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Ionic liquids based on 2-chloroethyltrimethylammonium chloride (CCC) as plant growth regulators

Research Article

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Abstract: Plant growth regulator - 2-chloroethyltrimethylammonium chloride (CCC) was converted into ionic liquid in the metathesis reaction. New forms of CCC as ILs were stable in air as well in contact with water and organic solvents. The biological action of the cation (CCC) in ILs was preserved. Type of anion determined hydrophobicity and hydrophilicity of the whole molecule and it also influenced the biological activity of the plant growth regulator. All of the investigated salts retarded growth of winter wheat stems and most of them increased yield at the same time, when compared to control and standard 2-chloroethyltrimethylammonium chloride.

Keywords: *Ionic liquids • Metathesis reaction • Anti-gibberellins • Chlormequat chloride*

1. Introduction

Lodging in cereals refers to the displacement of the stem from its vertical position and leaning it towards the soil. Stem lodging is usually caused by wind, low stem resistance or weight of water accumulated in the mature ears. Plant growth retardants (PGRs) could be used to efficiently prevent this adverse phenomenon.

Most PGRs applied in field crops inhibit gibberellin (GA) biosynthesis. At present four different types of these inhibitors are known: onium-type compounds, which have a positively charged ammonium, phosphonium or sulphonium group (*e.g.* chlormequat chloride, mepiquat chloride, chlorphonium and AMO-1618), compounds with a N-containing heterocycle (*e.g.* ancymidol, flurprimidol, tetcyclacis, paclobutrazol, uniconazole), structural mimics of 2-oxoglutaric acid (*e.g.* acylcyclohexanediones compounds such as prohexadione-Ca and trinexapac-ethyl), 16,17-Dihydro-GAs which represent different structures, mostly GA5 derivatives [1]. The first type of PGRs is widely used in agronomic crops to increase resistance of cereals to lodging and to reduce excessive growth in cotton. Onium-type compounds block the cyclases copalyl-diphosphate synthase and ent-kaurene synthase in the early steps of gibberellins metabolism [1].

Chlormequat chloride – 2-chloroethyltrimethylammonium chloride (CCC), is a compound which possesses one positively charged nitrogen atom and has an activity in reducing longitudinal shoot growth without lowering the plant productivity. This quaternary ammonium chloride belongs to a group of popular chemical compounds also known as 'quats' [2]. Chlormequat chloride was prepared in 1910 [3] and was described as plant growth regulator by N. E. Tolbert in 1960 [4,5]. CCC increases resistance to lodging by inhibiting cell elongation, as a result producing shorter and stronger stems. It also intensifies chlorophyll

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formation and root development and is used to increase yields in wheat, rye, oats and triticale. Moreover, CCC is also applied to increase lateral branching and flowering in some ornamental plants and to increase fruit setting in pears, olives, vines and tomatoes [6].

In 1997 World Health Organization classified CCC as slightly hazardous - LD_{50} in rats, mice hamsters guinea-pigs and monkeys between 200-1000 mg kg^{-1} of bodyweight indicate its moderate oral toxicity [7]. Maximum residue levels (MRLs) have been established by European Union commission for CCC accordingly to its noxiousness. For instance, maximum residue limits of CCC in food products are 10 mg kg^{-1} in oat and pear, 3 mg kg^{-1} in wheat and rye, and 0.5 mg kg^{-1} in milk and milk products [8,9]. In 1989 Danielsen *et al*. [10] suggested that CCC may negatively affect mammalian reproduction. More recently, it was shown that CCC did not affect reproduction in female mice [11] whereas reproduction in male mice was compromised [6,10,11]. On the contrary, current reports revealed that chlormequat chloride could not be proven to be detrimental to the male pig reproduction [6].

Ionic liquids (ILs) can be defined as the ionic compounds consisting of a cation and anion. Their melting points are low and they exist in a liquid form below the temperature of 100°C [12-17]. Potentially, there is a possibility of selection of appropriate ions to obtain an ionic liquid having the desired properties. This justifies their wide range of applications as wood preservatives, surfactants, disinfectants, antistatic or softening substances, extractants or media of chemical reactions. It should be noted that, being non-volatile, non-flammable and thermally stable they pollute the environment less than the conventional solvents. Extensive research has already led to the emergence of the third generation of ionic liquids. In this group, in addition to certain physical (I generation) and chemical (II generation) properties, appears also an expected biological activity [18,19]. III generation of ILs has recently expanded to include herbicidal ionic liquids, that due to their high activity, are novel salts with a high potential of application [20-22].

2. Experimental procedure

2.1. General

^1H NMR spectra were recorded on a Mercury Gemini 300 spectrometer operating at 300 MHz with tetramethylsilane as the internal standard. ^{13}C NMR spectra were obtained with the same instrument at 75 MHz. CHN elemental analyses were performed at A. Mickiewicz University, Poznan (Poland).

2.1.1. Preparation of ionic liquids with 2-chloroethyltrimethylammonioum cation:

In a round-bottom flask, 0.03 mol of sodium tetrafluoroborate was dissolved in 15 mL of distilled water. Then a stoichiometric amount of 2-chloroethyltrimethylammonioum chloride was added and the mixture was stirred for 1 hour at room temperature. The product, which precipitated as crystalline, white solid, was separated and washed with distilled water until no chloride anions were present in water. Then the product was dried under reduced pressure at 45°C for 24 h.

In a round-bottom flask, equipped with a dropping funnel, 0.03 mol of sodium (or potassium) salt of acids: nicotinic, benzoic, salicylic and propionic was dissolved in 30 mL of distilled water. Then a stoichiometric amount of the aqueous solution of 2-chloroethyltrimethylammonioum chloride was added and the mixture was stirred for 3 hours at room temperature. The water was evaporated using a rotary evaporator and the residue was dissolved in isopropanol. The product, deposited in isopropanol phase, was separated from insoluble inorganic salts. After removal of isopropanol the product was dried under reduced pressure at 45°C for 24 h.

In a round-bottom flask, equipped with reflux condenser and dropping funnel, 0.01 mol of trisodium phosphate was dissolved in 30 mL of distilled water. Then a 0.03 mol of 2-chloroethyltrimethylammonioum chloride was added and the mixture was stirred for 3 hours at room temperature. The water was evaporated using a rotary evaporator and the residue was dissolved in methanol. The product, which deposited in methanol phase, was separated from insoluble inorganic salts. After removal of methanol the product was dried under reduced pressure at 45°C for 24 h.

In a round-bottom flask, 0.03 mol of dodecylbenzenesulfonic acid was dissolved in 30 mL of distilled water. Then a stoichiometric amount of 2-chloroethyltrimethylammonioum chloride was added and the mixture was stirred for 3 hours at room temperature. The product, which precipitated as flocky, white solid, was separated and washed with distilled water until no chloride anions were present in water. Then the product was dried under reduced pressure at 45°C for 24 h.

2-Chloroethyltrimethylammonium tetrafluoroborate (**1**) ^1H NMR (DMSO-d_6) δ (ppm) 3.21 (s, 9H), 3.91 (t, J = 6.8 Hz, 2H), 4.09 (t, J = 7.1 Hz, 2H); ^{13}C NMR δ (ppm) 35.9, 53.2, 65.6. Elemental analysis calcd (%) for $C_5H_{13}BClF_4N$ (209.42): C 28.68, H 6.26, N 6.69; found: C 28.97, H 6.01, N 6.96.

Tris(2-chloroethyltrimethylammonium) phosphate (**2**) ^1H NMR (D_2O, 298K, 300 MHz) δ (ppm) 3.17 (s, 27H), 3.85 (t, J = 7.2 Hz, 6H), 4.03 (t, J = 6.4 Hz, 6H); ^{13}C NMR (D_2O, 298K, 75 MHz) δ (ppm) 36.5, 54.0, 66.1; ^{31}P NMR (D_2O, 298K, 121 MHz) δ (ppm) 1.5(s); Elemental analysis calcd (%) for $C_{15}H_{39}Cl_3N_3O_4P$ (462.82): C 38.93, H 8.49, N 9.08; found: C 38.57, H 8.21, N 8.82.

2-Chloroethyltrimethylammonium propionate (**3**) ^{1}H NMR (DMSO-d_6) δ (ppm) 0.95 (t, J = 6.9 Hz, 3H), 2.29 (q, J = 5.4 Hz, 2H), 3.22 (s, 9H), 3.84 (t, J = 6.7 Hz, 2H), 4.13 (t, J = 7.0 Hz, 2H); ^{13}C NMR δ (ppm) 9.7, 27.6, 35.9, 52.5, 65.7, 180.3. Elemental analysis calcd (%) for $C_8H_{18}ClNO_2$ (195.69): C 49.10, H 9.27, N 7.16; found: C 49.48, H 9.03, N 6.89.

2-Chloroethyltrimethylammonium benzoate (**4**) ^{1}H NMR (DMSO-d_6) δ (ppm) 3.30 (s, 9H), 3.93 (t, J = 6.8 Hz, 2H), 4.19 (t, J = 7.2 Hz, 2H), 7.53 (d, J = 7.7 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H), 7.98 (dd, $J^{1,2}$ = 6.8 Hz, $J^{1,3}$ = 1.2 Hz, 1H) ; ^{13}C NMR δ (ppm) 36.9, 53.8, 65.9, 128.2, 128.6, 129.1, 129.3, 131.2, 132.7, 167.3. Elemental analysis calcd (%) for $C_{12}H_{18}ClNO_2$ (243.73): C 59.13, H 7.44, N 5.75; found: C 58.86, H 7.23, N 5.47.

2-Chloroethyltrimethylammonium 2-hydroxybenzoate (**5**) ^{1}H NMR (DMSO-d_6) δ (ppm) 3.18 (s, 9H), 3.80 (t, J = 6.9 Hz, 2H), 4.12 (t, J = 6.9 Hz, 2H), 6.60 (t, J = 8.7 Hz, 1H), 6.62 (d, J = 6.9 Hz, 1H), 7.14 (t, J = 6.3 Hz,1H), 7.68 (dd, $J^{1,2}$ = 7.5 Hz, $J^{1,3}$ = 1.8 Hz, 1H); ^{13}C NMR δ (ppm) 36.4, 52.7, 65.0, 115.8, 115.9, 120.7, 129.9, 131.3, 163.0, 171.5. Elemental analysis calcd (%) for $C_{12}H_{18}ClNO_3$ (259.73): C 55.49, H 6.99, N 5.39; found: C 55.73, H 7.22, N 5.04.

2-Chloroethyltrimethylammonium pyridine-3-carboxylate (**6**) ^{1}H NMR (DMSO-d_6) δ (ppm) 3,26 (s, 9H), 3.87 (t, J = 6.4 Hz, 2H), 4.15 (t, J = 6.5 Hz, 2H), 7.55 (t, J = 7.7 Hz, 1H), 8.41 (dd, $J^{1,2}$ = 7.4 Hz, $J^{1,3}$ = 1.6 Hz, 1H), 8.99 (d, J = 5.0 Hz, 1H), 9.23 (s, 1H); ^{13}C NMR δ (ppm) 37.1, 53.7, 65.5, 123.5, 131.4, 137.0, 152.4, 155.6, 175.2. Elemental analysis calcd (%) for $C_{11}H_{17}ClN_2O_2$ (244.72): C 53.99, H 7.00, N 11.45; found: C 53.65, H 7.32, N 11.19.

2-Chloroethyltrimethylammonium dodecylbenzenesulfonate (**7**) ^{1}H NMR (DMSO-d_6) δ (ppm) 0.88 (t, J = 6.8 Hz, 3H), 1.26 (m, 18H), 1.69 (m, 2H), 2.61 (t, J = 6.3 Hz, 2H), 3.22 (s, 9H), 3.83 (t, J = 7.4 Hz, 2H), 4.16 (t, J = 6.5 Hz, 2H), 7.18 (d, J = 6.1 Hz, 2H), 7.51 (d, J =6.3 Hz, 2H); ^{13}C NMR δ (ppm) 13.9, 22.1, 25.5, 28.7, 28.8, 29.1, 31.3, 36.2 36.7, 53.1, 65.1, 125.5, 128.0, 137.8, 145.4. Elemental analysis calcd (%) for $C_{23}H_{42}ClNO_3S$ (448.10): C 61.65, H 9.45, N 3.13; found: C 61.32, H 9.17, N 3.28.

2.2. Thermal analysis

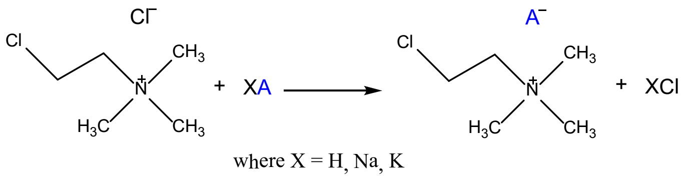
Thermal transitions of prepared salts were determined by DSC, with a Mettler Toledo Star^e TGA/DSC1 (Leicester, UK) unit, under nitrogen. Samples between 5 and 15 mg were placed in aluminum pans and heated from 25 to 120°C at a heating rate of 10°C min^{-1} and cooled with an intracooler at a cooling rate of 10°C min^{-1} to -100°C. Thermogravimetric analysis was performed using a Mettler Toledo Star^e TGA/DSC1 unit (Leicester, UK) under nitrogen. Samples between 2 and 10 mg were placed in aluminum pans and heated from 30 to 450°C at the heating rate of 10°C min^{-1}.

2.3. Biological activity

Field trials were performed in 2011 at Experimental Station in Winna Gora (western part of Poland) on winter wheat cultivar Zyta, which was cultivated according to the conventional agricultural practice. The experimental design was a randomized block with four replications and the plot size was 16.5 m^2. All tested salts were dissolved in water or alcohol and used at the dose rate of 400 g ha^{-1} of CCC. The standard product was a growth regulator containing 2-chloroethyltrimethylammonium chloride (CCC) – Antywylegacz Płynny 725 SL. All treatments were applied at the stage of the first detectable node of wheat growth (BBCH 31) using knapsack sprayer with XR 11003 flat-fan nozzles delivering 200 L ha^{-1} of spray solution at 0.3 MPa of an operating pressure. Shortly before harvesting the height of 25 randomly chosen winter wheat stems were measured within each plot from the ground to the top of ear. The effect of all tested salts was compared to the untreated sample. Grain yield from the plot was harvested using combine harvester. Grain from experimental plots was weighted and its moisture was determined. The yield obtained was converted to t ha^{-1} taking into account the standard grain moisture content of 14%. The data from field trials were analyzed by ANOVA. Tukey's test was used for the comparison of averages and LSD was determined on the level of 5%. All calculations were performed using Agriculture Research Manager (ARM) software.

3. Results and discussion

Using a 2-chloroethyltrimethylammonium chloride (CCC) as the source of the cation, 7 new quaternary ammonium salts were obtained in metathesis reaction (Scheme 1) with the yield exceeding 90%. As a result, chloride anion has been replaced by following anions: tetrafluoroborate (**1**), phosphate (**2**), propionate (**3**), benzoate (**4**), 2-hydroxybenzoate (salicylate) (**5**), pyridine-3-carboxylate (nicotinate) (**6**), dodecylbenzenesulfonate (**7**). The prepared salts are presented in Table 1. They were stable in air as well in contact with water or popular organic liquids and were non-flammable. At



Scheme 1. Synthesis of 2-chloroethyltrimethylammonium salts.

Table 1. Prepared salts.

Salt	Anion	Yield [%]	State at room temperature	Solubility in water
1	BF_4^-	97	solid	limited
2	PO_4^{3-}	96	solid	total
3	$C_2H_5COO^-$	97	liquid	total
4	$C_6H_5COO^-$	94	solid	total
5	$2\text{-}HOC_6H_4COO^-$	93	solid	total
6	$C_5H_4NCOO^-$	98	solid	total
7	$4\text{-}C_{12}H_{25}C_6H_4SO_3^-$	96	grease	insoluble

Table 2. The chemical shifts in ^{1}H NMR.

Salt	Cation – characteristic protons		
	$N(C\underline{H}_3)_3$	$NC\underline{H}_2$	$ClC\underline{H}_2$
1	3.21(s)	3.91(t)	4.09(t)
2	3.17(s)	3.85(t)	4.03(t)
3	3.22(s)	3.84(t)	4.13(t)
4	3.30(s)	3.93(t)	4.19(t)
5	3.18(s)	3.80(t)	4.12(t)
6	3.26(s)	3.87(t)	4.15(t)
7	3.22(s)	3.83(t)	4.16(t)
CCC	3.21(s)	3.84(t)	4.12(t)

s – singlet, t – triplet, shift in ppm

Table 3. Thermal properties of CCC derivatives.

Salt	T_g[a] (°C)	T_{cyst}[b] (°C)	T_m[c] (°C)	$T_{onset5\%}$[d] (°C)	T_{onset}[e] (°C)
1	-21	---	209	256	282
2	---	---	199	189	228
3	-84	---	---	145	240
4	---	---	84	175	255
5	15	---	99	188	233
6	---	---	88	150	232
7	---	---	---	270	373
CCC	---	---	---	250	254

[a] T_g - glass transition temperature; [b] T_{cryst} – temperature of crystallization; [c] T_m - melting point; [d] $T_{onset5\%}$ - decomposition temperature of 5% sample; [e] T_{onset} - decomposition temperature of 50% sample

elevated temperature, the synthesized salts were found to dissolve in short-chain alcohols and water (except salt **7** and **1**) but not in hexane or ether. Type of anion determined solubility of the salt in water. CCC is very soluble only in water and methanol and practically insoluble in many organic solvents such as isopropanol, acetone, acetonitrile or chloroform. Through the exchange reaction of the chloride anion obtained salts gained a solubility in some organic solvents such as isopropanol (**3**-**6**), acetone (**2**,**4**,**5**,**7**) and acetonitrile (**2**-**5**,**7**).The prepared salts were characterized by ^{1}H and ^{13}C NMR and elemental analysis. The ^{1}H NMR spectra of the starting chloride and the other salts indicated various chemical shifts (Table 2). The differences reached to 0.13 ppm for $N(CH_3)_3$ protons, 0.13 ppm for NCH_2 protons and 0.16 ppm for $ClCH_2$ protons, respectively. Thermal stability of salts **1**-**7** is presented in the Table 3. The studied salts were thermally stable between 145°C and 270°C. Such a broad range indicates how strong was the effect of anion on thermal stability of synthesized compounds. The most stable proved to be tetrafluoroborate (**1**) and dodecylbenzenesulfonate (**7**). Salts with the organic anion **3**-**7** could be qualified as ILs , because their melting points were below 100°C. CCC unlike the 2 chloroethyltrimethylammonium tetrafluoroborate (**1**), propionate (**3**) and salicylate (**5**) does not have a glass transition temperature. It also has no melting point on the contrary to the obtained salts (**1**,**2**,**4**-**6**), and is decomposed at a temperature of 250°C.

The newly synthesized forms of CCC retarded the growth of winter wheat stems. This effect varied depending on the type of anion. The plants treated with salts **1**-**7** were by 2.3 cm to 7.8 cm (3.1-10.4%)

Table 4. The influence of salts with 2-chloroethyltrimethylammonium cation on the stems length and yield of the winter wheat.

Salt	Dose (kg ha^{-1})	Stem length (cm)	Yield (t ha^{-1})
1	0.7	68.5 b*	6.26 cde
2	0.5	70.0 a	6.57 bcd
3	0.6	69.2 b	6.09 e
4	0.8	71.9 a	6.95 ab
5	0.8	69.5 b	6.87 ab
6	0.8	72.6 a	6.93 ab
7	1.5	67.1 b	6.65 bc
CCC (commercial)	0.6	70.7 a	6.13 de
Untreated check	-	74.9 a	6.28 cde

** the same letter in a column means no significant differences between treatments*

shorter than the control. Salt **1** and ILs **3**, **5**, **7** turned out to be the most active substances (Table 4) and were more active than the CCC-containing commercial product. Winter wheat plants from the plots treated with these compounds were by 5.4 to 10.4 percent shorter compared to the untreated check and by 1.7 to 5.1 percent shorter as compared to the plants treated with the commercial product. It should be noted that to effectively limit the growth of winter wheat, the dose of active part (2-chloroethyltrimethylammonium cation) in ILs was 400 g ha^{-1} while recommended doses for present formulation of CCC are between 700 and 1400 g ha^{-1}. The higher biological activity, which we found for the new forms of CCC, made possible the reduction of their dose, which is highly important from a point of view of integrated crop protection system.

The structure of the anion also had an influence on the yield of winter wheat. The yield from the untreated control amounted to 6.28 t ha^{-1}, while the yield from plots treated with new forms of CCC ranged from 6.09 to 6.95 t ha^{-1}. ILs **4**, **5** and **6** were the most active. The treatments with ILs provided an increase of yield by 0.59-0.67 t ha^{-1} (9.4-10.7%) compared to the control. Generally it can be concluded that the yield from treatments with salts containing organic anions was higher compared to the results obtaining for salts with inorganic anions. We can say that the best biological properties exhibited ILs with phenyl anion (salts **4**, **5**, **7**). None of the new forms of CCC caused damage to crop plants.

4. Conclusions

Popular plant growth regulator - 2-chloroethyltrimethylammonium chloride (CCC) can be converted into ionic liquid in the metathesis reaction. New forms of CCC as ILs were stable in air as well as in contact with water and organic solvents. The biological action of the cation in ILs has been preserved. The type of anion determines hydrophobicity and hydrophilicity of the whole molecule and dictates the activity as a plant growth regulator. The positive effect of ILs treatments on the yield of winter wheat was noted.

Acknowledgements

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