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Research Article

Synthesis of Some Organic Ammonium Formate Salts and Study of Their Antifungal Properties

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Abstract:

This work is concerned with the preparation of a number of organic ammonium formate salts (or ionic liquids; ILs) throughout a reaction between the formic acid and some aliphatic and aromatic amines including the ammonia. Five organic ammonium formate salts were formed, some of them were in the form of solid salts and others were as the ionic liquids (ILs) at room temperature. The yields of the prepared organic salts ranged from (21%) to (79%). However, some attempts towards the preparation of other fifteen organic salts were not successful. The resulting five organic ammonium formate salts/ionic liquids were characterized using a number of spectroscopic techniques such as the infrared (IR), the nuclear magnetic resonance spectroscopy (^{1}HNMR) and the mass spectrometry (ms). The collected spectroscopic data confirmed the formation of theses organic ammonium formate salts. The antifungal properties of the prepared organic ammonium formate salts were investigated against the aspergillus niger (*A. niger*), and the antifungal results were compared with the antifungal effect of the commercially available antifungal agent Daktarin, which was used as a reference in this study.

Keywords: organic ammonium formate salts; ionic liquids; spectroscopic; antifungal properties

Introduction:

Ionic liquids (ILs) at room temperature (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 or the anion. These properties are important for enzymatic reactions [1]. Ionic liquids were dated back to 1914, which the first ethyl ammonium nitrate **1** was identified (**Fig. 1**) [2].

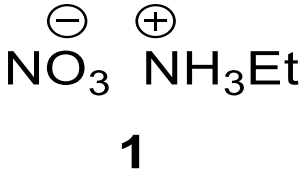


Figure 1: *The first reported ionic liquid*

They generally consist of an organic cation (ammonium derivative) and inorganic anion 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].

Experimental

1. Materials

Formic acid, ammonia, methyl amine, triethyl amine, ethylene diamine and chloroform were purchased from EMSURE and CDH.

2. Instrumentation

Melting point was measured on a Barnstead electrothermal IA 9100. The pH was measured using Jenway pH meter 3505. ^{1}HNMR spectrum was recorded on a JEOL 500 spectrometer. Residual proton signal from the deuteriated solvents were used as references [DMSO (^{1}H, 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.

3. Methods

3.1 Procedure for the preparation of the organic salts

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

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 drop wise 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.

4. The analytical data for the formic acid ammonium salts

Ammonium formate:

Yellow oil (3.28 g, 52.00 mmol, 21% yield); FT-IR ν_{max} 2527 cm^{-1} (N^+-H), 1704 cm^{-1} (C=O); δ_H (500 MHz; DMSO) 8.23 (H, s, *H*-COO), 7.38 (4H, br s, $^+$N*H*$_4$); m/z (CH_5O_2N, Mwt. 63.06) $[M]^+$ 63.05 (6%), 62.05 (2.02%).

Methylammonium formate:

Yellow oil (3.30 g, 42.90 mmol, 69% yield); FT-IR ν_{max} 2527 cm^{-1} (N^+-H), 1704 cm^{-1} (C=O); δ_H (500 MHz; DMSO) 8.23 (3H, s, $^+$N*H*$_3$), 7.96 (H, s, *H*-COO), 2.33 (3H, s, C*H*$_3$); m/z ($C_2H_7O_2N$, Mwt. 77.08) $[M]^+$ 77.05 (1.8%), 62.90 (0.21%), 59.05 (100%).

Triethylammonium formate:

Yellow oil (6.23 g, 42.39 mmol, 68% yield); FT-IR ν_{max} 2679 cm^{-1} (N^+-H), 1797 cm^{-1} (C=O); δ_H (500 MHz; DMSO) 8.28 (H, s, *H*-COO), 6.05 (1H, br s, $^+$N*H*), 2.92 (6H, q, J = 7.60 Hz, 3 × C*H*$_2$CH$_3$), 1.10 (9H, t, J = 6.70, 3 × CH$_2$C*H*$_3$); m/z ($C_7H_{17}O_2N$, Mwt. 147.22) $[M]^+$ 147.15 (0.01%), 101.15 (21.87%), 87.86.15 (100%).

Ethylenediammonium monoformate:

Yellow oil (5.25 g, 49.55 mmol, 80% yield); FT-IR ν_{max} 3346 cm^{-1}, 3286 cm^{-1} (free NH_2), 2695 cm^{-1} ($^+NH_3$), 1565 cm^{-1} (C=O); δ_H (500 MHz; DMSO) 8.43 (H, s, *H*-COO), 7.97 (3H, s, $^+$N*H*$_3$), 3.08 (2H, t, J = 5.70 Hz, -C*H*$_2$), 2.56 (2H, t, J = 10.45 Hz, -C*H*$_2$), 2.47 (2H, s, N*H*$_2$); m/z ($C_3H_{10}O_2N_2$, Mwt. 106.12) $[M]^+$ 106.10 (0.08%), 61.05 (10.11%), 60.05 (19.65%), 59.00 (100%).

Ethylenediammonium diformate:

Yellow oil (6.88 g, 45.24 mmol, 72% yield); FT-IR ν_{max} 2104 cm^{-1} (2 × $^+NH_3$), 1565 cm^{-1} (2 × C=O); δ_H (500 MHz; DMSO) 8.25 (2H, s, 2 × *H*-COO), 3.11 (6H, s, 2 × $^+$N*H*$_3$), 2.77 (4H, s, 2 × -C*H*$_2$); m/z ($C_4H_{12}O_4N_2$, Mwt. 152.12) $[M]^{+1}$ 153.10 (0.08%), 71.00 (83.72%), 58.95 (100%).

5. Determining the antifungal properties of the resulting organic salts: [10]

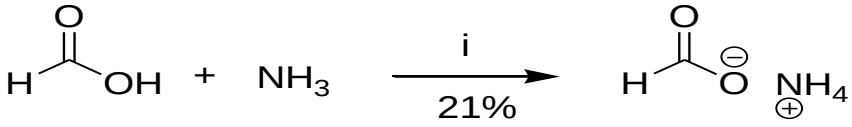
The agar dilution technique was used to measure the *in vitro* of the antifungal effect of the synthesized organic salts against the test fungi (*aspergillus niger*). In this method, graded concentrations of organic salts were incorporated in agar plates and inoculated in spots with the *As niger*. If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

Results and Discussion

1. The resulting ammonium formate salts (or Ionic liquids)

1.1 Ammonium formate:

The ammonium formate was obtained from the reaction of the formic acid and an excess of ammonia. The reaction gave only 21 % of the ammonium formate as a yellow oil **(Scheme 1)**.



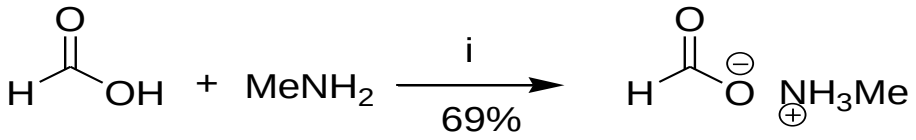
Reaction reagents and conditions: (i) 70 - 80 °C, 3 hrs

Scheme 1: *The preparation of ammonium formate*

The spectroscopic data revealed that the ($^+NH_4$) ammonium group appeared at 2527 cm^{-1} in the IR spectrum as a slightly weak broad band. The carbonyl group (C=O) was clearly seen at 1704 cm^{-1}. The ^{1}HNMR of the product showed the expected two signals. Additionally, the mass spectrometer also gave the exact mass for the molecular ion of (Mwt 63).

1.2 Methylammonium formate:

The methylammonium formate resulted from the reaction between the formic acid and methylamine. The methylammonium formate was obtained in a good yield 69 % as a yellow oil **(Scheme 2)**.



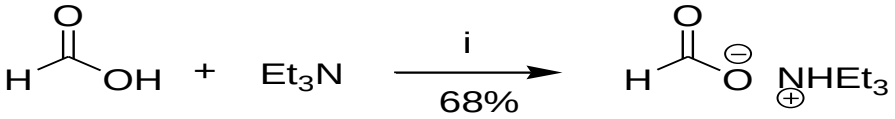
Reaction reagents and conditions: (i) 70 - 80 °C, 3 hrs

Scheme 2: *The preparation of methylammonium formate*

The IR data showed the ($^{+}NH_3$) ammonium group at 2527 cm^{-1} as a broad band. The carbonyl group (C=O) was seen at 1704 cm^{-1}. The ^{1}HNMR of this ammonium salt showed the expected three signals for the ammonium group, the hydrogen of the formate (*H*-COO^{-}) and the methyl group (CH_3NH_3) at 8.23, 7.96 and 2.33 ppm respectively. The mass spectrometry gave a further prove for the formation of the methylammonium formate by showing the exact mass for the molecular ion of (Mwt 77).

1.3 Triethylammonium formate:

The triethylammonium formate was obtained throughout the reaction between the formic acid and triethylamine. This triethylammonium formate was produced in a good yield 68 % as a yellow oil **(Scheme 3)**.



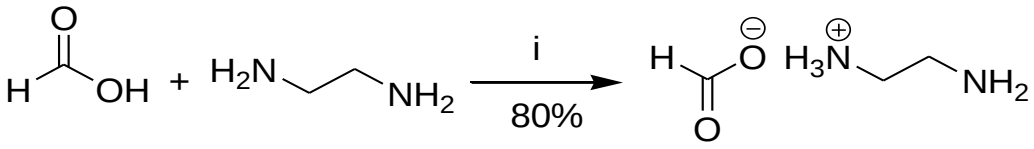
Reaction reagents and conditions: (i) 70 - 80 °C, 3 hrs

Scheme 3: *The preparation of triethylammonium formate*

The IR spectrum showed the (^{+}NH) ammonium group at 2679 cm^{-1} a broad band. The carbonyl group (C=O) was observed at 1797 cm^{-1}. The ^{1}HNMR of this ammonium salt showed the expected three signals for the ammonium group, the hydrogen of the formate (*H*-COO^{-}) and the ethyl groups (Et_3NH) at 8.28 ppm for (s, *H*-COO), 6.05 ppm (br s, ^{+}NH), 2.92 ppm for the methylene of the ethyl group (q, 3 × CH_2CH_3) and at 1.10 ppm for methyl of the ethyl group (t, 3 × CH_2CH_3) respectively. The mass spectrometry showed the formation of the triethylammonium formate as the exact mass for the molecular ion of (Mwt 147) was observed.

1.4 Ethylenediammonium monoformate:

The ethylenediammonium monoformate was prepared from the reaction between the formic acid and the ethylene diamine in a ratio of (1:1 mole/mole) respectively. This formate salt was produced in a very good yield 80 % as a yellow oil **(Scheme 4)**.



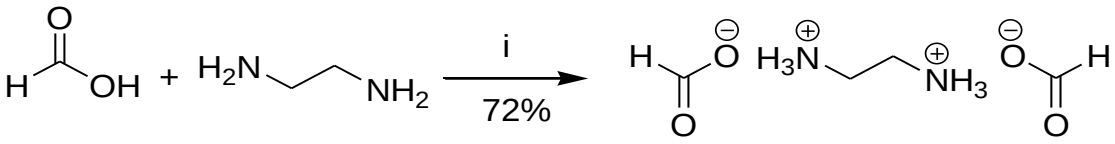
Reaction reagents and conditions: (i) 70 - 80 °C, 3 hrs

Scheme 4: *The preparation of ethylenediammonium monoformate*

The IR spectrum showed the free amine group (NH_2) at 3346 cm^{-1}, 3286 cm^{-1} as a W-shaped absorption band, which is a characteristic band for the free amino groups. The ^{+}NH bond of the ammonium group appeared at 2695 cm^{-1} as a broad band. The carbonyl group (C=O) was observed at 1565 cm^{-1}. The ^{1}HNMR data, showed the expected signlet signal for the hydrogen of the formate unit (*H*-COO^{-}) at 8.43 ppm. The ammonium group $^{+}NH_3$ appeared as a singlet at 7.79 ppm, whereas the two methylene groups $(CH_2)_2$ showed up at 3.08 ppm and 2.56 ppm as two triplets. However, the free amino group was observed at 2.47 as a singlet. The mass spectrometric data confirmed the formation of the ethylenediammonium monoformate as the exact mass for the molecular ion of (Mwt 106) was seen.

1.5 Ethylenediammonium diformate:

The ethylenediammonium diformate was obtained throughout the reaction between the formic acid and the ethylene diamine in a ratio of (2:1 mole/mole) respectively. This diformate salt was obtained in a good yield 72 % as a yellow oil **(Scheme 5)**.



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

Scheme 5: *The preparation of ethylenediammonium diformate*

The disappearance of the W-shaped absorption band for the primary amino groups was a good sign for the completion of the reaction and the formation of the ethylenediammonium diformate salt. The $^{+}$N-H bond of the two ammonium groups (2 × $^{+}NH_3$) appeared at 2104 cm^{-1} as a broad band. The two carbonyl groups (2 × C=O) were seen at 1565 cm^{-1}. The ^{1}HNMR data, revealed that the appearance of the singlet signal for the hydrogens of the two formate groups (*H*-COO^{-}) at 8.25 ppm. The two ammonium groups ($(^{+}NH_3)_2$ appeared as a singlet at 3.11 ppm, however the two methylene groups $(CH_2)_2$ showed up at 2.77 ppm as one singlet. The mass spectrometric data confirmed the formation of the ethylenediammonium monoformate as the molecular ion mass plus 1 (Mwt 153) was observed.

2. The effect of the ammonium salts of the formic acid (ionic liquids) on the growth of aspergillus niger:

Five ammonium salts of the formic acid were tested for their antifungal potentials against the *A. niger*. Their levels of inhibition ranged from 12.63% to 21.05% after the two days of testing in which the ethylene diammonium monoformate showed the highest inhibitory level (21.05%), whereas the triethylammonium formate had the lowest inhibition level (12.63%) within the first two days of the testing period. However, the triethylammonium formate has been found to have the highest inhibitory level (22.07%) on the fourth day of testing, whereas the methlammonium formate showed the lowest level of inhibition (8.70%) on the fourth day **(Table 1)**.

Organic Salt Name	**Testing period/inhibition levels %**		
	Two days	Four days	Six days
Ammonium formate	14.21	16.39	39.90
Methlammonium formate	17.89	8.70	33.65
Triethylammonium formate	12.63	22.07	31.25
Ethylene diammonium monoformate	21.05	9.70	34.38
Ethylene diammonium diformate	17.89	13.04	37.50

Table 1: *The inhibition levels of the ammonium formate salts against the aspergillus niger. The results after six days of testing were compared with that for the reference Daktarin (63%) after the same period of testing.*

On the sixth day of the testing period, the ammonium formate showed the highest level of inhibition against the A. niger reaching 39.90%. Whereas triethylammonium formate recorded the lowest inhibition level (31.25%) on the final day of the testing period **(Figure 2)**. The collected results after six days of testing for the inhibition levels of the resulting organic were compared with that for the reference daktarin (63%) after the same period of testing.

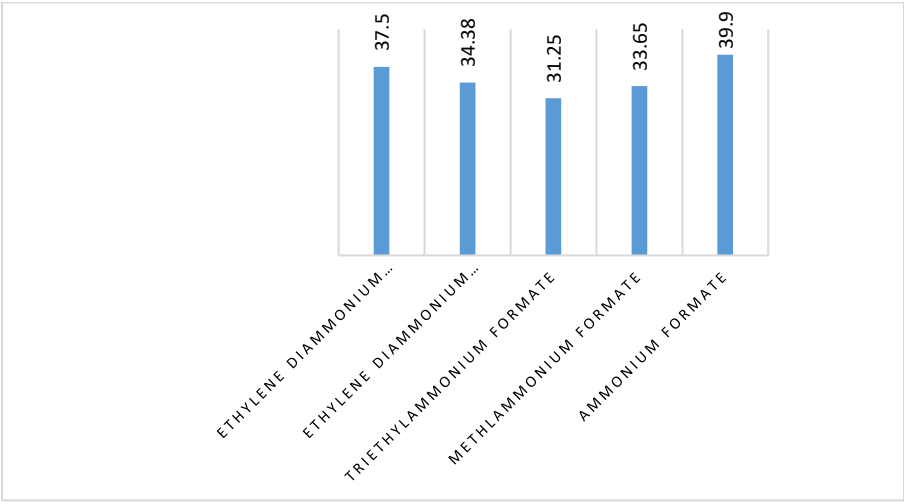


Figure 2: *The inhibition percentages on the sixth day of testing of the ammonium salts of the formic acid*

Conclusion

Five organic ammonium formate salts (or ionic liquids; ILs) have been synthesized and characterized using a number of spectroscopic techniques. These ammonium formate salts were formed as solids, whereas, others were formed as liquids (ILs) at room temperature, with yields ranging from (19.83%) to (99.00%). The antifungal properties of the resulting organic ammonium formate salts were investigated against the aspergillus niger (*A. niger*), and the antifungal results were compared with the antifungal effect of the commercially available antifungal agent the daktarin, which was used as a reference in this study. The highest level of inhibition was recorded for the ammonium formate reaching 39.9% after six days of the testing period, which was lower than the inhibition capacity of the daktarin (63%).

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