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Substituent effects of *cis*-cinnamic acid analogues as plant growh inhibitors

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ABSTRACT

1-*O*-*cis*-Cinnamoyl-β-D-glucopyranose is one of the most potent allelochemicals that has been isolated from *Spiraea thunbergii Sieb* by Hiradate et al. It derives its strong inhibitory activity from *cis*-cinnamic acid (*cis*-CA), which is crucial for phytotoxicity. By preparing and assaying a series of *cis*-CA analogues, it was previously found that the key features of *cis*-CA for lettuce root growth inhibition are a phenyl ring, *cis*-configuration of the alkene moiety, and carboxylic acid. On the basis of a structure–activity relationship study, the substituent effects on the aromatic ring of *cis*-CA were examined by systematic synthesis and the lettuce root growth inhibition assay of a series of *cis*-CA analogues having substituents on the aromatic ring. While *ortho*- and *para*-substituted analogues exhibited low potency in most cases, *meta*-substitution was not critical for potency, and analogues having a hydrophobic and sterically small substituent were more likely to be potent. Finally, several *cis*-CA analogues were found to be more potent root growth inhibitors than *cis*-CA.

1. Introduction

1-*O*-*cis*-Cinnamoyl-β-D-glucopyranose (**1**) (Fig. 1) was isolated from *Spiraea thunbergii* (Hiradate et al., 2004) as a potent allelochemical and was found in 56 species of woody plants grown in Japan by a bioassay of growth-inhibitory activity on root elongation of the germinated seedlings of lettuce (*Lactuca sativa L.*) (Morita et al., 2001). An essential structure for the bioactivity of *cis*-cinnamic acid (*cis*-CA) (*cis*-**2**) is thought to be the aglycone of the glycosyl ester **1** because *cis*-CA inhibited lettuce root growth as effectively as **1**, while *trans*-cinnamic acid (*trans*-CA) (*trans*-**2**) inhibited growth much less than the *cis*-isomer (Hiradate et al., 2005). Generally, the *trans*-**2** isomer is considered to be physiologically inactive and a weak antagonist of auxin, a plant hormone that regulates growth in the roots and stem (Koepfli et al., 1938; van Overbeek et al., 1951; Ferro et al., 2010). On the other hand, since the *cis*-**2** isomer inhibits the root growth of *Avena sativa*, *Triticum aestivum*, and *Arabidopsis thaliana*, and also induces epinastic curvature in *Solanum lycopersicum* seedlings (Koepfli et al., 1938; van Overbeek et al., 1951; Yang et al., 1999; Wong et al., 2005), 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 described. Nevertheless the first chemical synthesis of the glycosyl ester **1** was previously achieved, which confirmed its proposed structure and determined its optical rotation (Matsuo et al., 2011). A structure activity relationship study of *cis*-**2** was reported, this establishing the which revealed that essential structural features for its bioactivity being the *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). If additional units can be introduced in the *cis*-**2** isomer without a loss in activity, stronger bioactive compounds or functional analogues such as molecular probes for mechanistic investigations can be developed. Based on our previous reports, additional units on the *cis*-alkene moiety caused a significant loss in bioactivity, while the introduction of substituents on the aromatic ring core may be possible without a loss in bioactivity.

Describe herein are the synthesis and evaluation of the *cis*-CA analogues **3**–**58** having substituent(s) on the aromatic ring, the purpose of which was to identify substituent effects to clarify structure–activity relationship. These will be useful in the preparation of chemical probes (Fig. 2) as well as more active compounds,

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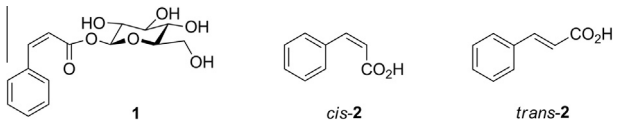


Fig. 1. Structures of 1-*O*-*cis*-cinnamoyl-β-D-glucopyranose (**1**) and *cis*- and *trans*-cinnamic acids (*cis*- and *trans*-**2**).

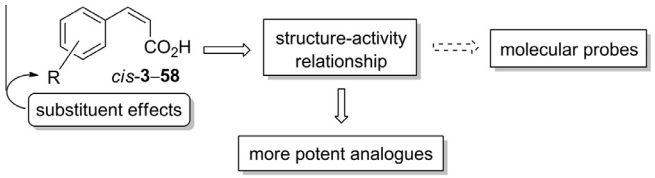


Fig. 2. An overview of this research.

leading to novel agrochemicals. To assay the bioactivity of the *cis*-CA analogues, inhibitory activity of these compounds against lettuce root growth were tested as described previously (Hiradate et al., 2005; Abe et al., 2012).

2. Results and discussion

2.1. Design and synthesis of cis-cinnamic acid analogues

A variety of *cis*-CA analogues bearing a substituent on the phenyl group at the *ortho*, *meta*, and *para* positions were systematically designed and synthesized. As a substituent, one of the alkyl, aromatic, alkoxy, halogeno, nitro, and trifluoromethyl groups were selected. Furthermore, a few multi-substituted analogues and polycyclic aromatic analogues including heteroaromatics, in place of the phenyl group, were also synthesized.

Their syntheses were mainly performed *via cis*-selective olefination of the corresponding aldehydes **59–114** with the modified Horner–Wadsworth–Emmons reaction (Ando, 1997; Ando et al., 2000), followed by hydrolysis of the ester of *cis*-olefins **115–170**, as depicted in Scheme 1.

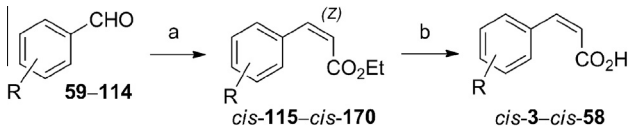
The commercially unavailable starting aldehydes **63**, **68**, **74–76**, **100**, **108** and **109** were prepared as shown in Scheme 2 (Wang et al., 2009; Li et al., 1998; Zhou and Zhao, 2009; Saitoh et al., 2009; Tasker et al., 1997).

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 the Tables.

2.2.1. Effects of alkylation or alkoxylation of the aromatic ring

The *ortho*-methylated *cis*-CA analogue *cis*-**3** showed a more pronounced inhibitory activity than *cis*-CA **2** (Table 1, entry 2). However, the *meta*-methylated analogue *cis*-**4** and *para*-methylated *cis*-**5** were slightly less active. The analogues *cis*-**6**–*cis*-**13** having larger alkyl and aryl substituents, such as ethyl and phenyl groups, were markedly less active (entries 5–12). *Meta*-alkylation may have less of an effect on bioactivity than *ortho*- and *para*-alkylation, and sterically hindered substituents were more likely to reduce the activity. While the *meta*-methoxy analogue *cis*-**15** was more potent, the *para*-methoxy and *meta*-ethoxy analogues *cis*-**16** and *cis*-**17** were slightly less active and the *ortho*-methoxy one *cis*-**14** was much less active (entries 13–16). The presence of a larger alkoxyl group at the *meta* position led to inactive compounds (entries 17–19).



Scheme 1. Synthesis of the substituted *cis*-CA analogues **3**–**58**: (a) ethyl 2-[bis(2-isopropylphenoxy)phosphoryl]acetate, Triton B, THF, −78 °C, 45–99%, (b) 10% NaOH aq., EtOH, rt, 53–99%.

2.2.2. Effects of halogenation of the aromatic ring

Halogenated analogues (Table 1, entries 20–31) showed a similar level of activity to *cis*-**2**, although *cis*-**21** and *cis*-**32** were slightly less active. It was found that *meta*-substituted ones were preferred, providing compounds with EC_{50} values in the range of 1.0–2.5 μM, in which the order of activity was F < Cl < Br < I (entries 20–31). As a result, the *meta*-iodo analogue *cis*-**31** was found to be more active than *cis*-CA.

2.2.3. Effects of trifluoromethylation and nitration of the aromatic ring

While *ortho*-nitrated *cis*-**33** and *para*-nitrated *cis*-**35** were inactive, *meta*-nitrated *cis*-**34** was slightly less active than *cis*-CA **2** (Table 1, entries 32–34). It is noteworthy that *meta*-trifluoromethylated *cis*-**37** improved the activity, while *ortho*-substituted *cis*-**36** and *para*-substituted *cis*-**38** reduced it (entries 35–37). These results indicated that strong electron-withdrawing groups may markedly affect the activity.

In total, for mono-substituted *cis*-CAs, the *meta*-substitution of sterically small alkyl, alkoxy, and halogeno groups was more likely to maintain strong bioactivity, in which hydrophobic substituents were somewhat preferred. Steric factors were more important than inductive and electronic effects. On the other hand, inhibitory activity was very sensitive to *para*- and *ortho*-substitutions, and sterically bulky and strong electron withdrawing substituents showing a low Hammett σ value at these positions significantly reduced the activity.

2.2.4. Effects of disubstitution of the aromatic ring

Disubstituted analogues were subjected to the inhibitory activity test (Table 1, entries 38–44). Additional *para*-methylated *cis*-**3** analogue *cis*-**39** and *para*-methoxylated *cis*-**3** analogue *cis*-**40** exhibited similar activity to *cis*-**3** (entries 38 and 39). However, the polymethoxylated *cis*-**2** analogues (*cis*-**41** and *cis*-**42**) significantly diminished the activity (entries 40 and 41). The *o*,*p*-dichloro analogue (*cis*-**43**) slightly improved activity, maybe due to higher hydrophobicity (entry 42).

If functional moieties were introduced in *cis*-**2** without a loss in inhibitory activity, a hydrophobic and relatively small substituent at the *meta*-position was preferred, regardless of its electronic properties such as the Hammet σ value.

2.2.5. Effects of the ring structure

The fused aromatic ring analogues *cis*-**46**-*cis*-**58** were examined as shown in Table 2. The 1-naphthyl, 9-phenanthryl-, 9-anthranyl-, and dibenzofuranyl analogues *cis*-**46**–*cis*-**49** markedly reduced the activity (entries 1–4). In contrast, the 2-naphthyl-, 5-benzofuranyl-, 5-(2,3-dihydro)benzofuranyl, 6-benzofuranyl, and benzothiophene-5-yl *cis*-**2** analogues *cis*-**50**–*cis*-**54** retained more potent inhibitory activity than *cis*-**2** (entries 5–9). The 5-benzofuranyl analogue *cis*-**52** also improved the activity. The potency of the 1-naphthyl analogue *cis*-**46** was markedly lower than that of the 2-naphthyl analogue *cis*-**50**. These results indicate that the "*ortho*, *meta*-disubstituted" type *cis*-CA analogues *cis*-**46**–*cis*-**49** were