**NOTE**

**Synthesis of 6-O-Octanoyl-1,2-O-isopropylidene-α-D-glucofuranose by Lipase-catalyzed Esterification in an Organic Solvent**

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Abstract: The synthesis of 6-O-octanoyl-1,2-O-isopropylidene-α-D-glucofuranose (C8-IpGlc) via the lipase-catalyzed esterification of 1,2-O-isopropylidene-α-D-glucofuranose (IpGlc) with octanoic acid was evaluated using ketones, nitriles, and tertiary alcohols as reaction media. Among the solvents assessed, acetone was the most effective solvent for the synthesis of C8-IpGlc at 50°C, without the formation of a by-product (a diester of IpGlc); the optimum molar ratio of octanoic acid:IpGlc was 2:1. Notwithstanding early-stage substrate inhibition by IpGlc at initial IpGlc concentrations greater than 250 mmol/L, an optimal product concentration of ca. 210 mmol/L (conversion = 53 % at 7 d) was achieved at longer reaction times by changing the initial IpGlc concentration from 25 to 800 mmol/L.

Key words: esterification, lipase, organic solvent, sugar acetal, sugar ester

1 INTRODUCTION

Sugar esters are non-ionic surfactants comprising a hydrophilic sugar and a hydrophobic fatty acid, and possess high stability, detergency and emulsifying capacity. The biodegradability of these esters under aerobic or anaerobic conditions in conjunction with other characteristics such as a low stimulatory effect, and no taste or odor has led to their widespread use in foods, cosmetics, detergents, and pharmaceutical products. One attractive feature of sugar esters is that the hydrophilic-lipophilic balance (HLB) can be easily manipulated by changing the type of constituent fatty acid and sugar moieties in the ester.

Conventional chemical synthesis of sugar esters involves transesterification in the presence of alkali catalysts at high temperature among other methods. However, the conventional chemical methods suffer from the limitation of being energy inefficient and the products become brown over time. An alternative synthesis that represents an attractive solution to the aforementioned issues employs the use of enzymes having high substrate specificity. Certain lipases have been widely used to catalyze the acylation of alcohols in organic solvents. Similarly, sugar esters can also be synthesized with generally high regioselectivity by employing lipase. Because lipase exhibits catalytic activity under moderate conditions, side reactions can be relatively easily suppressed in comparison with the chemical synthesis. Based on these considerations, a number of studies on the lipase-catalyzed synthesis of sugar esters in organic solvents have been performed.

Many substrates including glucose or fructose, sucrose, etc., used as sugar substrates, and long or medium chain fatty acids such as palmitic acid and oleic acid, used as acyl substrates, have been applied to the synthesis of sugar esters. However, due to the low solubility of sugars in organic solvents (the solubility of glucose in acetone is 0.44 mmol/L at 22°C), the reaction rate is low, resulting in low productivity. One means of circumventing this limitation is to improve the solubility of the sugar substrate in the organic solvent by using a hydrophobic sugar derivative as the sugar substrate. Many hydrophobic sugar derivatives such as sugar acetals, alkyl glycosides, and phenyl boronic acid esters are known. Among these, sugar acetals offer the advantage of ease of synthesis, and relatively facile deprotection. Notably, the solubility of the sugar acetal, 1,2-O-isopropylidene-α-D-glucofuranose, is very high in acetone (ca. 200 mmol/kg at 40°C).

Furthermore, monosaccharide-based esters, which are used as hydrophilic emulsifiers, can be synthesized by es-
terifying monosaccharides with medium-chain fatty acids, such as octanoic acid. The objective of this study was to synthesize 6-O-octanoyl-1,2-O-isopropylidene-α-D-glucofuranose (C8-IpGlc) via the lipase-catalyzed esterification of octanoic acid and IpGlc. C8-IpGlc serves as an intermediate for the synthesis of 6-O-octanoyl-D-glucose by means of elimination of an isopropylidene group of C8-IpGlc using acetic acid.

2 MATERIALS AND METHODS

2.1 Materials

The immobilized lipase from *Candida antarctica* (fraction B; CALB) was purchased from Roche Diagnostics, Mannheim, Germany. Octanoic acid and 1,2-O-isopropylidene-α-D-glucofuranose were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Sigma-Aldrich (Tokyo, Japan), respectively. All other reagents were purchased from Wako Pure Chemical Industries.

2.2 Solubility of IpGlc

An excess amount of IpGlc was mixed with the various organic solvents in a screw-capped vial; the organic solvents evaluated were acetone, 2-butanone, acetonitrile, propionitrile, butyronitrile, t-butyl alcohol, and t-amyl alcohol. The vial was stored at a specified temperature (30–70°C) with vigorous shaking (120 rpm) for 3 h to prepare a saturated solution. The supernatant of the mixture (ca. 5-10 mL) was rapidly transferred to a pre-weighed round bottom flask, and the solvent was then completely removed via rotary reduced pressure evaporation. The solubility of IpGlc was measured based on the weight of the flask after evaporation.

2.3 Lipase-catalyzed esterification of IpGlc

Prior to the esterification reaction, the organic solvents were dehydrated over 3A molecular sieves for 2 d. Specific compositions of IpGlc and octanoic acid were weighed into a screw-capped vial; the ratio of octanoic acid:IpGlc (mol/mol) ranged from 1:1 to 5:1, and the initial concentration of IpGlc ranged from 25 to 800 mmol/L. The respective organic solvent (5 mL) was added to the vial, and the vial was stored at a specific temperature (30–60°C) with vigorous shaking at 120 rpm. Immobilized lipase (100 mg) was then added to start the reaction. At appropriate intervals, an aliquot of the reaction mixture was withdrawn and analyzed using HPLC. The HPLC analysis was performed using a YMC-Pack Pro C18 column (3.0 mm × 150 mm, YMC, Kyoto, Japan), LC-10 ADVP pump (Shimadzu, Kyoto), and SPD-10AVP UV spectrophotometer (Shimadzu) at 220 nm. The analytes were eluted with a 70/30 (v/v) mixture of methanol/water at a flow rate of 0.4 mL/min.

2.4 Purification and identification of product

The esterification was performed by mixing 1.5 mmol of IpGlc, 3.0 mmol of octanoic acid, and 600 mg of immobilized lipase in 30 mL of acetone at 50°C. After the reaction reached equilibrium, the immobilized lipase was removed by filtration. Acetone in the reaction mixture was then removed by evaporation, and the residue was dissolved in a small amount of methanol. Ethyl acetate (50 mL) and 0.1 mol/L sodium hydroxide aqueous solution (30 mL) were added to this solution; the mixture was then thoroughly shaken. Following phase separation, the organic phase was collected and dehydrated over an excess amount of sodium sulfate. The solvent was subsequently removed by evaporation, and the residue was dissolved in hexane at 70°C. The product (6-O-octanoyl-1,2-O-isopropylidene-α-D-glucofuranose) was obtained after recrystallization.

Identification of the product was performed with 1H NMR using an ECP-500 spectrometer (500 MHz, JEOL, Tokyo, Japan).

3 RESULTS AND DISCUSSION

3.1 Solubility of IpGlc

Figure 1 shows the temperature dependence of the solubility of IpGlc in various organic solvents. The solubility increased with increasing temperature in all of the appraised solvents.

![Fig. 1](image-url) "Temperature dependence of solubility of 1,2-O-isopropylidene-α-D-glucofuranose in various organic solvents. The symbols ▼, ◀, ▼, ▼, ▼, and ▼ represent acetonitrile, propionitrile, butyronitrile, acetone, 2-butanone, t-butyl alcohol, and t-amyl alcohol, respectively."
solvents. In the case of ketones and nitriles, the solubility decreased with increasing alkyl chain length. In contrast, the solubility increased with increasing alkyl chain length in the case of tertiary alcohols. Overall, high solubility was observed in the tertiary alcohols, acetone, and acetonitrile. Regardless of the type of solvent, the temperature-dependence of the solubility was linear, and the dissolution enthalpy could be estimated from the van’t Hoff’s equation:

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\frac{d \ln S}{d(1/T)} = -\frac{\Delta H}{R}
\]

where \(S\) is the solubility, \(\Delta H\) is the dissolution enthalpy, \(T\) is the absolute temperature, and \(R\) is the gas constant. The dissolution enthalpies of IpGlc were 34.7 kJ/mol in acetone, 38.0 kJ/mol in 2-butanone, 59.4 kJ/mol in acetonitrile, 24.7 kJ/mol in propionitrile, 45.4 kJ/mol in butyronitrile, 27.9 kJ/mol in \(t\)-butyl alcohol, and 48.7 kJ/mol in \(t\)-amyl alcohol. No definite relationship between the dissolution enthalpy and the alkyl chain length or the type of polar group in the molecules was observed.

### 3.2 Selection of reaction medium

The lipase-catalyzed esterification reaction was performed using the abovementioned solvents as reaction media. In all of the test solvents, the reactions approached equilibrium within 8 h. The product concentrations at 10 h were 29.3 mmol/L in acetone, 37.2 mmol/L in 2-butanone, 33.4 mmol/L in acetonitrile, 23.6 mmol/L in propionitrile, 33.1 mmol/L in butyronitrile, 13.8 mmol/L in \(t\)-butyl alcohol, and 9.3 mmol/L in \(t\)-amyl alcohol. Because the solubilities of IpGlc in 2-butanone and in butyronitrile at 50°C were less than 30 mmol/L, achieving a high product concentration in these solvents was problematic. Therefore, 2-butanone and butyronitrile were not considered suitable solvents for the synthesis. Acetonitrile and propionitrile gave rise to relatively high product concentrations; nevertheless, a certain by-product was formed in these nitriles. Given that the retention time of this by-product in reversed-phase HPLC was longer than that for C8-IpGlc, the by-product is suggested to be hydrophobic in nature. In addition, a previous report indicated that a diester of IpGlc is easily formed in acetonitrile\(^9\). Based on these data, the by-product is assigned as the diester of IpGlc that was further acylated at the 5-O-position. The product concentrations in \(t\)-butyl alcohol and \(t\)-amyl alcohol were low, and those in acetone and 2-butanone were relatively high. Based on these results, acetone was chosen as the optimal reaction medium for the ensuing experiments.

The optimum molar ratio of the substrates and the effect of temperature on the reaction were subsequently investigated. The conversion (where conversion is defined based on IpGlc, the limiting substrate) increased with an increase in the molar ratio of octanoic acid:IpGlc from 1:1 to 2:1 and leveled off when the molar ratio of octanoic acid:IpGlc increased from 2:1 to 5:1. Therefore, an octanoic acid:IpGlc ratio of 2:1 (mol/mol) was employed in subsequent experiments. The reaction temperature was optimized by evaluating the esterification in the range of 30-60°C. The initial reaction rate and the product concentration at 10 h increased with increasing temperature within the range of 30-50°C. However, at 60°C, the initial reaction rate was lower than that at 50°C, even though the product concentration at 10 h was higher at 60°C. This may be due to the slight inactivation of lipase in acetone at 60°C. Based on these results, the reaction temperature was set at 50°C.

### 3.3 Identification of product

The \(^1\)H NMR spectrum of the product was as follows: δ (ppm, 500 MHz, CDCl\(_3\)): 0.88 (t, \(J = 6.7\) Hz, 3H), 1.25-1.34 (m, 8H), 1.67 (m, 2H), 2.37 (t, \(J = 7.5\) Hz, 2H), 3.19 (d, \(J = 3.7\) Hz, 1H), 3.34 (d, \(J = 3.7\) Hz, 1H), 4.08 (m, 1H), 4.25 (m, 2H), 4.37 (m, 1H), 4.44 (dd, \(J_1 = 9.0\) Hz, \(J_2 = 5.5\) Hz, 1H), 4.54 (d, \(J = 3.7\) Hz, 1H), 5.96 (d, \(J = 3.7\) Hz, 1H). The product comprised one molecule of octanoic acid and one molecule of IpGlc. Furthermore, lipase from *Candida antarctica* preferentially recognizes the primary hydroxyl group of a molecule, indicating that C8-IpGlc was formed by the reaction.

### 3.4 Optimization of product concentration

Based on the aforementioned results, the reaction was performed at initial IpGlc concentrations of 25-800 mmol/L (octanoic acid:IpGlc = 2:1, mol/mol) to examine the applicability of the method to the quantitative synthesis of C8-IpGlc. Figure 2 shows the time courses for the synthesis of C8-IpGlc at various initial IpGlc concentrations. The dependences of the product concentration at 24 h and conversion at 24 h on the initial IpGlc concentration are also shown in Fig. 3. Reaction equilibrium was attained within 12-72 h when the initial IpGlc concentration was less than 200 mmol/L (Fig. 2). The product concentration increased in proportion to the initial IpGlc concentration (equilibrium conversion = 45% – 55%)\(^7\). There was a gradual decline in the conversion within the range of 25-200 mmol/L of IpGlc as the IpGlc concentration increased (Fig. 3).

A significant decrease in the product concentration and conversion at 24 h was observed with increasing IpGlc concentration for initial IpGlc concentrations at the higher end of the 250-800 mmol/L range, possibly due to substrate inhibition by IpGlc; the product concentration was only 20 mmol/L (conversion = 2.5% ; 24 h) at 800 mmol/L IpGlc. This markedly low conversion stems from the fact that some of the IpGlc remained undissolved after 24 h for initial IpGlc concentrations of 250-800 mmol/L. A similar phenomenon was previously observed when the ester was synthesized using IpGlc in a mixture of acetone and \(t\)-butyl alcohol\(^9\), and it is likely that IpGlc generally has an inhibitory effect on lipase from *Candida antarctica* (fraction B). Although the product concentration was low during the
early stage of the reaction, an increase in the product concentration was observed during the middle stage of the reaction. Therefore, the time courses in Fig. 2 were sigmoid. This increase in product concentration during the middle stage of the reaction is attributed to the fact that lipase inhibition is relaxed as the IpGlc concentration decreases at longer reaction periods. In addition, this fact also shows that the inhibition is reversible. Finally, a product concentration of ca. 210 mmol/L (conversion = 53%) could be achieved at 7 d using the initial IpGlc concentration of 400 mmol/L. Based on these results, large quantities of C8-IpGlc can be synthesized at a high initial IpGlc concentration.

In conclusion, C8-IpGlc can be successfully synthesized by the esterification of IpGlc with octanoic acid using lipase from Candida antarctica. Although substrate inhibition was observed at high initial IpGlc concentrations in the early stage, a high product concentration could nonetheless be achieved by adopting a high initial IpGlc concentration at longer reaction times.

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References

