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Design and synthesis of conformationally constrained analogues of *cis*-cinnamic acid and evaluation of their plant growth inhibitory activity

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ABSTRACT

1-*O*-*cis*-Cinnamoyl-β-D-glucopyranose is known to be one of the most potent allelochemical candidates and was isolated from *Spiraea thunbergii Sieb* by Hiradate et al. (2004), who suggested that it derived its strong inhibitory activity from *cis*-cinnamic acid, which is crucial for phytotoxicity. In this study, key structural features and substituent effects of *cis*-cinnamic acid (*cis*-CA) on lettuce root growth inhibition was investigated. These structure–activity relationship studies indicated the importance of the spatial relationship of the aromatic ring and carboxylic acid moieties. In this context, conformationally constrained *cis*-CA analogues, in which the aromatic ring and *cis*-olefin were connected by a carbon bridge, were designed, synthesized, and evaluated as plant growth inhibitors. The results of the present study demonstrated that the inhibitory activities of the five-membered and six-membered bridged compounds were enhanced, up to 0.27 μM, and were ten times higher than *cis*-CA, while the potency of the other compounds was reduced.

1. Introduction

1-*O*-*cis*-Cinnamoyl-β-D-glucopyranose (**1**) (Figure 1), isolated from *Spiraea thunbergii Sieb* (Hiradate et al., 2004) as a potent allelochemical candidate, has been identified in 56 species of woody plants grown in Japan by growth-inhibitory activity tests on root elongation in germinated seedlings of lettuce (*Lactuca sativa L.*) (Morita et al., 2001). The part of the *cis*-cinnamic acid (*cis*-CA) structure (*cis*-**2**) considered essential for bioactivity is the aglycone of **1**, because *cis*-CA inhibited lettuce root growth as effectively as **1**, whereas inhibition of growth by *trans*-cinnamic acid (*trans*-CA) (*trans*-**2**) was markedly less than that by the *cis*-isomer (Hiradate et al., 2005). The *trans*-**2** isomer has generally been considered to be physiologically inactive and is also a weak antagonist of auxin, a plant hormone that regulates growth in the roots and stems (Koepfli et al., 1938; van Overbeek et al., 1951; Ferro et al., 2010). On the other hand, the *cis*-**2** isomer was shown to inhibit the root growth of *Avena sativa*, *Triticum aestivum*, and *Arabidopsis thaliana*, and also induced epinastic curvature in *Solanum lycopersicum* seedlings (Koepfli et al., 1938; van Overbeek et al., 1951; Yang et al., 1999; Wong et al., 2005). Therefore, it is widely considered to be an auxin agonist. Although mechanistic studies based on molecular biology have been reported (Chen et al., 2005; Guo et al., 2011), the molecular mechanisms of these activities have not yet been elucidated. In this study, herein, the glycosyl ester **1** (Matsuo et al., 2011) was synthesized, and the structure–activity relationship of *cis*-**2** subsequently investigated. It was found that the structural features essential for bioactivity were a *cis*-configuration of the alkene or cyclopropane, a carboxylic acid or its esters, and a planar ring including a phenyl group (Abe et al., 2012). Furthermore, the substituent effect on the aromatic ring of *cis*-**2** was also established, in which *meta*-substituents, especially sterically unhindered hydrophobic ones, were not critical for potency, whereas several *cis*-**2** analogues **3**–**5**, such as *m*-iodo, *m*-methoxy, or *m*-trifluoromethyl substituted ones, were more potent than that of *cis*-CA (Figure 2) (Nishikawa et al., 2013).

During the course of examining the structure–activity relationship (SAR) of *cis*-**2**, the planar ring and the carboxylic acid moiety were found to be essential for potency. However, these moieties could not be in the same plane, due to steric interference between the *ortho*-substituent and the carboxylate. Therefore, although the three-dimensional arrangement between the aromatic ring and carboxylate was expected to be crucial for bioactivity, the most effective three-dimensional arrangement could not be determined readily (Figure 3) because the C–C bond connecting the aromatic

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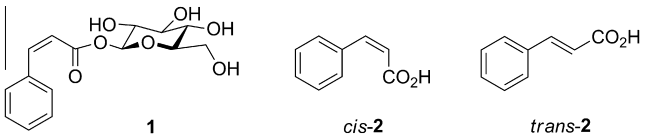


Fig. 1. Structures of 1-*O*-*cis*-cinnamoyl-β-D-glucopyranose (**1**) and *cis*- and *trans*-CA (*cis*- and *trans*-**2**).

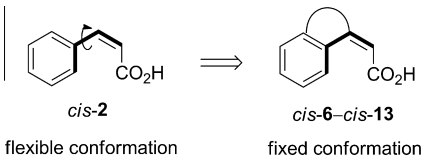


Fig. 3. Design of conformationally constrained analogues *cis*-**6** to *cis*-**13**.

ring and olefin could rotate freely. Therefore, conformationally constrained *cis*-**2** analogues may provide useful information on the conformation critical for potency. Herein is described the synthesis and evaluation of various kinds of conformationally constrained bridged *cis*-**2** analogues **6**–**13**.

2. Results and discussion

2.1. Design and synthesis of cis-cinnamic acid analogues

Bridged *cis*/*trans*-CA analogues **6**–**9** were designed to understand the spatial relevance between the aromatic ring and carboxylic acid, because these were expected to be conformationally constrained by bridging of the aromatic ring and the olefin. Analogues *cis*-**6**–*cis*-**9** were thus synthesized *via* olefination with the Horner–Wadsworth–Emmons reaction of the corresponding ketones **14**–**17**, followed by separation and hydrolysis of the *cis*/*trans*-isomers **18**–**21** (Scheme 1). Benzocyclobutenone (**14**) was prepared as shown in Scheme 2 (Aidhen and Ahuja, 1992). Whereas the *endo-isomer* **7** was also obtained as a by-product of olefination of the corresponding ketone **15** and its subsequent hydrolysis.

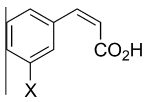
The *non*-bridged analogue *cis*-**24**, which had the same number of carbons as that in **8**, was also synthesized from 2-methylbenzaldehyde (**25**) by the following transformations (Scheme 3): (1) addition of EtMgI to the aldehyde group of **25**, (2) Jones oxidation of secondary alcohol **26**, (3) Peterson olefination of ketone **27** (Novák et al., 1992), (4) separation of *cis*- and *trans*-**28**, and (5) hydrolysis.

From results of the previous study, the *cis*-alkenyl group could be replaced by a *cis*-cyclopropyl group without significant loss in activity (Abe et al., 2012). The cyclopropyl analogues *cis*- and *trans*-**10** of the bridged CA were also prepared by Rh-catalyzed cyclopropanation (Cordi et al., 2001) of 1-methylene-1,2,3,4-tetrahydronaphthalene (**30**), followed by hydrolysis (Scheme 4). The stereochemistries of *cis*- and *trans*-**10** were then determined by NOE experiments.

Also designed and synthesized were the "doubly bridged" compounds *cis*- and *trans*-**11**, as shown in Scheme 5 (Cordi et al., 2001). These compounds were bridged by a dotted bond indicated by an arrow. The stereochemistries of *cis*- and *trans*-**11** were also determined by NOE experiments.

The *meta*-iodinated bridged CA analogues **12** and **13** were prepared as follows (Scheme 6): phenylbutyric acid **34** underwent iodination (Plati et al., 1943), and the resulting mixture of isomers **35** and **36** was subjected to a Friedel–Crafts reaction to afford a mixture of 7-iodo and 5-iodotetralones (**37** and **38**) (Cui et al., 2003). After separation of these products (**37** and **38**) with column chromatography, each iodotetralone was olefinated *via* Peterson olefination (Novák et al., 1992) to afford a mixture of *cis*- and *trans*-olefins along with an *endo*-olefin. After these products (**39** and **40**) were separated by column chromatography, the pure esters were individually hydrolyzed to provide the corresponding carboxylic acids **12** and **13**, respectively.

The *endo*-alkenyl bridged analogue **16** was also synthesized *via* a Reformatsky reaction of **29** with the bromoacetic acid ethyl ester (Johnson and Glenn, 1949; Miyashi et al., 1986; Chavan et al., 1992), followed by the dehydration and hydrolysis of ethyl ester **16** (Scheme 7).



X = I (*cis*-**3**)
X = OMe (*cis*-**4**)
X = CF_3 (*cis*-**5**)

Fig. 2. More potent *cis*-CA analogues **3**–**5**.

2.2. Bioassay and discussion

The growth inhibitory activity of the *cis*-CA analogues against the root-growth of lettuce (*Lactuca sativa* cv.) was measured as described previously (Hiradate et al., 2005). EC_{50} values, which indicate the effective concentration required to induce a half-maximum effect, are shown in Tables 1–3.

Bridged CA analogues **6**–**13** were conformationally constrained compounds that were expected to provide useful information on the conformation critical for the inhibitory activity of *cis*-CA (Table 1). The dihedral angle (°) between the aromatic plane and alkenyl plane in particular may be fixed by bridging (Fig. 4). Conformation searches and dihedral angles of bridged analogues were calculated with MMFF using the CONFLEX module to locate the global minimum (Goto and Osawa, 1993). As shown in Table 1, *cis*-**7** and *cis*-**8** were found to be markedly stronger inhibitors than *cis*-**2** itself, with *cis*-**7** in particular showing bioactivity that was ten times higher than that of *cis*-**2**. The four-membered ring analogue *cis*-**6**, which had a 0 dihedral angle, also exhibited potent activity, while that of the seven-membered ring analogue *cis*-**9**, the dihedral angle of which was 66°, was markedly reduced. These calculated dihedral angles were 66°, 34°, 18°, and 0° as the bridged ring size decreased from 7 to 4, which indicated that the optimal angle may be located between 0° and 18°. The potencies of *trans*-cinnamic acid analogues **6**–**9**, bridged by four, six, and seven-membered rings, were also reduced. Since *non*-bridged analogue *cis*-**24**, which had the same carbon number, did not exhibit inhibitory activity, the high potency of the six-membered bridged analogue may only be slightly related to the hydrophobic factor.

It was previously shown in a SAR study on *cis*-CA (Abe et al., 2012) that the *cis*-alkene could be replaced with the *cis*-cyclopropane skeleton, indicating the importance of the spacial relationship between the aromatic ring and carboxylate. Therefore, the alkene in the bridged analogues could be replaced by cyclopropane in a similar manner to that described above. Based on this hypothesis, two kinds of bridged *cis*- and *trans*-cyclopropane analogues **10** and **11**, as shown in Table 2, were designed and their growth inhibitory activities were examined. However, these compounds, including either the *cis*- or *trans*-form, were not potent, which may have been due to an unsuitable spatial relationship.

Based on the aforementioned substituent effects, both *meta*-iodo-substituted and six-membered bridged analogues improved