

SYNTHESIS OF QUATERNARY AMMONIUM SALTS HAVING PHENOXY ACETIC ACID MOIETY AND THEIR PLANT GROWTH RETARDANT ACTIVITY

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ABSTRACT

Eight new quaternary salts of ammonia (1a-d and 2a-d), from different phenols were synthesized and tested as plant growth retardants. The structure of various tertiary amines and esters was established with the help of IR and NMR spectral studies. Biological activity of these compounds was tested using seed germination and seedling growth as bioassay of *Oryza sativa*. All the compounds showed plant growth retardant activity comparable to cycocel (CCC) at higher concentrations.

KEYWORDS: Cycocel, *Oryza Sativa*, Tertiary Amine, Quaternary Ammonium Salts

INTRODUCTION

Plant growth retardants have been used for many years to manipulate the size, shape and overall quality of various crops. These compounds have been used to control the lodging, increasing the yield and producing compact plant in different crops. Quaternary ammonium salts have been found to possess plant growth retardant activity. In the present work eight new quaternary salts of ammonia from different phenols have been synthesized and tested as plant growth retardants

MATERIAL AND METHODS

Purity of analytical samples was checked by TLC. Silica gel G(13% gypsum) was employed for TLC plates of size(7.5 x2.5). The plates were developed in ether, benzene and ethyl acetate solvents of varying proportions. The spots were visualized by placing the plates for some time in chamber saturated with iodine .Drying of all organic extracts was done over anhydrous Na₂SO₄.

Preparation of Phenylchloroacetate (7)

A mixture of phenol (4.7g, 0.05 mol), chloroacetic acid (4.7g, 0.05 mol) and benzene (30 ml) in the presence of acid catalyst (0.5ml, conc. H₂SO₄) was refluxed with the help of Dean and Stark's apparatus till the separation of water is stopped. The cooled reaction mixture was treated with saturated solution of NaHCO₃ and washed with water and extracted with benzene. Extract was dried over anhydrous Na₂SO₄ and solvent was removed by distillation. The residue was distilled under reduced pressure to give (7). Yield ;6.8 g(90%) ,b.p 70-75°C/ 10-15 mm. ν_{\max} 3060, 3005, 2959, 1761, 1595, 1494, 1404, 1314, 1217, 1160, 1068,1022, 1004, 924, 902, 842, 767, 699, 550 and 496 cm⁻¹. δ ; 4.25 (2H, s, -Cl-CH₂-COO-), 7.1-7.4 (5H, Ar-H)

Preparation of N-Butylchloroacetate (8)

n-Butylchloroacetate was prepared as above. Yield ; 6.6 g, (85%), b.p 130 – 135°C.

ν_{\max} ; 2963, 2875, 1756, 1464, 1415, 1386,1310,1186,1086,1019,958,844,787,698 and 575 cm^{-1} . δ ; 0.9 (3H, t, $J=7\text{Hz}$, -OCH₂ CH₂ CH₂ CH₃), 1.3 (2H,m,-CH₂ CH₂ CH₂ CH₃) 1.6 (2H, m,-CH₂ CH₂ CH₂ CH₃), 3.9(2H, s, -ClCH₂-CO), 4.1 (2H,t, $J=7\text{ Hz}$, -O-CH₂CH₂CH₂CH₃).

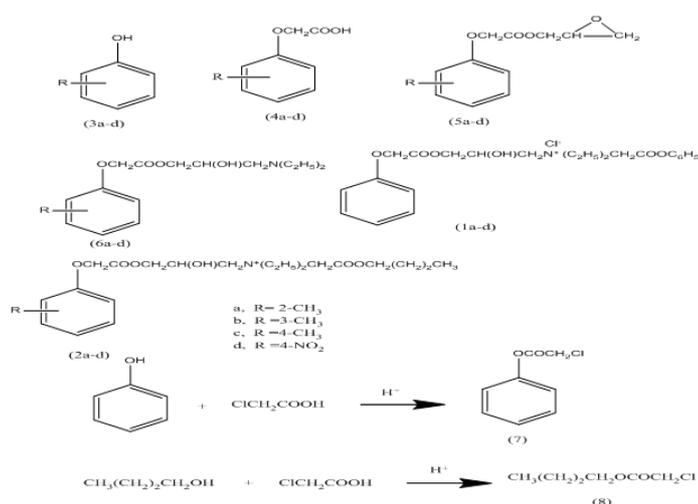


Figure 1

Preparation of 2-(2-Methylphenoxy) Ethanoic Acid (4a)

O-cresol (5.4g, 0.05mol) and chloroacetic acid (5.7g, 0.06 mol) were melted together on water bath; NaOH (5.4g in 22.5 ml of H₂O, 6N) was added to it drop wise slowly with stirring and then the mixture was heated (60-70°C) for 3h with occasional shaking. It was diluted with water and acidified with conc. HCl. The precipitates thus formed were filtered, washed and dried to give 2-(2-methylphenoxy) ethanoic acid. Yield; 6.6g (80%), m.p 124-126°C It gave characteristic tests for carboxylic acid group.

Compound 2-(3-methylphenoxy) ethanoic acid (4b) was also prepared by similar procedure from m-cresol. Yield 7.4g (90%), m.p 98-99°C.

Compound 2-(4-methylphenoxy) ethanoic acid (4c) was obtained similarly from p-cresol. Yield 7.1g (86%) m.p 139-141°C (lit 140-142°C)

Compound 2-(4-nitrophenoxy) ethanoic acid (4d) was also procured from 4-nitrophenol in the similar manner. Yield 8.8g (90%).

Preparation of (3-N,N-Diethylamino-2-Hydroxypropyl)-2-(2-Methylphenoxy) Ethanoate (6a)

2-(2-Methylphenoxy) ethanoic acid (4a, 6.4g, 0.038 mol), anhydrous K₂CO₃ (11.4g) and epichlorohydrin (120 ml) was refluxed under stirring for 13 h. The reaction mixture was filtered, filtrate concentrated and the residue taken up in benzene. The organic phase was washed successively with 10% NaOH, water, saturated aqueous NaCl, dried and concentrated to afford the epoxide compound (5a).

The above epoxide (1.6g) and diethylamine (5ml) was refluxed in absolute alcohol (50ml) for 4h cooled and alcohol was distilled off. The residual oil thus obtained was distilled under reduced pressure to give (6a). Yield 1.76g (81%), b.p. 108-110°C/7-8mm.

ν_{\max} (cm⁻¹); 3364, 2927, 1754, 1604, 1494, 1464, 1227, 1190, 1124, 1065, 930, 827, 756 and 700. δ ; 1.3 [6H, t, j=7 Hz, -N(CH₂CH₃)₂], 2.2 (3H, s, Ar-CH₃), 2.9 [6H, m, -CH₂N(CH₂CH₃)₂], 3.65 [3H, m, -OCH₂CH(OH)-], 4.4 (2H, s, -OCH₂COO-), 6.7-7.0 (4H, Ar-H).

(3-N,N-Diethylamino-2-hydroxypropyl)-2-(3-methylphenoxy) ethanoate (6b') was also prepared similarly by taking (4b', 6.4g, 0.045mol). Yield; 3.52g (81%) b.p 104- 106°C/5-6 mm.

ν_{\max} (cm⁻¹); 3435, 2970, 2815, 1735, 1669, 1469, 1386, 1292, 1202, 1163, 1064 and 773.

δ ; 1.15 [6H, t, j = 7Hz; N(CH₂CH₃)₂], 2.3 (3H, s, Ar-CH₃), 2.69-2.76 [6H, m, CH₂N(CH₂CH₃)₂], 3.8 [1H, m, -CH(OH)], 4.25 (2H, s, ArOCH₂), 4.45 (2H, d, j=7.5Hz, -COOCH₂CH(OH)), 6.5-8.5 (4H, Ar-H).

(3-N,N-Diethylamino 2-hydroxypropyl)-2-(4-methylphenoxy) ethanoate (6c) was also prepared similarly by taking (4c, 14.7g, 0.045 mol) Yield; 3.7g (80%), b.p 114-116°C/8-9mm.

(3-N, N-Diethylamino-2-hydroxypropyl)-2-(4-nitrophenoxy) ethanoate (6d) was also prepared similarly by taking (4d, 7.5g, 0.045 mol). Yield; 3.7g (80%), b.p 114-116°C/8-9mm

Quaternary Salt of (3-N, N-Diethylamino - 2- Hydroxypropyl) - 2- (2- Methylphenoxy) Ethanoate (1a)

A mixture of tertiary amine (6a, 0.5g) and phenylchloroacetate (7, 0.5g) dissolved in acetonitrile (30ml) was refluxed for 14h. Excess of the solvent was distilled off and residue was scratched by adding anhydrous ether. The unreacted tertiary amine and chloroester (7) being soluble in dry ether were decanted to get quaternary salt of ammonia a thick liquid which was stored in vacuum desiccators. Being hygroscopic in nature, the spectral studies of the salt (1a) could not be done. However it gave a positive test with copper wire .

Quaternary salts of (3-N,N-diethylamino-2- hydroxypropyl) -2-(3-methylphenoxy) ethanoate (1b) , (3- N,N-Diethylamino -2- hydroxypropyl) -2-(4-methylphenoxy) ethanoate (1c) and (3-N,N-Diethylamino -2 -hydroxypropyl -2-(4-nitrophenoxy) ethanoate (1d) were obtained similarly from corresponding tertiary amine (6b-d) in quantitative yield (about 90%).

Quaternary Salt of (3-N, N-Diethylamino- 2-Hydroxypropyl) -2- (2-Methylphenoxy) Ethanoate (2a)

A mixture of tertiary amine (7, 0.5 g) and n-butylchloroacetate (18, 0.5g) dissolved in acetonitrile (30 ml) as refluxed for 14h. Excess of the solvent was distilled off and residue was scratched by adding anhydrous ether. The unreacted tertiary amine and chloroester (18) being soluble in dry ether were decanted to get quaternary salt of ammonia as a thick liquid which was stored in vacuum desiccators. Being hygroscopic in nature, the spectral studies of the salt (2a) could not be done. However it gave a positive test with copper wire. Yield; 0.68g (89%)

Quaternary salts of (3-N,N- diethylamino -2- hydroxypropyl) -2-(3-methylphenoxy) ethanoate (2b), (3-N,N-diethylamino -2 -hydroxypropyl) -2-(4-methylphenoxy) ethanoate (2c) and (3- N,N-diethylamino -2- hydroxypropyl)-2-(4- nitrophenoxy) ethanoate (2d) were also prepared similarly by taking tertiary amines (6a-d) in 90% yield.

BIOLOGICAL TESTING

Quaternary salts of ammonia were tested on *Oryza sativa* (PR 116). To test on rice, twenty seeds in each replication were placed on a filter paper laid on bottom of a petridish of 9cm diameter to which 7ml of test solution of different concentration (25, 50, 100, 200, 400,500 micro g /ml) of the compounds had been poured. Each dish was kept at $28 \pm 2^\circ\text{C}$ for 7 days and there after the seed germination (%), root length and shoot length were measured. Separate control treatments with water, cycocel and ABA were also recorded simultaneously.

RESULTS AND DISCUSSIONS

Plant growth retardants have been used for many years to manipulate the size, shape and overall quality of various crops. These compounds have been used to control the lodging, increasing the yield and producing compact plant in different plant growth retardant activity in continuation of our previous work on quaternary salts of ammonia, this work includes the synthesis of eight new quaternary ammonium salts and their biological study as plant growth retardants. The synthesis of (1a-d) and (2a-d) compounds were carried out from different phenols.

Tertiary amines having glycidyl ester moieties were prepared by the reaction of epichlorohydrin with phenoxy acetic acids; the acids in turn were synthesized from different phenols, viz. 2-methylphenol, 3- methylphenol, 4-methylphenol and 4-nitrophenols were separately reacted with chloroacetic acid in the presence of two moles of sodium hydroxide followed by its workup and regeneration of acid with dilute hydrochloric acid to give corresponding phenoxy acetic acids (4a-d). These acids were characterized by the carboxylic acid group tests and melting points. The phenoxy acetic acids prepared above were refluxed with excess of epichlorohydrin in the presence of anhydrous potassium carbonate for thirteen hours. The epoxy compounds (5a-d) prepared in the above step were refluxed with diethylamine and absolute alcohol for four hours separately. Removal of alcohol followed by vacuum distillation of the residue under reduced pressure afforded the tertiary amines (6a-d).

The IR spectra of (6a) showed a characteristic peak at 3364 cm^{-1} (-OH), 1754 cm^{-1} (-COOR). Its NMR spectra depicts a characteristic triplet at $\delta 1.3$ for six protons N $(\text{CH}_2\text{CH}_3)_2$ and a multiplet at $\delta 2.7-3.0$ for six protons $[\text{CH}_2\text{N}(\text{CH}_2\text{CH}_3)_2]$. Phenylchloroacetate (7) and n-butylchloroacetate (8) were prepared by refluxing the equimolar mixture of a hydroxy compound (phenol and n-butylalcohol) and chloroacetic acid separately with benzene in the presence of a drop of conc. sulphuric acid as catalyst using Dean and Stark's apparatus till the separation of water continued. After the neutralization of the reaction mixture with saturated solution of sodium bicarbonate, it was extracted with benzene and benzene was distilled off and then dried over anhydrous sodium sulphate. The residue give the esters (7and 8) in quantitative yield. IR spectra of these esters (7and 8) depicts characteristic peaks at 1761 and 1755 cm^{-1} respectively for (-COOR). NMR spectra of phenylester (7) gave a singlet at $\delta 4.3$, for two protons ($\text{ClCH}_2\text{-COO}$) and at $\delta 7.1 - 7.4$, for aromatic protons. NMR spectra of n-butylester (8) gave a triplet at $\delta 0.9$ for three protons, two multiplets at $\delta 1.34$ and 1.6 for two protons each ($-\text{OOCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$) and singlet at $\delta 3.96$ for $-\text{ClCH}_2\text{COO}$ and a triplet at $\delta 4.1$ for two protons ($\text{ClCH}_2\text{COOCH}_2\text{-}$)

The tertiary amines (6a-d) prepared above were quaternized by refluxing the respective amines separately with phenylchloroacetate (7) and n-butylchloroacetate (8) using acetonitrile as solvent for 14 hours. Acetonitrile was distilled off and the respective residue was scratched with anhydrous ether. Ether was then decanted to get quaternary ammonium

salt (1a-d and 2a-d).

The biological activity of quaternary salts of ammonia having phenylchloroacetate (1a-d) and n-butylchloroacetate (2a-d) functionalities was tested using seed germination assay. Seeds of *Oryza sativa* (PR -116) were used for these studies. The effect of different concentrations of chemicals were studied on percent germination and seedling growth in terms of root length, shoot length and changes in fresh and dry mass of root and shoot, were also studied. Cycocel and ABA were used as standard for calibrating the potential tested compounds as retardants or inhibitors. Seeds germinated in water served as control.

Seed germination of newly synthesized compounds resulted seed germination comparable to CCC. The inhibitory effect was found to be maximum at higher concentration (500µg/ml). Synthesized compounds when tested for their effect on length of root and shoot, showed significant effect as compared to control, The inhibitory effect of 1a and 2a having ortho methyl was observed to be highest comparable with cycocel. Shoot length was also reduced by all concentrations of tested compounds. The maximum inhibitory effect was observed with 500µg/ml concentration of compound 1a followed by 2a. 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.

Biological studies of synthesized compounds revealed that compounds with methyl groups at ortho position (1a and 2a) were found to exhibit more growth retarding activity as compared to compounds with methyl groups at Meta and para position and nitro group at para position. This enhanced growth retardant effect may be due to ortho effect (which is combined effect of both steric as well as electronic). Further the salts prepared with phenylchloroacetate(1a-d) showed pronounced growth retarding effect as compared with that of prepared with n-butylchloroacetate(2a-d). This suggests that groups attached to quaternary nitrogen also have significant role in growth retardant activity of the molecule.

Table 1: Effect of Quaternary Salts of Ammonia Containing Phenyl Chloroacetate / N-Butyl Chloroacetate Moiety on Root Length (Cm) of *Oryza Sativa* (PR-116)

Compounds	Concentration (UG MI ⁻¹)						
	0	25	50	100	200	400	500
H ₂ O	11.5±0.08	-	-	-	-	-	-
1a	-	6.6±0.61	4.9±0.38	3.0±0.65	1.4±0.13	0.72±0.35	0.46±0.08
1b	-	6.7±0.37	5.6±0.63	4.2±0.64	1.8±0.29	1.3±0.56	0.7±0.09
2a	-	10.6±0.50	10.0±0.21	8.2±1.12	7.4±0.85	3.6±0.32	2.9±1.08
3a	-	9.4±0.82	8.7±0.61	6.3±0.27	4.4±0.54	2.4±0.38	1.8±0.28
3b	-	10.5±0.95	8.9±0.90	6.9±0.48	4.8±0.63	2.9±0.97	2.5±0.21
4a	-	9.7±0.30	9.0±0.52	8.6±0.26	8.5±1.08	7.9±0.47	6.2±0.35
4b	-	10.8±1.25	9.5±1.32	9.1±0.12	9.1±0.50	8.0±0.35	7.1±0.26
CCC	-	6.0±0.30	4.8±0.65	3.1±0.42	1.8±0.52	0.96±0.54	0.4±0.56
ABA(5ug/ml)	-	6.5±0.28	-	-	-	-	-

CD at 5% (compound) A = 1.19

CD at 5% (compound) B = 0.97

CD at 5% (compound x concentration) A X B = 1.15

Table 2. Effect of Quaternary Salts of Ammonia Containing Phenylchloroacetate/N – Butylchloroacetate Moiety on Shoot Length (Cm) Of Oryza Sativa (Pr-116)

Compounds	Concentration ($\mu\text{g MI}^{-1}$)						
	0	25	50	100	200	400	500
H ₂ O	7.9±0.56	-	-	-	-	-	-
1a	-	5.38±0.85	4.70±0.76	3.66±0.76	3.64±0.63	3.56±0.53	2.01±0.25
1b	-	6.33±0.58	5.30±0.57	4.95±0.42	4.20±0.62	4.16±0.41	2.23±0.72
2a	-	6.75±0.65	6.5±0.94	6.32±0.70	6.23±0.50	4.57±0.62	4.07±1.12
2b	-	6.95±0.84	6.80±0.57	6.34±1.02	6.10±0.65	5.30±1.14	4.66±1.12
3a	-	7.85±0.52	6.88±0.81	6.08±0.58	5.25±0.95	3.63±1.10	3.30±0.40
3b	-	6.25±1.04	6.10±0.65	5.89±1.19	5.75±0.86	4.50±0.54	3.16±1.05
4a	-	7.26±0.75	6.95±0.65	6.87±1.12	6.12±0.42	6.05±0.35	5.27±0.94
4b	-	7.02±0.47	6.50±0.41	6.41±0.42	6.15±1.08	6.0±0.91	5.18±0.99
CCC	-	5.46±0.30	4.38±0.12	3.36±0.81	2.89±0.08	2.01±0.50	1.79±0.06
ABA (5 $\mu\text{g/ml}$)	4.85±0.56	-	-	-	-	-	-

CD at 5% (compound) A = 0.56

CD at 5% (concentration) B = 0.46

CD at 5% (compound x concentration) A x B = 0

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