Preparation and Antifungal Study of Some Ammonium Salts Derived from Acetic Acid and Trichloroacetic Acid

Ramadan A. Bawa, Amany H. Elgroshi
Department of Chemistry, Faculty of Science, Misrata University.
* Corresponding author: r.bawa@sci.misuratau.edu.ly

Submission date : 26-09-2022 Acceptance date : 31-10-2022 Electronic publisher date: **12-11-2022**

Abstract: This work is concerned with the preparation of a number of organic ammonium acetate and trichloroacetate salts (ionic liquids; ILs) throughout a reaction of the acetic acid and trichloroacetic acid with some aliphatic and aromatic amines including the ammonia. Ten organic ammonium carboxylate salts were obtained, some of them were in the form of solid salts, whereas others were as liquids (ionic liquids; ILs) at room temperature. The yields of the resulting organic salts ranged from (21%) to (79%). However, some attempts towards the preparation of other organic salts were not successful. The obtained organic ammonium carboxylate salts/ionic liquids were characterized using spectroscopic techniques such as the infrared (IR), the nuclear magnetic resonance spectroscopy (^{1}HNMR) and the mass spectrometry (ms). The antifungal properties of these organic ammonium carboxylate salts were studied against the aspergillus niger (A. niger). The antifungal results were compared with the daktarin (a commercially available antifungal agent), which was used as a reference.

Keywords: Organic ammonium carboxylate salts, ionic liquids, spectroscopic, antifungal properties.

1. Introduction

Ionic liquids (ILS) (liquid organic salts) have received great attention from scientists, due to their various useful properties. Although they are composed of cation and anion, they exist as liquids at room temperature, therefore, they have been used broadly as moderators for interactions. They appeared as non-aqueous media in the enzymatic reactions. Furthermore, their chemical and physical properties (density, viscosity, melting point, etc.) could be adjusted through the cation and/or the anion. These properties are important for enzymatic reactions. [1] Ionic liquids were dated back to 1914, which the first ethyl ammonium nitrate was identified. [2] They generally consist of an organic cation (ammonium derivative) and inorganic or organic anion. Therefore, they belong to the salts, usually in the form of viscous liquids with melting points below 100 °C and some of them are liquids at temperatures below 400 °C. [2] Ionic liquids are important compounds, since they have a number of applications in various fields of science and research. Their biological activities attracted the interest of scientists in the field of biochemistry and medicine. The first report on liquid salts "Air and humidity at room temperature" was made by Wilkes and Zaworotko in 1921. Studies have shown that ionic liquids possess great effectiveness in the chemical synthesis and catalysis. Ionic liquids are molten salts that are liquids at temperatures below 100°C, the antimicrobial activity of ionic liquids greatly attracted researchers' interest. Many ILS inhibit the growth of different types of bacteria and fungi, thus, ionic liquids have been used as anti-bacterial and anti-fungal agents. [3,4] The ionic liquids have several applications in the food, environmental and biological fields. [5] Since ionic liquids consist of ions, they have various physical characteristics of low volatility, viscosity and electrical conductivity, making them very important type of slats possessing numerous applications in

chemical analysis and other fields. [6] Ionic liquids are organic salts that have melting points of 100°C or less. [7] They are environmentally friendly chemical compounds and their physical properties could be adjusted for specific applications. [8]

2. Experimental:

2.1. Materials:

Acetic acid, trichloroacetic acid, ammonia, methyl amine, triethyl amine, ethylene diamine, α-naphthyl amine and chloroform were purchased from (EMSURE and CDH). DMSO was purchased from (CARLO ERBA). The special food medium (PDA) was purchased from (OXOID).

2.2 . Instrumentation:

Melting point was measured on a Barnstead electrothermal IA 9100. The pH was measured using Jenway pH meter 3505. 1HNMR spectrum was recorded on a JEOL 500 spectrometer. Residual proton signal from the deuteriated solvents were used as references [DMSO (1H, 2.50 ppm)]. Coupling constants were measured in Hz. Infrared spectrum was recorded on Jasco FT/IR-4100 Fourier transform infrared spectrometer. Mass spectrum was recorded on a Shimadzu Qp–2010 Plus spectrometer.

2.3. Methods:

General procedures for the preparation of the ammonium carboxylate salts (ILs):

A literature procedure for the preparation of the organic salts was adapted to obtain the targeted organic salts. [9]

Solvent free procedure (A):

The liquid amine (1 mmol) was placed in a round-bottomed flask being immersed in a water bath. The carboxylic acid (1 mmol) was then added dropwise to the amine at a temperature of 70°C over 1 hour while stirring. The temperature was raised to 80°C after the addition of the carboxylic acid was complete. The reaction mixture was stirred at this temperature for further 2 hours, cooled to room temperature and dried.

Solvent included procedure (B):

Amine (1 mmol) was placed and dissolved in chloroform (10 – 15 cm3) in a round-bottomed flask being immersed in a water bath. The carboxylic acid (1 mmol) was then added portion wise to the amine solution at a temperature of 70°C over 1 hour while stirring. The temperature was raised to 80°C after the addition of the carboxylic acid was complete. The reaction mixture was stirred at this temperature for further 2 hours, cooled to room temperature and the solvent was removed under vacuum.

The analytical data for the acetic acid ammonium salts:

Ammonium acetate: procedure A

White solid (6.75 g, 87.6 mmol, 35 % yield) m.p 112 - 114 ºC (lit[10] 113 ºC); FT-IR vmax 2603 cm-1 (+NH3), 1764 cm-1 (C=O); δH (500 MHz; DMSO) 8.30 (4H, s, +NH4), 1.80 (3H, s, CH3-COO); m/z (C4H12O4N2, Mwt. 77.08) [M]+ 77.05 (0.25%), 59.05 (9.05%), 60.00 (100%).

Methylammonium acetate: procedure A

Colorless oil (14.37 g, 157.7 mmol, 63 % yield) FT-IR vmax 2604 cm-1 (+NH3), 1704 cm-1 (C=O); δH (500 MHz; DMSO) 7.99 (3H, s, +NH3), 1.77 (3H, s, CH3-+NH3), 1.74 (3H, s, CH3-COO); m/z (C3H9O2N, Mwt. 91.11) [M]+ 91.15 (0.03%), 59.05 (4.49%), 60.05 (47.76%), 61.05 (100%).

Triethylammonium acetate: procedure A

Yellow oil (25.36 g, 157.5 mmol, 63 % yield); FT-IR vmax 2470 cm-1 (N+-H), 1797 cm-1 (C=O); δH (500 MHz; DMSO) 8.20 (1H, br s, +NH), 3.69 (6H, q, J = 5.00 Hz, 3 × CH2CH3), 2.94 (9H, t, J = 5.00, 3 × CH2CH3), 1.16 (3H, s, CH3).

2-Aminoethan-1-ammoniumacetate : procedure A

Yellow semisolid (26.15 g, 217.6 mmol, 87 % yield); FT-IR vmax 3351 cm-1, 3286 cm-1 (free NH2), 2685 cm-1 (+NH3), 1539 cm-1 (C=O); m/z (C4H12O2N2, Mwt. 120.15) [M]+ 120.25 (0.02%), 71.00 (83.72%), 61.10 (95.07%), 60.05 (100%), 59.10 (42.12%).

Ethylenediammonium diacetate: procedure A

Beige solid (31.50 g, 175 mmol, 70 % yield) m.p 99 – 108 ºC; FT-IR vmax 2171 cm-1 (2 × +NH3), 1641 cm-1 (2 × C=O); δH (500 MHz; DMSO) 5.16 (6H, s, 2 × +NH3), 2.68 (4H, s, 2 × -CH2), 1.73 (6H, s, 2 × CH3-COO).

The analytical data for the trichloroacetic acid ammonium salts:

Ammonium trichloroacetate: procedure B

Green oil (6.00 g, 33.3 mmol, 95 % yield); FT-IR vmax 2603 cm-1 (+NH4), 1762 cm-1 (C=O); δH (500 MHz; DMSO) 8.31 (4H, s, +NH4); m/z (C2H4O2Cl3N, Mwt. 180.42) [M]+ 180.15 (0.02%), 127.00 (57.19%), 116.95 (35.49%), 83.95 (23.07%), 81.95 (100%).

Methylammonium trichloroacetate: procedure B

Colorless Semisolid (4.63 g, 23.8 mmol, 68 % yield) FT-IR vmax 2677 cm-1 (+NH3), 1742 cm-1 (C=O); δH (500 MHz; DMSO) 8.29 (3H, s, +NH3), 2.51 (3H, s, CH3-+NH3); m/z (C3H6O2Cl3N, Mwt. 194.44) [M]+ 194.20 (0.02%), 81.95 (100%).

Triethylammonium trichloroacetate: procedure B

Colorless oil (3.32 g, 12.5 mmol, 20 % yield); FT-IR vmax 2695 cm-1 (N+-H), 1619 cm-1 (C=O); δH (500 MHz; DMSO) 8.29 (1H, s, +NH), 2.47 (6H, q, J = 10.00 Hz, 3 × CH2CH3), 0.93 (9H, t, J = 10.00, 3 × CH2CH3); m/z (C8H16O2Cl3N, Mwt. 264.58) [M]+ 264.10 (0.02%), 101.15 (16.85%), 86.10 (100%), 58.05 (21.36%).

α-Naphthylammonium trichloroacetate: procedure B

Gray semisolid (5.41 g, 17.6 mmol, 98 % yield); FT-IR vmax 2597 cm-1 (+NH3), 1742 cm-1 (C=O); δH (500 MHz; DMSO) 8.33 (3H, s, +NH3), 7.89 – 7.87 (4H, m, 4 × Ar-CH), 7.49 – 7.47 (3H, m, 3 × Ar-CH); m/z (C12H10O2Cl3N, Mwt. 306.57) [M]+ 306.80 (0.31%), 162.15 (1.92%), 144.05 (2.29%), 113.50 (42.29%).

2-Aminoethan-1-aminium 2,2,2-trichloroacetate: procedure B

Yellow solid (2.78 g, 12.4 mmol, 69 % yield) mp 101 – 107 ºC; FT-IR vmax 3352 cm-1, 3287 cm-1 (free NH2), 2127 cm-1 (+NH3), 1652 cm-1 (C=O); m/z (C4H9O2Cl3N2, Mwt. 223.49) [M]+ 223.10 (0.79%), 162.30 (2.18%), 57.85 (57.50%).

Ethylenediammonium ditrichloroacetate: procedure B

Gray solid (6.41g, 16.6 mmol, 92 % yield) mp 150 – 155 ºC; FT-IR vmax 2172 cm-1 (2 × +NH3), 1749 cm-1 (2 × C=O); δH (500 MHz; DMSO) 8.28 (6H, s, 2 × +NH3), 2.93 (4H, s, 2 × -CH2); m/z (C6H10O4Cl6N2, Mwt. 386.87) [M]+ 386.00 (2.79%), 223.00 (3.86%), 82.00 (100.00%).

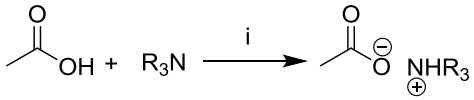
Determining the antifungal properties of the resulting organic salts: [11]

The agar dilution technique will be employed to measure the in vitro effect of an antifungal effect of all obtained organic salts against the test fungi (aspergillus niger). In this method, graded concentrations of antibiotics will be incorporated in agar plates and inoculated in spots with the As niger. If the microorganism under study is affected notably by the incorporated antifungal agent (the organic salts), no fungal growth is expected in agar plates with higher amounts of this antifungal drug. Fungal growth is observed as the antibiotic concentration in the agar plate diminishes. Inhibition of growth at the minimum concentration of the antibiotic is considered as the end point.

3. Results and Discussion:

The ammonium acetate salts:

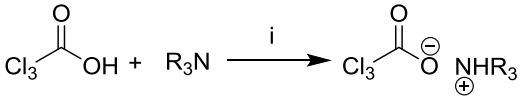
The ammonium acetate salts were obtained throughout the reaction between the acetic acid and an excess of ammonia or the amine derivative. The reaction gave the desired ammonium acetate salts in yields ranging from 35% to 87% (**Scheme 1**). However, no formation for the α-naphthylammonium acetate.



Reaction reagents and conditions: (i) 70 - 80 °C, 3 hrs

R_3N	Product	Yield
ammonia	ammonium acetate	35%
methylamine	methylammonium acetate	63%
triethylamine	triethylammoniumacetate	63%
α-napthylamine	α-naphthylammonium acetate	0%
ethylene diamine	2-aminoethan-1-ammonium acetate	87%
ethylene diamine	ethylene diammoium diacetate	70%

Scheme 1: the preparation of ammonium acetate salts



Reaction reagents and conditions: (i) 70 - 80 °C, 3 hrs

R_3N	Product	Yield
ammonia	ammonium trichloroacetate	95%
methylamine	methylammonium trichloroacetate	68%
triethylamine	triethylammoniumtrichloroacetate	20%
α-napthylamine	α-naphthylammonium trichloroacetate	98%
ethylene diamine	2-aminoethan-1-ammonium trichloroacetate	69%
ethylene diamine	ethylene diammoium ditrichloroacetate	92%

Scheme 2: The preparation of the ammonium trichloroacetate salts

The ammonium groups, in the obtained ammonium acetate salts, were appeared in the IR spectra at the range of 2685 to 2171 cm-1. The carbonyl groups were seen in the range between 1797 and 1539 cm-1. The 1HNMR of these resulting salts showed all expected chemical shifts. The mass spectrometry data gave the exact masses for all molecular ions of every obtained ammonium acetate salt along with other fragments.

The ammonium trichloroacetate salts:

The ammonium trichloroacetate salts were obtained throughout the reaction between the trichloroacetic acid and an excess of ammonia or the amine derivative. The desired ammonium trichloroacetate salts yielded between 20% and 98% **(Scheme 2).** It is worthwhile mentioning that the α-naphthylammonium trichloroacetate was formed in excellent yield.

The IR data showed the appearance of the

ammonium group, in the obtained ammonium trichloroacetate salts, at the range of 2695 to 2127 cm-1 as a rather broad band. The carbonyl groups, in these ammonium trichloroacetate salts, were seen in the range between 1762 and 1619 cm^{-1}. The 1HNMR of these resulting salts showed all expected chemical shifts. The mass spectrometry data gave the exact molecular ion masses for the obtained ammonium trichloroacetate salt along with other fragments.

The use of organic salts as antifungal agents against the aspergillus niger:

A fixed and specific concentration 30 ppm, (0.003 g, 2.0 cm3 DMSO in 100 cm3 media), was taken for all the organic salts that were prepared in this study. The control has been prepared (2.00 cm3 DMSO in 100 cm3 media). The antifungal agent, Daktarin, that has been used as a reference was prepared in a concentration of 30 ppm (0.003 g, 2.0 cm3 DMSO in 100 cm3 media). After the process of pouring the media, planting the aspergillus niger under study, and incubating the samples for six days, the average readings were taken on the second day, the fourth day, and the sixth day of testing. The inhibition rates were calculated for the second, fourth and sixth day of testing. The first reading of inhibition rates after two days of testing were found to be (0%) for the control and (52.63%) for the antifungal reference (daktarin). The second reading of inhibition rates after four days of testing were found to be (0%) for the control and (49.50%) for the antifungal reference (daktarin). Whereas, the third reading of inhibition rates after six days of testing were found to be (0%) for the control and (63.22%) for the antifungal reference (daktarin).

The five ammonium salts of the acetic acid were tested for their antifungal behaviour towards the A. niger showing inhibition effects ranging from (14.21%) to (24.74%) after the first two days of testing. The 2-aminoethan-1-ammoium acetate exhibited the highest inhibitory level (24.74%), however, the ethylene diammoium diacetate showed the lowest inhibition level (14.21%) within the same testing period. Unexpectedly, the ammonium acetate recorded the highest inhibition level (34.66%) on the fourth day of testing. Unpredictably, the 2-aminoethan-1-ammoium acetate showed dramatic drop in its inhibition behaviour recording only (2.33%) which was the lowest inhibitory level on the fourth day. The sixth day of the testing period showed that the ammonium acetate was still having the highest level of inhibition against the A. niger reaching (36.78%). Whereas, the organic salts containing the trichloroacetate group had the highest inhibition rates of all the salts that were obtained, this is due to the high electronegativity of chlorine atoms in the organic salt. Where the highest inhibition rate for this group was α-naphthyl ammonium trichloro-acetate (61.54%), which is the highest inhibition rate for all the prepared salts. The antifungal activity of the organic salts was similar to that of daktarin, and the drug similarity data of the obtained compounds make them encouraging substances for future development of antifungal agents **(Table 1).**

Conclusion:

Eleven ammonium salts of the acetic and trichloro-cetic acids were synthesized. The yields were ranging between 20 % and 98 %. The antifungal effects of all resulting ammonium salts were tested against the *A. niger*. The ammonium trichloroace-tate salts showed the highest inhibition levels of all the salts that were obtained. The highest inhibitory level was found to be for the *α*-naphthyl ammonium trichloroacetate (61.54%). The antifungal activity of the organic salts was similar to that of daktarin, and the drug similarity data of the synthesized compounds make them promising indicators for the future development of antifungal agents.

Table 1: Inhibition levels of ammonium salts of acetic and trichloroacetic acids against the aspergillus niger

Organic salt name	Testing period/inhibition levels %		
	Two days	Four days	Six days
Ammonium acetate	23.16%	34.45%	36.78%
Methylammonium acetate	21.05%	23.08%	26.68%
Triethylammonium acetate	15.79%	11.04%	25.48%
2-Aminoethan-1-ammonium acetate	24.74%	2.01%	28.37%
Ethylene diammonium diacetate	14.21%	15.38%	33.65%
Ammonium trichloroacetate	23.16%	41.47%	57.93%
Methylammonium trichloroacetate	21.05%	32.11%	50.72%
Triethylammonium trichloroacetate	17.89%	28.76%	48.08%
α-Naphtylammonium trichloroacetate	23.16%	46.49%	61.54%
2-Aminoethan-1-ammonium trichloroacetate	14.21%	33.44%	51.92%
Ethylene diammonium ditrichloroacetate	17.89%	39.80%	56.73%

References

[1] M. Naushad, Z. A. Alothmana, A. B. Khan and M. Ali; "Effect of ionic liquid on activity, stability and structure of enzymes": A review; *International Journal of Biological Macromolecules*, 2012, **51**, 555 – 560.

[2] W. Gao, F. Zhang, G. Zhangb and C. Zhou; "Key factors affecting the activity and stability of enzymes in ionic liquids and novel applications in biocatalysis" *Biochemical Engineering Journal*, 2015, **99**, 67 – 84.

[3] K. S. Egorova, E. G. Gordeev, and V. P. Ananikov; "Biological activity of ionic liquids and their application in pharmaceutics and medicine" *Chemical. Reviews*, 2017, **117**, 7132 – 7189.

[4] J. Zhang, W. Tan, F. Luan, X. Yin, F. Dong, Q. Li and Z. Guo; "Synthesis of quaternary ammonium salts of chitosan bearing halogenated acetate for antifungal and antibacterial activities" *Polymers* 2018, **10**, 530.

[5] X. Han and D. W. Armstrong; "Ionic liquids in separations" *Acc. Chem. Res.* 2007, **40**, 1079 – 1086.

[6] G. M. Merone, A. Tartaglia, E. Rosato, C. D'Ovidio, A. Kabir, H. I. Ulusoy, F. Savini, M. Locatelli; "Ionic liquids in analytical chemistry: applications and recent trends" *Current Analytical Chemistry*, 2021, **17**, 1340 – 1355.

[7] P. Sun and D. W. Armstrong; "Ionic liquids in analytical chemistry"; *Analytica Chimica Acta*, 2010, **661**, 1 – 16.

[8] T. D. Ho, C. Zhang, L. W. Hantao, and J. L. Anderson; "Ionic liquids in analytical chemistry: fundamentals, advances, and perspectives", *Anal. Chem.* 2014, **86**, 262 – 285.

[9] S. D. Kumar and J. S. Sandhu;"An efficient green protocol for the production of 1,8-dioxo-octahydroxanthenes in triethylamm-onium acetate (teaa) a recyclable inexpensive ionic liquid"*Rasayan J. Chem*., 2009, **2**, 937 – 940

[10] A. W. Davidson; W. H. McAllister; "Solutions of salts in pure acetic acid: solubilities of acetates". *Journal of the American Chemical Society*., 1930, **52**, 507 – 519.

[11] R. A. Bawa and M. M. Friwan "Synthesis and antifungal study of some acetophenone oximes and their terphthaloyl oxime esters", *Academic Journal of Chemistry*, 2019, **5**, 96 – 101.