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Synthesis and anti-microbial activity of hydroxylammonium ionic liquids

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ABSTRACT

Eight hydroxylammonium-based room temperature ionic liquids (ILs) have been synthesized by acid–base neutralization of ethanolamines with organic acids. The ILs were characterized by infrared and nuclear magnetic resonance spectroscopies and elemental analysis. Their anti-microbial activities were determined using the well-diffusion method. All eight ILs were toxic to *Staphylococcus aureus*, while *2-hydroxyethylammonium lactate* and *2-hydroxy-N-(2-hydroxyethyl)-N-methylethanaminium acetate* showed high anti-microbial activity against a wide range of human pathogens.

1. Introduction

Room temperature ionic liquids (ILs) are low melting point organic salts with many interesting properties including potential as 'green' substitutes for volatile organic compounds in process chemistry and the food industry (Welton, 1999). Specific application of ILs include catalysis (Kalkhambkar et al., 2011), CO_2 absorption (Yokozeki et al., 2008), synthesis of nanoparticles (Antonietti et al., 2004) and usage as industrial solvents (Welton, 1999; Luo et al., 2009). Typically, ILs involve a combination of heterocyclic organic cations with inorganic or organic anions, which provides their unique properties. However, the high solubility of some ILs in water, coupled with several studies (Bernot et al., 2005; Pretti et al., 2009) highlighting their toxicity to aquatic organisms, raises questions about their long-term utility.

Bacteria are a good starting point to examine IL toxicity as they have short generation times (Pham et al., 2010) compared with other living organisms. This has indirectly led to the realization that some ILs exhibit anti-microbial characteristics. For example, Pernak et al. (2003, 2004) and Roslonkiewicz et al. (2005) have reported a trend of increasing toxicity towards a range of bacteria (including rods, cocci and fungi) with increasing chain length of alkyl substituents in pyridinium, imidazolium and quaternary ammonium salts. Quaternary ammonium compounds (QACs) are generally considered to be bioactive and have been used for environmental and medical equipment disinfection (Demberelnyamba et al., 2004). On this basis, it is reasonable to assume that hydroxylammonium ILs should also exhibit anti-microbial characteristics.

The objectives of this study are therefore to synthesize and characterize a series of hydroxylammonium ILs and to investigate their anti-microbial activities. Five types of human pathogens were selected to assess the potential toxicities of these ILs and their effectiveness as anti-microbial agents.

2. Materials and methods

2.1. Synthesis and characterization of hydroxylammonium ILs

Eight hydroxylammonium ILs (hereafter referred to as **ILs 1–8**) were synthesized by acid–base neutralization of ethanolamines (**ILs 1–3** from 2-ethanolamine; **ILs 4–6** from 2,2′-iminodiethanol; **ILs 7** and **8** from bis(2-hydroxyethyl)methylamine) with three carboxylic acids (formic, acetic or lactic acid). Ethanolamines (ACS reagent grade) and acids (AR grade) were obtained from Merck (USA). In a typical preparation, a stoicheometric amount of the acid was added drop-wise to the ethanolamine contained in a round-bottomed flask equipped with a reflux condenser, a magnetic stirrer and an inlet and outlet for N_2 gas. The purpose of this procedure was to reduce the production of heat since the reaction is strongly exothermic. After the acid had been added the mixture was maintained at room temperature for 2 h with stirring then heated to 60 °C to ensure complete reaction. Reaction progress was moni-

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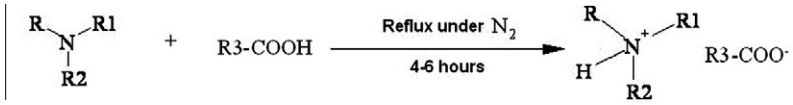

R = -H or, $-CH_3$
R1 = -H or, $-CH_2CH_2OH$ R3 = -H, $-CH_3$ or $-CHOHCH_3$
R2 = $-CH_2CH_2OH$

Fig. 1. Reaction scheme for the synthesis of the ammonium-containing ILs.

Table 1
Characterization data for the synthesized ILs.

Ionic liquid	Formula	Molecular weight	Yield (%)	IR (cm^{-1})	^{1}H-NMR	Elemental analysis	
						Experimental (%)	Calculated (%)
1	$C_4H_{11}O_3N$	121	86	3350, 2970, 2930, 1594, 1390	δ_H 1.88 (s, 3H, $-CH_3$ acetate), 3.00 (t, 2H, $-N-CH_2-$), 3.30 (s, 3H, $-NH_3$), 3.74 (t, 2H, $-O-CH_2-$), 5.37 (s, 1H, OH)	C 40.07 H 9.27 N 12.12	C 39.67 H 9.09 N 11.57
2	$C_3H_9O_3N$	107	82	3290, 3058, 2939, 2879, 1650, 1531, 1382, 1174, 1062	δ_H 3.10 (t, 2H, $-N-CH_2-$), 3.50 (s, 3H, $-NH_3$), 3.74 (t, 2H, $-O-CH_2-$), 5.60 (s, 1H, O)	C 32.98 H 8.34 N 12.56	C 33.67 H 8.41 N 13.08
3	$C_5H_{13}O_4N$	151	78	2970, 2931, 2877, 1566, 1411, 1355, 1309, 1117, 1070, 1022	δ_H 1.29 (s, 3H, $-CH_3$), 2.97 (t, 2H, $-N-CH_2$), 3.27 (q, 1H, $-CH$), 3.71 (t, 2H, $-O-CH_2$), 5.02 (m, 3H, $-OH$)	C 40.21 H 8.45 N 9.38	C 39.73 H 8.61 N 9.27
4	$C_6H_{15}O_4N$	165	90	3400, 2050, 1670, 1590, 1415,1080, 955	δ_H 1.91 (s, 3H, $-CH_3$ acetate), 3.14 (t, 4H, $-N-CH_2-$), 3.60 (s, 2H, $-NH_2$), 3.80 (t, 4H, $-O-CH_2-$), 5.37 (s, 2H, $-OH$)	C 42.85 H 9.25 N 7.96	C 43.64 H 9.09 N 8.48
5	$C_5H_{13}O_4N$	151	80	3188, 2923, 2852, 1554, 1395, 1334, 1066, 1043, 1016, 956	δ_H 1.30 (s, 3H, $-CH_3$), 3.08 (t, 4H, $-N-CH_2$), 3.80 (t, 4H, $-O-CH_2$), 3.98 (q, 1H, $-CH$),5.02 (m, 4H, $-OH$)	C 40.51 H 9.24 N 9.15	C 39.73 H 8.61 N 9.27
6	$C_7H_{17}O_5N$	195	81	3208, 2893, 2852, 1564, 1395, 1344, 1026, 1011	δ_H 1.91 (s, 3H, $-CH_3$), 2.88 (s, 3H, $-CH_3$), 3.26 (t, 4H, $-N-CH_2$), 3.87 (t, 4H, $-O-CH_2$), 5.27 (m, 4H, $-OH$)	C 42.69 H 8.59 N 7.65	C 43.08 H 8.71 N 7.18
7	$C_7H_{17}O_4N$	179	70	3217, 2788, 2696, 1587, 1463, 1375, 1340, 1137, 1074, 1008, 759	δ_H 1.87 (s, 3H, $-CH_3$), 2.80 (t, 4H, $-N-CH_2$), 3.27 (s, 3H, $-NMe$), 3.70 (bs, 1H, NH), 3.90 (t, 4H, $-O-CH_2$), 5.21 (m, 4H, $-OH$)	C 45.62 H 9.58 N 7.56	C 46.93 H 9.50 N 7.82
8	$C_6H_{15}O_4N$	165	84	3225, 2680, 2190, 1470, 1120, 956	δ_H 2.56 (t, 4H, $-N-CH_2$), 3.31 (s, 3H, $-NMe$), 3.60 (bs, 1H, NH), 3.79 (t, 4H, $-O-CH_2$), 5.38 (m, 4H, $-OH$)	C 44.21 H 9.26 N 8.47	C 43.64 H 9.09 N 8.48

tored by thin layer chromatography, using aluminium sheets coated with silica gel with methanol as the mobile phase. The resultant colourless, strongly hygroscopic, viscous liquids were kept at 80 °C under vacuum overnight to remove volatiles (the present ILs are thermally stable to ~300 °C). The synthesized ILs were characterized via infrared (Shimadzu 8400S) and ^{1}H-nuclear magnetic resonance (Bruker, 400 MHz) spectrometry and elemental analyses (Leco 932). The water content of the ILs was ~1000 ppm, determined with a coulometric Karl Fischer Titrator DL 39 (Mettler Toledo).

2.2. Anti-microbial activity

The ILs were assayed for anti-microbial activity against five registered microbial isolates obtained from the Institute of Medical Research (IMR), Kuala Lumpur, Malaysia. These were: gram-positive *Staphylococcus aureus* S 1426 and *Listeria monocytogenes* L 49 as well as gram-negative *Salmonella typhi* S 1180, *Vibrio cholera*e V 116 and *Klebsiella pneumonia* K 41. This test was conducted at the Department of Cell and Molecular Biology, University Putra Malaysia, using the well-diffusion method (Magaldi et al., 1998, 1999). Test plates were prepared as follows. Muller Hinton agar (20 mL) (Merck, Germany) was melted and cooled to 55 °C and then inoculated with 1 mL of the bacterial suspension. The inoculated agar was transferred onto a petri-plate and allowed to cool. Upon solidification of the medium, 6 mm diameter holes were created in the central part of the agar plate and 20 μL of IL solution, at concentrations 1%, 10% and 20% (v/v) in deionized water, were poured into the wells. The plates were then incubated at 37 °C, for 24 h or until visible growth was established, and the diameter of the inhibition-cleared zone around each well was determined. The screening results were compared with a standard antibiotic: gentamicin (Atlantic Laboratories, Thailand).

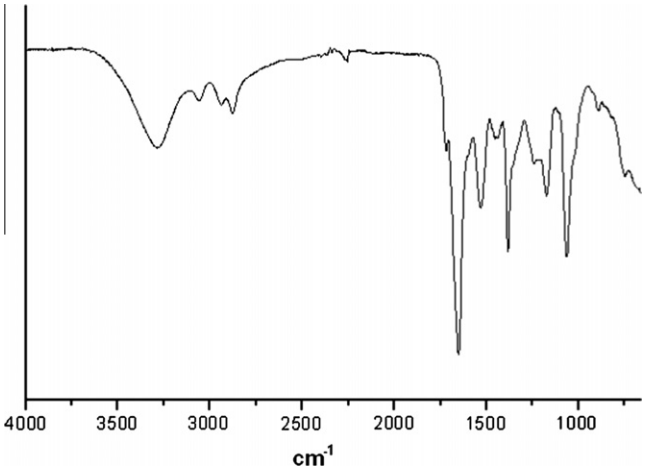

Fig. 2. IR spectrum of IL**2** (2-hydroxylethylammonium formate).