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# Biotransformation of Underutilized Natural Resource to Valuable Compounds in Ionic Liquid: Enzymatic Synthesis of Caffeic Acid Phenethyl Ester Analogues from Immature Coffee Beans

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## 1. Introduction

Caffeic acid esters are widely distributed in plants and propolis (Tagashira & Ohtake, 1998; Ysrael & Nonato, 1999). Caffeic acid phenethyl ester (CAPE) especially has been found in propolis and has a broad spectrum of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and antitumor activities (Bankova, 2005); it also has an inhibitory effect on HIV-1 integrase, cyclooxygenase, and lipoxygenase (Fesen et al., 1993; Michaluart et al., 1999; Nicklaus et al., 1997; Sud'ina et al., 1993). It has been reported that the ester part of CAPE is important for its antiproliferative effect on various human tumor cells (Nagaoka et al., 2003). Additionally, it was suggested that conversion of the phenyl group to a cyclohexyl group in a CAPE analogue enhanced the antiproliferative effect (Kadota & Tezuka, 2004).

Immature green coffee beans are not marketed as coffee because contamination of these beans negatively affects the flavor. However, they contain appreciable amounts of various caffeoylquinic acids, for example, 4.8–5.8 g of 5-caffeoylquinic acid/100 g of immature green coffee beans (Kishimoto et al., 2005a). These immature beans are notable among unused agricultural resources, and we are therefore currently investigating the enzymatic conversion of their caffeoylquinic acids to valuable products.

In recent work, we synthesized CAPE using 5-caffeoylquinic acid and 2-phenylethanol as substrates with chlorogenate hydrolase by a transesterification reaction in a biphasic aqueous-alcohol state and elucidated the antibacterial, antimutagenic, and anti-influenza virus activities of CAPE (Kishimoto et al., 2005b). The procedure using chlorogenate hydrolase provided various CAPE analogues, but the maximum conversion yield of CAPE was 50%. The insufficient yield was probably due to the hydrolysis of 5-caffeoylquinic acid by the enzyme to caffeic acid in the aqueous phase. Therefore, a new procedure for the synthesis of CAPE analogues superior to that method in terms of the conversion yield remained to be developed.

Ionic liquids (ILs), which are composed of a bulky asymmetric cation and a small anion, are easily modified with respect to the combination of cation and anion, and therefore,

numerous IL compositions are possible (Sureshkumar & Lee, 2009). Unlike conventional organic solvents used for biocatalytic reactions, ILs are able to dissolve many compounds, have a wide temperature range for the liquid phase, and possess no vapor pressures. Thus, ILs have good properties for use as reaction solvents, and extensive studies of enzymatic synthesis using ILs as the solvents have been carried out (Moniruzzaman et al., 2010; van Rantwijk & Sheldon, 2007).

Hydrolases, especially lipases, are noted for their tolerance of organic solvents, and are obvious candidates for the enzymatic synthesis in ILs (Sureshkumar & Lee, 2009). Indeed, lipases from *Candida antarctica*, *Burkholderia cepacia* (formerly *Pseudomonas cepacia*), and *Alcaligenes* sp. are catalytically active in ILs (Itoh et al., 2001; Nara et al., 2002). Additionally, lipases mediate transesterification reactions in these ILs with an efficiency comparable to that in tert-butyl alcohol, dioxane, or toluene (Lau et al., 2000; Nara et al., 2002; Park & Kazlauskas, 2001).

In this chapter, we describe alcoholysis with chlorogenate hydrolase (EC 3.1.1.42, Kikkoman, Chiba, Japan) from *Aspergillus japonicus* and transesterification with *C. antarctica* lipase B (Novozyme 435, Novozymes, Bagsvaerd, Denmark). Both reactions were performed with various ILs as the reaction solvent. Using chlorogenate hydrolase, various caffeoylquinic acids prepared from coffee beans were converted to methyl caffeate (Fig. 1). Using Novozyme 435, on the other hand, methyl caffeate was converted to CAPE analogues with various alcohols (Fig. 5). With consecutive reactions comprised of alcoholysis and transesterification, 5-caffeoylquinic acid from immature coffee beans was converted to a CAPE analogue, 3-cyclohexylpropyl caffeate (Fig. 9). Additionally, the CAPE analogues produced, namely, 2-cyclohexylethyl caffeate and 3-cyclohexylpropyl caffeate, were tested for their antiproliferative activities by MTT assay using four different human tumor cells, namely, colon carcinoma HT-29 cells, cervical carcinoma HeLa S3 cells, breast cancer MCF-7 cells, and chronic myeloid leukemia K-562 cells (Table 4).

## 2. Conversion of caffeoylquinic acids to methyl caffeate with chlorogenate hydrolase

The aim of this study was the development of conversion system of caffeoylquinic acids to valuable compounds. When an IL, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([bmim][NTf<sub>2</sub>]) was used as a reaction solvent, we found that immobilized chlorogenate hydrolase (Kikkoman) catalyzed the conversion of 5-caffeoylquinic acid to methyl caffeate with methanol (Fig. 1). The immobilized enzyme was prepared with chlorogenate hydrolase using quaternary ammonium sepabeads (Mitsubishi Chemical Co., Tokyo, Japan) (Kurata et al., 2011). To synthesize valuable compounds from caffeoylquinic acids, we attempted to develop a method for the conversion of caffeoylquinic acids to CAPE analogues *via* methyl caffeate. In section 2, we describe the properties of immobilised chlorogenate hydrolase in ILs. Using various caffeoyl quinic acid prepared from immature coffee beans, we developed a system to produce methyl caffeate.

### 2.1 Effect of aqueous solution on chlorogenate hydrolase in IL

When [bmim][NTf<sub>2</sub>] was used as the reaction solvent, the enzymatic conversion with immobilized chlorogenate hydrolase proceeded under a non-aqueous condition (Kurata et al., 2011). In various enzymatic syntheses, water can shift the equilibrium in the direction of hydrolysis; therefore, in methods using lipase, the water produced as a by-product was

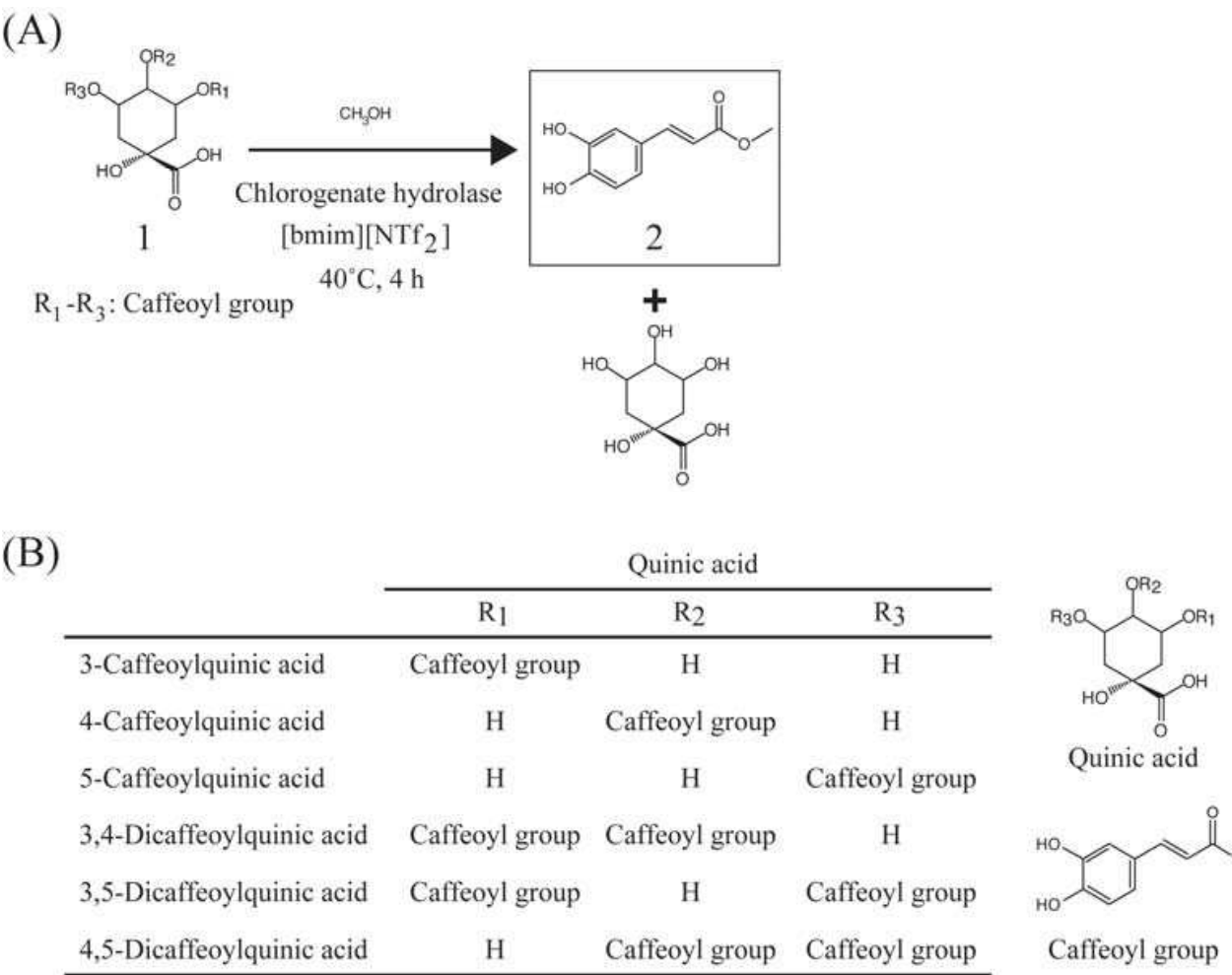


Fig. 1. Conversion of caffeoylquinic acids to methyl caffeate by chlorogenate hydrolase. (A) Alcoholysis of various caffeoyl quinic acids (compound 1) was catalysed by chlorogenate hydrolase with methanol and [bmim][NTf<sub>2</sub>] as the reaction solvent, and methyl caffeate (compound 2) was produced. (B) Structures of substrates for synthesis of methyl caffeate with chlorogenate hydrolase are shown.

removed (Bélafi-Bakó et al., 2002; Gubicza et al., 2000). However, the catalytic activity in non-aqueous solvents depends on the amount of water associated with the enzyme conforming to its native conformation (Barahona et al., 2006). Thus, we examined the effect of the addition of an aqueous solution on the production of methyl caffeate in [bmim][NTf<sub>2</sub>] by varying the additional volume of 50 mM sodium phosphate buffer (pH 6.5) from 0 to 5% (v/v) (Fig. 2).

When the concentration of the aqueous solution was 1% or lower, the production of methyl caffeate increased. However, the production of methyl caffeate was decreased in aqueous solutions greater than 2%. The production of caffeic acid was increased with addition of the buffer, indicating that the enzyme probably catalyzed hydrolysis of 5-caffeoylquinic acid to produce caffeic acid rather than alcoholysis to produce methyl caffeate. Thus, the result indicated that the addition of 1% aqueous solution was suitable for the production of methyl caffeate. It was suggested that ILs are able to maintain active structures of the enzymes with a monomolecular layer of water (Fehér et al., 2007). Thus, chlorogenate hydrolase would maintain the active structure with the layer of the buffer in [bmim][NTf<sub>2</sub>].

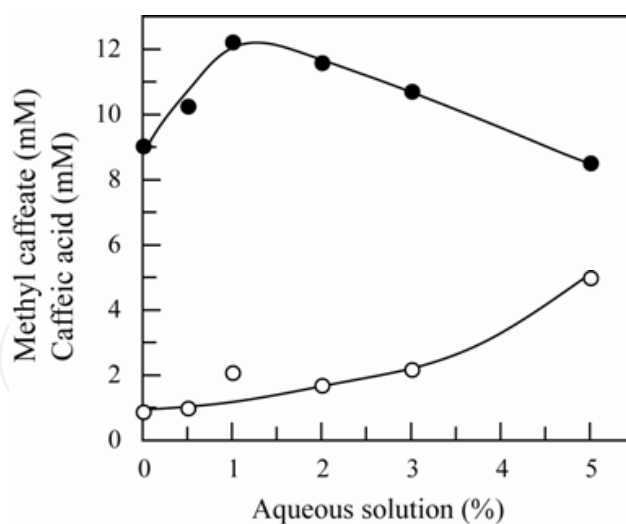


Fig. 2. Effects of water concentration on conversion of 5-caffeoylquinic acid to methyl caffeate by chlorogenate hydrolase (Kurata et al., 2011). The reaction was performed at 40°C using chlorogenate hydrolase and [bmim][NTf<sub>2</sub>] as the reaction solvent with a 0–5% (v/v) aqueous solution of 50 mM sodium phosphate (pH 6.5). Each symbol indicates methyl caffeate (closed circle) and caffeic acid (open circle).

## 2.2 Selection of IL for chlorogenate hydrolase

In order to investigate the activities of chlorogenate hydrolase in various ILs, the alcoholysis of 5-caffeoylquinic acid with methanol was examined (Fig. 3).

The reactions were performed in five ILs, namely, [bmim][NTf<sub>2</sub>], two [bmim] cation-containing ILs, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF<sub>4</sub>]) and 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([bmim][CF<sub>3</sub>SO<sub>3</sub>]), and two [NTf<sub>2</sub>] anion-containing ILs, *N*-methyl-*N*-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide ([MPPro][NTf<sub>2</sub>]) and *N*-methyl-*N*-propylpiperidinium bis(trifluoromethylsulfonyl)imide ([MPPip][NTf<sub>2</sub>]).

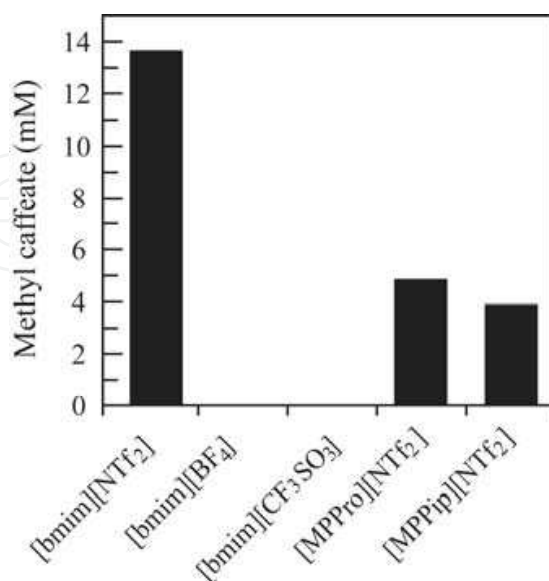


Fig. 3. Selection of IL for chlorogenate hydrolase. The reaction was performed with various ILs as the reaction solvent.

Although chlorogenate hydrolase catalyzed the alcoholysis reaction in [bmim][NTf<sub>2</sub>] to produce 13.7 mM methyl caffeate, the reaction scarcely proceeded in [bmim][BF<sub>4</sub>] and [bmim][CF<sub>3</sub>SO<sub>3</sub>]. Lipase from *Candida rugosa* was found to be active in [bmim][PF<sub>6</sub>] for the transesterification, but inactive in ILs including [bmim][acetate] and [bmim][nitrate] (Kaar et al., 2003). Lipase from *Pseudomonas aeruginosa* was more stable in [bmim][PF<sub>6</sub>] than in [bmim][BF<sub>4</sub>] (Singh et al., 2009). Thus, the nature of the anion in IL plays a critical factor in determining the enzyme activity and stability. It was reported that the hydrogen-bond basicities of [bmim][BF<sub>4</sub>] and [bmim][CF<sub>3</sub>SO<sub>3</sub>] are larger than that of [bmim][NTf<sub>2</sub>] (Anderson et al., 2002; Kaar et al., 2003). Additionally, it was suggested that the [BF<sub>4</sub>] and [CF<sub>3</sub>SO<sub>3</sub>] anions are more nucleophilic than the [NTf<sub>2</sub>] anion, and that the [BF<sub>4</sub>] and [CF<sub>3</sub>SO<sub>3</sub>] anions coordinate more strongly to positively charged sites in the structure of an enzyme. In consequence, the enzyme is deactivated by a conformation change in the enzyme structure due to these anions. As shown Figure 3, chlorogenate hydrolase was deactivated in [bmim][BF<sub>4</sub>] and [bmim][CF<sub>3</sub>SO<sub>3</sub>]. Additionally, the ability to decrease the enzyme activity was in the order of cations: [MPPip]<sup>+</sup> > [MPPro]<sup>+</sup> > [bmim]<sup>+</sup>. In the case of chlorogenate hydrolase, the enzyme activity was affected by the anions and cations. As shown in Figure 3, [bmim][NTf<sub>2</sub>] was suitable for the alcoholysis of caffeoylquinic acids to produce methyl caffeate.

### 2.3 Effect of temperature

The effect of temperature on the chlorogenate hydrolase activity of the alcoholysis reaction with 5-caffeoylquinic acid and methanol was examined in [bmim][NTf<sub>2</sub>] at temperatures from 20°C to 100°C (Fig. 4).

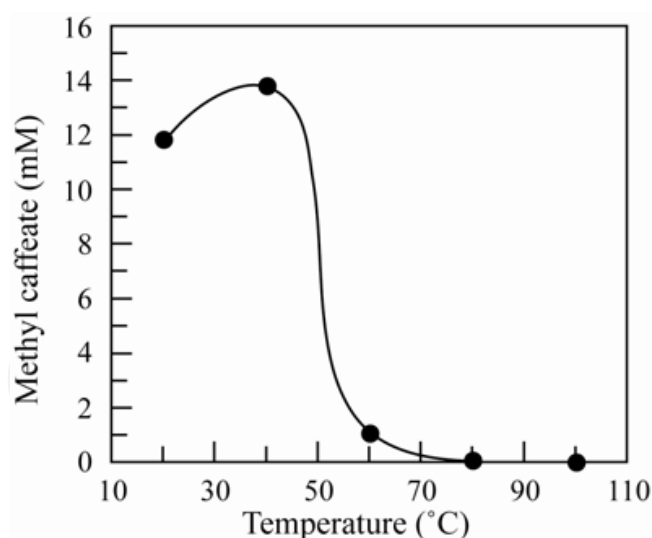


Fig. 4. Effect of temperature on conversion of 5-caffeoylquinic acid to methyl caffeate by chlorogenate hydrolase (Kurata et al., 2011). The reaction was performed at 20°C to 60°C for 4 h with immobilized chlorogenate hydrolase and [bmim][NTf<sub>2</sub>] as the reaction solvent.

With increased temperature, the amounts of the product initially increased. However, at temperatures higher than 40°C, the amounts of the product decreased, indicating that 40°C is the optimum temperature for chlorogenate hydrolase. Additionally, the enzyme is deactivated at temperatures higher than 80°C.



2.4 Substrate specificity for chlorogenate hydrolase

We examined the production of methyl caffeate using the alcoholysis reaction by chlorogenate hydrolase in [bmim][NTf<sub>2</sub>] (Table 1). Methyl caffeate was produced using various caffeoylquinic acids (15 μmol) and methanol (2200 μmol). Using 3-caffeoylquinic acid, 4-caffeoylquinic acid, 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid, methyl caffeate was synthesized at concentrations of 9.0 mM (9.0 μmol), 9.2 mM (9.2 μmol), 12.9 mM (12.9 μmol), 13.9 mM (13.9 μmol), and 17.1 mM (17.1 μmol), respectively (Kurata et al., 2011).

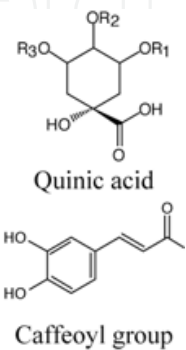
	Quinic acid			Yield (%)	
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>		
3-Caffeoylquinic acid	Caffeoyl group	H	H	60.0	
4-Caffeoylquinic acid	H	Caffeoyl group	H	61.3	
5-Caffeoylquinic acid	H	H	Caffeoyl group	86.0	
3,5-Dicaffeoylquinic acid	Caffeoyl group	H	Caffeoyl group	92.7	
4,5-Dicaffeoylquinic acid	H	Caffeoyl group	Caffeoyl group	114.0	

Table 1. Conversion yields of caffeoylquinic acids to methyl caffeate.

Because dicaffeoylquinic acid and caffeoylquinic acid have two and one caffeoyl groups, respectively, the volumes of methyl caffeate prepared from 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid were greater than those of methyl caffeate prepared from 3-caffeoylquinic acid, 4-caffeoylquinic acid, and 5-caffeoylquinic acid. In the cases of 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, both caffeoyl groups would be used for synthesis of methyl caffeate.

Additionally, using a mixture of 3,4-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, which is a crude fraction prepared from coffee beans, HPLC analysis showed that all peaks of caffeoylquinic acids disappeared and that the peak of methyl caffeate occurred after a 4-h reaction with chlorogenate hydrolase in [bmim][NTf<sub>2</sub>] (Kurata et al., 2011).

Chlorogenate hydrolase acted on caffeoylquinic acids and dicaffeoylquinic acids. Thus, methyl caffeate was produced from various caffeoylquinic acids prepared from coffee beans using this procedure.

3. Conversion of methyl caffeate to CAPE analogues with Novozyme 435

In section 2, we described success in converting various caffeoylquinic acids to methyl caffeate with good yields. Next, we tried to convert methyl caffeate to valuable compounds, namely, CAPE analogues, using IL as the reaction solvent (Fig. 5).

3.1 Selection of lipase for transesterification in IL

We initially selected methyl caffeate and 3-cyclohexyl-1-propanol as substrates for comparative study of the enzyme’s performance in [bmim][NTf<sub>2</sub>]. Four commercially available lipases, *C. antarctica* lipase B (Novozyme 435, Novozymes, 30 U mg<sup>-1</sup>), *Rhizomucor miehei* lipase (RMIM, Novozymes, 1370 U mg<sup>-1</sup>), *B. cepacia* lipases (PS-CI, Wako Pure Chemical, Osaka, Japan, 2560 U mg<sup>-1</sup>), and *Thermomyces lanuginosus* lipase (TLIM, Novozymes, 1850 U mg<sup>-1</sup>) were tested (Table 2).

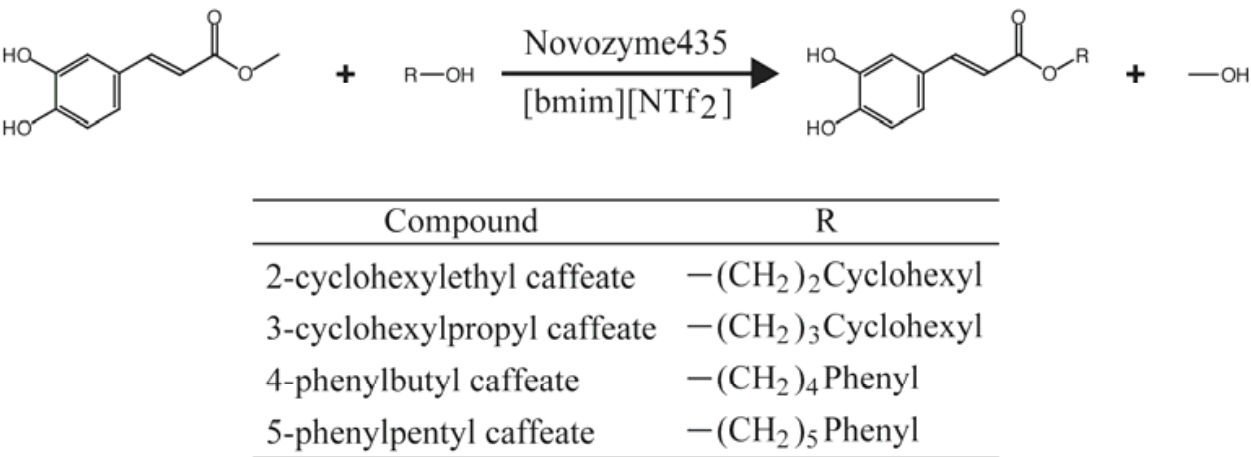


Fig. 5. Convesion of methyl caffeate to CAPE analogues by Novozyme 435.

3-Cyclohexylpropyl caffeate (mM)	
Novozyme 435	30.8
RMIM	11.6
PS-CI	6.6
TLIM	1.8

Table 2. Selection of lipase for conversion of methyl caffeate (Kurata et al., 2010). The reaction was performed with 1,200,000 U of lipases.

Among these enzymes, Novozyme 435 was the most suitable for the procedure. PS-CI and TLIM hardly catalyzed the reaction, although RMIM catalyzed it relatively well. The difference in the recognition of the substrate by lipases is probably in accordance with the difference in the substrate orientation in the active center (Pleiss et al., 1998). With one possible exception, the substrates easily accessed the active center of Novozyme 435. Additionally, it is suggested that the substrates hardly accessed the active sites of RMIM, PS-CI, and TLIM.

3.2 Selection of IL for Novozyme 435

In order to investigate the activity of Novozyme 435 in various ILs, the transesterification of 2-cyclohexylethanol was examined (Fig. 6). The reactions were performed in eight ILs, namely, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([bmim][NTf<sub>2</sub>]), three [bmim] cation-containing ILs, 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF<sub>6</sub>]), 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF<sub>4</sub>]), and 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([bmim][CF<sub>3</sub>SO<sub>3</sub>]), and four [NTf<sub>2</sub>] anion-containing ILs, 1-propyl-2,3,5-trimethylpyrazolium bis(trifluoromethylsulfonyl)imide ([PMPPra][NTf<sub>2</sub>]), N-methyl-N-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide ([MPPPro][NTf<sub>2</sub>]), N-methyl-N-propylpiperidinium bis(trifluoromethylsulfonyl)imide ([MPPip][NTf<sub>2</sub>]), and N,N,N-trimethyl-N-propylammonium bis(trifluoromethylsulfonyl)imide ([TMPA][NTf<sub>2</sub>]).



Although Novozyme 435 catalyzed the transesterification in [bmim][NTf<sub>2</sub>] and [bmim][PF<sub>6</sub>] to produce 2-cyclohexylethyl caffeate (40.1 mM and 22.9 mM, respectively), the transesterification scarcely proceeded in [bmim][CF<sub>3</sub>SO<sub>3</sub>] and [bmim][BF<sub>4</sub>] (Fig. 6, closed bar). The amounts of 2-cyclohexylethyl caffeate produced in all [NTf<sub>2</sub>] anion-containing ILs were smaller to that in [bmim][NTf<sub>2</sub>], but the reactions proceeded effectively in these ILs (Fig. 6, open bars).

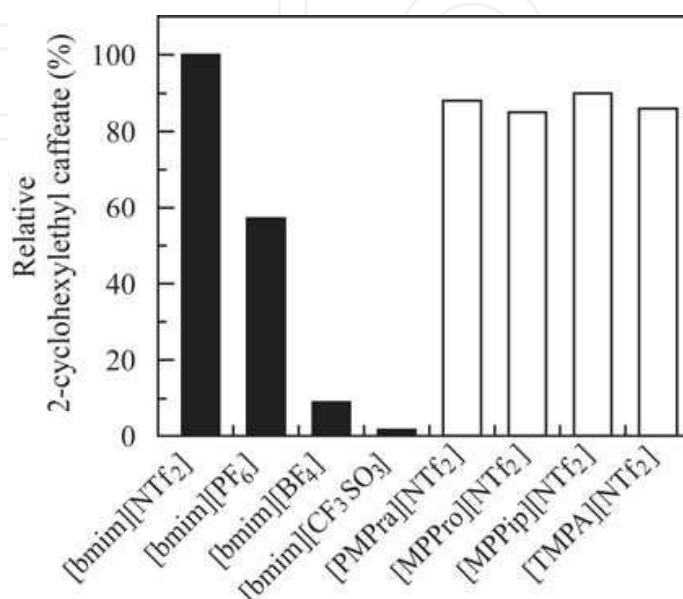


Fig. 6. Comparison of transesterifications in ILs. The reaction was performed with Novozyme 435 and [bmim][NTf<sub>2</sub>], which was used as a reaction medium. The amount of 2-cyclohexylethyl caffeate (40.1 mM) in [bmim][NTf<sub>2</sub>] was taken as 100%.

The results indicated that Novozyme 435 activity in ILs is anion dependent, like chlorogenate hydrolase (Fig. 3). As described in section 2. 2, chlorogenate hydrolase would be deactivated by conformation changes in the enzyme structure due to the [BF<sub>4</sub>] and [CF<sub>3</sub>SO<sub>3</sub>] anions. Similarly, Novozyme 435 was deactivated in [bmim][BF<sub>4</sub>] and [bmim][CF<sub>3</sub>SO<sub>3</sub>] (Fig. 6). In addition, Novozyme 435 activity was higher in [bmim][NTf<sub>2</sub>] than in [bmim][PF<sub>6</sub>]. It was reported that the pH of the reaction mixture with [bmim][PF<sub>6</sub>] was decreased by hydrogen fluoride, which was produced by the hydrolytic decomposition of the [PF<sub>6</sub>] anion (Hernández-Fernández et al., 2007). Novozyme 435 activity in [bmim][PF<sub>6</sub>] would be decreased with the decrease in pH value. Because the concentrations of 2-cyclohexylethyl caffeate produced in [bmim][NTf<sub>2</sub>] and [bmim][PF<sub>6</sub>] were 40.1 mM and 22.9 mM, respectively, [bmim][NTf<sub>2</sub>] was suitable for the transesterification of methyl caffeate.

### 3.3 Effect of temperature

The effect of temperature on the activity of Novozyme 435 for the transesterification with 2-cyclohexylethanol and 3-cyclohexyl-1-propanol was examined in [bmim][NTf<sub>2</sub>] by varying temperatures ranging from 60°C to 110°C (Fig. 7).

With increased temperature, the amounts of the product were initially increased. However, at higher than 90°C, the amounts of the product were decreased, indicating that 80°C is the optimum temperature for the transesterification.

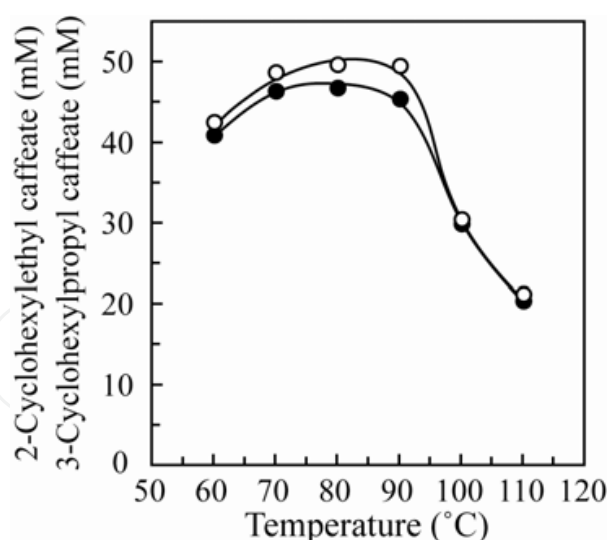


Fig. 7. Effect of temperature on productions of 2-cyclohexylethyl caffeate and 3-cyclohexylpropyl caffeate (Kurata et al., 2010). The reaction was performed at 60°C–110°C with Novozyme 435 and [bmim][NTf<sub>2</sub>], which was used as a reaction medium.

### 3.4 Reusability of IL and lipase

We evaluated the repeated use of Novozyme 435 in [bmim][NTf<sub>2</sub>] (Fig. 8). After the reaction, we added diethyl ether to the reaction mixture to form a diethyl ether-IL two-phase state. The great advantage of the state was the easy removal of the upper diethyl ether phase containing the product and unreacted substrate, while the lower phase containing the enzyme and IL could be used again.

After three cycles of repeated use, Novozyme 435 retained its ability to produce about 40 mM 2-cyclohexylethyl caffeate with a conversion yield of about 80%. After four and five cycles, the amounts of 2-cyclohexylethyl caffeate were decreased to 28.7 mM and 27.4 mM, respectively.

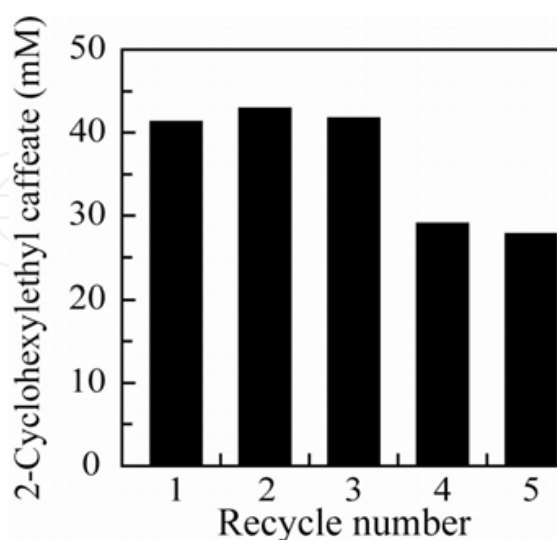


Fig. 8. Reuse of lipase in [bmim][NTf<sub>2</sub>] solvent system (Kurata et al., 2010). The reaction mixture was comprised of Novozyme 435, methyl caffeate, and 2-cyclohexylethanol in [bmim][NTf<sub>2</sub>]. The reaction was repeated five times.

3.5 Substrate specificity for Novozyme 435

We examined the production of CAPE analogues using transesterification with [bmim][NTf<sub>2</sub>] (Kurata et al., 2010). CAPE analogues were produced using 50 mM methyl caffeate (50 μmol) and 300 mM alcohols (300 μmol). We synthesized 48.8 mM 2-cyclohexylethyl caffeate (48.8 μmol), 46.9 mM 3-cyclohexylpropyl caffeate (46.9 μmol), 49.4 mM 4-phenylbutyl caffeate (49.4 μmol), and 42.0 mM 5-phenylpentyl caffeate (42.0 μmol). The conversion yields are shown in Table 3.

In terms of the conversion yield, this procedure using Novozyme 435 is superior to the previously reported procedure affording CAPE with the maximum conversion yield of 50% (Kishimoto et al., 2005a). CAPE was obtained by transesterification catalyzed by Novozyme 435 using isooctane as the solvent with the conversion yield of 91.65% (Chen et al., 2010). The conversion yields of the CAPE analogues produced by the [bmim][NTf<sub>2</sub>] system are comparable to that of CAPE produced by the isooctane system.

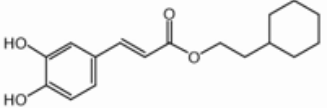
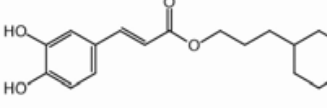
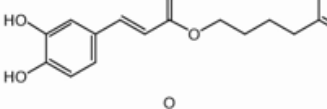
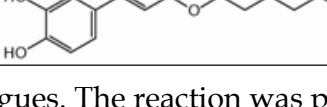
CAPE analogues		Yield (%)
2-cyclohexylethyl caffeate		97.6
3-cyclohexylpropyl caffeate		93.8
4-phenylbutyl caffeate		96.7
5-phenylpentyl caffeate		84.0

Table 3. Conversion yields of CAPE analogues. The reaction was performed with methyl caffeate, each of alcohol, Novozyme 435, and [bmim][NTf<sub>2</sub>], which was used as a reaction medium. For production of 2-cyclohexylethyl caffeate, 3-cyclohexylpropyl caffeate, 4-phenylbutyl caffeate, and 5-phenylpentyl caffeate, alcohols used were shown as follows: 2-cyclohexylethanol, 3-cyclohexyl-1-propanol, 4-phenyl-1-butanol, 5-phenyl-1-pentanol, respectively (Kurata et al., 2010).

4. Consecutive conversions of 5-caffeoylquinic acids to 3-cyclohexylpropyl caffeate

We described the alcoholysis of various caffeoylquinic acids with methanol to produce methyl caffeate in section 2, and the transesterification of methyl caffeate to various CAPE analogues in section 3. Both reactions was performed in the same IL, namely, [bmim][NTf<sub>2</sub>], with good production yields. In section 4, we investigated a one-pot consecutive conversion of 5-caffeoylquinic acid to a CAPE analogue, 3-cyclohexylcaffeate, *via* methyl caffeate (Fig. 9). In the case of a one-pot two-step reaction, purification of the reaction intermediate is not required, so that the intended product was expected to be obtained with high conversion yield.

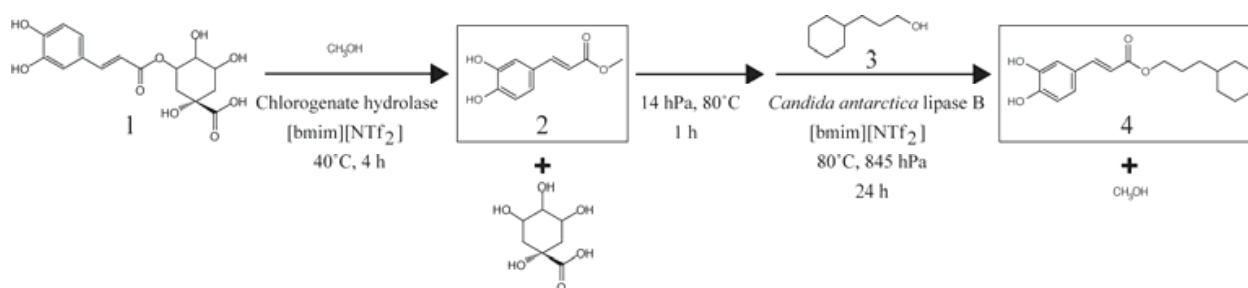


Fig. 9. Consecutive enzymatic reactions for synthesis of 3-cyclohexylpropyl caffeate from 5-caffeoylquinic acid. In all reaction steps, [bmim][NTf<sub>2</sub>] was used as the solvent. Compound 1: 5-caffeoylquinic acid, 2: methyl caffeate, 3: 3-cyclohexyl-1-propanol, and 4: 3-cyclohexylpropyl caffeate.

#### 4.1 Conversion of 5-caffeoylquinic acid with chlorogenate hydrolase

We initially examined the production of methyl caffeate or 3-cyclohexylpropyl caffeate using immobilized chlorogenate hydrolase in [bmim][NTf<sub>2</sub>] (Fig. 10).

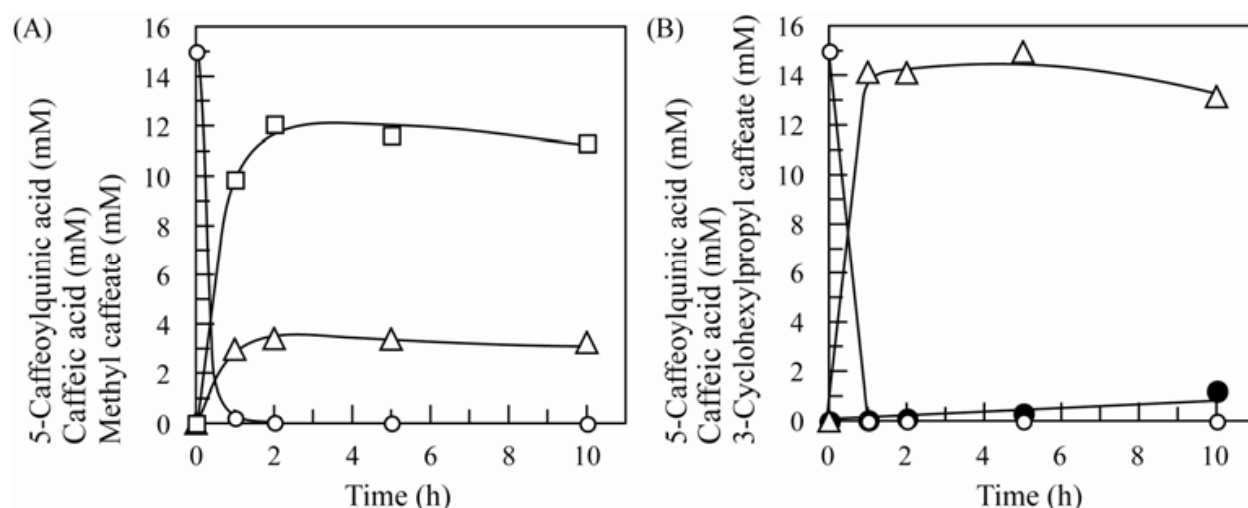


Fig. 10. Conversion of 5-caffeoylquinic acid to methyl caffeate and caffeic acid (A) and 3-cyclohexylpropyl caffeate and caffeic acid (B) by chlorogenate hydrolase (Kurata et al., 2011). The reaction was performed with 5-caffeoylquinic acid, methanol (A) or 3-cyclohexyl-1-propanol (B), chlorogenate hydrolase, and [bmim][NTF<sub>2</sub>]. Symbols indicate 5-caffeoylquinic acid (open circle), caffeic acid (open triangle), methyl caffeate (open square), and 3-cyclohexylpropyl caffeate (closed circle).

The reaction mixture was comprised of 5-caffeoylquinic acid, alcohols, 50 mM sodium phosphate (pH 6.5), chlorogenate hydrolase, and [bmim][NTf<sub>2</sub>] as the reaction solvent. After a 10-h reaction using 15 mM 5-caffeoylquinic acid and 2200 mM methanol, 11.3 mM methyl caffeate and 3.2 mM caffeic acid were produced with conversion yields of 75.4% and 21.5%, respectively (Fig. 10A), whereas 13.1 mM caffeic acid and 1.21 mM 3-cyclohexylpropyl caffeate were produced with conversion yields of 87.6% and 8.1%, respectively, using 15 mM 5-caffeoylquinic acid and 2200 mM 3-cyclohexyl-1-propanol (Fig. 10B). The result showed that methanol is a better substrate for chlorogenate hydrolase than 3-cyclohexyl-1-propanol to produce methyl caffeate with a high conversion yield. Additionally, Novozyme 435 did not catalyze the conversion of 5-caffeoylquinic acid to 3-cyclohexylpropyl caffeate or

methyl caffeate (data not shown), but efficiently catalyzed the conversion of methyl caffeate to 3-cyclohexylpropyl caffeate with a conversion yield of 93.8%, as described in section 3.5. Thus, consecutive conversions of 5-caffeoylquinic acid to 3-cyclohexylpropyl caffeate *via* methyl caffeate using chlorogenate hydrolase and Novozyme 435 would be a more effective procedure than a single conversion of 5-caffeoylquinic acid to 3-cyclohexylpropyl caffeate.

#### 4.2 Effect of methanol on transesterification by Novozyme 435

During conversion of 5-caffeoylquinic acid with methanol, the reaction mixture probably contained unreacted methanol. Additionally, when methyl caffeate prepared from caffeoylquinic acids was used as the acyl donor to produce 3-cyclohexylpropyl caffeate, methanol was produced as a byproduct.

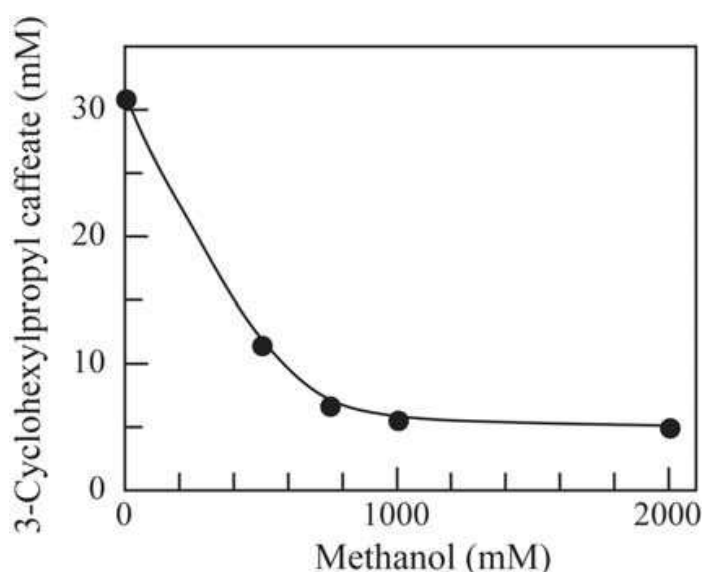


Fig. 11. Effect of methanol on conversion of methyl caffeate to 3-cyclohexylpropyl caffeate by Novozyme 435 (Kurata et al., 2011). Transesterification of methyl caffeate was performed with methyl caffeate, 3-cyclohexyl-1-propanol, Novozyme 435, 0–2200 mM methanol, and [bmim][NTf<sub>2</sub>] as the reaction solvent.

In the conversion of methyl caffeate to 3-cyclohexylpropyl caffeate in the consecutive synthesis, therefore, the reverse reaction with methanol would occur easily to decrease the amount of the desired product. We tested the effect of methanol on Novozyme 435, and observed that methanol had an inhibitory effect on the production of 3-cyclohexylpropyl caffeate (Fig. 11).

For development of the two-step procedure, we attempted to avoid this difficulty by removing the unreacted methanol *in vacuo* (14 hPa) and performing the transesterification of methyl caffeate by Novozyme 435 under reduced pressure (845 hPa), so that methanol could be removed immediately from the reaction mixture (Itoh, 2007).

#### 4.3 Consecutive enzymatic reactions for synthesis of 3-cyclohexylpropyl caffeate

For the development of a convenient procedure for synthesis of 3-cyclohexylpropyl caffeate from 5-caffeoylquinic acid *via* methyl caffeate, we tested the consecutive enzymatic reactions by chlorogenate hydrolase and Novozyme 435 using [bmim][NTf<sub>2</sub>] as the solvent (Fig. 12).



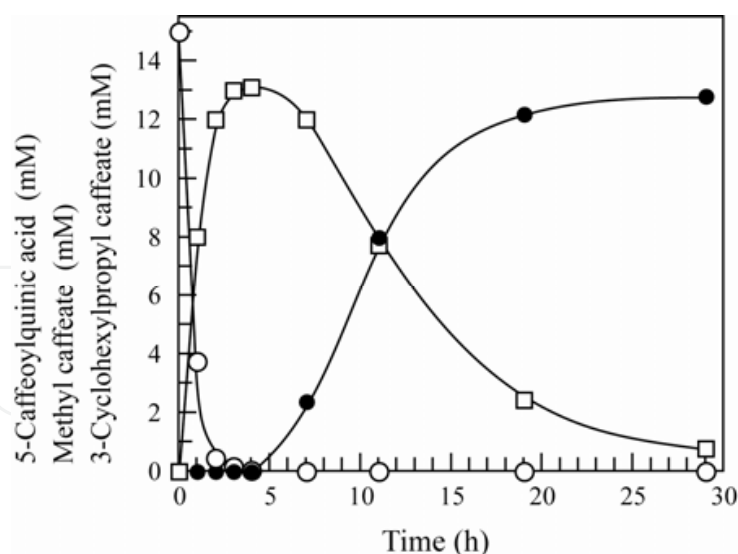


Fig. 12. Consecutive reactions for conversion of 5-caffeoylquinic acid to 3-cyclohexylpropyl caffeate *via* methyl caffeate (Kurata et al., 2011). The symbols indicate 5-caffeoylquinic acid (open circle), methyl caffeate (open square), and 3-cyclohexylpropyl caffeate (closed circle).

As shown in Figure 9, the first alcoholysis reaction for 4 h at 40°C with methanol using chlorogenate hydrolase produced methyl caffeate from 5-caffeoylquinic acid. Next, the unreacted methanol was removed under reduced pressure (14 hPa) for 1 h at 80°C, and chlorogenate hydrolase was deactivated at 80°C. In the subsequent transesterification reaction by Novozyme 435 with 3-cyclohexyl-1-propanol, the methyl caffeate produced was converted to 3-cyclohexylpropyl caffeate under reduced pressure (845 hPa) to remove the by-product methanol.

In the consecutive synthesis, the methyl caffeate was transesterified efficiently to 3-cyclohexylpropyl caffeate by Novozyme 435 with deactivation of chlorogenate hydrolase by taking advantage of the difference between the optimum temperatures for chlorogenate hydrolase (40°C, Fig. 4) and Novozyme 435 (80°C, Fig. 7).

In the one-pot two-step synthesis, 15 mM 5-caffeoylquinic acid (15 µmol) was converted *via* 13.1 mM methyl caffeate (13.1 µmol, 87.3%) to 12.8 mM 3-cyclohexylpropyl caffeate (12.8 µmol, 97.7%). The net conversion yield of 3-cyclohexylpropyl caffeate toward 5-caffeoylquinic acid was 85.3%.

In the synthesis of CAPE analogues, the conversion yield of 3-cyclohexylpropyl caffeate toward 5-caffeoylquinic acid (85.3%) was superior to that of CAPE toward 5-caffeoylquinic acid (50%) (Kishimoto et al., 2005a). CAPE analogues were obtained by single transesterification systems catalyzed by Novozyme 435 using [bmim][NTf<sub>2</sub>] or isooctane as the solvent with conversion yields of 93.8% and 91.65%, respectively (Chen et al., 2010; Kurata et al., 2010). The conversion yield of 3-cyclohexylpropyl caffeate produced by the consecutive conversion system was comparable to those of CAPE produced by single transesterification systems.

## 5. Antiproliferative effect against human tumor cells

In the study, we developed a convenient procedure for synthesis of CAPE analogues. As described in section 1, CAPE analogues have antimicrobial, anti-inflammatory, antioxidant, and antitumor activities (Kishimoto et al., 2005b). Finally, the CAPE analogues produced,



namely, 2-cyclohexylethyl caffeate and 3-cyclohexylpropyl caffeate, were purified and tested for their antiproliferative activities against human tumor cells *in vitro*.

5.1 Antiproliferative effect

Cellular growth in the presence of 2-cyclohexylethyl caffeate or 3-cyclohexylpropyl caffeate produced in [bmim][NTf<sub>2</sub>] was determined by an MTT assay (Carmichael et al., 1987). Rapidly growing human tumor cells, namely, colon carcinoma HT-29 cells, cervical carcinoma HeLa S3 cells, breast cancer MCF-7 cells, and chronic myeloid leukemia K-562 cells, were harvested, counted, and inoculated at appropriate concentrations (135 µl volumes) into a 96-well microtiter plate. After 24 h, 15 µl of CAPE analogues were applied to triplicate culture wells, and the culture was incubated at 37°C. After 72 h, 15 µl of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Nacalai Tesque, Kyoto, Japan) prepared at 5 mg/ml in PBS (Invitrogen, Carlsbad, CA) was added to microculture wells. After a 4-h incubation at 37°C, 200 µl of DMSO was added to the supernatant from each microculture well. After a thorough mixing with a mechanical plate mixer, absorbance at 540 nm was measured. IC<sub>50</sub> values represent the compound concentration required to reduce the proliferation of the tumor cell by 50%.

Cell viability was calculated using the following equation:

Cell viability (%) = [(A - B)/(C - B)] × 100,

where A is the absorbance of the cells in the culture medium treated with drugs, B is the absorbance of the culture medium (blank), and C is the absorbance of static cells (control). The MTT assay was repeated two times.

	IC <sub>50</sub> (µM)			
	HT-29	HeLa S3	MCF7	K562
2-Cyclohexylethyl caffeate	11.1	16.4	5.5	9.7
3-Cyclohexylpropyl caffeate	14.7	22.9	5.8	9.3
Caffeic acid	165.8	134.5	490.8	56.0
5-Fluorouracil	21.0	35.9	24.9	9.8

Table 4. Antiproliferative activities of CAPE analogues (Kurata et al., 2010).

Both 2-cyclohexylethyl caffeate and 3-cyclohexylpropyl caffeate halved the numbers of the four tumor cells at less than 25 µM. Although the IC<sub>50</sub> value of caffeic acid for K562 cells was 56.0 µM, the values for other three tumor cells, HT-29, HeLa S3, and MCF-7, were 165.8 µM, 134.5 µM, and 490.8 µM, respectively. 2-Cyclohexylethyl caffeate and 3-cyclohexylpropyl caffeate exhibited stronger antiproliferative activities than caffeic acid, and the IC<sub>50</sub> values of both compounds were comparable to that of 5-fluorouracil, which is a widely accepted anticancer drug (Yoshida et al., 2008).

It was also reported that CAPE inhibited the proliferation of human leukemic HL-60 cells and human colorectal cancer HCT116 cells and induced apoptosis (Chen et al., 2001; Wang et al., 2005). Thus, 2-cyclohexylethyl caffeate and 3-cyclohexylpropyl caffeate might induce apoptosis.

## 6. Conclusion

Generally, enzymes offer advantages in stereo and regioselectivity for synthesis of biologically active compounds (Luetz et al., 2008). Diminished yields, selectivity, and poor solubility of substrates in the aqueous phase may require that the enzymatic reactions be performed in the non-aqueous phase. In this regard, various solvents, such as organic solvents (Gubicza et al., 2000), ILs, and supercritical carbon dioxide (Oakes et al., 2001), were investigated to overcome the disadvantages of the aqueous solvent. Increasing environmental demands and legal restrictions currently necessitate the development of new types of green solvents and innovations for more sustainable enzymatic processes. ILs as solvents have at least four advantages for enzymatic reactions: 1) ILs are easily modified with cations and anions to dissolve polar and non-polar substrates, 2) the purification of products is very easy, 3) the enzymes can be used repeatedly with IL, and 4) the thermal stability and non-volatility of ILs may promote the reaction to obtain the intended product. In this study using various ILs as the reaction solvent, we examined the properties of chlorogenate hydrolase from *A. japonicus* and Novozyme 435 (*C. antractica* lipase B). Additionally, we produced 2-cyclohexylethyl caffeate and 3-cyclohexylpropyl caffeate, and showed that they had antiproliferative effect on various human tumor cells. We thus developed a convenient procedure for conversion of 5-caffeoylquinic acid to 3-cyclohexylpropyl caffeate *via* methyl caffeate. The procedure developed in this study would be useful for the exploitation of immature coffee beans that contain various caffeoylquinic acids (Fig. 13).

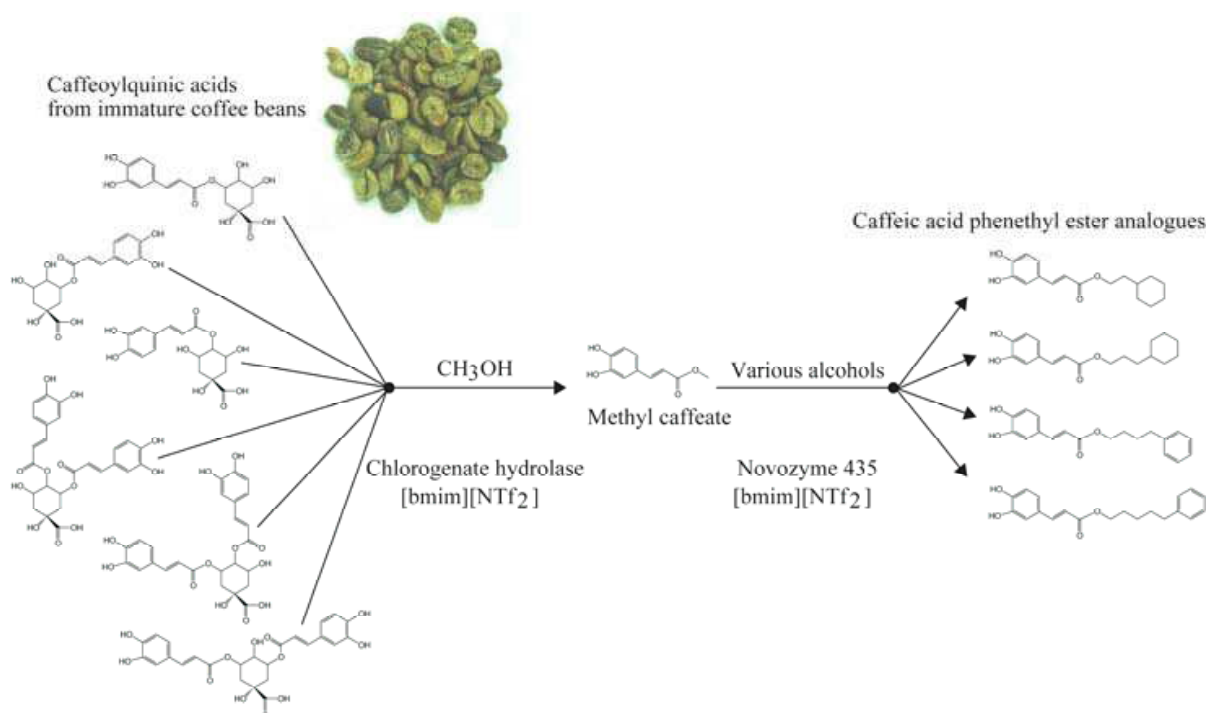


Fig. 13. One-pot two-step synthesis of various CAPE analogues from various caffeoylquinic acids prepared from coffee beans *via* methyl caffeate. Using chlorogenate hydrolase, Novozyme 435, and [bmim][NTf<sub>2</sub>] as the reaction solvent, caffeoylquinic acids from coffee beans were converted to various CAPE analogues, which have antimicrobial, anti-inflammatory, antioxidant, and antitumor activities.

Using various enzymes with ILs as the solvent, an underutilized natural resource was converted to a valuable bioactive compound. Investigation of combinations of enzymes and ILs could lead to development of a novel bioconversion system.

## 7. Acknowledgment

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## 8. References

- Anderson, J.L.; Ding, J.; Welton, T. & Armstrong, D.W. (2002). Characterizing ionic liquids on the basis of multiple solvation interactions. *J. Am. Chem. Soc.*, 124, 47, 14247-14254
- Bankova, V. (2005). Recent trends and important developments in propolis research. *eCAM.*, 2, 1, 29-32
- Barahona, D.; Pfromm, P.H. & Rezac, M.E. (2006). Effect of water activity on the lipase catalyzed esterification of geraniol in ionic liquid [bmim]pf<sub>6</sub>. *Biotechnol. Bioeng.*, 93, 2, 318-24
- Bélafi-Bakó, K.; Dörmö, N.; Ulbert, O. & Gubicza, L. (2002). Application of pervaporation for removal of water produced during enzymatic esterification in ionic liquids. *Desalination.*, 149, 1-3, 267-268
- Carmichael, J.; DeGraff, W.G.; Gazdar, A.F.; Minna, J.D. & Mitchell, J.B. (1987). Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of radiosensitivity. *Cancer. Res.*, 47, 4, 943-946
- Chen, H.C.; Ju, H.Y.; Twu, Y.K.; Chen, J.H.; Chang, C.M.; Liu, Y.C.; Chang, C. & Shieh, C.J. (2010). Optimized enzymatic synthesis of caffeic acid phenethyl ester by rsm. *N. Biotechnol.*, 27, 1, 89-93
- Chen, Y.J.; Shiao, M.S.; Hsu, M.L.; Tsai, T.H. & Wang, S.Y. (2001). Effect of caffeic acid phenethyl ester, an antioxidant from propolis, on inducing apoptosis in human leukemic hl-60 cells. *J. Agric. Food. Chem.*, 49, 11, 5615-5619
- Fehér, E.; Major, B.; Bélafi-Bakó, K. & Gubicza, L. (2007). On the background of enhanced stability and reusability of enzymes in ionic liquids. *Biochem. Soc. T.*, 35, 6, 1624-1627
- Fesen, M.R.; Kohn, K.W.; Leteurtre, F. & Pommier, Y. (1993). Inhibitors of human immunodeficiency virus integrase. *Proc. Natl. Acad. Sci. U S A.*, 90, 6, 2399-2403
- Gubicza, L.; Kabiri-Badr, A.; Keoves, E. & Bélafi-Bakó, K. (2000). Large-scale enzymatic production of natural flavour esters in organic solvent with continuous water removal. *J. Biotechnol.*, 84, 2, 193-196
- Hernández-Fernández, F.J.; de los Rios, A.P.; Rubio, M.; Gomez, D. & Villora, G. (2007). Enhancement of activity and selectivity in lipase-catalyzed transesterification in ionic liquids by the use of additives. *J. Chem. Technol. Biotechnol.*, 82, 882-887
- Itoh, T.; Akasaki, E.; Kudo, K. & Shirakami, S. (2001). Lipase-catalyzed enantioselective acylation in the ionic liquid solvent system: Reaction of enzyme anchored to the solvent. *Chem. Lett.*, 30, 3, 262

- Itoh, T. (2007). Biotransformation in ionic liquid, In: *Future directions in biocatalysis*, Matsuda, T., 3-20, Elsevier Bioscience, The Netherlands
- Kaar, J.L.; Jesionowski, A.M.; Berberich, J.A.; Moulton, R. & Russell, A.J. (2003). Impact of ionic liquid physical properties on lipase activity and stability. *J. Am. Chem. Soc.*, 125, 14, 4125-4131
- Kadota, S. & Tezuka, Y. (2004). Study on antimetastatic activity of a propolis constituent, cape, and its analogues. *Honeybee. Sci.*, 25, 3, 107-112
- Kishimoto, N.; Kakino, Y.; Iwai, K. & Fujita, T. (2005a). Chlorogenate hydrolase-catalyzed synthesis of hydroxycinnamic acid ester derivatives by transesterification, substitution of bromine, and condensation reactions. *Appl. Microbiol. Biotechnol.*, 68, 2, 198-202
- Kishimoto, N.; Kakino, Y.; Iwai, K.; Mochida, K. & Fujita, T. (2005b). *In vitro* antibacterial, antimutagenic and anti-influenza virus activity of caffeic acid phenethyl esters. *Biocontrol. Sci.*, 10, 4, 155-161
- Kurata, A.; Kitamura, Y.; Irie, S.; Takemoto, S.; Akai, Y.; Hirota, Y.; Fujita, T.; Iwai, K.; Furusawa, M. & Kishimoto, N. (2010). Enzymatic synthesis of caffeic acid phenethyl ester analogues in ionic liquid. *J. Biotechnol.*, 148, 2-3, 133-8
- Kurata, A.; Takemoto, S.; Fujita, T.; Iwai, K.; Furusawa, M. & Kishimoto, N. (2011). Synthesis of 3-cyclohexylpropyl caffeate from 5-caffeoylquinic acid with consecutive enzymatic conversions in ionic liquid. *J. Mol. Catal. B: Enzyme.*, 69, 161-167
- Lau, R.M.; van Rantwijk, F.; Seddon, K.R. & Sheldon, R.A. (2000). Lipase-catalyzed reactions in ionic liquids. *Org. Lett.*, 2, 26, 4189-4191
- Luetz, S.; Giver, L. & Lalonde, J. (2008). Engineered enzymes for chemical production. *Biotechnol. Bioeng.*, 101, 4, 647-653
- Michaluart, P.; Masferrer, J.L.; Carothers, A.M.; Subbaramaiah, K.; Zweifel, B.S.; Koboldt, C.; Mestre, J.R.; Grunberger, D.; Sacks, P.G.; Tanabe, T. & Dannenberg, A.J. (1999). Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. *Cancer. Res.*, 59, 10, 2347-2352
- Moniruzzaman, M.; Kamiya, N. & Goto, M. (2010). Activation and stabilization of enzymes in ionic liquids. *Org. Biomol. Chem.*, 8, 13, 2887-2899
- Nagaoka, T.; Banskota, A.H.; Tezuka, Y.; Midorikawa, K.; Matsushige, K. & Kadota, S. (2003). Caffeic acid phenethyl ester (cape) analogues: Potent nitric oxide inhibitors from the netherlands propolis. *Biol. Pharm. Bull.*, 26, 4, 487-491
- Nara, S.J.; Harjani, J.R. & Salunkhe, M.M. (2002). Lipase-catalysed transesterification in ionic liquids and organic solvents: A comparative study. *Tetrahedron Lett.*, 43, 16, 2979-2982
- Nicklaus, M.C.; Neamati, N.; Hong, H.; Mazumder, A.; Sunder, S.; Chen, J.; Milne, G.W. & Pommier, Y. (1997). Hiv-1 integrase pharmacophore: Discovery of inhibitors through three-dimensional database searching. *J. Med. Chem.*, 40, 6, 920-929
- Oakes, R.S.; Clifford, A.A. & Rayner, C.M. (2001). The use of supercritical fluids in synthetic organic chemistry. *J. Chem. Soc. Perkin. Trans. ,* 1, 917-941
- Park, S. & Kazlauskas, R.J. (2001). Improved preparation and use of room-temperature ionic liquids in lipase-catalyzed enantio- and regioselective acylations. *J. Org. Chem.*, 66, 25, 8395-8401

- Pleiss, J.; Fischer, M. & Schmid, R.D. (1998). Anatomy of lipase binding sites: The scissile fatty acid binding site. *Chem. Phys. Lipids.*, 93, 1-2, 67-80
- Singh, M.; Singh, R.S. & Banerjee, U.C. (2009). Stereoselective synthesis of (*r*)-1-chloro-3(3,4-difluorophenoxy)-2-propanol using lipases from *pseudomonas aeruginosa* in ionic liquid-containing system. *J. Mol. Catal. B: Enzyme.*, 56, 4, 294-299
- Sud'ina, G.F.; Mirzoeva, O.K.; Pushkareva, M.A.; Korshunova, G.A.; Sumbatyan, N.V. & Varfolomeev, S.D. (1993). Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. *FEBS. Lett.*, 329, 1-2, 21-24
- Sureshkumar, M. & Lee, C.K. (2009). Biocatalytic reactions in hydrophobic ionic liquids. *J. Mol. Catal. B: Enzyme.*, 60, 1-2, 1-12
- Tagashira, M. & Ohtake, Y. (1998). A new antioxidative 1,3-benzodioxole from *melissa officinalis*. *Planta. Med.*, 64, 6, 555-558
- van Rantwijk, F. & Sheldon, R.A. (2007). Biocatalysis in ionic liquids. *Chem. Rev.*, 107, 6, 2757-2785
- Wang, D.; Xiang, D.B.; He, Y.J.; Li, Z.P.; Wu, X.H.; Mou, J.H.; Xiao, H.L. & Zang, Q.H. (2005). Effect of caffeic acid phenethyl ester on proliferation and apoptosis of colorectal cancer cells *in vitro*. *World. J. Gastroenterol.*, 11, 26, 4008-4012
- Yoshida, K.; Yamaguchi, K.; Osada, S.; Kawaguchi, Y.; Takahashi, T.; Sakashita, F. & Tanaka, Y. (2008). Challenge for a better combination with basic evidence. *Int. J. Clin. Oncol.*, 13, 3, 212-219
- Ysrael, M.C. & Nonato, M.G. (1999). Biological activities of chemical constituents from *ipomoea muricata* [jacq] convolvulaceae. *Acta. Manilana. Ser. A.*, 47, 41-52

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## **Applications of Ionic Liquids in Science and Technology**

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This volume, of a two volume set on ionic liquids, focuses on the applications of ionic liquids in a growing range of areas. Throughout the 1990s, it seemed that most of the attention in the area of ionic liquids applications was directed toward their use as solvents for organic and transition-metal-catalyzed reactions. Certainly, this interest continues on to the present date, but the most innovative uses of ionic liquids span a much more diverse field than just synthesis. Some of the main topics of coverage include the application of RTILs in various electronic applications (batteries, capacitors, and light-emitting materials), polymers (synthesis and functionalization), nanomaterials (synthesis and stabilization), and separations. More unusual applications can be noted in the fields of biomass utilization, spectroscopy, optics, lubricants, fuels, and refrigerants. It is hoped that the diversity of this volume will serve as an inspiration for even further advances in the use of RTILs.

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