Functional Structure/Activity Relationships

Syntheses and Phytotoxicity of All Stereoisomers of 6-(2-Hydroxy-6-phenylhex-1-yl)-5,6-dihydro-2H-pyran-2-one and Determination of Effect of #,#-Unsaturated Carbonyl Structure and Hydroxy Group Bonding to Chiral Carbon

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Syntheses and Phytotoxicity of All Stereoisomers of 6-(2-Hydroxy-6-phenylhex-1-yl)-5,6-dihydro-2*H*-pyran-2-one and Determination of Effect of α,β-Unsaturated Carbonyl Structure and Hydroxy Group Bonding to Chiral Carbon

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ABSTRACT

All four stereoisomers of naturally occurring 6-(2-hydroxy-6-phenylhex-1-yl)-5,6-dihydro-2*H*-pyran-2-one (**1**) were synthesized by employing yeast-reduction products with high optical purity (95%ee–more than 99% ee), and then their phytotoxicities against lettuce and Italian ryegrass were evaluated. In the Italian ryegrass seedlings test, (6*S*,2′*R*)-**1** showed the most potent and stereospecific activity against the shoots (IC_{50} = 260 μM) and roots (IC_{50} = 43.2 μM) with a significant difference from other stereoisomers. The highest seed germination inhibitory activity against Italian ryegrass seed was also observed in (6*S*,2′*R*)-**1**, showing 53% germination ratio from control at 1000 μM. This advantageous (6*S*,2'*R*)-stereochemistry was employed in the syntheses of α,β-dihydro, 2′-dehydroxy, and 2′-methoxy derivatives **13-15**. By the test using these derivatives, the importance of α,β-unsaturated double bond and a hydroxy group bonding to a chiral center on the 6-alkyl chain of 5,6-dihydro-α-pyrone for phytotoxicity was determined. In the test against lettuce, 6*S*-configuration and (6*S*,2'*S*)-configuration were necessary for growth inhibition (IC_{50} = ca. 60 μM) and germination inhibition (63% germination ratio at 1000 μM), respectively.

Key words: 5,6-dihydro-α-pyrone; allelopathy; plant growth regulation; germination inhibitory activity; phytotoxicity

INTRODUCTION

5,6-Dihydro-α-pyrone is a secondary metabolite biosynthesized by plants. Antitumor activity and cytotoxicity,[1-12] antiproliferative,[13-14] antimicrobial,[15-17] larvicidal,[18] carbonic anhydrase inhibitor,[19] spasmolitic,[20] antiprotozoal,[21,22] antifungal,[23,24] and anti-inflammatory[11] activity have been reported. The effect of stereochemistry and structure-activity relationship have not been discussed in these experiments. With regarding to the plant growth regulation, the phytotoxicities of **2-5** have been reported (Figure 1).[25-28] We focused on the substituent of the alkyl chain at 6-position of the 5,6-dihydro-α-pyrone structure in this project on the phytotoxicity. Although the polyacetate compound, (+)-boronolide (**5**), showed weak activity in our previous study,[28] the phenyl group and alkenyl substituent seem to be tolerable for the phytotoxicity. The purpose of this project is to clarify the effect of the stereochemistry and hydroxy group in the 6-alkyl group on the phytotoxicity. To promote this project, the compound **1**,[23] which was isolated from *Ravensara crassifolia*, was selected as a target compound because of the presence of a hydroxy group bonding to a chiral carbon on the 6-alkyl chain. The stereochemistry of the natural compound **1** was revised to (6*S*,2'*S*)-configuration[29] after the first report. After syntheses of all four stereoisomers of **1**, the growth inhibitory and germination inhibitory activities against lettuce and Italian ryegrass were examined. The stereospecific phytotoxicity of 5,6-dihydro-α-pyrone is first described in this article.

The syntheses of (6*R*,2'*S*)-**1**[30], (6*S*,2'*R*)-**1**,[31-38] and both (6*R*,2'*R*)-**1** and (6*S*,2'*R*)-**1**[4] have been achieved. However, the synthetic report on (6*S*,2'*S*)-**1** can not be

found. We selected (1'*R*,2'*S*)-**6** and (1'*S*,2'*S*)-**6**,[17] which could be prepared from one racemic compound by yeast-reduction,[39] as the staring materials for the syntheses of all four stereoisomers. Furthermore, the importance of the α,β-unsaturated structure and the hydroxy group bonding to a chiral center on the 6-alkyl chain for the phytotoxicity was also determined by the preparations of the derivatives **13**-**15** and their biological tests against plants.

MATERIALS AND METHODS

All test compounds were synthesized by the methods described in the supporting information.

(*R*)-6-[(*R*)-2-Hydroxy-6-phenylhex-1-yl]-5,6-dihydro-2*H*-pyran-2-one ((6*R*,2'*R*)-1). colorless oil; $[\alpha]^{20}_{D}$ +56 (*c* 0.20, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3434, 2934, 1714, 1256, 1050 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.16 (m, 5H), 6.89 (ddd, *J* = 9.5, 4.3, 4.3 Hz, 1H), 6.01 (ddd, *J* = 9.7, 1.8, 1.8 Hz, 1H), 4.74 (m, 1H), 4.00 (m, 1H), 2.62 (t, *J* = 7.6 Hz, 2H), 2.34 (m, 2H), 2.13 (br, 1H), 1.87 (ddd, *J* = 14.5, 9.8, 2.2 Hz, 1H), 1.69–1.59 (m, 3H), 1.53–1.46 (m, 3H), 1.37 (m, 1H), 1.49 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 145.5, 142.4, 128.3, 128.2, 125.6, 121.2, 75.0, 66.9, 42.2, 37.8, 35.8, 31.4, 29.9, 25.1; MS (FAB) *m/z* 275 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{17}H_{23}O_3$ 275.1647 $(M+H)^+$, found 275.1650; >99% ee (AD-H, 5% *iso*-PrOH in hexane, 1.0 mL/min, detected at 254 nm, t_R30 min).

(6*R*,2'*S*)-1. colorless oil; $[\alpha]^{20}_{D}$ +84 (*c* 0.47, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3700, 3018, 1717, 1559 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.26 (m, 2H), 7.19-7.16 (m,

3H), 6.89 (m, 1H), 6.02 (ddd, J = 9.8, 1.9, 1.9 Hz, 1H), 4.65 (m, 1H), 3.85 (m, 1H), 2.63 (t, J = 7.7 Hz, 2H), 2.40 (m, 2H), 2.05 (br, 1H), 1.97 (ddd, J = 14.4, 8.6, 7.5 Hz, 1H), 1.79 (ddd, J = 14.4, 5.6, 3.6 Hz, 1H), 1.66 (m, 2H), 1.57–1.47 (m, 3H), 1.40 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 145.2, 142.4, 128.4, 128.3, 125.7, 121.2, 77.0, 69.1, 41.9, 37.5, 35.8, 31.4, 29.5, 25.1; MS (FAB) *m/z* 275 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{17}H_{23}O_3$ 275.1647 $(M+H)^+$, found 275.1647; >99% ee (AD-H, 5% *iso*-PrOH in hexane, 1.0 mL/min, detected at 254 nm, t_R41 min).

(6*S*,2'*S*)-1. colorless oil; $[\alpha]^{20}_D$ −56 (*c* 0.57, $CHCl_3$); 95% ee (AD-H, 5% *iso*-PrOH in hexane, 1.0 mL/min, detected at 254 nm, t_R22 min).

(6*S*,2'*R*)-1. colorless oil; $[\alpha]^{20}_D$ −84 (*c* 0.19, $CHCl_3$); 95% ee (AD-H, 5% *iso*-PrOH in hexane, 1.0 mL/min, detected at 254 nm, t_R34 min).

(*S*)-6-[(*R*)-2-Hydroxy-6-phenylhex-1-yl]-3,4,5-6-tetrahydro-2*H*-pyran-2-one (13). colorless oil; $[\alpha]^{20}_D$ +28 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.25 (m, 2H), 7.17–7.15 (m, 3H), 4.50 (m, 1H), 3.80 (m, 1H), 2.64–2.54 (overlapped, 1H), 2.62 (t, J = 7.7 Hz, 2H), 2.43 (ddd, J = 17.7, 8.7, 7.2 Hz, 1H), 2.40 (br, 1H), 2.00–1.79 (m, 4H), 1.72–1.59 (m, 3H), 1.58–1.44 (m, 4H), 1.39 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 142.4, 128.3, 128.2, 125.6, 79.6, 69.0, 43.0, 37.4, 35.8, 31.3, 29.3, 27.8, 25.0, 18.3; MS (FAB) *m/z* 277 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{17}H_{25}O_3$ 277.1796 $(M+H)^+$, found 277.9796.

(*R*)-6-(6-Phenylhex-1-yl)-5,6-dihydro-2*H*-pyran-2-one (14). colorless oil; $[\alpha]^{20}_D$ −121 (*c* 0.21, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.25 (m, 2H), 7.18–7.15 (m, 3H), 6.86 (ddd, J = 9.7, 5.0, 3.4 Hz, 1H), 6.01 (ddd, J = 9.9, 1.8, 1.8 Hz, 1H),

4.40 (m, 1H), 2.60 (t, J = 7.7 Hz, 2H), 2.33–2.29 (m, 2H), 1.79 (m, 1H), 1.67–1.59 (m, 3H), 1.52 (m, 1H), 1.43–1.34 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 145.0, 142.6, 128.3, 128.2, 125.6, 121.4, 77.9, 35.9, 34.8, 31.3, 29.4, 29.2, 29.1, 24.7; MS (FAB) *m/z* 259 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{17}H_{23}O_2$ 259.1697 $(M+H)^+$, found 259.1698.

(*S*)-6-[(*R*)-2-Methoxy-6-phenylhex-1-yl]-5,6-dihydro-2*H*-pyran-2-one (15). Colorless oil; $[\alpha]^{20}_D$ −93 (*c* 0.24, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.25 (m, 2H), 7.19–7.16 (m, 3H), 6.88 (m, 1H), 6.03 (ddd, J = 9.7, 1.8, 1.8 Hz, 1H), 4.55 (m, 1H), 3.42 (dddd, J = 5.9, 5.9, 5.9, 5.9 Hz, 1H), 3.30 (s, 3H), 2.62 (t, J = 7.7 Hz, 2H), 2.38 (m, 2H), 2.07 (ddd, J = 14.5, 6.7, 6.7 Hz, 1H), 1.76 (ddd, J = 14.3, 5.9, 5.9 Hz, 1H), 1.69–1.53 (m, 4H), 1.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 145.1, 142.4, 128.3, 128.4, 125.7, 121.4, 76.7, 75.3, 56.1, 38.3, 35.9, 32.9, 31.6, 29.5, 24.6. MS (FAB) *m/z* 289 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{18}H_{25}O_3$ 289.1804 $(M+H)^+$, found 289.1804.

Plant growth regulation activity and seed germination inhibitory activity. The plant growth regulation activity of our synthesized all stereoisomers of **1** and **13-15** were evaluated using lettuce (*Lactuca sativa* L. Green-wave (Takii Seed Co. Ltd., Kyoto, Japan)) and Italian ryegrass (*Lolium multiflorum* Lam. Wase-fudo (Takii Seed Co. Ltd.)) seedling. A sheet of filter paper (diameter = 90 mm) was put in a 90 mm Petri dish and wetted with 500 μL of test sample solution dissolved in acetone at concentrations from 6.0 to 0.15 mM or from 6.0×10^{-5} to 6.0×10^{-9} M. After the filter paper had dried, 3 mL of water was poured into the dish to adjust the final concentration from 1000 to 25 μM or from 10^{-5} to 10^{-9} M. Thirty seeds of each plant were placed on

111 the filter paper, and the Petri dishes were sealed with parafilm. The Petri dishes were
112 then incubated in the dark at 20°C. The lengths of roots and shoots were measured
113 after 3 days for lettuce seedlings and after 5 days for Italian ryegrass seedlings by using
114 an ordinary ruler. The shoot and root lengths of the control were ca. 1 and 2 cm for
115 lettuce seedlings and 2 and 3 cm for Italian ryegrass seedlings, respectively.
116 Experiments were performed in triplicate for each sample at 1000 to 25 μM, and
117 conducted in singlicate for each sample at 10^{-5} to 10^{-9} M. In addition, the seed
118 germination inhibitory activity of our synthesized all stereoisomers of **1** and **13-15** were
119 evaluated using Italian ryegrass and lettuce seed. Preparing the Petri dish including
120 thirty seeds at 1000 μM by employing the same method, the petri dish was then
121 incubated in the dark at 20°C. The germinated seeds were counted after 4 days for
122 ryegrass and 3 days for lettuce, and the seed germination ratio of each compound
123 against control were calculated. The seed germination ratio of the control was ca. 80%
124 for ryegrass and 100% for lettuce. Experiments were performed in triplicate for each
125 sample. The data are presented as percentage differences from the control,
126 respectively.

127 **Evaluation of biological activity**. Experiments were performed in triplicate
128 or more for each sample. Statistical analyses were conducted one-way ANOVA
129 followed by Tukey's multiple-comparison test by using PRISM software ver. 5.0
130 (GraphPad software Inc., San Diego, CA, USA), and the values of *p* were considered to
131 be statistically significant. The value of IC_{50} (effective concentration for inducing
132 50% growth inhibitory ratio compared with control) was calculated when the

concentration was 1000 to 25 μM. The data are presented as percentage differences from the control when the concentration was 10^{-5} to 10^{-9} M.

RESULTS AND DISCUSSION

To synthesize the stereoisomers (6*R*,2'*R*)- and (6*R*,2'*S*)-**1**, the synthetic sequence was started from the reported aldehyde (1'*R*,2'*S*)-**6**[17] (Scheme 1). The aldehyde **6** was treated with 4-phenylbutylmagnesium bromide to give alcohols (2*R*,1'*R*,2'*S*)-**7** (40%) and (2*S*,1'*R*,2'*S*)-**7** (38%). These secondary alcohols were converted to the *R*- and *S*-α-methoxy-α-(trifluoromethyl)phenylacetic acid esters, respectively, to determine the absolute configuration at the C2 positions by the modified Mosher method (Figure 2).[40] The $\Delta\delta_{S-R}$ values of their ^{1}H-NMR spectra of (*S*)-(2*R*,1'*R*,2'*S*)-**7** and (*R*)-(2*R*,1'*R*,2'*S*)-**7** were negative at triisopropylsilyl group and the C2' position, and the value was positive at the C6 position. On the other hand, the $\Delta\delta_{S-R}$ values of their ^{1}H-NMR spectra of (*S*)-(2*S*,1'*R*,2'*S*)-**7** and (*R*)-(2*S*,1'*R*,2'*S*)-**7** were positive at triisopropylsilyl group and the C2' position, and the value was negative at the C6 position (Figure 2). After the protections of the hydroxy groups of (2*R*,1'*R*,2'*S*)- and (2*S*,1'*R*,2'*S*)-**7** as 4-methoxybenzyl ethers by using 4-methoxybenzyl bromide and NaH, the desilylations with (*n*-Bu)$_4$NF were performed, respectively. Pyridinium chlorochromate oxidations of the resulting cyclopentanol derivatives (1*S*,2*R*,2'*R*)-**9** and (1*S*,2*R*,2'*S*)-**9** followed by Baeyer–Villiger oxidations by employing *m*-chloroperoxybenzoic acid afforded (6*R*,2'*R*)- and (6*R*,2'*S*)-**11**, respectively (Scheme 1). The introductions of the α,β-unsaturated double bonds to these lactones with

155 $[Ph(SeO)]_2O$ gave (6*R*,2'*R*)- and (6*R*,2'*S*)-**12**, respectively. Finally, the cleavages of
156 each *p*-methoxybenzyl ether by using trifluoroacetic acid gave (6*R*,2'*R*)- and (6*R*,2'*S*)-**1**.
157 The stereoisomers (6*S*,2'*S*)- and (6*S*,2'*R*)-**1** were synthesized by the same synthetic
158 method from (1'*S*,2'*S*)-**6**.[17] The enatiomeric excesses of **1**–**4** were determined as more
159 than 99%ee for (6*R*,2'*R*)- and (6*R*,2'*S*)-**1**. On the other hand, 95%ee was observed for
160 (6*S*,2'*S*)- and (6*S*,2'*R*)-**1**. The success of these syntheses enabled us to clarify the
161 phytotoxicities of stereoisomers of **1**.
162 The plant growth inhibitory activities of the synthesized stereoisomers **1**
163 against lettuce and Italian ryegrass were evaluated (Table 1). Against lettuce, all
164 stereoisomers showed growth inhibitory activities against both the shoots (IC_{50} = 227–
165 491 μM) and roots (IC_{50} = 58.4–95.2 μM). The activities of (6*S*,2'*R*)- and (6*S*,2'*S*)-**1**
166 were higher than (6*R*,2'*R*)-**1** with the significant difference, showing IC_{50} values of
167 200-300 μM against the shoots and around 60 μM against roots. Although the
168 difference of activity levels against the shoots and roots was not wide and less than
169 2-fold, the 6*S*-absolute configuration would be more important than the 2'-absolute
170 configuration for the higher activity against lettuce. Against Italian ryegrass, the
171 growth inhibitory activity was also shown against both the shoots (IC_{50} = 260–741 μM)
172 and roots (IC_{50} = 43.2–131 μM) in all stereoisomers. The phytotoxicity of (6*S*,2'*R*)-**1**
173 was most potent against the shoots (IC_{50} = 260 μM) and roots (IC_{50} = 43.2 μM),
174 showing 2-3-fold more potent than the other stereoisomers with the significant
175 difference. The IC_{50} values against Italian ryegrass was broader than against lettuce.
176 The chiral centers at both the 6- and 2'-positions would be important. The tests at

177 10^{-5}–10^{-9} M showed neither the growth inhibition nor promotion against both lettuce
178 and Italian ryegrass.

179 To clarify an another phytotoxicities of the test compounds, the seed
180 germination inhibitory activities of stereoisomers of **1** against lettuce and Italian
181 ryegrass seeds were examined at 1000 μM (Figure 3). Against lettuce, only (6*S*,2'*S*)-**1**
182 showed lower germination ratio (63%) with the significant difference from the other
183 stereoisomers. The same stereoisomer (6*S*,2'*S*)-**1** was effective for the growth
184 inhibition of lettuce. On the other hand, all stereoisomers exhibited the germination
185 inhibitory activity (germination ratio of 53%-70%) with the significant difference from
186 the control against Italian ryegrass. The (6*S*,2'*R*)-**1** was most potent to show the
187 significant difference from (6*R*,2'*R*)-**1** and (6*S*,2'*S*)-**1**. The (6*S*,2'*R*)-**1** was a most
188 effective stereoisomer in both the growth and germination inhibitory activity against
189 Italian ryegrass. Since the germination inhibitory activities were not observed at 0.6 M,
190 the growth inhibitions observed in these stereoisomers were not due to the germination
191 inhibitory activities.

192 Based on the stereochemistry of the most effective stereoisomer (6*S*, 2'*R*)-**1**,
193 derivatives **13**-**15** were synthesized (Scheme 2) to evaluate the importance of the
194 α,β-unsaturated structure and the hydroxy group bonding to a chiral center for the
195 activity. The saturated derivative **13** was obtained by hydrogenolysis of (6*S*,2'*R*)-**11**
196 under H_2 gas in the presence of $Pd(OH)_2$/C. After the Grignard reaction of (1'*S*,2'*S*)-**6**,
197 the resulting secondary hydroxy group was mesylated. The treatment of the crude
198 mesylate with $NaBH_4$ gave the dehydroxy product. This intermediate was converted

199 to 2'-dehydroxy derivative **14** by the same synthetic method described above. To
200 prepare the 2'-methoxy derivative **15**, the secondary hydroxy group obtained by
201 Grignard reaction to (1'S,2'S)-**6** was methylated by using NaH and CH_3I. The desired
202 stereochemistry was separated by silica gel column chromatography. After the
203 desilylation by *n*-Bu_4NF, the resulting intermediate was converted to **15** by the same
204 method described above.
205 The plant growth inhibitory activities of derivatives **13-15** against lettuce and
206 Italian ryegrass were assessed (Table 1). Even if the stereochemistry was
207 (6*S*,2'*R*)-stereoisomer, the derivative **13** was inactive against both plants to suggest the
208 important role of the α,β-unsaturated carbonyl structure. Michael acceptor properties
209 of (*R*)-5,6-dihydro-2*H*-pyran-2-one was reported against cancer cell lines.[41] We found
210 this property in the plant growth inhibition. The allelopathic α,β-unsaturated carbonyl
211 compounds have been reported,[42,43] whose α,β-unsaturated structures are assumed to
212 act as Michael accepters. The absence of the 2'-hydroxy group decreased the activity,
213 the derivative **14** showing 9-fold less potent than (6*S*,2'*R*)-**1** against Italian ryegrass
214 roots. Against lettuce roots, more than 16-fold less potent activity was observed. As
215 for the effect on the growth of shoots, the only less than around 2-fold activity was
216 shown against lettuce and Italian ryegrass. Furthermore, the activity of 2'-methoxy
217 derivative **15** was 3-fold and more than 16-fold less potent than (6*S*,2'*R*)-**1** against
218 lettuce shoots and roots, respectively. Against Italian ryegrass shoots and roots, 3-fold
219 and 8-fold less potent activities were shown. Even if the α,β-unsaturated structure is
220 presence, the activities of **14** and **15** decreased. Both the α,β-unsaturated carbonyl

structure and hydroxy group bonding to a chiral carbon affect the growth inhibition of roots than shoots. Comparing with polyacetate compound, (+)-boronolide (**5**), the activity of (6*S*,2'*R*)-**1** was dramatically increased. This fact suggested that the presence of 2'-hydroxy group, chiral center, and phenyl group on the 6-alkyl chain was necessary for the higher activity. These results suggest the molecular target of 6-(2-hydroxy-6-phenylhex-1-yl)-5,6-dihydro-2*H*-pyran-2-one **1** in the plant cells. It seems that the 6-oxygen, 2'-hydroxy group, and phenyl group would react to an active site after binding of enone part to a binding site. The (6*S*,2'*R*)-stereochemistry would be most advantageous for the reaction to an active site of Italian ryegrass. In the germination inhibitory activity against Italian ryegrass, all derivatives **13**-**15** exhibited lower activity than (6*S*,2'*R*)-**1** as shown in the growth test, demonstrating the significant role of α,β-unsaturated carbonyl structure, 2'-hydroxy group bonding to the 2'-chiral center for the higher activity (Figure 3). These facts also suggest the role of the binding site of enone part and active site of the chiral positions.

In summary, we synthesized all stereoisomers of 6-(2-hydroxy-6-phenylhex-1-yl)-5,6-dihydro-2*H*-pyran-2-one with high optical purities (95%ee–more than 99% ee). For the growth inhibition against lettuce, the 6S-configuration was important. As for the germination inhibition against lettuce, (6*S*,2'*S*)-**1** was most potent. Additionally, we clarified that (6*S*,2′*R*)-**1** was most effective for the growth inhibitory and the seed germination inhibitory activities against Italian ryegrass. The crucial roles of the α,β-unsaturated carbonyl structure and the hydroxy group bonding to 2'-chiral carbon for these activities were revealed. It was

suggested that the phytotoxicity of (6*S*,2′*R*)-**1** is higher than that of phytotoxic natural lignans.[44-47] The stronger stereospecific activity of 5,6-dihydro-2*H*-pyran-2-one than that of lignans was also shown.

ASSOCIATED CONTENT

Supporting Information

Supporting Information is available free of charge on the ACS Publications website at DOI:

Syntheses of compounds. NMR and MS data of intermediates **6-12** and the final compounds **1**, **13-15**. NMR and MS spectra of the final compounds **1**, **13-15**. HPLC data of all stereoisomers of **1** (PDF).

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435 **Table 1**. Plant growth inhibitory activity of all stereoisomers and derivatives **13-15**

436 (IC_{50}: μM±SE, n = 3). In the case of IC_{50} >1000 μM, % from control is shown.

437 a-b: p<0.05, a-c: p<0.001

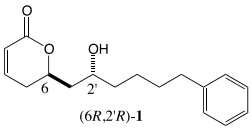
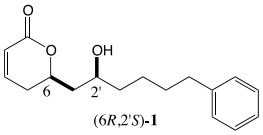
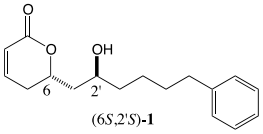
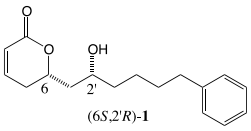
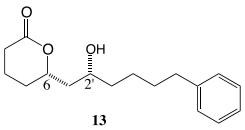
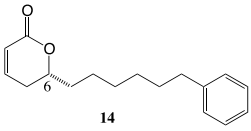
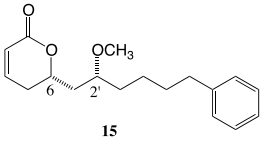
Compounds	Lettuce		Italian Ryegrass	
	Shoots	Roots	Shoots	Roots
 (6*R*,2'*R*)-**1**	491 ± 75[a]	95.2 ± 7.0[a]	625 ± 63[a]	127 ± 10[a]
 (6*R*,2'*S*)-**1**	363 ± 27[ab]	73.9 ± 3.8[ab]	741 ± 41[a]	114 ± 6.4[a]
 (6*S*,2'*S*)-**1**	227 ± 20[b]	58.4 ± 4.2[b]	548 ± 96[a]	131 ± 8.8[a]
 (6*S*,2'*R*)-**1**	303 ± 32[b]	62.3 ± 5.3[b]	260 ± 20[b]	43.2 ± 4.1[c]
 **13**	>1000 (-7%)	>1000 (-27%)	>1000 (-12%)	>1000 (-33%)
 **14**	549 ± 11	>1000 (-18%)	695 ± 98	409 ± 43
 **15**	>1000 (-34%)	>1000 (-27%)	772 ± 12	377 ± 55

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Figure caption

Figure 1. All stereoisomers of 6-(2-hydroxy-6-phenylhexyl)-5,6-dihydro-2*H*-pyran-2-one **1** and phytotoxic 6-substituted 5,6-dihydro-α-pyrone **2**–**4**.

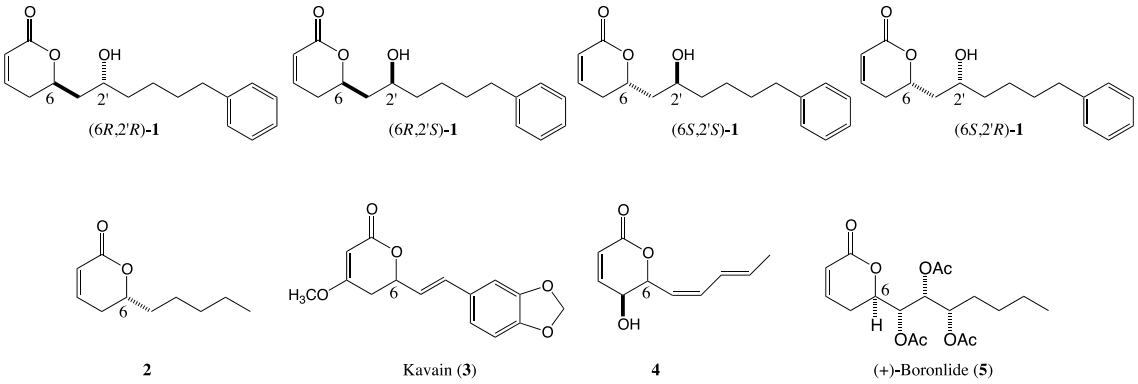
Scheme 1. Syntheses of all stereoisomers of 6-(2-hydroxy-6-phenylhexyl)-5,6-dihydro-2*H*-pyran-2-one **1**

Figure 2. Chemical shift differences ($\Delta\delta_{S-R}$) of (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid esters.

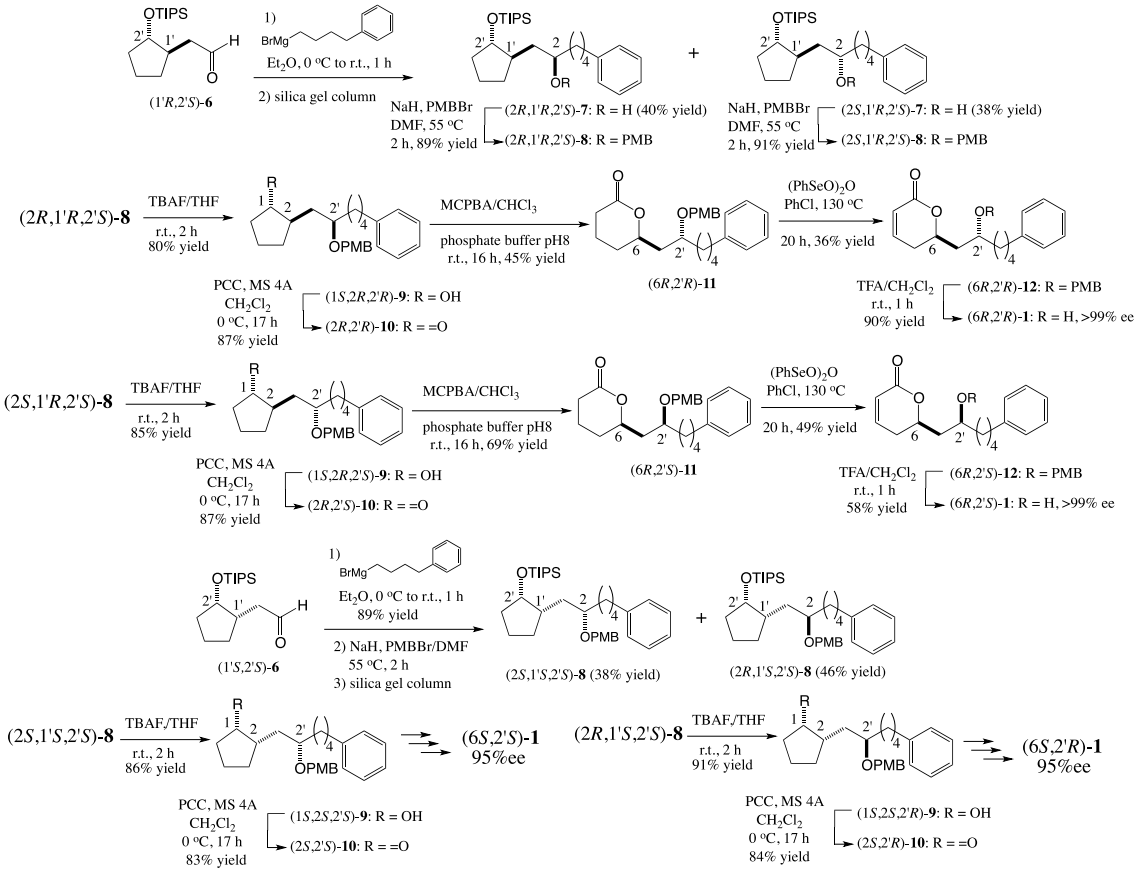
Figure 3. Seed germination ratio of lettuce and Italian ryegrass at 1000 μM (% ± SE, *n* = 3). Statistically significant differences are represented by different letters above the bars (on-way ANOVA, Tukey post-test, $P<0.05$).

Scheme 2. Syntheses of 6-substituted 5,6-dihydro-α-pyrone derivatives **13-15**.

Figure 1



Scheme 1


OTIPS
1)
BrMg
Et2O, 0 °C to r.t., 1 h
2) silica gel column
(1'R,2'S)-6
NaH, PMBBr
DMF, 55 °C
2 h, 89% yield
(2R,1'R,2'S)-7: R = H (40% yield)
(2R,1'R,2'S)-8: R = PMB
NaH, PMBBr
DMF, 55 °C
2 h, 91% yield
(2S,1'R,2'S)-7: R = H (38% yield)
(2S,1'R,2'S)-8: R = PMB
(2R,1'R,2'S)-8
TBAF/THF
r.t., 2 h
80% yield
MCPBA/CHCl3
phosphate buffer pH8
r.t., 16 h, 45% yield
(PhSeO)2O
PhCl, 130 °C
20 h, 36% yield
PCC, MS 4A
CH2Cl2
0 °C, 17 h
87% yield
(1S,2R,2'R)-9: R = OH
(2R,2'R)-10: R = =O
(6R,2'R)-11
TFA/CH2Cl2
r.t., 1 h
90% yield
(6R,2'R)-12: R = PMB
(6R,2'R)-1: R = H, >99% ee
(2S,1'R,2'S)-8
TBAF/THF
r.t., 2 h
85% yield
MCPBA/CHCl3
phosphate buffer pH8
r.t., 16 h, 69% yield
(PhSeO)2O
PhCl, 130 °C
20 h, 49% yield
PCC, MS 4A
CH2Cl2
0 °C, 17 h
87% yield
(1S,2R,2'S)-9: R = OH
(2R,2'S)-10: R = =O
(6R,2'S)-11
TFA/CH2Cl2
r.t., 1 h
58% yield
(6R,2'S)-12: R = PMB
(6R,2'S)-1: R = H, >99% ee
(1'S,2'S)-6
1)
BrMg
Et2O, 0 °C to r.t., 1 h
89% yield
2) NaH, PMBBr/DMF
55 °C, 2 h
3) silica gel column
(2S,1'S,2'S)-8 (38% yield)
(2R,1'S,2'S)-8 (46% yield)
(2S,1'S,2'S)-8
TBAF/THF
r.t., 2 h
86% yield
(6S,2'S)-1
95%ee
PCC, MS 4A
CH2Cl2
0 °C, 17 h
83% yield
(1S,2S,2'S)-9: R = OH
(2S,2'S)-10: R = =O
(2R,1'S,2'S)-8
TBAF/THF
r.t., 2 h
91% yield
(6S,2'R)-1
95%ee
PCC, MS 4A
CH2Cl2
0 °C, 17 h
84% yield
(1S,2S,2'R)-9: R = OH
(2S,2'R)-10: R = =O

492 **Figure 2**

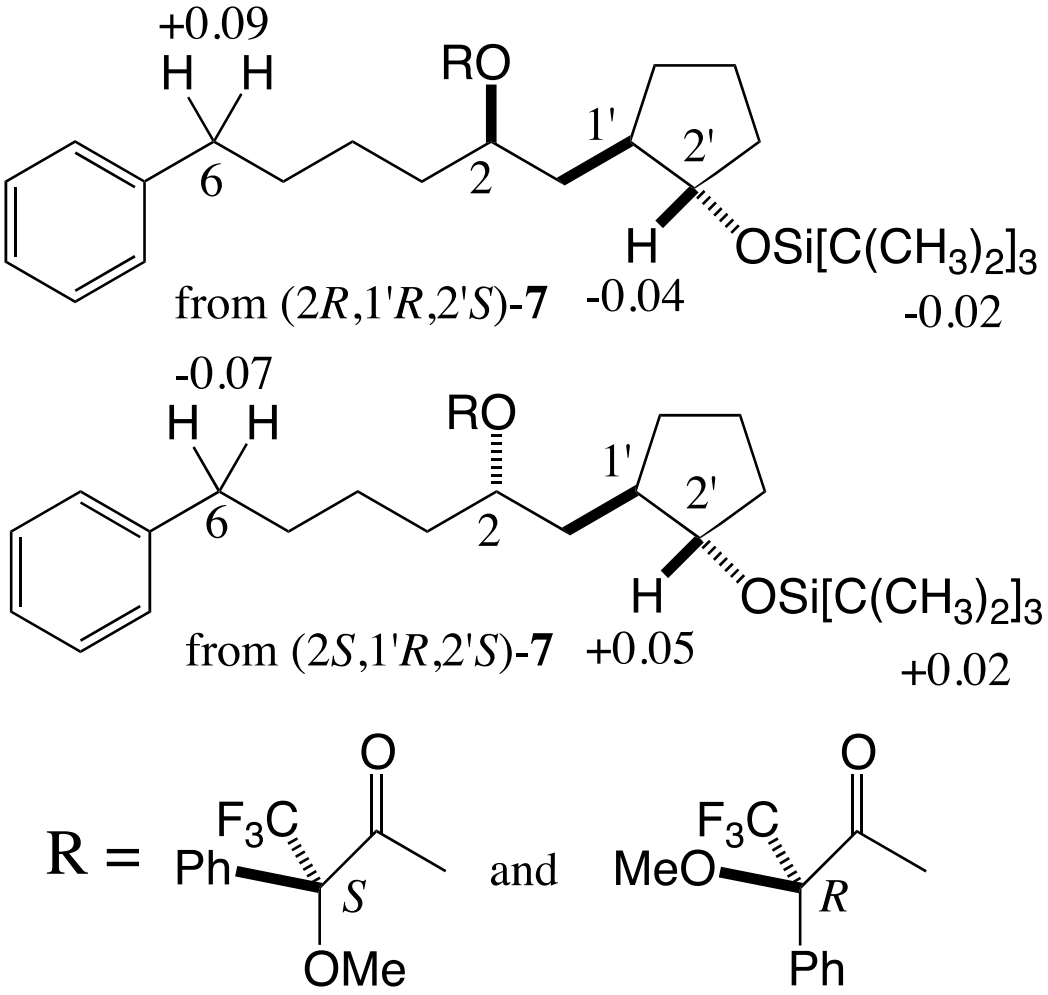


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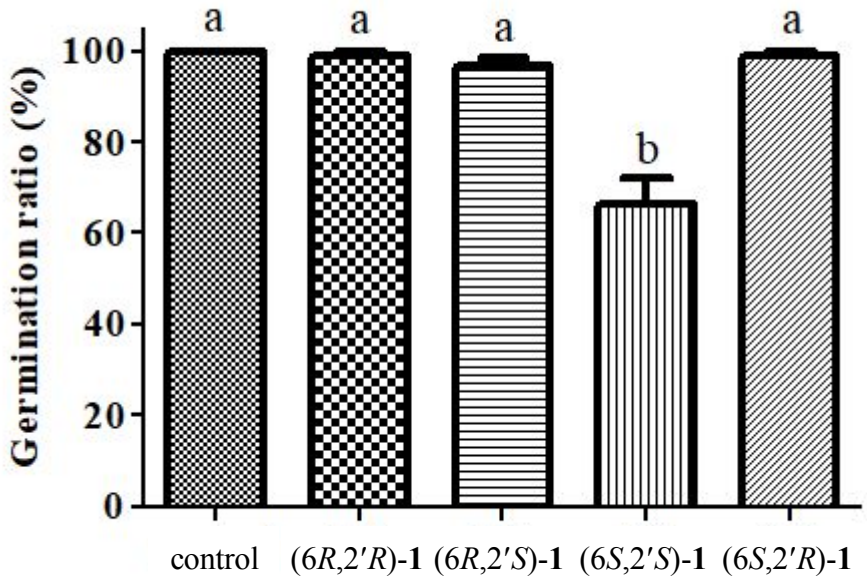
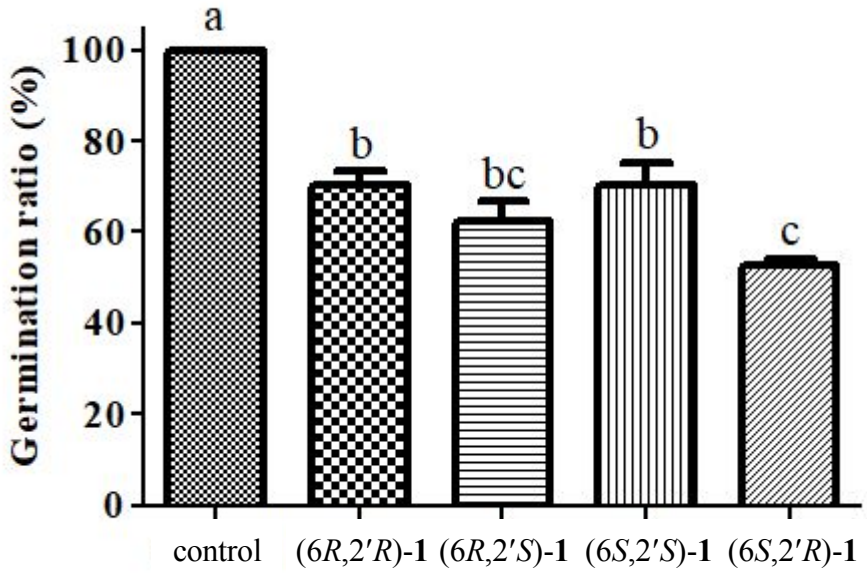
Figure 3

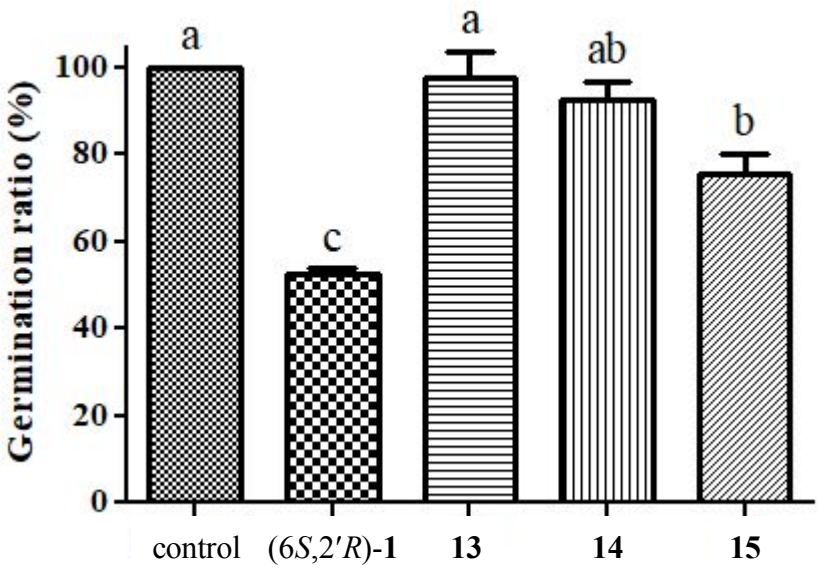
(A) Germination inhibitory activity of stereoisomers 1 against lettuce



(B) Germination inhibitory activity of stereoisomers 1 against Italian ryegrass



507 **(C) Germination inhibitory activity of derivatives 13-15 against Italian ryegrass**



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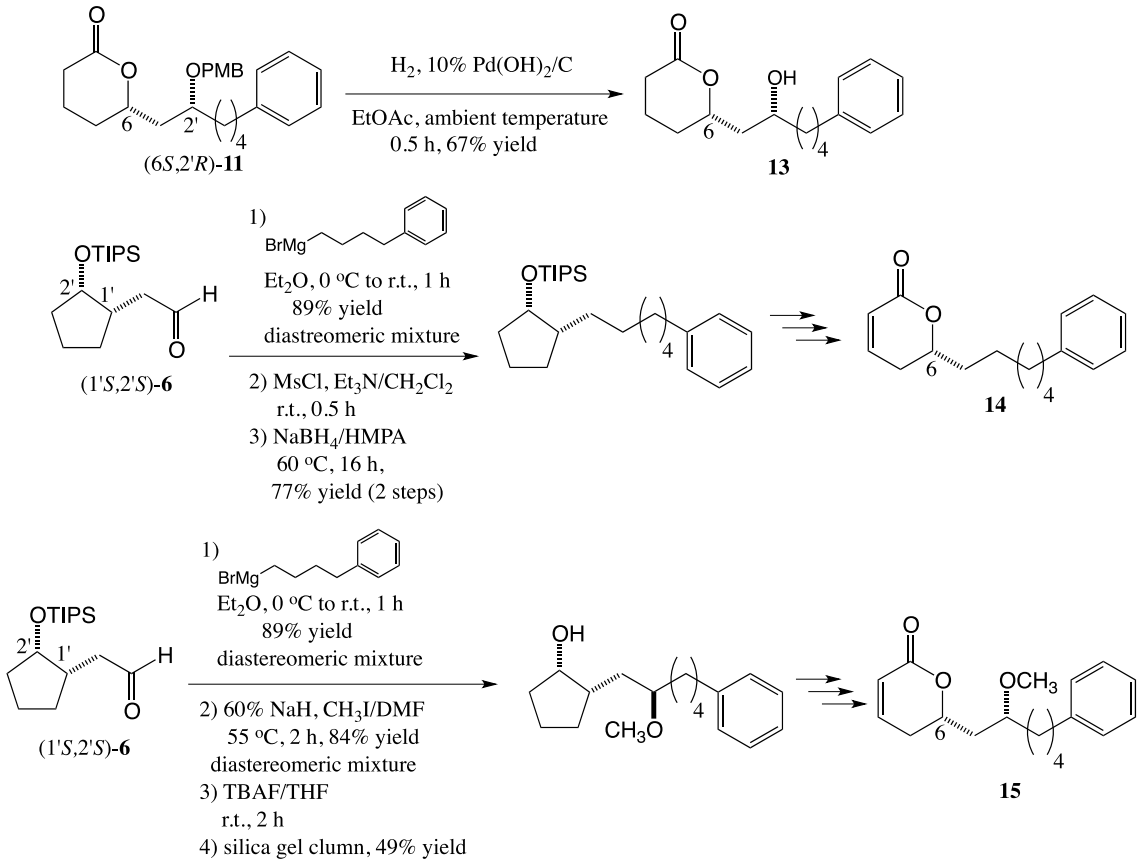
Scheme 2


OPMB
(6S,2'R)-11
H2, 10% Pd(OH)2/C
EtOAc, ambient temperature
0.5 h, 67% yield
OH
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OTIPS
(1'S,2'S)-6
1)
BrMg
Et2O, 0 °C to r.t., 1 h
89% yield
diastreomeric mixture
2) MsCl, Et3N/CH2Cl2
r.t., 0.5 h
3) NaBH4/HMPA
60 °C, 16 h,
77% yield (2 steps)
14
1)
BrMg
Et2O, 0 °C to r.t., 1 h
89% yield
diastereomeric mixture
2) 60% NaH, CH3I/DMF
55 °C, 2 h, 84% yield
diastereomeric mixture
3) TBAF/THF
r.t., 2 h
4) silica gel clumn, 49% yield
OCH3
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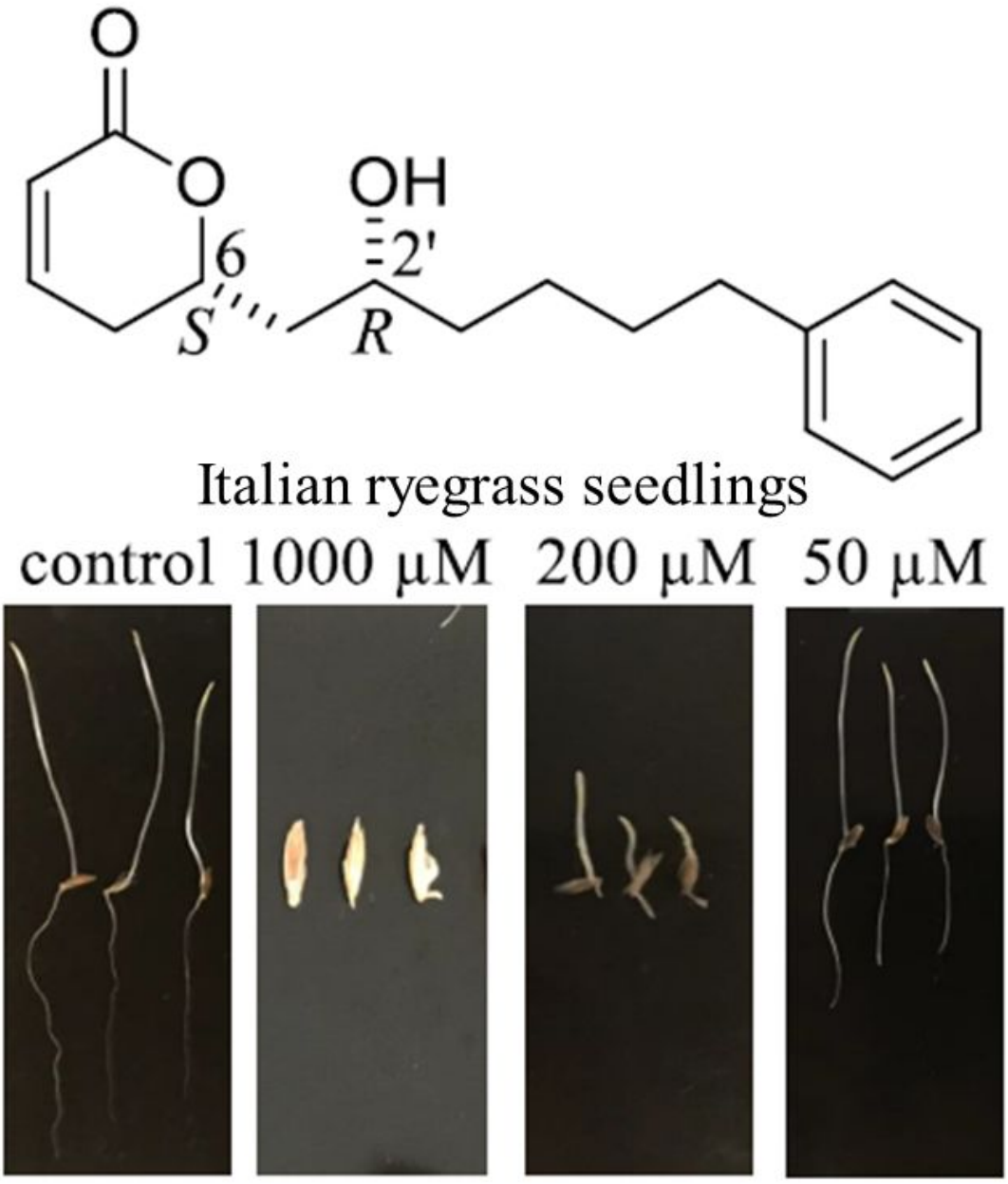
532 **TOC Graphic**



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