Practical Production of 6-O-Octanoyl-D-allose and Its Biological Activity on Plant Growth

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The transesterification of D-allose (the C-3 epimer of D-glucose) with vinyl octanoate using Candida antarctica lipase in tetrahydrofuran proceeded with high regioselectivity to produce 6-O-octanoyl-D-allose with nearly complete conversion. The growth-inhibiting activity of 6-O-octanoyl-D-allose on lettuce seedlings was about 6-fold greater than that of D-allose.

Key words: rare sugar; sugar fatty acid ester; Candida antarctica lipase; D-allose

The rare sugar, D-allose (1; the C-3 epimer of D-glucose) has been extensively studied1) and found to have significant biological activities: an immunosuppressive effect2) and protective effect against liver damage3) in addition to a suppressive effect on leukocyte formation in homeothermal animals.4) However, its biological activities on plants have only been described in a limited manner.5) We have reported the enzymatic synthesis of D-allose fatty acid esters via lipase-catalyzed transesterification6) because we expected that the introduction of unbranched alkyl chains to D-allose would provide not only good surface activity and enhanced membrane permeability, but also higher biological activity than that of D-allose itself on the basis of biosurfactant research on sugar fatty acid esters.7,8) Moreover, they are also non-ionic and biodegradable9) and, therefore, could be widely used in the food, cosmetic, and pharmaceutical industries. Although the same regioselective acylation of the C-6 primary hydroxy group as D-glucose was observed in that of D-allose, irrespective of the different configuration of the hydroxy group at C-3 of D-allose, a drawback in this process is the use of toxic acetonitrile which may not be compatible with food industrial purposes. Different environmentally benign procedures for preparing ordinary sugar fatty acid esters have been reported using acetone, which is recognized as safe for use in food manufacturing,10) ethyl methyl ketone and azeotropic distillation11) and supercritical carbon dioxide.12)

We describe in this paper the practical and environmentally benign production of 6-O-octanoyl-D-allose, using vinyl octanoate in a non-toxic organic solvent (Fig. 1), and its biological activity on plant growth.

The reactions of D-allose with vinyl octanoate, using Candida antarctica lipase in different organic solvents, were carried out as shown in Table 1. We first tried to use acetone (analytical grade) as an organic solvent, but found that D-allose reacted more slowly in acetone than in acetonitrile and that a small amount of the diester was produced from the second esterification as a by-product. We therefore examined the effect of the water content in acetone to increase the reaction rate. The use of HPLC grade acetone and dehydrated acetone (<50 ppm water) increased the reaction rate and decreased the diester to some extent. These results suggested that the water content in acetone is an important factor for the reaction rate and for the second esterification to produce the diester. In contrast, the transesterification of D-allose with vinyl octanoate in dehydrated tetrahydrofuran (THF, <50 ppm water) proceeded with high regioselectivity and reaction rate at 45 °C for 24 h to give 6-O-octanoyl-D-allose in a 95% conversion without producing the diester. This reaction rate was comparable to that when using acetonitrile. THF was therefore found to be the optimum solvent for transesterification with vinyl octanoate catalyzed by Candida antarctica lipase, producing 2 with nearly complete conversion.

Table 1. Effect of Organic Solvent on the 6-O-Octanoate 2 Production Catalyzed by Candida antarctica Lipase

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Time (h)</th>
<th>Conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetone (analytical)</td>
<td>48</td>
<td>67</td>
</tr>
<tr>
<td>acetone (HPLC)</td>
<td>48</td>
<td>74</td>
</tr>
<tr>
<td>acetone (dehydrated)</td>
<td>48</td>
<td>86</td>
</tr>
<tr>
<td>THF (dehydrated)</td>
<td>24</td>
<td>95</td>
</tr>
</tbody>
</table>

Reaction conditions: D-allose; 60 mg (0.33 mmol), vinyl octanoate; 192 µl (0.99 mmol), solvent; 3 ml, lipase; 60 mg.

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The biological activities of D-allose 1 and 6-O-octanoate 2 were determined by using lettuce seedlings (Fig. 2). D-Allose 1 inhibited the respective growth of lettuce roots and hypocotyls at concentrations greater than 3 mM and 10 mM, and 2 inhibited the respective growth of lettuce roots and hypocotyls at concentrations greater than 1 mM and 3 mM. When the length of the lettuce roots and hypocotyls was plotted against the logarithm of the concentration, all concentration-response curves were linear in the 20–80% inhibition ranges. The concentrations required for 50% inhibition of the lettuce hypocotyls in the assay (defined as $I_{50}$), as interpolated from the concentration-response curves, were 32.4 mM and 5.6 mM for 1 and 2, respectively, and those of the lettuce roots were 21.5 mM and 3.4 mM for 1 and 2, respectively. Comparing the $I_{50}$ values, the respective growth-inhibiting activity of 2 on the hypocotyls and roots was 5.8 and 6.3-fold greater than that of 1. Thus, the introduction of the octanoyl group to the O-6 position of 1 induced about a 6-fold increase in the inhibitory activity. It has been found that phosphorylation of D-mannose by hexokinase triggered a signal cascade resulting in the growth inhibition of lettuce. D-Allose 1 and octanoate 2 might have inhibited the growth of lettuce seedlings via the same signal cascade.

In summary, we established a one-step process for the enzymatic acylation of the primary hydroxy group (C-6) of the unprotected rare sugar, D-allose, with vinyl octanoate in a low-toxic organic solvent. This method enables the practical and environmentally benign production of 6-O-octanoate 2. The biological activity of octanoate 2 on lettuce seedlings was about 6-fold greater than that of 1, perhaps due to the surface activity (cmc = 26.9 mM). The production of different lengths of 6-O-acetyl-D-alloses and their growth-inhibiting tests on plants are in progress.

**Experimental**

Lipases from *Candida antarctica* (Novozym 435) immobilized on a macroporous acrylic resin were obtained from Novo Nordisk (Bagsvaerd, Denmark). D-Allose was provided by the Rare Sugar Research Center of Kagawa University. Dehydrated acetonitrile, dehydrated THF, and acetone (analytical, HPLC grade and dehydrated, respectively) were purchased from...
Wako Chemical Co. (Tokyo, Japan) and used without further dehydration. All other reagents were purchased from Wako Chemical Co. Qualitative analyses of the reaction mixtures were carried out by TLC on silica gel 60 (Merck, 0.25 mm), using a solvent mixture of ethyl acetate/methanol (20:1, v/v), and column chromatography was performed with silica gel (Merck, 23–400 mesh). The $^1$H- and $^{13}$C-NMR spectra were respectively measured at 400 and 100 MHz with a Jeol JNM-A400 spectrometer in CD$_3$OD at room temperature, using TMS as the internal standard. The mass spectra were recorded with a Jeol JMS-SX 102A mass spectrometer. The HPLC system consisted of a Shimadzu LC9A pump (Tosoh, Japan) and a Shimadzu SPD-A UV detector connected to an ODS-80Ts column (4.6 × 250 mm, Tosoh, Japan) and a Shimadzu SPD-A UV detector (214 nm), eluting with acetonitrile–methanol–water (25:25:50, v/v) at a flow rate of 1 ml/min. The retention time of the product was 12.9 min.

**General procedure for enzymatic synthesis of rare sugar esters.** Vinyl octanoate (192 µl, 0.99 mmol) was added to D-allose (60 mg, 0.33 mmol), Novozym 435 (60 mg), and the organic solvent (3 ml) in a test tube flask (ChemiStation PPS-2510; Eyela, Japan) at 45 °C. The suspension was stirred by a magnetic bar at 300 rpm. The enzyme reaction progress was monitored by HPLC. After 1–2 days, the filtrate through Celite was purified by column chromatography on silica gel, using a solvent mixture of ethyl acetate–methanol (20:1, v/v). The resulting sugar ester was further purified by recrystallization from ethyl acetate-hexane. The $^1$H- and $^{13}$C-NMR spectra corresponded to those of 6-O-octanoyl-β-D-allopyranose which had previously been reported.5)

**Bioassay.** D-Allose 1 and 6-O-octanooate 2, each dissolved in a small volume of water or methanol, were added to a sheet of filter paper (Toyo No. 2) in a 3.5-cm Petri dish and then dried. The filter paper in the Petri dish was then moistened with 0.8 ml of a 0.05% (v/v) aqueous solution of Tween 20, and 10 lettuce seeds were arranged on the filter paper and grown in the dark at 25 °C. The control seedlings were treated with only a solution of Tween 20. The lengths of the hypocotyls and roots of the lettuce seedlings were measured after 60 h.

**References**


