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Synthesis and inhibitory activity of deoxy-D-allose amide derivative against plant growth

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ABSTRACT

1,2,6-Trideoxy-6-amido-D-allose derivative was synthesized and found to exhibit higher growth-inhibitory activity against plants than the corresponding deoxy-D-allose ester, which indicates that an amide group at C-6 of the deoxy-D-allose amide enhances inhibitory activity. In addition, the mode of action of the deoxy-D-allose amide was significantly different from that of D-allose which inhibits gibberellin signaling. Co-addition of gibberellin GA_3 restored the growth of rice seedlings inhibited by the deoxy-D-allose amide, suggesting that it might inhibit biosynthesis of gibberellins in plants to induce growth inhibition.

Abbreviations: GA: gibberellin; DMF: *N*,*N*-dimethylformamide; TsCl: 4-toluenesulfonyl chloride

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KEYWORDS
Rare sugar; D-allose; gibberellin biosynthesis; inhibitory activity

Rare sugars are monosaccharides present in limited amounts in nature [1]. Although the biological activities of almost all rare sugars are largely unknown, some biological activities of D-allose (**1**), a C-3 epimer of D-glucose, have been reported. D-Allose showed an immunosuppressive effect [2] and a protective effect against liver damage in animals [3], anti-proliferative effect through cell cycle arrest and apoptosis [4–6], suppressive effect for development of salt-induced hypertension [7], and anti-oxidative effects [8,9]. D-Allose has also been demonstrated to scavenge reactive oxygen species (ROS) generated by the hypoxanthinexanthine oxidase system to inhibit the production of ROS from neutrophils [10], and retard plant growth [11]. However, its concentration required for inhibition of plant growth is relatively high (more than 3 mM). Therefore, it is necessary to improve its activity by chemical modification.

We previously reported that 6-*O*-acyl-D-allose showed six times higher growth-inhibitory activity against lettuce than D-allose [12]. Furthermore, we described the effect of fatty acid chain length on growth-retarding activity of 6-*O*-acyl derivatives of D-allose, D-gulose, and D-altrose on rice seedlings [13,14], and the role of hydroxyl groups at C-1 and C-2 of D-allose on the biological activity [15]. The inhibitory activity of 6-*O*-decanoyl-2-deoxy-D-allose was comparable to that of 6-*O*-decanoyl-D-allose (**2**), whereas 6-*O*-decanoyl-1,2-dideoxy-D-allose (**4**) showed little activity. In general, amide groups are more stable than ester groups that are susceptible to hydrolysis by esterases *in vivo* and, therefore, we expected that amide replacement would improve inhibitory activity on plant growth. However, we previously reported that the amide replacement of **2** resulted in lower inhibitory activity [16], which prompted us to investigate the amide replacement effect on the other allose ester. In this study, we synthesized a new deoxy derivative **6** having amide group at C-6 to examine the effect of replacement of the ester group of **4** with an amide group on plant growth-inhibitory activity (Figure 1).

Results and discussion

6-(Decanoylamino)-1,2,6-trideoxy-D-allose (**6**) was prepared from the known compound 1,2-dideoxy-D-allose (**3**) [17] as shown in Scheme 1. First, regioselective tosylation of **3** at low temperature gave a monotosylate **7** in 68% yield. Then, azidation of **7** with sodium azide in DMF followed by catalytic hydrogenation afforded an amine **5**. Finally, acylation of **5** with decanoyl chloride gave the amide **6** in 63% yield in three steps.

The biological activity of **6** was evaluated using lettuce, cress, Italian ryegrass, and rice seedlings (Figure 2). The amide **6** inhibited the growth of four plants in a concentration-dependent manner ranging from 0.03 to 1 mM, and interestingly, slight growth promotion (119%) for lettuce roots was observed at 0.1 mM (Figure 2(a)). Of the plant species tested, rice seedlings were most susceptible to **6**; the rice growth was completely inhibited by **6** at a concentration greater than 1 mM (Figure 2(d)). The concentrations required for

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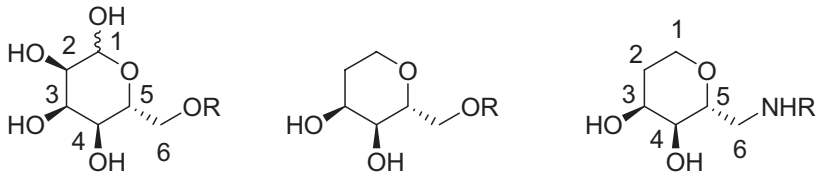


1: R = H
2: R = $CO(CH_2)_8CH_3$

3: R = H
4: R = $CO(CH_2)_8CH_3$

5: R = H
6: R = $CO(CH_2)_8CH_3$

Figure 1. Structures of D-allose and deoxy-D-allose derivatives **1–6**.

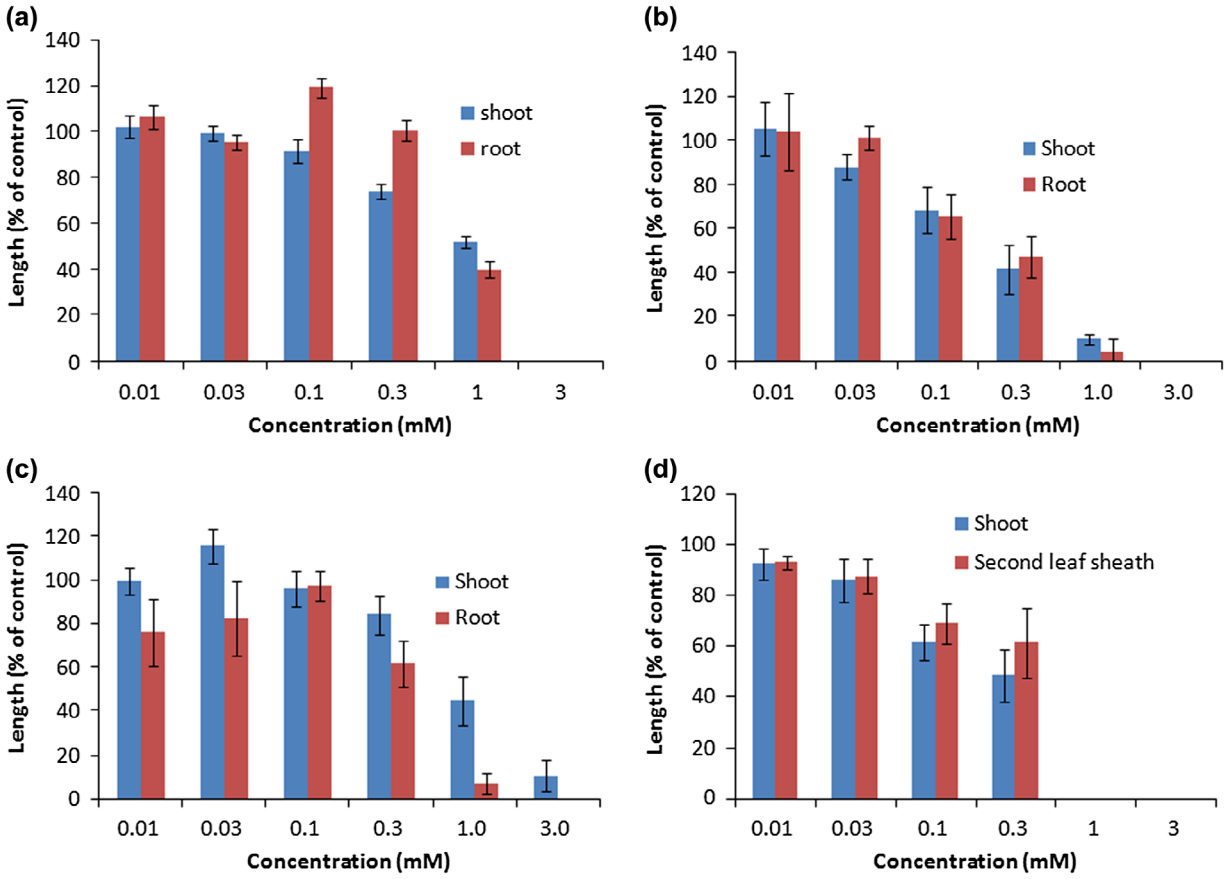


Figure 2. Biological activity of the amide **6** on (a) lettuce, (b) cress, (c) Italian ryegrass, and (d) rice seedlings in 0.01–3.0 mM. The plant growth was completely inhibited at 3 mM of (a) and (b), and 1 and 3 mM of (d). Values are mean ± SE from three independent experiments.

50% inhibition (IC_{50}) of lettuce, cress, Italian ryegrass, and rice hypocotyls growth are listed in Table 1. The IC_{50} values for the four plants range from 0.2 to 1 mM. Among the four species, **6** showed the highest activity for cress for both shoots and roots with IC_{50} values of 0.21 and 0.25 mM, respectively. The IC_{50} values of other D-allose derivatives for cress are summarized in Table 2. The activity of the amide **6** was at least 14 times higher than that of the amine **5**, which demonstrated the same effect of acylation with medium-chain fatty acids at the C-6 on inhibitory activity as the modification of D-allose **1** [12]. Furthermore, comparing the IC_{50} values of the amide **6** with that of the ester **4** having the same decanoyl group, **6** exhibited significantly higher activity than **4**. These results indicate that the replacement of an ester group with an amide group enhances inhibitory activity in the 1,2-dideoxy-D-allose series.

Further, we investigated the mode of action of **6** on plant growth. In plants, four different types of growth inhibitors are known. Of these, prohexadione-calcium, trinexapac-ethyl, daminozide (*N*-dimethylaminosuccinic acid), and 16,17-dihydro-GA_5 block 3β-hydroxylation of GA_{20} to GA_1 in gibberellin (GA) biosynthesis [18,19]. It is also reported that growth suppression by these compounds can be reversed by co-addition of the active gibberellin, GA_3 [20,21]. As reported previously, co-addition of GA_3

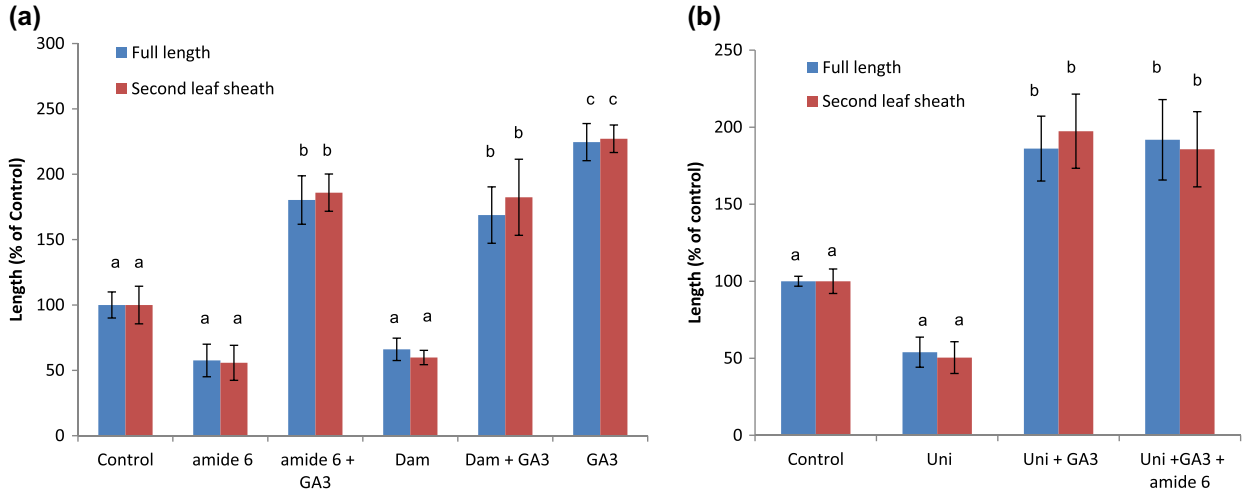
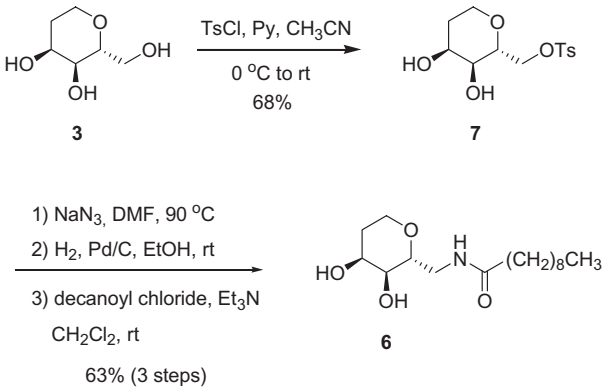


Figure 3. (a) Effect of the amide **6**, daminozide (Dam, 0.25 mM), and GA_3 (1 μM) on rice seedlings and (b) Effect of uniconazole (Uni, 0.001 mM), GA_3 (1 μM) and **6** (0.2 mM) on rice seedlings. Relative lengths (% of control) of shoot and second leaf sheath of rice at 7 days after treatment. Values are mean ± SEM ($n = 14$), and bars with different letters indicate significant difference as determined by Tukey's honestly significant difference comparison ($p < 0.05$).

also restored the growth of rice seedlings treated with D-allose ester [13,14], whereas the monosaccharide form of D-allose did not inhibit GA biosynthesis but inhibited GA-signaling pathway [11]. Therefore, we examined the effect of co-addition of GA_3 on growth inhibition by **6**. Daminozide was used as a positive control. As shown in Figure 3(a), rice growth was restored (183%) by co-addition of GA_3 (1 μM) with **6**. (0.25 mM). Neither withered shoots nor roots were observed. We found a similar tendency with daminozide. These results suggest that the amide **6** as well as the ester **2** inhibits gibberellin biosynthesis to cause growth suppression. Furthermore, we examined whether **6** affects a GA-signaling pathway in rice, using another known growth retardant, uniconazole, which inhibits the conversion of *ent*-karuene to *ent*-karuonic acid in an earlier stage of gibberellin biosynthesis [21] (Figure 3(b)). For this experiment, rice seeds were pre-treated with 0.001 mM of uniconazole at 30 °C in the dark for 24 h [22]. We observed similar growth recovery for both of uniconazole and GA_3-treated groups (210 and 213%, respectively, for shoot length) regardless of presence or absence of **6**. These results suggest that, unlike D-allose, the amide **6** does not inhibit GA signaling.

In summary, **6** was synthesized from 1,2-dideoxy-D-allose (**3**) and its biological activity on plant growth was evaluated. We found that the replacement of the ester group of **4** to an amide group led to enhancement of plant growth-inhibitory activity. Further, co-addition experiments with GA_3 imply that **6** may inhibit GA biosynthesis in rice seedling like daminozide. It is noteworthy that **6** exhibit higher inhibitory activity than D-allose **1** inhibiting GA signaling. Thus, the allose amide **6** could be a new class of plant growth regulators which has higher activity and more safety compared to commercially available daminozide having dimethylhydrazine-structure which is suspected of being carcinogenic in mice [23].



Scheme 1. Synthesis of deoxy-D-allose amide (**6**).

Table 1. IC_{50} values of the amide 6 for four plant species.

	IC_{50} (mM)	
Plant	Shoot	Root
Lettuce	1.0	0.82
Cress	0.21	0.25
Italian ryegrass	0.51	0.33
Rice	0.25	ND

ND = Not Determined.

Table 2. IC_{50} values of **1**, **2**, **4**, **5**, and **6** for cress.

	IC_{50} (mM)	
Compound	Shoot	Root
1	1.5	0.71
2	0.32	0.36
4	>3.0	2.7
5	>3.0	>3.0
6	0.21	0.25

Experimental

D-Allose was provided by the Rare Sugar Research Center at Kagawa University, Japan. ^{1}H and ^{13}C NMR spectra were measured at 600 MHz and 150 MHz, respectively, with a Jeol JNM-ECA600 spectrometer in CD_3OD at room temperature using TMS as an internal standard. Optical rotation data were measured with a Jasco P-1010 optical rotation polarimeter using methanol. High resolution mass spectrums (HRMS) were taken using a Waters Xevo G2-XS-TOF mass spectrometer.

Synthesis of 6-O-(4-toluenesulfonyl)-1,2-dideoxy-D-allose (7)

Tosyl chloride (77 mg) and pyridine (50 µL) were added to 1,2-dideoxy-D-allose (**3**, 19.5 mg, 0.13 mmol) in CH_3CN (0.9 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated and the residue was then purified by column chromatography on silica gel to give a monotosylate **7** [24] as a colorless liquid (26.6 mg, 68%). ^{1}H-NMR (600 MHz, CD_3OD) δ: 1.70 (1H, m), (1H, tdd, *J* = 12.4, 5.3, 2.4 Hz),2.46 (3H, s), 3.34 (1H, dd, *J* = 10.0, 2.9 Hz), 3.56 (1H, ddd, *J* = 10.0, 5.3, 1.4 Hz), 3.66 (1H, ddd, *J* = 12.4, 2.2, 2.4 Hz), 3.68 (1H, m), 3.99 (1H, br dd, *J* = 6.0, 2.9 Hz), 4.09 (1H, dd, *J* = 10.4, 6.0 Hz), 4.26 (1H, dd, *J* = 10.4, 2.0 Hz), 7.78 (1H, d, *J* = 8.3 Hz), 7.43 (2H, d, *J* = 8.3 Hz). ^{13}C-NMR (150 MHz, CD_3OD) δ: 21.75, 34.83, 66.57, 71.83, 72.91, 73.82, 79.48, 129.08, 130.96, 134.39, 146.43. R_f 0.51 (ethyl acetate/hexane = 4:1).

Synthesis of 6-(decanoylamino)-1,2,6-trideoxy-D-allose (6)

NaN_3 (34 mg, 0.52 mmol) was added to a solution of **7** (39.5 mg, 0.13 mmol) in DMF (1.0 mL) at 90 °C, and the reaction mixture was stirred for 48 h. The resulting mixture was extracted with ethyl acetate (5 mL × 3), and the residue was purified by column chromatography on silica gel to give an azide **8** (20.8 mg) as a colorless liquid, R_f 0.42 (ethyl acetate/methanol = 10:1), which was used without further purification. Pd/C (17.5 mg) was added to **8** in ethanol (1.0 mL), and the reaction mixture was stirred under a H_2 atmosphere for 48 h. The resulting mixture was filtered through Celite and concentrated *in vacuo* to give an amine **5** (16.6 mg) as a colorless liquid, R_f 0.05 (ethyl acetate/methanol = 10:1), which was used without further purification. Triethylamine (54 µL, 0.39 mmol) and decanoyl chloride (81 µL, 0.40 mmol) were added to **5** in dichloromethane (1.3 mL) at room temperature, and the reaction mixture was stirred for 5 h. After the usual work-up, the residue was purified by column chromatography on silica gel to give the amide (**6**) as a colorless liquid (24.6 mg, 63% in 3 steps). ^{1}H-NMR (600 MHz, CD_3OD) δ: 0.88 (3H, t, *J* = 6.9 Hz), 1.29 (12H, m), 1.58 (2H, br t, *J* = 6.9 Hz), 1.72 (1H, m), 1.80 (1H, tdd, *J* = 14.4, 5.5, 2.8 Hz), 2.19 (2H, t, *J* = 7.2 Hz), 3.22 (1H, dd, *J* = 9.6, 2.8 Hz), 3.27 (1H, dd, *J* = 14.4, 6.8 Hz), 3.52 (1H, dd, *J* = 14.4, 2.0 Hz,), 3.56 (1H, ddd, *J* = 9.6, 6.8, 2.8 Hz), 3.62 (1H, dd, *J* = 11.0, 5.5 Hz), 3.71 (1H, td, *J* = 12.4, 2.8 Hz), 4.02 (1H, br d, *J* = 2.8 Hz). ^{13}C-NMR (150 MHz, CD_3OD) δ: 14.42, 23.70, 27.07, 30.26, 30.39, 30.45, 30.62, 33.03, 33.73, 36.95, 42.36, 62.71, 68.00, 70.81, 75.87, 176.95. $[\alpha]^{22}_{D}$ + 24.2 (*c* 0.480, MeOH). R_f 0.45 (ethyl acetate/methanol = 30:1). HR-ESI-TOF-MS *m/z* $(M + Na)^+$: calculated for $C_{16}H_{31}NO_4Na$, 324.2151; found, 324.2150.

Biological assay

The biological activity of amide (**6**) was tested on four different plant species: lettuce (*Lectuca sativa*), cress (*Lepidium sativum*), Italian ryegrass (*Lolium multiflorum*), and rice (*Oryza sativa* L. cv. Nihonbare) seedlings. Statistical tests were carried out using R (version 3.3.3) statistical software.

Bioassay on lettuce, cress, and Italian ryegrass: The amide **6** was dissolved in a small volume of methanol, which was added to a sheet of filter paper (Toyo No. 2) in a 3.5 cm Petri dish and 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. Ten sets of test plants were arranged on the filter paper and grown in the dark at 25 °C. The control groups were treated only with a solution of Tween 20. The lengths of the hypocotyls or shoots and roots of the lettuce, cress, and Italian ryegrass seedlings were measured after 48 h and the percentage of shoot length and root length were calculated with reference to the shoot and root lengths of the seedlings in the control groups.

Bioassay on rice seedlings: Following Ref. [22], rice seeds were sterilized with ethanol for 5 min and then washed with water. The seeds were then sterilized for 30 min with 1% sodium hypochlorite and washed again with water. The sterilized seeds were soaked in water for 2 d at 30 °C under fluorescent light. Seven germinated seeds were transplanted into tubes containing a test solution of 0.05% Tween 20 (2 mL each). After incubating for 7 d under light, the lengths of shoot and the second leaf sheath of each rice seedling were measured and the growth ratios against control were calculated.

Author contributions

M.T.I.C. and Y.K. designed the synthetic route and wrote the manuscript with the aid of R.C.Y. M.T.I.C. and H.A. conducted the synthetic experiment and bioassay.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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