
<b>9f</b>	–	95	93	93	91	90	79
<b>9g</b>	–	98	97	95	92	90	90
<b>9h</b>	–	92	91	85	82	80	80
<b>CCC</b>	–	95	90	86	80	79	72
<b>ABA(5<math>\mu\text{g ml}^{-1}</math>)</b>	–	85	–	–	–	–	–

**Table 2: Effect of Bis-Quaternary Salts of Ammonia Containing Benzyl/Cinnamic Acid Moiety on Length Root (Cm) of Oryza Sativa (PR-116)**

Compounds	Concentration ( $\mu\text{g MI}^{-1}$ )							
	0	25	50	100	200	400	500	CD
H <sub>2</sub> O	14.3 $\pm$ 0.07	-	-	-	-	-	-	-

**With Benzyl Moiety**

<b>1a</b>	–	6.7 $\pm$ 0.62	6.3 $\pm$ 0.45	5.7 $\pm$ 0.38	2.6 $\pm$ 0.55	1.7 $\pm$ 0.34	0.52 $\pm$ 0.28	0.51
<b>1b</b>	–	11.0 $\pm$ 0.33	9.7 $\pm$ 0.42	9.2 $\pm$ 0.60	6.8 $\pm$ 0.75	6.5 $\pm$ 0.35	6.2 $\pm$ 0.62	1.20
<b>1c</b>	–	6.1 $\pm$ 0.82	5.7 $\pm$ 0.56	5.2 $\pm$ 0.64	2.0 $\pm$ 0.72	1.3 $\pm$ 0.47	0.4 $\pm$ 0.26	0.42
<b>1d</b>	–	10.4 $\pm$ 0.56	9.8 $\pm$ 0.65	7.4 $\pm$ 0.70	6.9 $\pm$ 0.66	6.4 $\pm$ 0.28	5.8 $\pm$ 0.23	1.09

**With Cinnamic Acid Moiety**

<b>9e</b>	–	12.0 $\pm$ 0.60	10.0 $\pm$ 0.45	7.5 $\pm$ 0.30	6.2 $\pm$ 0.44	5.1 $\pm$ 0.72	3.7 $\pm$ 0.25	1.30
<b>9f</b>	–	9.2 $\pm$ 0.24	7.5 $\pm$ 0.75	4.4 $\pm$ 0.82	3.8 $\pm$ 0.81	2.6 $\pm$ 0.61	1.4 $\pm$ 0.54	0.92
<b>9g</b>	–	13.1 $\pm$ 0.61	10.9 $\pm$ 0.79	9.2 $\pm$ 0.21	9.0 $\pm$ 0.64	8.6 $\pm$ 0.58	5.3 $\pm$ 0.44	1.40
<b>9h</b>	–	10.8 $\pm$ 0.51	9.1 $\pm$ 0.83	8.9 $\pm$ 0.88	8.8 $\pm$ 0.72	8.2 $\pm$ 0.59	7.9 $\pm$ 0.72	0.99
<b>CCC</b>	–	7.2 $\pm$ 0.30	4.3 $\pm$ 0.65	3.2 $\pm$ 0.42	2.0 $\pm$ 0.52	1.5 $\pm$ 0.54	0.3 $\pm$ 0.56	0.55
<b>ABA (5 <math>\mu\text{g ml}^{-1}</math>)</b>	–	6.5 $\pm$ 0.26	–	–	–	–	–	–

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