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ARTICLE

Ionic liquids with a theophyllinate anion

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Ammonium and piperidinium theophyllinate-based ILs were synthesized and characterized. Physicochemical properties, such as thermal stability, phase transition temperatures, viscosity, density, refractive index, as well as surface activity, feeding deterrence, antifungal activity and also biodegradability were determined. The synthesized theophyllinate-based ILs were surface active compounds. They exhibited insect-feeding deterrent activities. At the same time, the studied salts are also efficient fungicides and potentially pesticidal ionic liquids. The obtained data suggest that certain structural modifications may increase the biodegradability in order to provide an environmentally friendly product.

Introduction

Ionic liquids (ILs, defined as organic salts with a melting below 100 °C) have been investigated for an increasing number of applications due to their unique properties[1,2]. The evaluation of ILs has proceeded very quickly from the first generation (with unique, designable physical properties), through the second generation (with targeted chemical properties, combined with selected physical properties), to the third generation (with targeted biological properties, combined with physical and chemical properties)[3]. ILs with biologically active ions were synthesized and shown to retain the original biological activity of both the cation and anion. They can be directly prepared from pharmaceutically-active ingredients[4-7] and also from commonly used pesticides[8-14]. Regulations of the European Parliament state that low-risk plant protection products should be identified and placed on the market[15]. Regulatory pressure to eliminate the most toxic, older synthetic pesticides in order to reduce the environmental impact is helping to drive the development of pesticides based on natural product.

Recently, herbicidal ionic liquids seem to be an interesting proposal[8,10-14]. They allow for reducing the herbicide dose per hectare, while controlling its toxicity (toxic phenoxy-based herbicides may become nontoxic as herbicidal ionic liquids)[8].

Theophylline (3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione also known as 1,3-dimethylxanthine) is a member of the xanthine family. This compound bears structural and pharmacological similarity to caffeine and is used in therapy of respiratory diseases. Theophylline is naturally found in cocoa beans and trace amounts are present in brewed tea. The high concentration of caffeine in young tea leaves protects the plant from virulent microbes[16] as well as insects[17], therefore theophylline was selected as an anion with promising properties.

ILs starting from natural compounds have recently attracted an increasing attention. The possibility to use anions originating from natural or renewable materials to prepare ILs has been marginally investigated. Natural amino acids have been used to develop ILs, in which the anion is a natural compound[18]. Additionally, ILs with lactate[19,20] and mandelate[21] as an anion have also been prepared. Herein, we present novel active ILs with theopyllinate as an anion. Ammonium cations with a sp^3 hybridisation and a long alkyl substituent were selected as counter ions based on the assumption, that hydrophobic, non-crystal and biologically active salts will be obtained. The aim of the study was to search for efficient and non-hazardous pesticidal ionic liquids.

Results and Discussion

Ammonium (**1**-**12**) with various substituents and piperidinium (**13**-**14**) theophyllinates were synthesized with a high yield 98-92% (Table 1). The structures of the 14 synthesized theophyllinate salts were shown in Figure 1. The majority of the tested salts are fundamentally novel, as only a single compound (**9**) has been tested in previous studies[22]. All salts are waxes or liquids at room temperature, except **1**, which is a solid with a melting point above 100 °C (116 °C, Table 2). The above-mentioned liquids and waxes might, in some cases, be considered to represent ILs. The synthesized ILs were stable in air and in contact with water and popular organic solvents. They were hygroscopic and require drying under reduced pressure at elevated temperature. The ILs could be made anhydrous by heating at 80 °C in vacuum and storing them

over P_4O_{10}. Upon such treatment of salts, the water content was determined to be less than 500 ppm by colorimetric Karl-Fischer titration. The obtained ILs were insoluble in hexane and ethyl acetate but were soluble in acetone, dichloromethane, DMSO and low molecular weight alcohols. All of the synthesized salts, with the exception of salts **7** and **8**, were exquisitely soluble in water. The purity of ILs with a long alkyl substituent was determined by a direct two-phase titration technique EN ISO 2871-2: 2010 and expressed as the content of the cationic active substance (Table 1). The purity of the obtained ILs was high (> 97%). All of the prepared ILs were characterized by ^{1}H NMR and ^{13}C NMR and elemental analysis. The chemical shift of the proton between the two nitrogen atoms in the imidazole ring reached lower values and covered a range of 0.65-0.84 ppm. The proton chemical shift value for theophylline was at 7.97 ppm, while for 9 the value reached 7.12 ppm. The chemical shifts of the proton for piperidinium theophyllinates (**13**-**14**) were lower, reaching 7.31 and 7.32 ppm accordingly.

Table 1. Prepared theophyllinates

Salt	R^1	R^2	R^3	State[a]	Surfactant content[b]
1	CH_3	$CH_2C_6H_5$	$C_{12}H_{25}$/ $C_{14}H_{29}$	solid	98
2	CH_2CH_2OH	CH_2CH_2OH	coco[c]	wax	99
3	CH_2CH_2OH	CH_2CH_2OH	oleyl[d]	wax	99
4	$(CH_2CH_2O)_x$[e]	$(CH_2CH_2O)_y$[e]	H-tallow[f]	liquid	98
5	CH_3	CH_3	oleyl[d]	wax	97
6	CH_3	CH_3	coco[c]	wax	97
7	CH_3	coco[c]	coco[c]	liquid	98
8	CH_3	H-tallow[f]	H-tallow[f]	wax	99
9	CH_3	$C_{10}H_{21}$	$C_{10}H_{21}$	liquid	99
10	CH_3	CH_2=$CHCH_2$	$CH_2C_6H_5$	liquid	-
11	$C_{10}H_{21}$	-	-	wax	99
12	$C_{12}H_{25}$	-	-	liquid	99
13	$C_{10}H_{21}$	-	-	liquid	99
14	$C_{12}H_{25}$	-	-	wax	99

[a] at 25 °C, [b] in %, [c] cocoalkyl chain distribution C_8 – 5, C_{10} – 6, C_{12} – 50, C_{14} – 19, C_{16} – 10, C_{18} – 10%, [d] oleyl chain distribution C_{12} – 1, C_{14} – 4, C_{16} – 12, C_{18} – 82%, [e] x + y = 15,[f] alkyl hydrogenated tallow chain distribution C_{12} – 1, C_{14} – 4, C_{16} – 31, C_{18} – 64%.

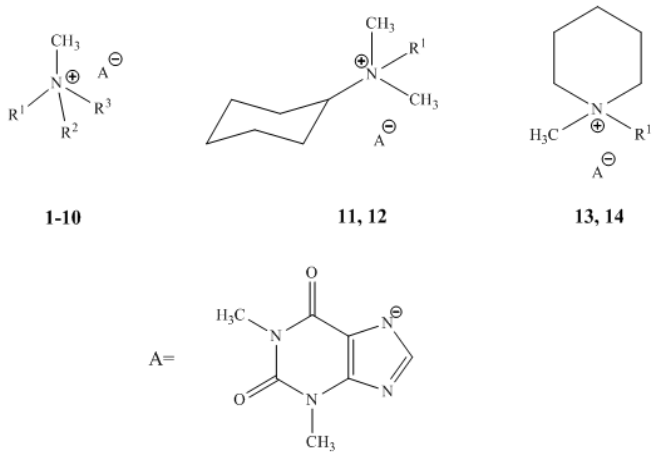

Figure 1. Structure of prepared theophyllinates

Data presented in Table 2 show that the prepared salts were thermally stable. They manifested glass transition temperatures in the range from -6 to -48 °C. Salts **1**, **6**, and **8** manifested no glass transition temperature. In case of **1**, **6** and **8** the crystallization and melting temperatures were observed. Decomposition temperatures for all tested salts were very diverse and changed in the range of 139 - 250 °C for $T_{onset\ 5\%}$ and 224 - 390 °C for $T_{onset\ 50\%}$, respectively.

Table 2. Thermal transitions and decomposition temperatures[a] of synthesized theophyllinates

Salt	T_g[b]	T_c[c]	T_m[d]	$T_{onset\ 5\%}$[e]	$T_{onset\ 50\%}$[e]
1	-	76	116	161	269
3	-6	-	-	250	357
4	-48	-	-	208	390
5	-28	-	-	196	288
6	-	-10,5	-0,7	206	236
7	-35	-	-	195	253
8	-	22	22	248	392
9	-46	-	-	184	224
10	-10	-	-	139	288

[a] in °C, [b] glass transition temperature,[c] crystallization temperature,[d] melting point on heating,[e] decomposition temperatures as $T_{onset5\%}$ to 5% and $T_{onset50\%}$ to 50% mass loss.

The obtained room temperature ionic liquids (RTILs) were subjected to the determination of density, viscosity and refractive index values in the temperature range of 20 - 80 °C. The highest density value reached 1.2119 g·cm^{-3} for **10**, whereas the lowest value reached 0.9448 g·cm^{-3} for **7** (Table 3).

Table 3. Density[a] of obtained RTILs

RTIL	d_{20}	d_{40}	d_{60}	d_{80}
4	1.0945	1.0805	1.0662	1.0517
7	0.9448[b]	0.9354	0.9228	0.9101
9	0.9699	0.9575	0.9449	0.9320
10	1.2119	1.1987	1.1852	1.1710
12	1.0575	1.0447	1.0316	1.0181
13	1.0973	1.0841	1.0709	1.0577

[a] in g·cm^{-3}, at 20, 40, 60 and 80°C, [b] at 25 °C.

The viscosity values for RTILs at 20 °C varied notably depending on the structure of the cation (Table 4). The highest value of 87.635 Pa·s was observed for **10**, while the lowest value of 0.370 Pa·s was observed for **4**. The differences in viscosity values diminished with increasing temperature and reached a similar level ranging from 0.074 to 0.012 Pa·s at 80 °C. Values of refractive index for the obtained RTILs varied in the range of 1.5290 - 1.4760. The dependence between the refractive index and temperature was linear - with the increase of temperature the refractive index values decreased, which can be seen in Figure 2 for **9**.

Table 4. Viscosity[a] of obtained RTILs

RTIL	η_{20}	η_{40}	η_{60}	η_{80}
4	0.370	0.078	0.026	0.012
7	0.755[b]	0.218	0.054	0.020
9	1.089	0.164	0.042	0.017
10	87.635	4.126	0.407	0.074
12	2.587	0.303	0.073	0.026
13	14.157	0.611	0.104	0.035

[a] Pa·s, at 20, 40, 60 and 80°C, [b] at 25 °C.

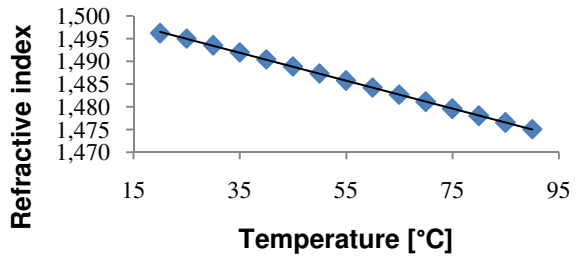

Figure 2. Refractive index of didecyldimethylammonium theophyllinate (**9**)

Table 5 presents parameters of surface activity: critical micelle concentration CMC, surface tension at CMC γ_{CMC}, surface excess concentrations at the saturated interface Γ_{max}, the minimum surface occupied by a molecule at the interface A_{min} and the Gibbs free energy of adsorption layer ΔG^0_{ads}. It was found, that the type of cation was decisive for CMC of aqueous solutions of the synthesized salts. In case of the same anion, the cation exchange caused a decrease of CMC value, e.g. from 38.9 and 11.8 mmol dm^{-3} for piperidinium (**13**-**14**) to 6.92 and 0.34 mmol dm^{-3} for ammonium theophyllinates. It was observed that elongation of alkyl substituents in the cation contributed to the decrease of CMC values. In ammonium theophyllinates water solutions (**1**-**7**, **9**) process of micellization began at lower concentrations than in piperidinium IL solutions. This was due to the presence of alkyl chain substituents longer than **8** carbon atoms in the cation of ammonium theophyllinates (**1**-**7**, **9**). The surface tension γ_{CMC} was reduced to a minimum for theophyllinate (**9**), reaching 29.59 mN m^{-1}. The negative values of ΔG^0_{ads} for all studied theophyllinates indicate that the process proceeds spontaneously.

Table 5. Surface activity of prepared theophyllinates

Salt	CMC[a]	γ_{CMC}[b]	Γ_{max}[c]	A_{min}[d]	$-\Delta G^0_{ads}$[e]
1	2.69	35.38	3.27	5.07	26.02
2	2.10	34.52	3.60	4.61	25.48
3	0.79	38.86	1.93	8.61	34.27
4	0.34	49.61	2.44	6.81	28.79
5	0.65	41.80	4.05	4.10	24.57
6	1.99	33.19	4.39	3.78	21.65
7	0.37	30.07	3.57	4.65	30.21
9	1.23	29.59	4.33	3.84	26.20
11	6.92	36.57	7.90	2.10	16.28
12	5.62	37.81	7.02	2.36	17.95
13	38.9	32.00	13.4	1.24	10.86
14	11.8	39.86	11.5	1.44	13.66

[a] mmol dm^{-3}, [b] mN m^{-1}, [c] 10^6mol m^{-2}, [d] 10^{19}m^2, [e] kJ m^{-1}.

Table 6. Criteria for the estimation of feeding deterrent activity based on the total coefficient

Total coefficient	Deterrent activity
200 – 151	very good
150 – 101	good
100 – 51	medium
50 – 0	week

Insect-feeding deterrent activities of synthesized theophyllinates towards *Tribolium confusum* (larvae and adults), *Sitophilus granarius* (adults), and *Trogoderma granarium* (larvae) were presented on the basis of the amount of food consumed. The activities were estimated by the criteria listed in Table 6. The results are presented in Table 7. All of the studied theophyllinates exhibited insect-feeding deterrent activities (from very good to medium, predominantly very good ones). Their activity was higher compared to theophylline or potassium theophyllinate. Compared to the standard (azadirachtin), their efficiency was lower, with the exception of **3**. By selecting the most appropriate cation in combination with the theophyllinate as an anion, a novel and effective insect-feeding deterrent may be obtained. The obtained results are at a similar level of activity to those obtained for the previously described ILs based on stored product insect antifeedants, which consist of tripolyphoshate, dihydrogen citrate and propionate anions[23].

Table 7. Feeding deterrent activities, total coefficient of prepared theophyllinates

Compound	*Tribolium confusum* (larvae)	*Trogodema granarium* (larvae)	*Tribolium confusum* (weevils)	*Sitophilus granaries* (adults)
1	163.3[a]	181.8[a]	180.7[a]	166.0[a]
2	169.3[a]	160.7[a]	130.4[b]	141.3[b]
3	178.2[a]	186.0[a]	153.6[a]	168.9[a]
4	146.7[b]	178.3[a]	167.2[a]	169.3[a]
7	158.2[a]	194.7[a]	168.6[a]	142.8[b]
9	187.8[a]	191.3[a]	143.8[b]	147.4[b]
11	155.3[a]	190.6[a]	106.7[b]	139.8[b]
12	155.3[a]	186.8[a]	159.7[a]	141.4[b]
13	131.3[b]	187.4[a]	88.7[c]	150.0[b]
14	161.6[a]	174.6[a]	121.2[b]	150.0[b]
theophylline	73.2[c]	149.1[b]	87.9[c]	66.1[c]
potassium theophyllinate	62.9[c]	145.3[b]	106.4[b]	65.8[c]
azadirachtin	188.4[a]	194.2[a]	185.0[a]	174.3[a]

[a] very good, [b] good, [c] medium.

Table 8. Inhibition of the growth of *Fusarium culmorum*, *Microdochium nivale*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* due to prepared ILs

IL	The growth of *F. culmorum* mycelium [cm]			The growth of *M. nivale* mycelium [cm]			The growth of *B. cinerea* mycelium [cm]			The growth of *S. sclerotiorum* mycelium [cm]		
	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm
control	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
2	4.4	2.7	1.8	4.2	2.0	0.4	4.6	4.6	0.1	4.6	1.2	0.1
3	4.6	2.8	2.3	1.9	1.4	0.4	4.6	4.6	1.2	4.6	2.3	0.2
4	4.6	3.0	2.2	3.2	0.7	0.2	4.6	2.5	0.0	4.6	2.4	0.1
5	4.2	4.3	2.2	3.1	1.7	0.3	4.6	4.6	1.2	4.6	2.4	0.3
6	4.2	2.1	0.0	4.0	2.1	0.1	4.4	4.4	0.0	4.6	0.6	0.0
7	4.6	4.6	2.6	4.6	3.3	1.2	4.6	4.6	1.7	4.2	4.6	0.8
8	4.6	4.6	4.6	4.6	4.6	3.6	4.6	4.6	4.6	4.6	4.6	4.6
11	4.6	4.6	0.0	4.0	1.6	0.1	4.6	3.0	0.0	4.6	2.0	0.0
12	4.6	3.8	0.9	1.7	0.6	0.0	4.6	1.8	0.0	4.6	0.6	0.0
13	4.6	4.6	0.2	4.6	2.9	0.5	4.6	4.6	0.0	4.6	4.6	0.0
14	4.6	4.6	0.0	3.5	1.8	0.0	4.6	3.7	0.0	4.6	0.3	0.0
Tebu 250 EW	0.1	0.0	0.0	1.2	0.0	0.0	3.4	1.0	0.0	0.3	0.0	0.0
LSD $_{(P=0.05)}$	0.18	0.59	0.33	0.28	0.35	0.21	0.08	0.33	0.20	0.28	0.37	0.16

Antifungal activity of the tested ILs was evaluated in respect to four species of pathogenic fungi: *Fusarium culmorum*, *Sclerotinia sclerotiorum*, *Microdochium nivale* and *Botrytis cinerea*. In the preliminary experiment with **9** the mycelial growth of the tested

fungi was significantly inhibited at concentrations of 10, 100 and 1000 ppm. All the tested ILs (except **8**) showed fungistatic activity at a concentration of 1000 ppm, which can be seen in Table 8.

A significant part of the salts also inhibited the growth of mycelia at a concentration of 100 ppm. The fungus of *M. nivale* was most sensitive to the applied ILs at a concentration of 10 ppm. The tested theophyllinates, particularly at a concentration of 10 and 100 ppm, were less effective in comparison to the commercial fungicide Tebu 250 EW containing tebuconazole. At a concentration of 1000 ppm their efficiency was most often very high. This suggests that the obtained salts are novel and efficient antifungal ILs. The selection of the ammonium cation, which exhibits notable antibacterial and antifungal activity even as a chloride, was relevant and ensured high activity against fungi. The high activity of ILs with an anion originating from natural resources towards fungi leads to a question regarding their biodegradability.

The determination of residues after biodegradation tests revealed that the theophylline anion was not biodegraded in all of the salts. Furthermore, the biodegradation of the cation for theophyllinates **1**-**6**, **9** and **10** was also marginal. ILs **7** and **8** were characterized by low polarity and hence it was not possible to determine their residues with the employed method. The progress of biodegradation processes was only observed for the cation of a single IL (**2**). Cocoalkyl substituent of this cation is a mixture of linear, saturated alkyls with various chain length from 8 to 18 carbon atoms and unsaturated alkyls with 18 carbon atoms. The biodegradation efficiency for the cation of **2** was shown in Table 9. The highest susceptibility to biodegradation was observed for the longest alkyl chain (C_{18}), whereas the shortest alkyl chain (C_8) was characterized by the lowest biodegradability. The biodegradation experiments carried out with the ammonium theophyllinates (**1**-**10**) highlight the crucial role of substituents at the quaternary nitrogen atom. The studied salts possessed different substituents, such as: methyl, benzyl, a mixture of saturated and unsaturated straight-chain alkyls (coco, oleyl, hydrogenated tallow and $C_{12}H_{25}/C_{14}H_{29}$), decyl, 2-hydroxyethyl, polyoxyethylene and allyl. The number and length of substituents in the cation should be taken into consideration during the design of biodegradable ammonium theophyllinates. Some reports suggest, that *Pseudomonas putida* is capable of utilizing this compounds as a sole carbon source[24], however this was not observed in the framework of this study. The foundation of this phenomenon may be related to the fact, that theophyllinates exhibit higher resistance compared to theophylline.

Table 9. Biodegradation efficiency of linear alkyl substituents with various chain length in the cation of theophyllinate (**2**)

Cocoalkyl chain substituent Chain length	Content[a]	Biodegradation efficiency[a]
C_8	5	13.7
C_{10}	6	16.0
C_{12}	50	16.9
C_{14}	19	33.8
C_{16}	10	66.9
C_{18}	10	73.1

[a] in %.

The employed PCR method was used to evaluate whether the presence of the studied salts contributed to a facilitation or inhibition of microbial growth. The results were shown in Figure 3.
The PCR assay indicated that the studied theophyllinates had a negative impact on the bacterial species in the consortium, since a strong inhibition of microbial growth could be observed in general. It is worth noticing that some of the salts exhibit selective inhibitory activity against certain bacterial species – i.e. ILs **3** and **4** were most efficient at decreasing the growth of *Variovorax* species (VariP), **8** was effective against *Sphingobacterium* (SphiP), while **10** notably limited the growth of *Achromobacter* species (AchrP). Facilitation of microbial growth occurred exclusively for theophyllinate **2**. In this IL, the highest increase in the relative abundance was observed for *Variovorax* (VariP), while the lowest increase was observed for *Sphingobacterium* (SphiP).

The results obtained with the use of Real Time PCR were in accordance with the determination of biodegradation residues via HPLC-MS. The majority of the studied theophyllinates were characterized by a marginal susceptibility to biological degradation and exhibited a negative impact on microbial growth.

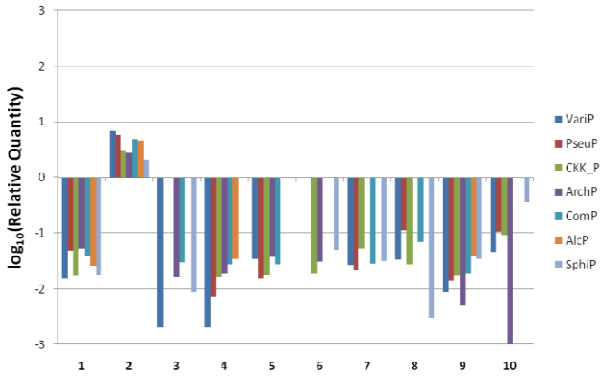

Figure 3. Analysis of qualitative and quantitative changes in the relative abundance of species in the bacterial consortium after biodegradation tests with the selected teophillinates (**1** – **10**)

Experimental

Materials

Theophylline, didecyldimethylammonium chloride, benzalkonium chloride were obtained from Sigma Aldrich/Fluka and used without further purification. Quaternary ammonium chlorides with oleyl, hydrogenated tallow and coco alkyl group are products of AkzoNobel. Allylbenzyldimethylammonium bromide, 1-alkyl-1-methylpiperidinium bromides and alkylcyclohexyldimethyl ammonium bromides were synthesized according to the procedure described recently[25].

General

^{1}H NMR spectra were recorded on a Mercury Gemini 300 spectrometer operating at 300 MHz with TMS as the internal standard. ^{13}C NMR spectra were obtained with the same instrument

at 75 MHz. CHN elemental analyses were performed at the Adam Mickiewicz University, Poznan (Poland). The stability of the synthesized salts in contact with air and water as well as their solubility in various solvents was tested (detailed description given in Electronic Supplementary Information). The water content was determined using an Aquastar volumetric Karl-Fischer titration with Composite 5 solution as the titrant and anhydrous methanol as a solvent. Density was measured with an Automatic Density Meter DDM2911 using the mechanical oscillator method. Density of the samples (approx. 2.0 cm^3) was measured with respect to temperature controlled via Peltier, from 20 to 90 °C. The uncertainty of measurements was estimated to be less than 10^{-5} $g \cdot cm^{-3}$. Viscosity was determined using a rheometer (Rheotec RC30-CPS) with cone-shaped geometry (C50-2). The viscosity of the samples (approx. 1.5 cm^3) was measured with respect to temperature, from 20 to 90 °C. The uncertainty of the viscosity measurement was estimated to be less than 10^{-4} Pa·s. Refractive index was determined using Automatic Refractometer J357 with electronic temperature control from 20 to 90 °C. The uncertainty of measurements was estimated to be less than 10^{-5}.

Synthesis

Quaternary ammonium chlorides or bromides or 1-alkyl-1-methylpiperidinium bromides (0.05 mol) was dissolved in methanol and a stoichiometric amount of saturated methanol solution of KOH was added. The solutions were stirred at room temperature for 5 min, after which the precipitated KCl or KBr was filtrated off. Then, a stoichiometric amount of an appropriate theophylline was added. The solutions were stirred again at room temperature for 15 min and after evaporation of the solvent at a temperature of 40 °C, the product was dissolved in 20 cm^3 of anhydrous acetone. KCl or KBr (residue of the exchange reaction) was separated by filtering and acetone was evaporated to obtain the product, which was finally dried under reduced pressure at 50 °C.

Benzalkonium theophyllinate (1) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.85 (m, 3H; CH_3), 1.24 (s, 18H; CH_2), 1.77 (s, 2H; CH_2), 3.01 (s, 6H; CH_3), 3.21 (s, 3H; CH_3), 3.41 (s, 3H; CH_3), 4.64 (s, 2H; CH_2), 7.25 (s, 1H; CH), 7.51 (t, ^3J(H,H)=12.4 Hz, 2H; CH), 7.60 (m, 3H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.9, 21.8, 22.1, 25.8, 27.3, 28.6, 31.3, 48.9, 63.3, 65.9, 114.3, 128.2, 128.7, 130.1, 132.9, 146.0, 149.6, 151.7, 157.2.

Cocodi(2-hydroxyethyl)methylammonium theophyllinate (2) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.86 (t, ^3J(H,H)=6.6 Hz, 3H; CH_3), 1.25 (m, 20H; CH_2), 1.65 (s, 2H; CH_2), 3.19 (s, 6H, CH_3), 3.37 (t, ^3J(H,H)=7.9 Hz, 5H; CH_3, OH), 3.58 (t, ^3J(H,H) = 3.9 Hz, 4H; CH_2), 3.99 (t, J = 4.5 Hz, 4H; CH_2), 7.21 (s, 1H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ= 13.9, 21.9, 22.3, 22.6, 26.3, 29.2, 29.3, 29.5, 29.6, 31.8, 48.8, 54.6, 63.6, 64.1, 111.6, 146.2, 149.7, 151.7, 157.2.

Oleyldi(2-hydroxyethyl)methylammonium theophyllinate (3) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.86 (t, ^3J(H,H)=11.8 Hz, 3H; CH_3), 1.24 (m, 22H; CH_2), 1.67 (s, 2H; CH_2), 1.98 (m, 3H; CH_3), 3.07 (s, 3H; CH_3), 3.21 (s, 3H; CH_3), 3.38 (m, 2H; CH_2), 3.43 (m, 11H; CH_3, CH_2, OH), 3.82 (d, ^3J(H,H)=4.9 Hz, 2H; CH_2), 5.27 (s, 2H; CH), 7.27 (s, 1H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.9, 21.7, 22.1, 25.8, 26.8, 27.6, 28.7, 28.8, 29.1, 29.7, 31.3, 48.9, 54.8, 63.2, 114.5, 129.6, 146.1, 149.7, 151.7, 157.3.

Polyoxyethylene (15) (hydrogenated tallow)methylammonium theophyllinate (4) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.86 (t, ^3J(H,H) =6.7 Hz, 3H), 1.24 (m, 30H; CH_2), 1.63 (s, 2H; CH_2), 3.07 (s, 3H; CH_3), 3.22 (s, 3H; CH_3), 3.43 (m, 5H; CH_3, OH), 3.51 (m, 2H; CH_2), 3.55 – 3.6 (m, 52H; CH_2), 3.82 (s, ^3J(H,H)=4.6 Hz, 4H; CH_2), 4.00 (s, 4H; CH_2), 7.26 (s, 1H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.9, 21.7, 22.1, 28.7, 28.8, 29.1, 31.3, 48.8, 60.2, 62.7, 69.5, 69.4, 69.8, 72.4, 114.4, 146.2, 149.6, 151.8, 157.5.

Oleyltrimethylammonium theophyllinate (5) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.86 (t, ^3J(H,H)=11.8 Hz, 3H, CH_3), 1.24 (m, 22H; CH_2), 1.67 (s, 2H; CH_2), 2.01 (m, 3H; CH_3), 3.07 (s, 9H; CH_3), 3.21 (s, 3H; CH_3), 3.43 (m, 3H; CH_3), 3.82 (d, ^3J(H,H)=4.9 Hz, 2H; CH_2), 5.27 (s, 2H; CH), 7.27 (s, 1H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.9, 21.7, 22.1, 25.8, 26.9, 27.6, 28.7, 28.8, 29.1, 29.7, 31.5, 48.6, 63.2, 114.5, 129.7, 146.3, 149.6, 151.5, 157.1.

Cocotrimethylammonium theophyllinate (6) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.86 (t, ^3J(H,H)=6.7 Hz, 3H; CH_3), 1.25 (m, 20H; CH_2), 1.60 (m, 2H; CH_2), 3.21 (s, 3H; CH_3), 3.27 (s, 9H; CH_3), 3.30 (t, ^3J(H,H)=8.5 Hz, 2H; CH_2), 3.43 (m, 3H; CH_3), 7.24 (s, 1H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.9, 22.7, 23.1, 26.2, 29.25, 29.32, 29.4, 29.5, 29.6, 31.9, 49.8, 62.5, 114.7, 146.6, 149.4, 151.7, 157.3.

Dicocotrimethylammonium theophyllinate (7) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.86 (t, ^3J(H,H)=8.7 Hz, 6H; CH_3), 1.25 (s, 44H; CH_2), 1.61 (m, 4H; CH_2), 3.02 (s, 6H; CH_3), 3.19 (s, 7H; CH_2, CH_3), 3.38 (s, 6H; CH_3), 7.16 (s, 1H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.9, 21.7, 22.1, 25.7, 27.2, 28.5, 28.7, 28.9, 29.7, 31.3, 49.9, 62.7, 114.8, 146.3, 149.7, 151.8, 157.4.

Di(hydrogenatedtallowalkyl)dimethylammonium theophyllinate (8) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.87 (t, ^3J(H,H)=6.7 Hz, 6H; CH_3), 1.26 (m, 48H; CH_2), 1.61 (m, 4H; CH_2), 3.23 (s, 3H; CH_3), 3.31 (s, 6H; CH_3), 3.39 (t, ^3J(H,H)=6.3 Hz, 7H; CH_2, CH_3), 7.19 (s, 1H; CH). ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=14.0, 22.5, 26.1, 27.2, 29.1, 29.25, 29.29, 29.37, 29.50, 29.55, 29.59, 31.8, 51.03, 63.2, 114.8, 146.3, 149.7, 151.8, 157.4.

Allylbenzyldimethylammonium theophyllinate (10) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=2.92 (s, 6H; CH_3), 3.22 (s, 3H; CH_3), 3.41 (s, 3H; CH_3), 3.97 (d, ^3J(H,H)=7.3 Hz, 2H; CH_2), 4.52 (m, 2H; CH_2), 6.15 (m, 1H; CH), 7.22 (s, 1H; CH), 7.57 (m, 5H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=27.4, 29.5, 48.6, 65.8, 66.5, 125.7, 127.9, 128.7, 130.2, 132.9, 114.2, 146.2, 149.5, 151.6, 157.3; elemental analysis calcd (%) for $C_{19}H_{25}N_5O_2$ C 64.20, H 7.09, N 19.70; found: C 64.41, H 7.28, N 19.41.

Cyclohexyldecyldimethylammonium theophyllinate (11) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.89 (t, ^3J(H,H)=6.4 Hz, 3H; CH_3), 1.12 (m, 1H; CH), 1.39-1.49 (m, 20H; CH_2), 1.69-1.74 (m, 1H; CH), 2.02 (m, 2H; CH_2), 2.19 (m, 2H; CH_2), 3.30 (s, 6H; CH_3), 3.48 (t, ^3J(H,H)=6.2 Hz, 2H; CH_2), 3.50-3.53 (m, 1H;

CH), 3.40 (s, 6H; CH_3), 7.28 (s, 1H; CH). ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.2, 19.2, 24.0, 24.1, 24.7, 25.8, 28.7, 28.8, 28.9, 29.1, 30.6, 48.2, 62.0, 71.5. 114.22, 145.91, 149.56, 151.74, 157.17; elemental analysis calcd (%) for $C_{25}H_{45}N_5O_2$: C 67.08, H 10.13, N 15.64; found: C 67.30, H 10.27, N 15.36.

Cyclohexyldodecyldimethylammonium theophyllinate (12) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.86 (t, ^3J(H,H)=6.0 Hz, 3H; CH_3), 1.07-1.14 (m, 1H; CH), 1.24-1.32 (m, 20H; CH_2), 1.40-1.46 (m, 2H; CH_2), 1.57-1.66 (m, 3H; CH, CH_2), 1.81-1.85 (m, 2H; CH_2), 2.03-2.07 (m, 2H; CH_2), 2.96 (s, 6H; CH_3), 3.24 (m, 1H; CH), 3.27-3.31 (m, 2H; CH_2), 3.40 (s, 6H; CH_3), 7.22 (s, 1H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.93, 21.54, 22.11, 24.38, 24.83, 25.29, 25.84, 27.3, 28.52. 28.73, 28.87, 28.95, 29.03, 29.04, 29.77, 31.31, 47.60, 61.67, 70.58, 114.22, 145.91, 149.56, 151.74, 157.17; elemental analysis calcd (%) for $C_{27}H_{49}N_5O_2$: C 68.17, H 10.38, N 14.72; found: C 68.01, H 10.62, N 14.51.

1-Decyl-1-methylpiperidinium theophyllinate (13) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.86 (t, ^3J(H,H)=5.2 Hz, 3H; CH_3), 1.23 (m, 14H; CH_2), 1.51-1.56 (m, 2H; CH_2), 1.61-1.64 (m, 2H; CH_2), 1.77 (m, 4H; CH_2), 3.01 (s, 3H; CH_3), 3.22 (s, 3H; CH_3), 3.27-3.31 (m, 6H; CH_2), 3.42 (s, 3H; CH_3), 7.31 (s, 1H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.89, 19.27, 20.65, 20.91, 22.15, 25.83, 27.27, 28.51, 28.69, 28.82, 28.96, 29.04, 29.68, 31.26, 46.85, 59.79, 62.44, 113.42, 145.45, 149.38, 151.68, 156.91; elemental analysis calcd (%) for $C_{23}H_{41}N_5O_2$: C 65.83, H 9.85, N 16.69; found: C 65.57, H 9.65, N 17.01.

1-Dodecyl-1-methylpiperidinium theophyllinate (14) ^{1}H NMR (300 MHz, [D_6]DMSO, 20°C, TMS): δ=0.86 (t, ^3J=5.9 Hz, 3H; CH_3), 1.24 (m, 18H; CH_2), 1.51-1.56 (m, 2H; CH_2), 1.61-1.65 (m, 2H; CH_2), 1.78 (m, 4H; CH_2), 3.01 (s, 3H; CH_3), 3.21 (s, 3H; CH_3), 3.28-3.30 (m, 6H; CH_2), 3.41 (s, 3H; CH_3), 7.32 (s, 1H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 20°C, TMS): δ=13.90, 19.28, 20.68, 20.96, 22.10, 25.87, 27.33, 28.56, 28.74, 28.86, 28.96, 29.03, 29.04, 29.76, 31.31, 46.81, 59.84, 62.44, 113.48, 145.41, 149.37, 151.68, 156.93; elemental analysis calcd (%) for $C_{25}H_{45}N_5O_2$: C 67.08, H 10.13, N 15.64; found: C 67.29, H 10.27, N 15.96.

Thermal analysis

Thermal transition temperatures of the obtained salts were determined by DSC, with a Mettler Toledo Star^e TGA/DSC1 (Leicester, UK). 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 a heating rate of 10 °C·min^{-1}. Nitrogen was used as carrier gas.

Surface Activity

The surface tension was determined using the shape drop method. The measurements were carried out by the use of a DSA 100 analyzer (Krüss, Germany, the accuracy of 0.01 mN·m^{-1}) at 25 °C (temperature controlled using a Fisherbrand FBH604 thermostatic bath – Fisher, Germany, with the accuracy of 0.1 °C). The principle of this method is to form an axisymetric drop at a tip of a syringe needle. The image of the drop (3 cm^3) from a CCD camera was digitized. The surface tension (γ_{CMC}, mN·m^{-1}) was calculated by analyzing the profile of the drop according to the Laplace's equation. The values of the critical micelle concentration (CMC) and the surface tension at the CMC (γ_{CMC}) were determined from the intersection of the two straight lines drawn in low and high concentration regions in surface tension curves (γ_{CMC} vs log C curves) using a linear regression analysis method.

Assay of biological activity as potential fungicides

Four species of fungi were used: *Fusarium culmorum* (KZF-5), *Sclerotinia sclerotiorum* (KZF-14), *Microdochium nivale* (KZF-7) and *Botrytis cinerea* (KZF-12) (obtained from the Institute of Plant Protection-NRI collection).

The sample of tested salts was dissolved in 4 cm^3 of water, then added to sterile medium (PDA – *Potato Dextrose Agar*, Difco^{TM}) and cooled to 50 ºC. The salt concentration in the medium was 10, 100 and 1000 ppm. Liquid medium containing the tested salts was distributed on the Petri dishes of diameter 50 mm. The 4 mm disks of the examined fungi were placed in the center of the Petri dish. In the control sample the fungi were grown on PDA with the addition of sterile water. The tested compounds were compared with a commercial fungicide (Tebu 250 EW) containing tebuconazole as an active substance. The plates were incubated in room temperature until the mycelium in the control reached the edge of Petri dish. Afterwards, the diameter of mycelium was measured, subtracting the initial diameter of the disc with the fungus (4 mm). For each experimental sample four replications were performed. The results were subjected to Student-Newman-Keuls's analysis to test for significant differences between control and samples with addition of ILs.

Microorganisms

A bacterial consortium with a high biodegradation potential towards studied salts was selected from the group of 218 consortia isolated from petroleum-contaminated soil[26]. The genetic characterization of the bacterial consortium based on the analysis of 16s rRNA sequences revealed the following taxa: *Alcaligenes* (AlcP); *Sphingobacterium* (SphiP); *Citrobacter* (CKK); *Achromobacter* (AchrP); *Comamonadaceae* (ComP); *Pseudomonas* (PseuP); *Variovorax* (VariP). Preparation of the inoculum and subsequent cultivation conditions were carried out as described recently[27] (detailed description given in Electronic Supplementary Information).

Preparation of biodegradation tests

The biodegradation tests were carried out in loosely closed Erlenmeyer flasks, with 50 cm^3 of the mineral medium and approx. 0.025 g of the studied salt, which was a sole source of carbon and energy. The initial inoculum was adjusted to reach an OD_{600} value of 0.1±0.01 by adding approx. 1 cm^3 of dense cell suspension from the

aerobically grown preculture. The cultivation was carried out at 25 °C and 120 rpm for 30 days. The samples were prepared in triplicates. Samples lacking biomass served as control to account for potential abiotic losses. After finishing the biodegradation tests, the biomass was separated by centrifugation (10.000 g for 10 min.) and was rinsed three times with the mineral medium (5 cm^3). The aliquotes were combined with the supernatant. About 10 cm^3 of the supernatant were subjected to ultrasound-assisted extraction with methanol (3 x 1 cm^3). The extracts were combined, filtered through a 0.2 µm PTFE syringe filter, diluted with a methanol:water solution (80:20 v/v) and subjected to determination of residual salts with the use of HPLC-MS (UltiMate 3000 RSLC, Dionex, with a Hypersil GOLD column 100 mm x 2.1 mm I.D.; 1.9 µm and an API 4000 QTRAP triple quadrupole mass spectrometer, AB Sciex).

Analysis of qualitative and quantitative changes in the relative abundance of species in the bacterial consortium with the use of Real Time PCR

Bacterial DNA extracted from the biomass obtained after the biodegradation tests was subjected to Real-time PCR with the ddCt method for relative quantification to study bacterial community dynamics, as described recently[27].

Feeding deterrent activity test

The bioassay experiments were conducted with *Tribolium confusum* Duv. (larvae and adults), *Sitophilus granarius* L. (adults), and *Trogoderma granarium* Ev. (larvae). The insects were grown on a wheat grain or whole-wheat meal diet in laboratory colonies, which were maintained at 26 ± 1 °C and 60 ± 5% relative humidity. Choice and no-choice tests for insect-feeding were conducted following a previously described procedure[28]. Wheat wafer discs (1 cm in diameter, 1 mm thick) were saturated by dipping either in ethanol only (control) or in an ethanol solution of the studied salts (1%). After evaporation of the solvent (30 min of air-drying) the wafers were weighed and offered to the insects in plastic boxes as the sole food source for 5 days. In all variants, three deterrent coefficients were calculated as follows: absolute coefficient of deterrency, A = (CC - TT)/(CC + TT) x 100; relative coefficient of deterrency, R = (C - T)/(C + T) x 100; the total coefficient of deterrency, the sum of the absolute and the relative coefficients. In these 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. Each of the three experiments was repeated five times with 3 adults of *Sitophilus granarius*, 20 adults 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 adults used for the experiments were unsexed, 7–10 days old, and the larvae were 15–30 days old. After 5 days the discs were reweighed and the average weight of eaten food was calculated.

Conclusions

Third generation ionic liquids were synthesized by employing a natural product, theophylline, as an anion. The physical state of the synthesized theophyllinates depends on the size of the cation. Salts with a melting point above 100 °C and ionic liquids in the forms of liquids and waxes may be obtained. They are stable in air as well as in contact with water and popular organic solvents. At the same time they are thermally stable and display surface activity. All synthesized theophyllinates exhibited insect-feeding deterrent activities (from very good to medium, predominantly very good). Very efficient antifungal properties could be observed at a concentration of 1000 ppm, hence the obtained salts are novel fungicidal and potentially pesticidal ionic liquids. High biological resistance of the tested theophyllinate-based ILs may be advantageous in terms of applicability. They may be used in specific biocidal agents and remain active even after prolonged time periods. The obtained data also suggest that certain structural modifications may increase the biodegradability in order to provide an environmentally friendly product. On the basis of IL **2**, it can be established that long, saturated and unsaturated alkyl substituents in the cation enhance the biodegradability of theophyllinate, which corresponds well with the observations of other researchers[29]. The synthesized theophyllinate-based ILs are surface active compounds, however it is difficult to correlate the surface activity with the established biological activities.

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Notes and references

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