Ionic liquids with dual biological function: sweet and anti-microbial, hydrophobic quaternary ammonium-based salts†

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Received (in Montpellier, France) 30th July 2008, Accepted 29th August 2008
First published as an Advance Article on the web 22nd October 2008
DOI: 10.1039/b813213p

The dual nature of ionic liquids has been exploited to synthesize materials that contain two independent biological functions by combining anti-bacterial quaternary ammonium compounds with artificial sweetener anions. The synthesis and physical properties of eight new ionic liquids, didecyldimethylammonium saccharinate ([DDA][Sac]), didecyldimethylammonium acesulfamate ([DDA][Ace]), benzalkonium saccharinate ([BA][Sac]), benzalkonium acesulfamate ([BA][Ace]), hexadecylpyridinium saccharinate ([HEX][Sac]), hexadecylpyridinium acesulfamate ([HEX][Ace]), 3-hydroxy-1-octyloxymethylpyridinium saccharinate ([1-(OctOMe)-3-OH-Py][Sac]), and 3-hydroxy-1-octyloxymethylpyridinium acesulfamate ([1-(OctOMe)-3-OH-Py][Ace]), are reported, as well as the single crystal structures for [HEX][Ace] and [1-(OctOMe)-3-OH-Py][Sac]. Determination of anti-microbial activities is described for six of the ILs. While some exhibited decreased anti-microbial activity others showed a dramatic increase. For two of the ionic liquids, [DDA][Sac] and [DDA][ACE], oral toxicity, skin irritation, and deterrent activity was also established. Unfortunately, both ILs received a Category 4 (harmful) rating for oral toxicity and skin irritation. However, deterrent activity experiments point to use as an insect deterrent, as both ILs scored either "very good" or "good" against several types of insects.

Introduction

Ionic liquids (ILs) are currently defined as salts that are composed solely of cations and anions which melt below 100 °C. These salts have been studied for a variety of applications such as in electrochemistry,[1–3] separation science,[4–7] chemical synthesis,[8–13] and catalysis,[14–16] however, until recently, very few, if any, ILs had been used as liquid materials themselves.[17,18] In addition, those material applications which have appeared, typically concentrated on a single desirable property brought by either the cation or the anion. But ILs, by definition, have at least two discrete types of ions, both of which can provide a unique property or function. Thus, our goal has been to explore how to exploit the dual nature of ILs by preparing materials that possess two functions, particularly two biological functions. Here, we present the combination of anti-bacterial quaternary ammonium compounds (QACs) with artificial sweeteners.

The anti-bacterial properties of QACs were first discovered during the late 19th century, amongst carbonium dye compounds, such as auramin, methyl violet, and malachite green.[19] Initially, QACs were found to be most effective against gram-positive organisms, until Jacobs and Heidelberger[20–23] further exploited their anti-bacterial properties against other types of organisms. It was not until 1935 that the full potential of QACs was recognized by the chemical community, when the synthesis of benzalkonium chloride, a long-chain QAC, by Domagk[24] and further characterization of its anti-bacterial activities, proved that QACs were effective against a wider variety of bacterial strains.

Later, in the 20th century, researchers became more interested in the synthesis of water-soluble QACs for potential applications as surfactants,[25,26] anti-electrostatic agents,[27] anti-corrosive agents,[28] disinfectants,[29] and phase-transfer catalysts.[30] These newly developed water-soluble QACs showed anti-bacterial action against not only gram-positive and gram-negative bacteria, but also pathogen species of fungi and protozoa.[31] These discoveries led to applications for

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† Electronic supplementary information (ESI) available: Characterization data. Fig. S1: ORTEP (50% probability thermal ellipsoids) of the asymmetric unit of [HEX][Ace]. Fig. S2: Close contacts around the cations in [HEX][Ace]. Fig. S3: π-Stacking modes of the polymeric cation in [HEX][Ace]. Fig. S4: π-Stacking mode of the dimeric cation in [HEX][Ace]. Fig. S5: ORTEP (50% probability thermal ellipsoids) of the asymmetric unit of [1-(OctOMe)-3-OH-Py][Sac]. CCDC reference numbers 687477 and 687478. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b813213p

QACs in wood preservation[32–34] and as preservatives in common household products,[35] especially for general environmental sanitation in hospitals and food production facilities. Furthermore, QACs have been used as penetration enhancers for transnasal and transbuccal drug delivery, such as nasal vaccinations.[36] The ability of QACs to penetrate and open cell membranes has been widely used in drug delivery such as liposomes, which consists of long alkyl chain QACs, and non-viral gene delivery.[37]

We have had specific interest in employing the IL concept to pair the biological activity of a class of compounds such as QACs, with a second biological activity inherent in the counterion.[38] One such class of ions, which has also seen independent use in preparing 'edible' ILs, includes non-nutritive sweeteners such as saccharinate and acesulfame.[39,40] Salts of these anions are currently used in food products and are approved as food additives by most national and global health agencies. Yet, only a handful of quaternary ammonium saccharinates and acesulfamates have been reported in the literature.[41] Here we demonstrate the concept of preparing ILs by pairing the biological activity inherent in the cation with a separate biological function possessed by the anion with the synthesis, physical properties, anti-microbial activities, toxicity, and deterrent activity of new QAC-based ILs.

Results and discussion

Synthesis and characterization

Synthesis. Didecyldimethylammonium saccharinate ([DDA][Sac]), didecyldimethylammonium acesulfamate ([DDA][Ace]), benzalkonium saccharinate ([BA][Sac]), benzalkonium acesulfamate ([BA][Ace]), hexadecylpyridinium saccharinate ([HEX][Sac]), hexadecylpyridinium acesulfamate ([HEX][Ace]), 3-hydroxy-1-octyloxymethylpyridinium saccharinate ([1-(OctOMe)-3-OH-Py][Sac]) and 3-hydroxy-1-octyloxymethylpyridinium acesulfamate ([1-(OctOMe)-3-OH-Py][Ace]) (Fig. 1) were prepared in high yield as hydrophobic salts from commercially available QACs benzalkonium chloride ([BA][Cl]), didecyldimethylammonium chloride ([DDA][Cl]), and hexadecylpyridinium chloride ([HEX][Cl]), and from one pyridinium salt, 3-hydroxy-1-octyloxymethylpyridinium chloride [1-(OctOMe)-3-OH-Py][Cl], which was prepared by a nucleophilic substitution reaction of 3-hydroxypyridine by octyl chloromethyl ether under anhydrous conditions. Each of the cations was paired with saccharinate or acesulfamate by a stoichiometric metathesis reaction in aqueous solution, using sodium saccharin ([Na][Sac]) or potassium acesulfame ([K][Ace]). The hydrophobic

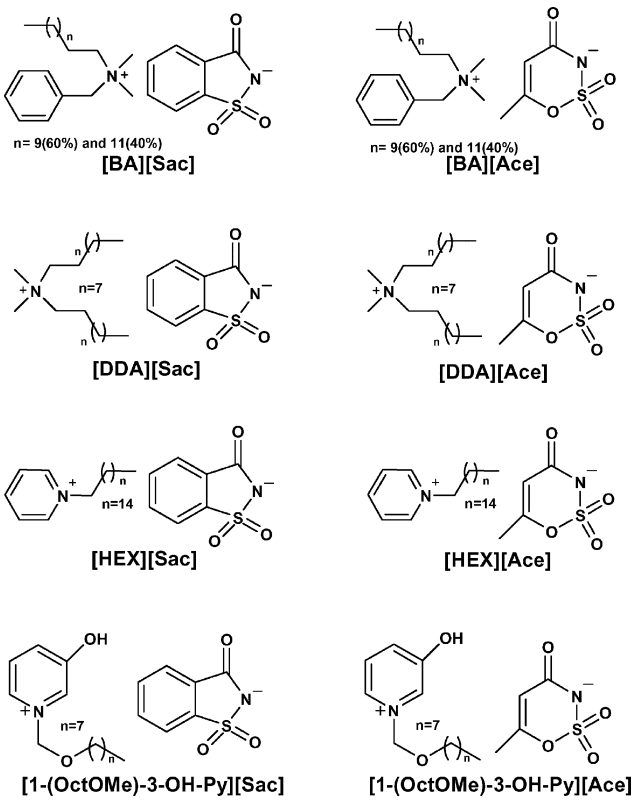

Fig. 1 Structures of the synthesized ILs.

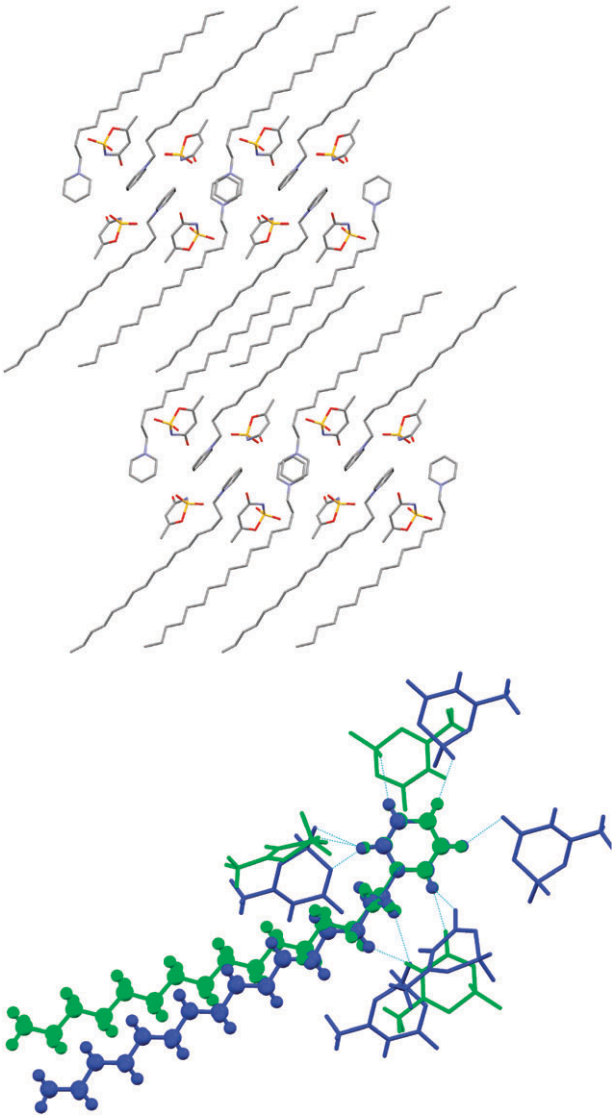

Fig. 2 Packing diagram along the *a* crystallographic axis for [HEX][Ace] (top) and overlay of the two cations in the asymmetric unit including the anions with close contacts to each (bottom).

nature of these salts allowed them to be easily extracted from the aqueous phase into chloroform.

All of the newly prepared ILs were found to be low melting solids at room temperature with the exception of [DDA]-containing salts, the only cation without an aromatic ring, which were found to be liquid at room temperature. The salts studied are only sparingly soluble in cold and hot water, but freely soluble and stable in many organic solvents (*e.g.*, chloroform, methanol, ethanol, ethyl acetate, *N*,*N*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO)).

Crystal structures. Single-crystal structures for two of the compounds, [HEX][Ace] and [1-(OctOMe)-3-OH-Py][Sac], also confirmed the syntheses. Although not the focus of this paper, interesting packing behavior was observed which may provide clues to the low melting nature of these compounds in particular and QAC ILs in general.

The packing diagram for [HEX][Ace] (Fig. 2) reveals that the cation tails interdigitate to create charge-rich and hydrophobic regions. Closer examination indicates that the two unique cations are not equivalent with slight differences in the orientation of the hexadecyl tail groups. This modest difference leads to completely different packing environments. One cation π-stacks in a polymeric fashion (Fig. 3) and has only three close contacts with the anions. The second cation forms a π-stacked dimer with anions capping each open face. These cations have five close contacts with the anions.

Fig. 4 illustrates the packing in the structure of [1-(OctOMe)-3-OH-Py][Sac]. Here the strong hydrogen bonding between the cation and anion dominates and a single cation/anion pair is found in the asymmetric unit. These hydrogen bonded ion pairs stack in alternate directions.

Thermal behavior. The thermal properties of the ILs (Table 1) were determined by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). All of the synthesized salts exhibited melting points below 100 °C, allowing their classification as ILs. Interesting phase transition behavior was observed for [DDA][Sac], [DDA][Ace] and [HEX][Ace] which was not found for the other ILs. These three ILs had a detectable glass transition-type transformation at −33, −53 and −11 °C, respectively. Following glass transition, samples [DDA][Sac] and [Hex][Ace] exhibited consecutive crystallization and melting transitions. On the contrary, [DDA][Ace] was the only IL obtained that did not exhibit any other thermal transition besides a glass transition.

As seen in Table 1, all the ILs were found to be thermally stable to temperatures ranging between 160 and 210 °C. One-step decomposition was found for [BA][Sac], [DDA][Sac], [1-(OctOMe)-3-OH-Py][Sac] and [1-(OctOMe)-3-OH-Py][Ace]. The anions $[Sac]^-$ and $[Ace]^-$ normally display a two-step decomposition, suggesting that the cations, $[BA]^+$, $[DDA]^+$ and $[1\text{-(OctOMe)-3-OH-Py}]^+$, play a role in the decomposition of these ILs resulting in the single decomposition step observed.

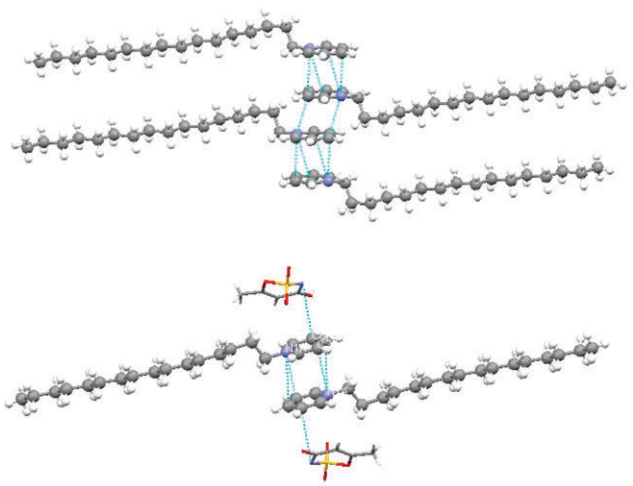

Fig. 3 One cation in [HEX][Ace] π-stacks in a polymeric fashion (interplanar spacing 3.5 and 3.6 Å) (top), while the second cation forms π-stacked dimers (interplanar spacing 3.4 Å) with acesulfamate anions capping both sides (bottom).

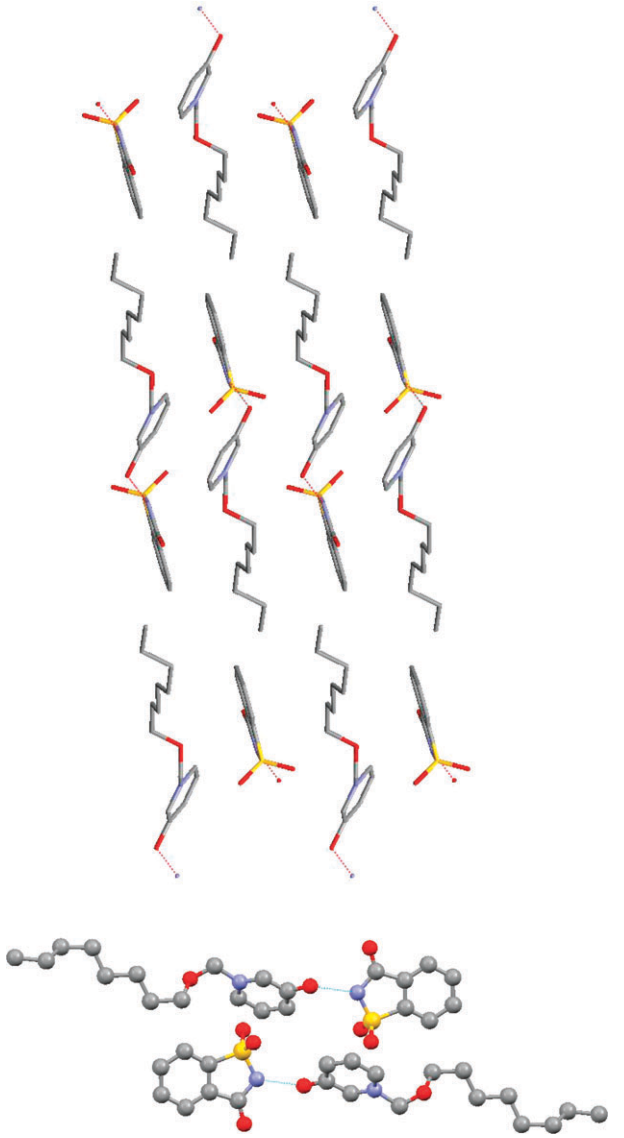

Fig. 4 Packing diagram along the *a* crystallographic axis for [1-(OctOMe)-3-OH-Py][Sac] (top) and close up of the hydrogen bonding and alternate stacking of the ion pairs (bottom).

Table 1 Thermal properties[a]

	T_g	T_c	T_{s-s}	T_m	$T_{onset5\%}$	T_{onset}
Ionic liquids						
[BA][Sac]	—	16[b]	—	74	164	204
[DDA][Sac]	−33	15[c]	—	16	187	214
[HEX][Sac]	—	30[c]	—	66	207	253/412[g]
[1-(OctOMe)-3-OH-Py][Sac]	—	—	—	95–98[e]	206	301
[BA][Ace]	—	30[c]	−36	90	184	187/249/394[g]
[DDA][Ace]	−53	—	—	—	189	232/426[g]
[HEX][Ace]	−11	5[b]	—	57	212	267/494[g]
		18[c]				
[1-(OctOMe)-3-OH-Py][Ace]	—	—	—	79–81[e]	203	267
Starting materials						
Na[Sac]	—	98[c]	—	120	431	459/541[g]
K[Ace]	—	—	—	68	190	192/260[g]
[BA][Cl]	—	16[bd]	—	—	143	169
[DDA][Br][f]	—	—	—	—	166	196
[HEX][Cl]	—	45[c]	—	73	184	213
[1-(OctOMe)-3-OH-Py][Cl]	—	—	—	68–70[e]	178	247

[a] Phase transition points (°C) were measured from transition onset temperatures determined by DSC from the second heating cycle at 5 °C min^{-1}, after initially heating and then cooling of the samples to −100 °C unless otherwise indicated: T_g = glass transition temperature; T_c = crystallization temperature; T_{s-s} = solid–solid transition temperature on heating; T_m = melting point on heating. Decomposition temperatures were determined by TGA, heating at 5 °C min^{-1} under air atmosphere and are reported as ($T_{onset\ 5\%}$) onset to 5 wt% mass loss and (T_{onset}) onset to total mass loss. [b] Transition measured on heating cycle. [c] Transition measured on cooling cycle. [d] Transition only during first heating [e] Visual melting point range *via* hot-plate apparatus. [f] Multiple transitions due to presence of water in starting material. [g] Multiple decomposition steps.

Two-step decomposition was observed for samples [HEX][Sac], [DDA][Ace] and [HEX][Ace]. Increase in the thermal stability (first decomposition step) in these salts, over the thermal stabilities of the starting materials may indicate an anion stabilizing effect on the parent cations; $[HEX]^+$ and $[DDA]^+$. Similarly, the stabilizing effect of the anion can be observed for the sample of [BA][Ace], which is the only sample that exhibits a three-step decomposition pathway.

Biological properties

Anti-microbial, anti-bacterial and anti-fungal activities. The minimum inhibitory concentration (MIC) (Table 2) and minimum bactericidal or fungicidal concentration (MBC) (Table 3) were determined for [BA][Sac], [DDA][Sac], [BA][Ace] and [DDA][Ace]. (The starting materials, [BA][Cl] and [DDA][Cl], which inherently exhibit anti-microbial, anti-bacterial and anti-fungal activities, included in Tables 2 and 3 for comparison.) The activities of the ILs approach those of commercially available [BA][Cl] and [DDA][Cl], although the ILs were not found to be limited to a specific class of bacteria or fungi. These same observations have been seen in previous literature,[42] where it was found that the anti-microbial activities for imidazolium chlorides, tetrafluoroborates, and hexafluorophosphates were independent of the counterion.

It is thought that 1-alkoxymethylpyridinium chlorides are strongly active against microbes, yet in previous research,[43] it was concluded that the antimicrobial activities depended on the substituent at the 3-position of the pyridine ring. Unfortunately, [1-(OctOMe)-3-OH-Py][Cl] and the ILs, [1-(OctOMe)-3-OH-Py][Sac] and [1-(OctOMe)-3-OH-Py][Ace] exhibited no antimicrobial activity.

Acute oral toxicities. The acute oral toxicities of [DDA][Ace] and [DDA][Sac] were determined in three male and three female Wistar rats, where the rats received a dosage of 300 mg/kg b.w. (mg of substance per kg of body weight) and 2000 mg/kg b.w. of each IL. The ILs were suspended in water

Table 2 MIC values[a]

	Ionic liquid				Starting materials	
Strain	[BA][Sac]	[DDA][Sac]	[BA][Ace]	[DDA][Ace]	[BA][Cl]	[DDA][Cl]
S. aureus	4	4	4	8	2	2
S. aureus (MRSA)	4	4	4	4	2	2
E. faecium	8	8	8	8	4	4
E. coli	16	16	31	16	8	8
M. luteus	8	4	8	8	4	2
S. epidermidis	4	4	4	4	2	2
K. pneumoniae	4	4	8	4	4	4
C. albicans	16	16	16	16	8	8
R. rubra	16	16	16	16	8	4
S. mutans	0.1	31	1	16	2	2
Mean value	**8.0**	**10.7**	**10.0**	**10.0**	**4.4**	**3.8**

[a] In ppm.

Table 3 MBC values[a]

Strain	Ionic liquids				Starting materials	
	[BA][Sac]	[DDA][Sac]	[BA][Ace]	[DDA][Ace]	[BA][Cl]	[DDA][Cl]
S. aureus	31.2	62.5	31.2	16	62.5	31.2
S. aureus (MRSA)	31.2	31.2	31.2	31.2	31.2	31.2
E. faecium	16	16	31.2	31.2	31.2	31.2
E. coli	62.5	16	125	62.5	62.5	31.2
M. luteus	62.5	31.2	62.5	62.5	31.2	31.2
S. epidermidis	31.2	16	62.5	31.2	16	31.2
K. pneumoniae	62.5	16	31.2	31.2	31.2	16
C. albicans	31.2	16	31.2	31.2	16	16
R. rubra	62.5	31.2	62.5	62.5	31.2	31.2
S. mutans	0.5	62.5	16	125	16	16
Mean value	**39.1**	**29.9**	**48.5**	**48.5**	**32.9**	**26.6**

[a] In ppm.

Table 4 Criteria for the estimation of the deterrent activity based on the total coefficient

Total coefficient	Deterrent activity
200–151	Very good
150–101	Good
100–51	Medium
50–0	Weak

prior to intragastric administration. After receiving the dosage of 300 mg/kg b.w. for [DDA][Ace] or [DDA][Sac], one male rat died during the first 24 h, while the other 5 rats remained alive. But when the dosage was increased to 2000 mg/kg b.w., all of the rats died between 24 and 96 h after administration. Death was preceded by decrease in spontaneous motor activity, excessive excretion from nostrils, and difficulty of breathing. The above results indicate the acute toxicity range for both ILs is between 300–2000 mg/kg b.w. in male and female rats. Thus, these ILs would be classified as category 4 (harmful) toxins according to standard OECD grading.[44]

Skin irritation. Skin irritation of [DDA][Ace] and [DDA][Sac] was determined on New Zealand albino rabbits. All of the exposed animals exhibited defined erythema after 1 h. The erythema had increased to severe and severe eschar formation was also observed after 24 h. Although no edema occurred, the skin irritation of these ILs is defined as category 4 (the highest) by standard OECD grading.[45]

Deterrent activity. The deterrent activity of [DDA][Ace] and [DDA][Sac] toward *Tribolium confusum* (larvae and beetles), *Sitophilus granarius* (beetles) and *Trogoderma granarium* (larvae) was determined by using a known method, in which the amount of food consumed is monitored over a specific time interval. Three deterrent coefficients had to be calculated from the average amount of food consumed: (a) the absolute coefficient of deterrency, $A = (CC - TT)/(CC + TT) \times 100$, (b) the relative coefficient of deterrency, $R = (C - T)/(C + T) \times 100$, and (c) the total coefficient of deterrency, which is the sum of the absolute and the relative coefficients, $T = A + R$.[46] In these

Table 5 Feeding deterrent activity

Ionic liquid	Relative coefficient	Absolute coefficient	Total coefficient	Deterrent activity
Sitophilus granarius (beetles)				
[DDA][Ace]	97.5	57.9	155.5	Very good
[DDA][Sac]	57.8	56.6	114.5	Good
Azadirachtin[a]	100.0	74.3	174.3	Very good
$LSD_{0.05}$[b]	57.8	28.8	60.1	
Trogoderma granarium (larvae)				
[DDA][Ace]	94.0	85.0	179.0	Very good
[DDA][Sac]	94.2	86.1	180.3	Very good
Azadirachtin[a]	100.0	94.2	194.2	Very good
$LSD_{0.05}$[b]	0.3	7.6	7.8	
Tribolium confusum (beetles)				
[DDA][Ace]	96.2	19.1	115.3	Good
[DDA][Sac]	95.0	90.7	186.6	Very good
Azadirachtin[a]	100.0	85.0	185.0	Very good
$LSD_{0.05}$[b]	0.6	9.2	9.0	
Tribolium confusum (larvae)				
[DDA][Ace]	95.0	64.1	159.1	Very good
[DDA][Sac]	95.3	88.8	184.1	Very good
Azadirachtin[a]	100.0	88.4	188.4	Very good
$LSD_{0.05}$[b]	2.1	29.4	29.1	

[a] Natural deterrent. [b] The least significant differences at the 5% level of significance.

equations, CC is the average weight of the food consumed in the control, TT is the average weight of the food consumed in the no-choice test, and T and C are the average weights of the food consumed in the choice test.

The total coefficient value T is compared to standard values for deterrent activity in Table 4, where a value of 0 equals neutral activity and a value of +150 to +200 corresponds to very high deterrent activity. The results of deterrent activity for [DDA][Ace] and [DDA][Sac] are compared to a natural deterrent, azadirachtin, in Table 5. The ILs received either 'very good' or 'good' deterrent activity for all tested insects. In particular, [DDA][Sac] exhibited the same deterrent activity toward *Tribolium confusum* (larvae and beetles) as azadirachtin and thus, could be classified as a potential synthetic insect deterrent.

Conclusions

We have prepared ILs of two biologically active ions by combining anti-microbial QACs cations with sweetener anions. Some of these ILs demonstrate properties such as limited water solubility, high thermal stability, and good deterrent activity against insects; which suggest potential application as an insecticide. Although oral toxicity and skin irritation values were higher than hoped, there is still potential use, not only for these ILs, but also for new, related ILs which can be prepared by tuning the composition in such a manner to reduce toxicity.

In general, research in the IL field has begun to shift from random combinations of ions to a design scheme in which both the cation and anion are chosen based on the desired physical, chemical, and biological properties. All procedures performed on these animals were in accordance with established guidelines and were reviewed and approved by the University of Alabama's Institutional Animal Care and Use committee. As our fundamental understanding of IL behavior increases, more control over the resultant properties of the salts will be possible, and the number of potential applications, such as those presented here, will continue to grow.

Experimental

Chemicals and microorganisms

Benzalkonium chloride [BA][Cl] (molecular formula $C_6H_5CH_2N(CH_3)_2RCl$ where R = $C_{12}H_{25}$ (60%) and $C_{14}H_{29}$ (40%)), didecyldimethylammonium bromide [DDA][Br] (tech., 75 wt% gel in water), hexadecylpyridinium chloride [HEX][Cl] (monohydrate, minimum 99%) and sodium saccharinate Na[Sac] (hydrate, minimum 98%) were purchased from Sigma Aldrich. Potassium acesulfamate K[Ace] ($\geq$ 99%) was purchased from Fluka.

The following microorganisms were used: bacteria *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* (MARSA) ATCC 43300, *Enterococcus faecium* ATCC 49474, *Escherichia coli* ATCC 2592,2 *Micrococcus luteus* ATCC 9341, *Staphylococcus epidermidis* ATCC 12228, *Klebsiella pneumoniae* ATCC 4352, and fungi *Candida albicans* ATCC 10231, *Rhodotorula rubra* PhB and *Streptococcus mutans* PCM (Polish Collection of Microorganisms) 2502. The *Rhodotorula rubra* was obtained from the Department of Pharmaceutical Bacteriology, Poznań University of Medical Sciences, Poland.

General synthesis[47]

Solid (0.001 mol) Na[Sac] or K[Ace] was dissolved in distilled water and added to hot aqueous solutions containing 0.001 mol of [BA][Cl], [DDA][Br] or [HEX][Cl]. The mixtures were stirred at 60 °C for 1 h and then cooled to room temperature. The hydrophobic product was extracted with chloroform and purified using distilled water washes, until chloride or bromide ions were no longer detected in the product phase using $AgNO_3$. The chloroform was evaporated and the IL was dried under vacuum.

The starting material 3-hydroxy-1-octyloxymethylpyridinium chloride was prepared according to previous literature.[43] Solid (0.03 mol) K[Ace] or Na[Sac] was dissolved in distilled water and then added to an aqueous solution containing 0.03 mol [1-(OctOMe)-3-OH-Py][Cl]. The reaction was completed by gentle heating and stirring in a water bath for 2 h. The heat was removed and stirring was continued at room temperature for 24 h. The mixture was filtered, and the precipitate was washed with cold distilled water (3 × 20 mL) to give an oil or solid IL. The IL was dried under vacuum, and recrystallized from ethyl acetate and then dried again under vacuum. Karl–Fischer analysis indicated the water content of all dried ILs to be less than 500 ppm.

Thermal analysis

Melting points and other thermal transitions of the ILs were determined by DSC, with a TA Instruments model 2920 Modulated DSC (Newcastle, DE), cooled with a liquid nitrogen cryostat. The calorimeter was calibrated for temperature and cell constants using indium standard (mp 156.61 °C, ΔH = 28.71 J g^{-1}). Data were collected at constant atmospheric pressure where the ILs were placed in aluminum pans with sample sizes from 5 to 15 mg. An empty sample pan was used as reference. All experiments were performed at a heating rate and a cooling rate of 5 °C min^{-1}. The DSC was adjusted so zero heat flow was between 0 and −0.5 mW, and the baseline drift was less than 0.1 mW over the temperature range 0–180 °C.

Thermal decomposition temperatures were measured in the dynamic heating regime using a TGA, 2950 TA Instrument, under air atmosphere. The amount of IL used was between 2 and 10 mg in each case, and the samples were heated from 40 to 800 °C at a constant heating rate of 5 °C min^{-1}. Decomposition temperatures ($T_{5\%dec}$) were determined from onset to 5 wt% mass loss; this provides a more realistic representation of thermal stability at elevated temperatures.

X-Ray diffraction

Crystalline samples of [HEX][Ace] and [1-(OctOMe)-3-OH-Py][Sac] were mounted on a glass fiber on a goniometer head of a Siemens SMART CCD diffractometer equipped with a Mo-Kα source (λ = 0.71073 Å) and a graphite monochromator. Data collection was conducted at −100 °C which was achieved by streaming cold nitrogen over the

crystal. Final unit cell parameters were determined by least-squares refinement of the hemispherical data set obtained from 20 s exposures. Data were corrected for Lorentz and polarization effects and absorption using SADABS.[48] The initial structure solution was carried out using the direct methods option in SHELXTL version 5.[49] The positions of all non-hydrogen atoms were refined anisotropically. The hydrogen atoms were added and allowed to refine unconstrained in order to obtain proper close contact interactions.

Crystal data for [HEX][Ace]. $C_{25}H_{42}N_2O_4S$; M_r = 466.67; triclinic, space group $P\bar{1}$; T = 173 K; a = 7.921(3), b = 13.374(5), c = 25.689(10) Å, α = 76.755(7), β = 82.225(7), γ = 89.260(7)°; Z = 4; V = 2624.2(17) Å^3; D_c = 1.181 g cm^{-3}; 7459 independent (R_{int} = 0.0249) and 5755 observed ([$I > 2\sigma(I)$]) reflections; GooF = 1.070; R_1, wR_2 [$I > 2\sigma(I)$] = 0.0462, 0.1208; R_1, wR_2 (all data) = 0.0641, 0.1411

Crystal data for [1-(OctOMe)-3-OH-Py][Sac]. $C_{21}H_{28}N_2O_5S$; M_r = 420.51; triclinic, space group $P\bar{1}$; T = 173 K; a = 8.1626(15), b = 8.7141(16), c = 16.756(3) Å, α = 81.872(3), β = 80.780(3), γ = 62.850(3)°; Z = 2; V = 1043.7(3) Å^3; D_c = 1.338 g cm^{-3}; 2969 independent (R_{int} = 0.0170) and 2515 observed ([$I > 2\sigma(I)$]) reflections; GooF = 1.033; R_1, wR_2 [$I > 2\sigma(I)$] = 0.0362, 0.0871; R_1, wR_2 (all data) = 0.0472, 0.0937

Antimicrobial characteristics

Anti-microbial activity was determined by the tube dilution method. Bacteria strains were cultured in Mueller–Hinton broth for 24 h and fungi were cultured on Sabouraud agar for 48 h. Suspensions of the above microorganisms, at a concentration of 10^6 cfu mL^{-1}, were prepared from each culture. Two milliliters of serial twofold dilutions of IL were inoculated with the above-mentioned suspension to obtain a final concentration of (1–5) × 10^5 cfu mL^{-1}.

Growth of the microorganism (or its lack) was determined visually after incubation for 24 h at 35 °C (bacteria) or 48 h at 22 °C (fungi). The lowest concentration at which there was no visible growth (turbidity) was determined to be the minimal inhibitory concentration (MIC). Then, from each tube content, 10 mL (calibrated loop) was smeared on an agar medium with inactivates (0.3% lecithin, 3% polysorbate 80, and 0.1% L-cysteine) and incubated for 48 h at 35 °C (bacteria) or for 5 days at 22 °C (fungi). The lowest concentration of the IL that killed 99.9% or more of the microorganism was defined as the minimum biocidal concentration (MBC).

Acute oral toxicity test

The toxicity was tested according to the method of acute toxic class.[44] Three male (250 ± 25 g) and three female (170 ± 17 g) Wistar rats were used for each IL tested. The ILs were first suspended in distilled water and then administered intragastrically at doses of 300 mg/kg b.w. and 2000 mg/kg b.w. After the dose was administered, the rats were observed for 14 days.

Skin irritation tests

Each IL was tested on 3 male New Zealand albino rabbits, where the fur was previously removed from the back of the rabbit. Half a milliliter of the ILs (100%, pure) was distributed on two 6 cm^3 sites of the same animal. The application site was then covered with a porous gauze dressing and secured in place with tape. After a 4 h exposure, the dressing was removed and the application site was gently washed with water. Observations were then conducted at 1, 24, 48, and 72 h, where the test sites were evaluated for erythema and edema using a prescribed scale.[45]

Feeding deterrent activity tests

Three species of insects were selected for testing: *Tribolium confusum* Duv. (larvae and beetles), *Sitophilus granarius* L. (beetles), and *Trogoderma granarium* Ev. (larvae). Insects were grown on a wheat grain or whole-wheat meal diet in laboratory colonies which was maintained at 26 ± 1 °C and 60 ± 5% relative humidity. The laboratory assay was conducted according to the method developed and standardized for storage insects feeding activity for both choice and no-choice test.[46]

Wheat wafer discs (1 cm in diameter × 1 mm thick) were saturated by dipping in either ethanol (96%) only (control) or in a 1% ethanol solution of [DDA][Ace] or [DDA][Sac]. After evaporation of the solvent by air-drying (30 min), the wafers were weighed and offered as the only food source for the insects over a five day period. The feeding of the insects was recorded under three conditions: (a) control test (two control discs (CC)), (b) choice test (a choice between one treated disc (T) and one control disc (C)), and (c) no-choice test (two treated discs (TT)). Each of the three experiments was repeated five times with 3 beetles of *Sitophilus granarius*, 20 beetles and 10 larvae of *Tribolium confusum*, and 10 larvae of *Trogoderma granarium*. The number of individual insects depended on the intensity of their food consumption. The beetles utilized in the experiments were unsexed, 7–10 days old, and the larvae were 5–30 days old. After five days of feeding, the discs were reweighed. The data from the experiments have been statistically corrected by an analysis of variance.

Acknowledgements

This work was supported by Poznań University of Technology, BW 32-222/2008.

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