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Note

Retarding Activity of 6-*O*-Acyl-D-alloses against Plant Growth

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The retarding activity of 6-*O*-dodecanoyl-D-allose against rice growth was higher than that of the octanoate and the decanoate. The activities of 6-*O*-dodecanoyl-D-glucose, -D-mannose, and -D-galactose against rice seedlings were examined. 6-*O*-Dodecanoyl-D-allose exhibited the highest activity, suggesting the importance of the α-axial hydroxy group at C-3 of D-allose.

Key words: rare sugar; D-allose; sugar fatty acid ester; *Candida antarctica* lipase; plant-growth retardant

The rare sugar, D-allose (a C-3 epimer of D-glucose) has been extensively studied[1] and found to have significant biological activities: an immunosuppressive effect[2] and protective effect against liver damage,[3] in addition to a suppressive effect on leukocyte formation in homeothermic animals.[4] However, its biological activities against plants have only been described in a limited manner. We have hypothesized that D-allose fatty acid esters could enhance membrane permeability and show higher biological activity than that of the sugar alone, because sugar fatty acid esters are known to have good surface activity,[5] as well as glucosyltransferase inhibition[6] and antimicrobial properties.[7] We have already reported that the growth-inhibiting activity of 6-*O*-octanoyl-D-allose against lettuce seedlings was about 6-fold greater than that of D-allose.[8] This significant improvement in plant growth-inhibiting activity makes it interesting to examine the retarding activities of different lengths of 6-*O*-acyl-D-alloses against plant growth. There is also a need to establish whether the α-axial hydroxy group at C-3 in D-allose is important for this retarding activity.

We demonstrate in this study the effect of the length (C_8–C_{12}) of 6-*O*-acyl-D-alloses and the importance of the C-3 axial hydroxy group of D-allose to the rice growth-retarding activity, in comparison with the 6-*O*-dodecanoate of D-glucose, D-mannose (its C-2 epimer), and D-galactose (its C-4 epimer) (Fig. 1).

6-*O*-Decanoyl- and 6-*O*-dodecanoyl-D-alloses (**2** and **3**) were prepared as reported previously,[9] and the effect of the length (C_8–C_{12}) of the carbon chain of the fatty acid moiety on rice growth-retarding activity was investigated (Fig. 2). The retarding activity of 6-*O*-dodecanoyl-D-allose **3** against rice growth was significantly higher than that of the octanoate **1** and the decanoate **2**, indicating that the longer length of the carbon chain of the fatty acid moiety, the greater retarding potency, probably due to their hydrophobicity. However, longer 6-*O*-tetradecanoyl-D-alloses showed lower activity (115% of the control at 0.1 mM), but had too poor solubility in water to examine further. Thus, the activity of 6-*O*-dodecanoyl-D-allose against rice seedlings was significantly greater than that of D-allose (All). It is known that gibberellin biosynthesis inhibitors retard rice shoot elongation, and that such inhibition can be recovered by treating with gibberellin (GA_3).[10,11] As shown in Fig. 3, the recovery of rice growth (200%) was apparent when GA_3 (100 ppm) was co-added with a solution of 6-*O*-dodecanoyl-D-allose **3** (100 ppm, 280 μM) that retarded more than half the growth of the control. This result implies that 6-*O*-dodecanoyl-D-allose might inhibit gibberellin biosynthesis as well as the known plant growth regulator.[10,11]

Syntheses of 6-*O*-dodecanoyl-D-glucose, -D-mannose, and -D-galactose (**4**, **5**, and **6**) were then carried out according to the previously reported procedure.[8] The transesterification of D-glucose with vinyl dodecanoate catalyzed by *Candida antarctica* lipase in dehydrated tetrahydrofuran (THF) proceeded with high regioselectivity at 45 °C for 48 h to give the 6-*O*-dodecanoate **4** with an 76% yield. However, under the same reaction conditions, the transesterification of D-mannose and D-galactose afforded the 6-*O*-dodecanoate (**5** and **6**) in only low to moderate yields, due to the production of the diester as reported.[12] The retarding activities of epimeric 6-*O*-dodecanoates (**4**, **5**, and **6**) against rice growth are shown in Fig. 2. 6-*O*-Dodecanoyl-D-glucose **4** had almost no effect on the growth of rice at concentrations from 0.1 to 1 mM, like the lack of effect of glucose. On the other hand, the dodecanoates **5** and **6** retarded rice growth in a concentration-dependent manner at concentrations from 0.1 to 0.3 mM and completely inhibited it at a concentration of 1 mM, although mannose had almost no effect (110% of the control) and galactose exhibited a promoting effect (153% of the control) at a concentration of 3 mM (data were not shown), in contrast with allose. Thus, the introduction of a fatty acid moiety to mannose and galactose brought about their retarding activity against rice growth, but their dodecanoates (**5** and **6**) exhibited lower activity than 6-*O*-dodecanoyl-D-allose **3**. These results suggest that the α-axial hydroxy group at C-3 of allose played an important role in the retarding activity, since mannose and galactose are the C-2 and C-4 epimers of glucose.

We could demonstrate that the introduction of a medium-chain-length fatty acid moiety to the C-6

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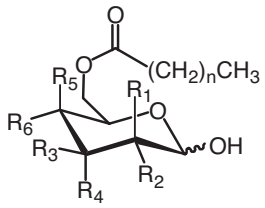

1: $R_1 = R_3 = R_5 = H$, $R_2 = R_4 = R_6 = OH$, n = 6
2: $R_1 = R_3 = R_5 = H$, $R_2 = R_4 = R_6 = OH$, n = 8
3: $R_1 = R_3 = R_5 = H$, $R_2 = R_4 = R_6 = OH$, n = 10
4: $R_1 = R_4 = R_5 = H$, $R_2 = R_3 = R_6 = OH$, n = 10
5: $R_2 = R_4 = R_5 = H$, $R_1 = R_3 = R_6 = OH$, n = 10
6: $R_1 = R_4 = R_6 = H$, $R_2 = R_3 = R_5 = OH$, n = 10

Fig. 1. 6-*O*-Acyl-D-hexoses.

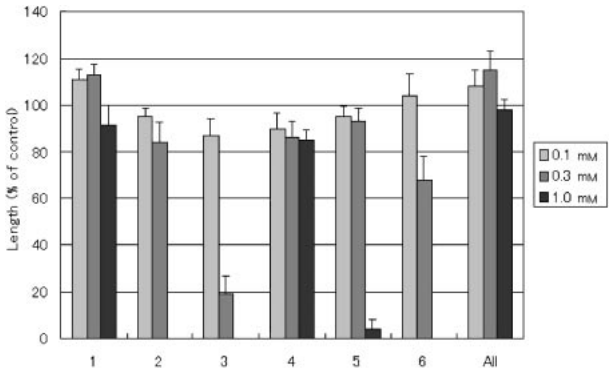

Fig. 2. Effects of 6-*O*-Acyl-D-hexoses **1**–**6** and D-Allose on Rice Seedling Growth.
The mean value ± SE from three independent experiments with 7 plants for each determination is shown.

hydroxy group of D-allose significantly increased the retarding activity against rice growth as well as lettuce growth. Furthermore, we confirmed the importance of the α-axial hydroxy group at C-3 of D-allose for the retarding activity against rice seedling growth. These results suggest that D-allose fatty acid esters could be a surprisingly *simple* and new class of lead compounds for plant-growth regulators, although their activity is not high and their mechanism of action is unclear. Furthermore, these rare sugar fatty acid esters are also biodegradable[5] and, therefore, may have lower toxicity when used for agricultural purposes.

Experimental

General procedure for the enzymatic synthesis of sugar esters. The lipase from *Candida antarctica* (Novozym 435) immobilized on a macroporous acrylic resin was obtained from Novo Nordisk (Bagsvaerd, Denmark). D-Allose was provided by the Rare Sugar Research Center of Kagawa University. Vinyl dodecanoate (520 µl, 2 mmol) was added to D-glucose, D-mannose or D-galactose (180 mg, 1 mmol), and Novozym 435 (180 mg) in THF (18 ml) in a test tube flask (Eyela, ChemiStation PPS-2510) at 45 °C. The suspension was stirred by a magnetic bar at 300 rpm, the enzyme reaction progress being monitored by TLC. The suspension was passed through Celite after 2 d, and the resulting filtrate was purified by column chromatography on silica gel, using a solvent mixture of ethyl acetate-chloroform (1:1, v/v), to afford the corresponding sugar esters in 76%, 45%, and 37% yields, respectively. The ^{1}H- and ^{13}C-NMR spectra (Jeol, JNM-A400) corresponded to those of 6-*O*-dodecanoyl-D-glucose **4**, -D-mannose **5** and -D-galactose **6** which had previously been reported.[12–14]

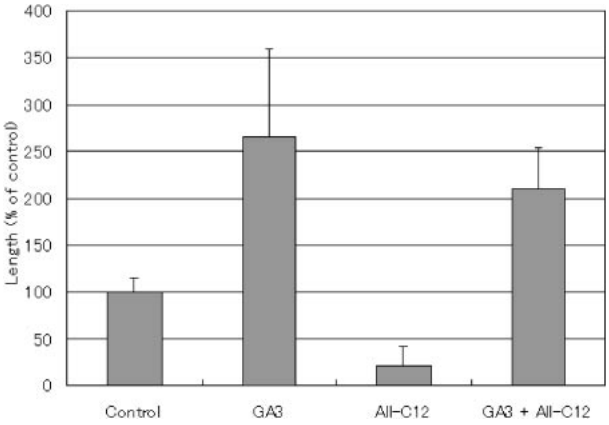

Fig. 3. Effects of 6-*O*-Dodecanoate **3** and GA_3 on Rice Seedling Growth.

Rice seedling bioassay.[15] Seeds of rice (*Oryza sativa* L. cv. Kinuhikari) were sterilized with ethanol for 5 min and then washed with tap water. The seeds were then sterilized for 30 min with 1% sodium hypochlorite and washed again with tap water. The sterilized seeds were soaked in water for 2 d at 30 °C under fluorescent light. Seven germinated seeds were transplanted into a glass tube (30 mm i.d.) containing 2 ml of a test solution, and the tubes were sealed with a parafilm. The control seeds were transplanted into a tube containing only water. After incubating for 7 d under continuous light, the length of the second leaf sheath of each rice seedlings was measured, and the growth ratio to the control value was calculated.

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