Enzymatic Regioselective Transesterification of Sugar Alcohols and Aromatic Esters in Organic Solvents

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INTRODUCTION

Esters of monosaccharides and disaccharides have a number of interesting and potentially useful properties, including surface activity, antitumor activity, and plant growth-inhibiting activity. This potential has not been fully explored because production of oligoesters (e.g., di-, tri-, and tetra-) of sugars is a difficult problem in organic chemistry due to the abundance of hydroxyl groups in sugar molecules and their similar reactivities. Even preferential acylation of primary over secondary hydroxyl groups can only rarely be carried out with free sugars; this usually requires protected sugars, thereby necessitating cumbersome protection and deprotection steps. Enzymatic regioselective acylation of sugars offers an alternative to the poor selectivity of chemical synthesis and, recently, there have been many reports on the enzymatic synthesis of sugar esters using lipases or proteases in organic media.

Very recently, we investigated the catalytic potential of the commercially available crude protease from Bacillus licheniformis, suspended in organic solvents, toward aromatic compounds. Our previous success in developing enzymatic synthesis of aromatic polyester is extended to regioselective transesterification of sugar alcohols and aromatic esters. In the present work, we report that enzymatic acylations were carried out for disaccharides, mainly sucrose, with an activated ester of terephthalic acid in pyridine by employing the same enzyme.

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MATERIALS AND METHODS

Materials

Protease from Bacillus licheniformis (Optimase M-440) was obtained from Solvay Enzyme (Elkhart, Indiana), and Sigma Sil-A was obtained from Sigma Chemical (St. Louis, Missouri) and used for the preparation of TMS derivatives of sugars. Bis(2,2,2-trifluoroethyl)terephthalate was synthesized from terephthaloyl chloride and 2,2,2-trifluoroethanol following the general methodology and had the same characteristics as previously described. All other chemicals and solvents used in this work were of analytical grade.

Analytical Methods

All sugar derivatives in this work were determined by gas chromatography using a 10-m Alltech AT-1 capillary column packed with polydimethylsiloxane and all reaction mixtures were subjected to precolumn derivatization with 1,1,1,3,3,3-hexamethyldisilazane according to the general methodology. The positions of acylation in all enzymatically prepared compounds were established by 13C-NMR (Bruker AMX 500).

Reactions

Our acylation strategy was to use enzyme-catalyzed transesterification of sugars with an activated ester donor [bis(2,2,2-trifluoroethyl)terephthalate; TFE-te] in anhydrous pyridine. The initial concentrations of TFE-te and sucrose were 0.1 and 0.4 M, respectively. The reaction was initiated by the addition of 100 mg/mL of Optimase M-440, and the suspension was magnetically stirred at 250 rpm. The reaction was terminated by filtering out the enzyme and evaporating the pyridine. The sucrose esters were purified by using silica gel chromatography with an eluant consisting of ethyl acetate/methanol/water (18:1.25:1).

RESULTS AND DISCUSSION

Comparison of the Reactivities for Various Disaccharides and TFE-te

The enzymatic transesterification experiments were carried out for various disaccharides: maltose, lactose, and sucrose. Cellobiose was excluded from the experiments due to its too-low solubility in pyridine. In all cases, the reaction readily took place and in no case was any appreciable conversion detected without enzyme. The data in Table 1 indicate that sucrose was the best substrate for the enzymatic transesterification with TFE-te. Furthermore, the low cost and ready availability of pure sucrose make this disaccharide a highly desirable starting material for the preparation of new, commercially significant products and materials.
Effect of Reaction Temperature on Enzymatic Acylation of Sucrose

The rate of the enzymatic transesterification between sucrose and TFE-te and the enzyme inactivation rate were studied in pyridine in the range from 27 °C to 50 °C. The enzymatic acylation of sucrose in pyridine accelerated upon an increase of the temperature (from 27 °C to 50 °C) as depicted in FIGURE 1. However, the inactivation rate also increased as the reaction temperature increased. After 1 day, there was no significant decrease in the enzyme activity at 30 °C (less than 10%), whereas the residual activity was only 50.1% at 50 °C. Comparison of the top and

<table>
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<th>Disaccharide</th>
<th>Conversion after 24 h (%)$^b$</th>
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<tbody>
<tr>
<td>maltose</td>
<td>19.5</td>
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<td>lactose</td>
<td>20.8</td>
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<td>sucrose</td>
<td>52.2</td>
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</table>

$^a$Conditions: 0.025 M disaccharides; 0.1 M TFE-te; Optimase M-440, 100 mg/mL; pyridine, 1 mL; 37 °C.

$^b$Determined by gas chromatography on the basis of the disappearance of the substrate disaccharide.

FIGURE 1. Effects of temperature on the acylation rate of sucrose (bottom) and on the inactivation of Optimase M-440 suspended in anhydrous pyridine (top). Crude enzyme (100 mg) was added to the solution (1 mL) in different vials and the suspensions were shaken at different temperatures. After 24 hours, pyridine was decanted and the residual activity was analyzed by GC with the same substrates at 37 °C.
bottom panels of FIGURE 1 reveals that the temperature had the opposite effects on the enzyme in organic solvent. Whereas the reaction rate increased as the temperature increased, the inactivation was much faster at high temperature.

**Sucrose Ester Synthesis**

In order to preparatively synthesize the sucrose esters, we scaled up the enzymatic process. Sucrose (0.1 M) and TFE-te (0.4 M) were dissolved in 100 ml of pyridine and the reaction was carried out at 37°C. After 20 days, the reaction was terminated by filtering out the enzyme and evaporating the organic solvent. The residual solids were loaded onto a silica column (see MATERIALS AND METHODS), and sucrose esters were separated into monoester and diester. No triester was ever formed, as evidenced by TLC and GC.

To determine the site of sucrose acylation, 13C-NMR analysis of the sucrose ester products was carried out. Following the general strategy developed by Yoshimoto et al., acylation at position 1' of sucrose should result in a downfield shift of the peak at C1 and in an upfield shift of the peak at C2'. Acylation at position 6 of sucrose should result in a downfield shift of the peak at C6 and in an upfield shift in the peak at C5. The chemical shifts in TABLE 2 enable us to assign structures to the monoester and diester products, with the sucrose monoester identified as sucrose 1'-terephthalate and the diester product identified as sucrose 6,1'-diterephthalate. Hence, sucrose was acylated at the 1' position followed by acylation at the 6 position (FIGURE 2).

The solubility of the sucrose ester products was tested in a variety of solvents. The monoester and diester were soluble in a variety of polar organic solvents, including DMSO, DMF, methanol, ethanol, acetone, tetrahydrofuran, and pyridine. The diester was insoluble in water, whereas the monoester was soluble, indicating that a monoester and diester mixture could be separated by simple extraction using water.

To summarize, protease has been employed in the present study for the regioselective acylation of sugars with activated terephthalate in pyridine. This work leads

<table>
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<tr>
<th>Carbon Number</th>
<th>Sucrose</th>
<th>Sucrose Monoterephthalate</th>
<th>Sucrose Diterephthalate</th>
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</table>
FIGURE 2. Scheme of sucrose acylation in anhydrous pyridine catalyzed by Optimase M-440.

to a straightforward synthetic methodology for the preparative production of aromatic monoesters and diesters of sugars.

REFERENCES
