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Effect of room temperature ionic liquid structure on the enzymatic acylation of flavonoids

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ABSTRACT

Enzymatic acylation reactions of flavonoids (rutin, esculin) with long chain fatty acids (palmitic, oleic acids) were carried out in 14 different ionic liquid media containing a range of cation and anion structures. Classification of RTILs according to flavonoid solubility (using COSMO-RS) was the basis for structural selection. Overall, anion selection had a far greater influence on lipase activity than choice of cationic moiety. RTILs containing TF_2N^-, PF_6^- and BF_4^- anions were most successful as reaction media while RTILs containing anions with stronger solvating properties (i.e. H-bonding ability) resulted in decreased yields, likely due to increased interactions with the protein structure of the lipase. Biosynthesis of rutin proceeded much slower than of esculin. All-in-all, judicious selection of RTILs was central to achieving high yields (>98% after 6 days for TOMA·TF_2N) since a balance must be struck that maximized flavonoid solubility with minimum negative impact on lipase activity. The process also benefitted from an increased reaction temperature which may have helped to reduced mass transfer limitations.

1. Introduction

Room temperature ionic liquids (RTILs) have been steadily gaining momentum over the last years as a more environmentally friendly alternative to traditional solvents. Besides having negligible vapour pressure, properties such as their wide liquid range, excellent thermal stability and tunable solvent properties add much to their appeal [1,2]. In fact, new and promising RTILs are continually being examined for their ability to improve reaction systems in order to obtain both higher purities, and yields of a wide range of specialty products.

One reaction system which could greatly benefit from an increased capacity is the acylation reaction of flavonoids. Flavonoids are naturally occurring bioactive compounds whose application in the food, pharmaceutical and even cosmetics industries could be drastically expanded through improved solubility and miscibility in hydrophobic environments. Presently, the lipase-catalyzed esterification/transesterification reactions used to produce these modified flavonoid compounds are typically carried out in organic solvent media, and generally take many days to reach equilibrium [3–5]. Moreover, as the number of organic solvents capable of solubilizing adequate amounts of both polar flavonoids and non-polar long chain fatty acid substrates is limited, long reaction times and low conversions often ensue.

As alternative reaction media, RTILs have enormous potential due largely to the ability to tailor their properties through appropriate cation, anion and substituent selection [1]. Resulting changes in physico-chemical properties, including changes to properties such as density, viscosity, solubility and miscibility may be used to advantage when designing a reaction system or set-up. In the case of flavonoid ester biosynthesis, flavonoid solubility is believed to be a major factor governing the overall rate and extent of the reaction. Employing RTILs as media in these acylation reactions could allow for greater dissolution of flavonoid substrates and reflect favourably on the productivity of the reaction system.

Acylation of flavonoids has already been shown to be possible employing commonly used RTILs, BMIM·PF_6 and BMIM·BF_4 [11,12]. While results were promising, other RTILs with different structures may have been much more efficient at catalyzing these reactions. In actual fact, with the enormous number of possible candidates (>10^{18}) to choose from, a viable strategy for RTIL selection is sorely needed. With this in mind, a computational approach, COSMO-RS (conductor-like screening model for real solvent) was selected for flavonoid solubility estimation in RTILs. Principle advantages to this COSMO-RS approach include elimination of impractical trial and error methods for RTIL selection and alleviation of the need for often unavailable experimental property data for creation of training sets. Briefly, this physically founded approach considered dominant interactions among RTIL systems (i.e. electrostatic, hydrogen bonding and van der Waals), based on the calculation of signal-moment descriptors. On this basis, COSMO-RS calculations made it possible to sort the RTILs into three groups (high, moderate and low) according to the extent of flavonoid solubility.

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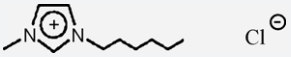
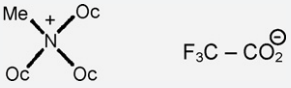
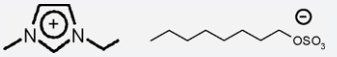
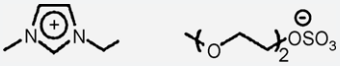
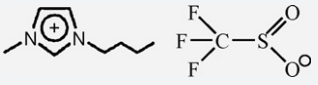
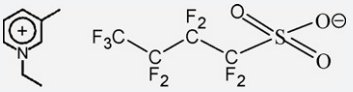
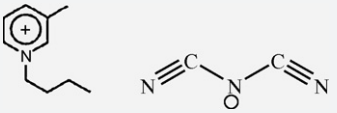
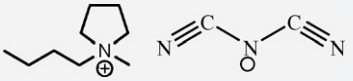
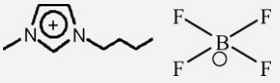
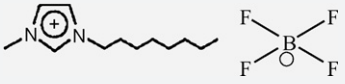
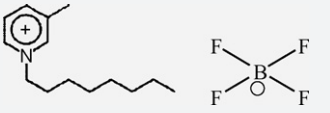
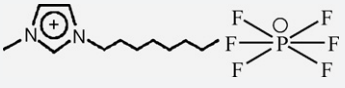
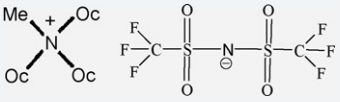
Group I consisted of hydrophilic RTILs with strong solvating properties while RTILs belonging to groups II and III had progressively weaker solvating properties [6]. COSMO-RS solubility predictions were verified experimentally in a total of 12 ionic liquids with fairly good results. However, esculin solubility predictions (root mean square deviation, RMSD 0.22–0.25 log-units) were more accurate than predictions for rutin (RMSD 1.10–1.51 log-units). Additionally, the COSMO-RS model predicted solubilities of flavonoids in strongly solvating RTILs more accurately than in those with lower solvating power [6].

Having determined with some certainty the solubility of selected flavonoids in a range of RTILs, the next logical step was to evaluate the instructive value of using this pre-screening method for the selection of promising RTIL candidates for the production of flavonoid esters. The main aim of this work was to therefore establish the validity of employing the COSMO-RS pre-screening approach in the selection of RTIL candidates. As such, RTILs from all three groups were employed as media in acylation reactions of flavonoids (esculin, rutin) with long chain fatty acids (palmitic, oleic) in order to assess the

Table 1
Selected properties of room temperature ionic liquids (RTILs).

No.	Name	Abbrev.	MW (g/mol)	mp (°C)	Miscib.[a]	ρ (g/cm^3)	Structure
I	1-Hexyl-3-methyl imidazolium chloride	HMIM·Cl	202.79	−85	Y (8.0)	1.040	
I	Trioctylmethylammon-ium trifluoroacetate	TOMA·TFA	481.73	n.d.	n.d.	n.d.	
II	1-Ethyl-3-methylimidazolium octylsulfate	EMIM·OctSO$_4$	320.45	−9	Y (2.9)	1.095	
II	1-Ethyl-3-methylimi-dazolium 2(2-methoxy ethoxy)ethylsulfate	EMIM·MDEGSO$_4$	310.37	<−65	Y (7.0)	1.210	
II	1-Butyl-3-methyl imidazolium trifluoro methanesulfonate	BMIM·CF$_3$SO$_3$	288.3	n.d.	Y (8.9)	1.305	
II	1-Ethyl-3-methyl pyridinium perfluoro butanesulfonate	EMPY·C$_4$F$_9$SO$_3$	421.3	−6	Y (5.2)	1.522	
II	1-Butyl-3-methyl pyridinium dicyanamide	BMPY·N(CN)$_2$	216.3	n.d.	Y (6.5)	1.052	
II	1-Butyl-1-methyl pyrrolidinium dicyanamide	BMPyrr·N(CN)$_2$	208.3	n.d.	n.d.	n.d.	
III	1-Butyl-3-methyl imidazolium tetrafluoroborate	BMIM·BF$_4$	226.0	<−65	Y (3.9)	1.212	
III	1-Methyl-3-octyl imidazolium tetrafluoroborate	OMIM·BF$_4$	282.1	<−65	N	1.105	
III	3-Methyl-1-octyl pyridinium tetrafluoroborate	OMPY·BF$_4$	293.2	n.d.	N	1.102	
III	1-Butyl-3-methyl imidazolium hexafluorophosphate	BMIM·PF$_6$	284.2	15	N	1.380	
III	1-Methyl-3-octylimidazolium hexafluorophosphate	OMIM·PF$_6$	340.3	<−65	N	1.245	
III	Trioctylmethylammon-ium bis(trifluoro methyl sulfonyl)imide	TOMA·TF$_2$N	648.3	<−65	N	1.101	

Abbreviations—No.: group number assigned according to COSMO-RS predicted ability to solubilize flavonoids: (I) high, (II) moderate, and (III) low [6]; MW: molecular weight; mp, melting point; miscib.: miscibility with water; ρ: density at room temperature; and n.d.: not determined.

[a] Where applicable, number in brackets represents the pH value of RTILs in water.