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Plant-growth inhibitory activity of heliannuol derivatives

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Abstract

In addition to (+)-, (−)- and (±)-heliannuol E, growth-inhibitory activities of five synthetic chromans and four tetrahydrobenzo[b]oxepins were examined against oat and cress. All heliannuol E isomers exhibited similar biological activities against cress, whereas when tested against oat roots, the unnatural optical isomer (+) showed no inhibitory activity. Four brominated chromans and two tetrahydrobenzo[b]oxepin derivatives also showed apparent inhibition against both cress and oat.

Keywords: *Helianthus annuus* L.; Compositae; Heliannuol; Plant-growth inhibition; Allelochemicals

1. Introduction

Heliannuols are a family of allelochemicals of sunflower origin (*Helianthus annuus* L.) (Macias et al., 1993, 1994, 1999a,b, 2002) (Fig. 1). Their biosynthesis might be through bisabolene-type terpene precursors, which undergo oxidation reactions to introduce oxygen-functionalities, followed by cyclization including ring-contraction, leading to heliannuols (Macias et al., 1994). Their herbicidal activities against a wide range of monocotyledons and dicotyledons were reported (Macias et al., 1994, 1999b), although detailed activities of heliannuol E **1** have not been described (Macias et al., 1999a). Such chemicals would be effective candidates for herbicide from the viewpoint of environmental concerns. Synthetic studies on heliannuols A (Grimm et al., 1994; Takabatake et al., 2000; Tuhina et al., 2002; Kishuku et al., 2003), C (Kamei et al., 2003), D (Vyvyan and Looper, 2000; Takabatake et al., 2000; Tuhina et al., 2002; Macias et al., 2003), E **1** (Sato et al., 2001) and its methyl derivative (Sabui and Venkatesmaran, 2003) have been reported utilising phenol derivatives in a biomimetic way or benzopyrone derivatives as intermediates. In this context, we have independently performed the total synthesis of (+)-, (−)- and (±)-heliannuol E **1** by employing the electrochemically generated spirodienone, with concomitant rearrangement to the corresponding chroman framework as key-steps (Doi et al., 2003a,b). During the investigation, a number of heliannuol-related compounds (**1**–**10**) were obtained as by-products or synthetic intermediates. We describe herein their growth-inhibition of oat (*Avena sativa* L.) and cress (*Lepidium sativum* L.) (Fig. 2).

2. Results and discussion

2.1. Synthesis

In addition to (+)-, (−)-, (±)-heliannuol E compounds **1**, **3** and **4** (Doi et al., 2003a,b), **2**, **5**–**10** were synthesized to understand structure–activity relationships. As can be seen in Schemes 1 and 2, their synthesis involved the electrochemical spirodienone-construction, followed by rearrangement (Mori et al., 2001) as the key process. Constant current electrolysis (CCE) of **11**, produced by a

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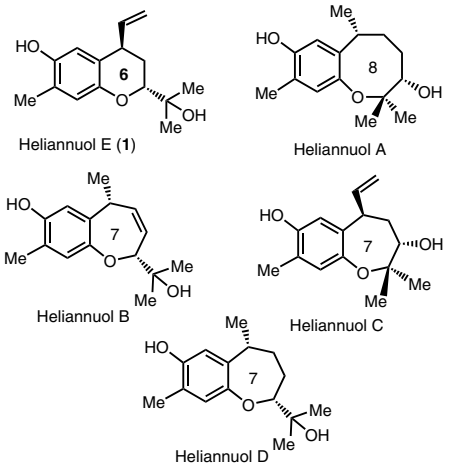


Fig. 1.

standard procedure from 4-hydroxy-3-methylbenzaldehyde through a cinnamic acid derivative, provided the spiro compound **12** in low yield, owing to a considerable amount of by-products including preowned polymers. This difficulty was circumvented by introduction of a bromine substituent at the *ortho*-position of a phenol group (Doi et al., 2003a) to accomplish our total synthesis of **1**. Exposure of **12** to $BF_3 \cdot OEt_2$ effected a 1,2-shift reaction, leading to **2** as the sole product in 62% yield, while a bromo derivative of **11** afforded a 5:1 mixture of **3** and **4** (Doi et al., 2003a). To synthesize **5** and **6**, partially masked **11** was oxidized to give **13** in 81% yield, which was submitted to the Wittig reaction. The resultant olefin was oxidized to give diol **14**, while subsequent removal of the protecting group followed by bromination gave **15**. Upon oxidation with [bis(trifluoroacetoxy)iodo]benzene (PIFA), the corresponding spiro derivative was obtained in 69% yield, whereas a yield of below 20% was observed under CCE conditions. The subsequent rearrangement reaction provided **5** and **6** in 36% and 14% yields, respectively.

Compounds **7**–**10** were synthesized to investigate the activities of the tetrahydrobenzo[b]oxepin framework, shared by heliannuols B, C and D (Fig. 2). The simple benzo[b]oxepin derivative **7** was produced by the above-mentioned procedure from **16** (Baird and Winstein, 1962) through the spirodienone **17**. Upon comparison of two methods to produce **17**, anodic oxidation provided a better yield (40%) than the 36% of PIFA oxidation.

To obtain the modified derivatives **8**–**10**, **18** produced by TBS protection of 4-bromo-2-methylphenol (Klarmann et al., 1933) was reacted with 6-methyl-5-hepten-2-one to give **19** (68%). The coupling product was converted into **21** through olefin **20**. Anodic oxidation of **21** gave the spiro derivative **22** in 62% yield, which was alternatively produced by PIFA, although the yield using the chemical oxidant (27%) was lower than that of the anodic oxidation, as in the case of **17**. Treatment of **22** with $BF_3 \cdot OEt_2$ gave diastereomeric **8** in 77% yield, which was successively converted by hydrogenolysis into **9** and **10** in 23% and 65% yields, respectively.

2.2. Bioassay

The chroman and benzo[b]oxepin derivatives, **2**–**10** along with (+)-, (−)- and (±)-heliannuol E **1**, were subjected to assessment of growth-inhibition against the shoot- and root-growth of cress (*L. sativum* L.) and oat seedlings (*A. sativa* L.). The doses required for 50% inhibition, as interpolated from the dose–response curve, are shown in Table 1. Natural (−)-**1** exhibited inhibitory activity at 10^{-3} M concentration against both cress and oat, while unnatural (+)- and (±)-**1** showed no effect

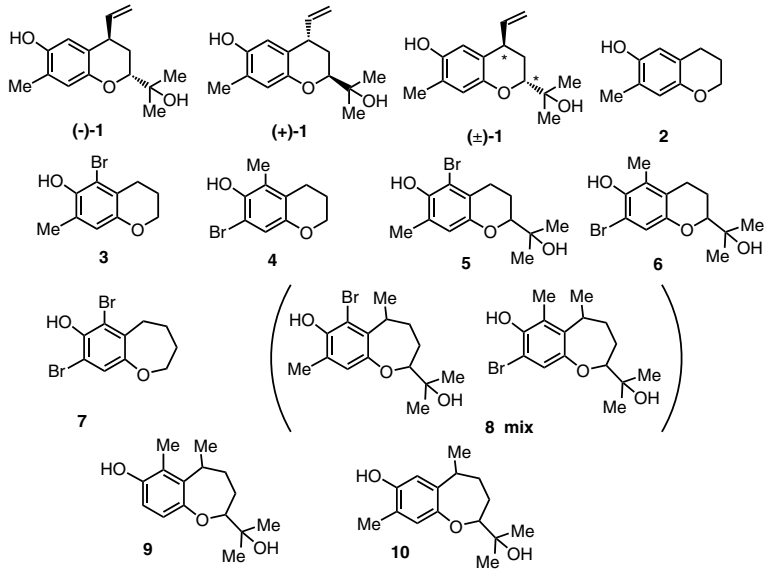
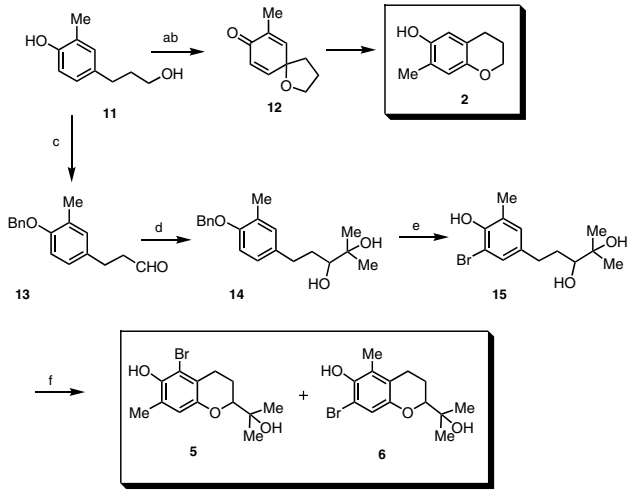
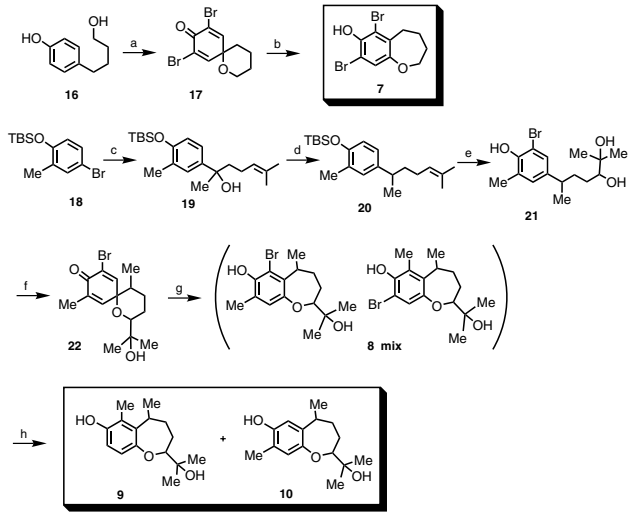


Fig. 2.



Scheme 1. Synthesis of **2**, **5** and **6**. *Reagents*: (a) CCE. (b) $BF_3 \cdot OEt_2$. (c) (i) K_2CO_3, BnBr; (ii) $SO_3 \cdot pyr$, DMSO, Et_3N. (d) (i) Ph_3PCHMe_2I, *n*-BuLi; (ii) OsO_4, NMO. (e) (i) H_2, l0% Pd–C; (ii) $PyrHBr_3$. (f) (i) CCE or PIFA; (ii) $BF_3 \cdot OEt_2$.

against roots of oat. The absolute configuration of **1** seems to be recognized by oat. Inhibitory activity of **2**, carrying the most simple chroman structure, were comparable to those of **1**. The brominated derivatives **3**–**8**, and the seven-membered ring derivatives **9** and **10**, exhibited remarkable inhibition against both hypocotyl and root of cress, as well as oat, with the exception of **6** and **9**. However, introduction of bromine substituents (**7** and **8**) give rise to inhibitory activity, although activity of the each isomer of **8** was unclear. Strong activity against both cress and oat was found in the case of **10**, which has the same carbon framework as that of heliannuol D. No activity of the regio-isomer of the aryl methyl group **9** was observed, indicating that the methyl substituent may play



Scheme 2. *Reagents:* (a) (i) Br_2; (ii) CCE or PIFA. (b) $BF_3 \cdot OEt_2$. (c) *n*-BuLi, then **6** methylhept-5-en-2-one. (d) Et_3SiH, $BF_3 \cdot OEt_2$. (e) (i) OsO_4, NMO; (ii) TBAF; (iii) Br_2. (f) CCE or PIFA. (g) $BF_3 \cdot OEt_2$. (h) H_2, 10% Pd–C.

an important role in the inhibitory effect against oat. This regio-recognition was apparent contrast to that of **3** and **4**, which showed similar inhibition.

In conclusion, heliannuol-derivatives were synthesized by employing the rearrangement of spirodienones under Lewis acid conditions. The synthetic chromans (**2**–**6**) and the tetrahydrobenzo[b]oxepins (**7**–**10**), along with (+)-, (−)- and ±-**1**, were submitted to growth inhibition assay against oat and cress. Bromine-substituted derivatives showed inhibitory activities at 10^{-3}–10^{-4} M concentration, which were better than those of natural (−)-**1**. Compound **10** was observed to possess the strongest activity against oat and cress.

3. Experimental

3.1. General

IR spectra were recorded on a JASCO Model A-202 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were obtained on JEOL JNM EX-270 and JEOL JNM GX-400 spectrometers in $CDCl_3$ using tetramethylsilane as an internal standard. HRMS were obtained on a Hitachi M-80 B GC–MS spectrometer operating at the ionization energy of 70 eV. Prep. and analytical TLC were carried out on silica gel plates (Kieselgel 60 PF_{254}, E. Merck AG, Germany) using UV light and/or 5% molybdophosphoric acid in ethanol for detection. Kanto Chemical Silica 60N (spherical, neutral, 63–210 μm) was used for cc. Anodic oxidation was performed under CCE conditions using a glassy carbon beaker as an anode and a platinum wire as a cathode in the presence of appropriate supporting salts.

3.1.1. 7-Methyl-1-oxaspiro[4.5]deca-6,9-dien-8-one (*12*)

A solution of **11** (53 mg, 0.32 mmol) in acetone (160 ml) in the presence of *n*-Bu_4NClO_4 (5.5 g) was electrolyzed (1.2–1.6 V vs SCE, 2 F/mol). The reaction mixture was evaporated and the residue was chromatographed on a silica gel column (hexane:EtOAc, l/l) to give **12** (l0.6 mg, 20%) as an oil. IR: δ_{max} 2952, 1671, 1643 cm^{-1}; ^1H NMR: δ 1.87 (3H, *d*, $J = 1.0$ Hz), 2.04 (2H, *t*, $J = 7.3$ Hz), 2.15 (2H, *tt*, $J = 6.3$, 7.3 Hz), 4.07 (2H, *t*, $J = 6.3$ Hz), 6.13 (1H, *d*, $J = 9.8$ Hz), 6.58 (1H, *dd*, $J = 1$, 3.4 Hz), 6.78 (1H, *dd*, $J = 3.4$, 9.8 Hz); ^{13}C NMR: δ 15.7, 26.9, 36.9, 69.0, 77.8, 127.1, 133.7, 144.9, 149.3, 186.2. Found: *m*/*z* 164.0816. Calc. for $C_{10}H_{l2}O_2$: M^+, 164.0836.

3.1.2. 7-Methylchroman-6-ol (*2*)

To a solution of **12** (l0.6 mg, 0.065 mmol) in CH_2C1_2 (1 ml) was added $BF_3 \cdot OEt_2$ (0.025 ml, 0.2 mmol); the mixture was reacted at ambient temp. for l.5 h. The resulting mixture was diluted with EtOAc, washed with H_2O and brine, and dried (Na_2SO_4). After evaporation,

Table 1
Inhibitory activity of heliannuol derivatives on the growth of cress and oat seedlings

Compounds	EC_{50} (M)*			
	Cress		Oat	
	Hypocotyl	Root	Coleoptile	Root
(−)-**1**	1.2×10^{-3}	**	2.0×10^{-3}	6.2×10^{-3}
(+)-**1**	1.3×10^{-3}	1.0×10^{-2}	**	$>10^{-2}$
(±)-**1**	1.7×10^{-3}	1.0×10^{-2}	5.0×10^{-3}	***
2	1.4×10^{-3}	7.2×10^{-3}	**	3.7×10^{-3}
3	8.5×10^{-4}	1.5×10^{-3}	1.1×10^{-3}	2.6×10^{-3}
4	8.4×10^{-4}	1.4×10^{-3}	2.3×10^{-3}	6.0×10^{-3}
5	7.1×10^{-4}	6.3×10^{-4}	2.4×10^{-3}	1.0×10^{-3}
6	6.5×10^{-4}	6.5×10^{-4}	$>10^{-2}$	9.4×10^{-4}
7	6.1×10^{-4}	4.8×10^{-4}	2.5×10^{-3}	1.6×10^{-3}
8	4.1×10^{-4}	4.7×10^{-4}	4.0×10^{-3}	1.1×10^{-3}
9	8.5×10^{-4}	1.0×10^{-3}	$>10^{-2}$	$>10^{-2}$
10	1.1×10^{-4}	1.1×10^{-3}	1.7×10^{-3}	2.3×10^{-3}

* EC_{50} represents the concentration of sample which causes 50% inhibition of the shoot and root growth of cress or oat seedlings, respectively.
** The dose–response curves of cress and oat seedlings for sample were not linear when the percentage elongation was plotted against for the logarithm of the dose, and EC_{50} was not estimated.
*** No inhibitory activity.

the residue was applied to a silica gel column (hexane:EtOAc, 2/1) to give **2** (6.6 mg, 62%): mp 126–127 °C (hexane–diethyl ether). IR: δ_{max} 3315 cm^{-1}; ^1H NMR: δ 1.96 (2H, *tt*, $J = 5.4$, 6.8 Hz), 2.17 (3H, *s*), 2.70 (2H, *t*, $J = 6.8$ Hz), 4.11 (2H, *t*, $J = 5.4$ Hz), 6.47 (1H, *s*), 6.56 (1H, *s*); ^{13}C NMR: δ 15.6, 22.6, 24.6, 66.3, 115.3, 118.4, 120.2, 122.9, 147.1, 148.4. Found: *m/z* 164.0848. Calc. for $C_{10}H_{12}O_2$: M^+, 164.0837.

3.1.3. 3-(4-Benzyloxy-3-methylphenyl)propanal (*13*)

To an ice-cooled solution of **11** (0.72 g, 4.3 mmol) in DMF (7.2 ml) were added K_2CO_3 (1.80 g, 13 mmol) and BnBr (0.77 ml, 6.5 mmol); the mixture was stirred at ambient temp. for 15 h. The reaction mixture was diluted with hexane/EtOAc (1:1, 200 ml), washed with H_2O and brine and then dried (Na_2SO_4). After evaporation, a crude product was subject to silica gel cc (hexane:EtOAc, 2/1) to give a benzyl ether (0.84 g, 76%).

A mixture of the benzyl ether (636 mg, 2.48 mmol), $SO_3 \cdot$pyr (1.18 g, 7.4 mmol) and Et_3N (2.06 ml, 15 mmol) in DMSO (10 ml) was stirred at ambient temp. for 10 min. The reaction mixture was diluted with EtOAc, washed with H_2O and brine, and then dried (Na_2SO_4). After evaporation, a crude product was applied to a silica gel column (hexane:EtOAc, 2/1) to give **13** (0.505 g. 81%) as an oil. IR: δ_{max} 2921, 1724 cm^{-1}; ^1H NMR: δ 2.73 (2H, *t*, $J = 7.3$ Hz), 2.87 (2H, *t*, $J = 7.3$ Hz), 5.05 (2H, *s*), 6.80 (1H, *d*, $J = 7.8$ Hz), 6.95 (1H, *dd*, $J = 2$, 7.8 Hz), 6.99 (1H, *brd*, $J = 2$ Hz), 7.31–7.44 (5H, *m*), 9.80 (1H, *s*); ^{13}C NMR: δ 16.4, 27.4, 45.6, 69.9, 111.5, 126.2, 127.0, 127.2, 127.6, 128.4, 130.7, 132.1, 137.4, 155.3, 201.7. Found: *m/z* 254.1256. Calc. for $C_{17}H_{20}O_2$: M^+, 254.1305.

3.1.4. 2-Methyl-5-(4-benzyloxy-3-methylphenyl)pentane-2,3-diol (*14*)

To an ice-cooled solution of isopropyltriphenylphosphonium iodide (3.01 g, 7.0 mmol) in THF (20 ml) was added *n*-BuLi (1.57 M solution in hexane, 3.8 ml). After 30 min, a solution of **13** (505 mg, 2 mmol) in THF (5 ml) was added, and the mixture was stirred for 5 min. The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried (Na_2SO_4). After evaporation, a crude product was subjected to a silica gel cc (hexane:EtOAc, 40/1) to give an olefin (87.3 mg, 16%) as an oil.

To a solution of the olefin (13.5 mg, 0.048 mmol) in acetone/H_2O (l0:l, 1 ml) were added NMO (11.3 mg) and OsO_4 (0.04 M solution in *t*-BuOH, 0.05 ml); the mixture was stirred at ambient temp. for 13 h. After the addition of sat. aq. Na_2SO_3, the resultant mixture was dilute with EtOAc, washed with 1 M HCl and brine, and then dried (Na_2SO_4). After evaporation, the residue was purified by silica gel cc (hexane:EtOAc, 3/1) to give **14** (11 mg 73%) as an oil. IR: δ_{max} 3407, 2925 cm^{-1}; ^1H NMR: δ 1.12 (3H, *s*), 1.19 (3H, *s*), 1.60–1.66 (H, m), 1.75 (1H, *m*), 2.27 (3H, *s*), 2.58 (1H, *ddd*, $J = 6.8$, 9.3, 14.7 Hz), 2.83 (2H, *ddd*, $J = 4.9$, 9.8, 14.7 Hz), 3.40 (1H, *brd*, $J = 10.3$ Hz), 5.06 (2H, *s*), 6.81 (1H, *d*, $J = 8.3$ Hz), 6.99 (1H, *dd*, $J = 2$, 8.3 Hz). 7.02 (1H, *brd*, $J = 2$ Hz), 7.29–7.45 (5H, *m*); ^{13}C NMR: δ 16.5, 23.2, 26.6, 32.1, 33.8, 69.9, 73.l, 77.9, 111.4, 126.3, 127.0, 127.6, 128.4, 128.6, 130.9, 133.9, 137.5, 155.0. Found: *m/z* 314.1853. Calc. for $C_{20}H_{26}O_3$: M^+, 314.1880.

3.1.5. 5-(3-Bromo-4-hydroxy-5-methylphenyl)-2-methylpentane-2.3-diol (*15*)

A solution of **14** (49 mg, 0.16 mmol) in MeOH (3 ml) in the presence of catalytic amounts of 10% Pd–C was

stirred at ambient temp. for 2 h under a hydrogen atmosphere. After filtration, the filtrate was evaporated, and the residue was purified by silica gel cc (hexane:acetone, 2/1) to give a phenol (34.4 mg, 98%) as an oil.

To a solution of the phenol (34.4 mg, 0.15 mmol) in $CHCl_3$ (5 ml) was added $PyrHBr_3$ (49 mg, 0.15 mmol) in $CHCl_3$ (5 ml)–pyridine (0.2 ml) at 0 °C. After addition of H_2O, the resulting mixture was diluted with $CHCl_3$, washed with 1 M HCl and brine, and dried (Na_2SO_4). After evaporation, the residue was applied to a silica gel column (hexane:EtOAc, 2/1) to give **15** (42 mg, 90%) as an oil. IR: δ_{max} 3399, 2974 cm^{-1}; ^1H NMR: δ 1.16 (3H, *s*), 1.19 (3H, *s*), l.55–2. 05 (2H, *m*), 2.27 (3H, *s*), 2.54 (1H, *ddd*, $J = 7.3, 9.3, 13.7$ Hz), 2.81 (2H, *ddd*, $J = 4.9, 9.7, 13.7$ Hz), 3.37 (1H, *brd*, $J = 10.8$ Hz), 6.92 (1H, *s*), 7.15 (1H, *s*); ^{13}C NMR: δ 16.7, 23.3, 26.6, 31.8, 33.6, 73.2, 77.6, 109.9, 125.6, 128.8, 130.5, 134.9, 148.4. Found: *m/z* 302.0555. Calc. for $C_{l3}H_{19}O_3{}^{79}Br$: M^+, 302.0517.

3.1.6. 5-Bromo-2-(1-hydroxyisopropyl)-7-methylchroman-6-ol (*5*) and 6-bromo-2-(1-hydroxyisopropyl)-8-methylchroman-7-ol (*6*)

A solution of **15** (42 mg, 0.14 mmol) in dioxane-60% aq. $HClO_4$ (5/1, 117 ml) in the presence of $LiClO_4$ (2 g) was electrolyzed (1.6–1.9 V vs SCE, 10 F/mol). The reaction mixture was neutralized by addition of sat. aq. $NaHCO_3$ and evaporated. The residue was dissolved in $CHCl_3$, washed with H_2O and brine, and dried (Na_2SO_4). After evaporation, a crude product was subjected to silica gel cc (hexane:EtOAc, 2/1) to give a spiro compound (7.6 mg, 18%) as an oil.

To a solution of the spiro compound (119.5 mg, 0.4 mmol) in CH_2Cl_2 (3 ml) was added $BF_3 \cdot OEt_2$ (0.15 ml, 1.2 mmol); the mixture was washed with H_2O, sat. aq. $NaHCO_3$ and dried (Na_2SO_4). After evaporation, the residue was applied to a silica gel column (hexane:EtOAc, 4/1) to give **5** (43.6 mg, 36%) and **6** (16.6 mg, 14%) as oils. **5**: IR: δ_{max} 3518, 2976 cm^{-1}; ^1H NMR: δ 1.27 (3H, *s*), 1.31(3H, *s*), 1.72 (1H, *dddd*, $J = 6.4, 11.7, 11.7, 15.1$ Hz), 2.07 (1H, *dddd*, $J = 2, 2, 4.9, 15.1$ Hz), 2.24 (3H, *s*), 2.64 (1H, *ddd*, $J = 6.4, 11.7, 17.1$ Hz), 2.84 (1H, *brdd*, $J = 4.9, 17.1$ Hz), 3.64 (1H, *dd*, $J = 2.0, 11.7$ Hz), 6.64 (1H, *s*); ^{13}C NMR: δ 16.6, 22.4, 24.5, 25.9, 26.7, 71.7, 81.8, 111.9, 117.9, 119.1, 127.7, 144.4, 148.6. Found: *m/z* 302.0383. Calc. for $C_{13}H_{17}O_3{}^{81}Br$: M^+, 302.0341. **6**: IR: δ_{max} 3408, 2976 cm^{-1}; ^1H NMR: δ 1 26 (3H, *s*), 1.31 (3H, *s*), 1.72 (1H, *dddd*, $J = 5.9, 11.7, 12.7, 13.7$ Hz), 2.09 (1H, *dddd*, $J = 2, 2, 6.9, 13.7$ Hz), 2.59 (1H, *ddd*, $J = 6.9, 12.7, 17.1$ Hz), 2.75 (1H, *ddd*, $J = 2, 5.9, 17.1$ Hz), 3.64 (1H, *dd*, $J = 2, 11.7$ Hz), 6 88 (1H, *s*); ^{13}C NMR: δ 12.4, 22.2, 23.5 24.4, 25.9, 71.7, 81.7, 107.5, 116.4, 121.9, 123.7, 143.9, 148.8. Found: *m/z* 302.0348. Calc. for $C_{l3}H_{l7}O_3{}^{81}Br$: M^+, 302.0341.

3.1.7. PIFA oxidation of (*15*)

To a solution of PIFA (640 mg, 1.5 mmol) in MeCN (5 ml) was added a solution of **15** (214 mg, 0.71 mmol) in MeCN (7 ml); the mixture was stirred at 0 °C for 15 min. The resulting mixture was diluted with EtOAc, washed with H_2O and brine, and then dried (Na_2SO_4). After removal of the solvent, the residue was subjected to a silica gel cc to give the corresponding spiro compound (147.5 mg, 69%).

3.1.8. 2,4-Dibromo-7-oxaspiro[5.5]undeca-1,4-dien-3-one (*17*)

To a solution of **16** (1.04 g, 6.2 mmol) in THF (83 ml) at −78 °C was added Br_2 (1.5 g, 9.4 mmol) in $CHCl_3$ (l00 ml); the mixture was stirred at ambient temperature for 2 days. The resulting mixture was washed with H_2O, aq. $Na_2S_2O_3$ and brine, and then dried (Na_2SO_4). After evaporation, a crude product was applied to a silica gel column (PhMe:EtOAc, 10/1) to give a dibromide (0.24 g, 12%), along with the corresponding monobromide (0.81 g, 53%).

A solution of the dibromide (33 mg, 0.1 mmol) in $MeNO_2$ (l00 ml) in the presence of *n*-Bu_4NClO_4 (1.7 g) was electrolyzed (3.3–3.5 V vs SCE, 6.2 F/mol). The reaction mixture was evaporated, and the residue was repeatedly purified by silica gel cc (hexane:EtOAc, 1/1, then 3/1) to give **17** (13 mg, 40%) as an oil. IR: δ_{max} 2940, 1684, 1595 cm^{-1}; ^1H NMR: δ 1.68–1.86 (6H, *m*), 3.88 (2H, *t*, $J = 5.4$ Hz), 7.60 (2H, *s*); ^{13}C NMR: δ 19.5, 24.7, 34.1, 62.8, 74.2, 121.8, 149.0, 171.9. Found: *m/z* 319.9053. Calc. for $C_{l0}H_{10}O_2{}^{79}Br_2$: M^+, 319.9048.

3.1.9. PIFA oxidation of the dibromo derivative of (*16*)

To a solution of PIFA (333 mg, 0.78 mmol) in MeCN (18 ml) was added a solution of the dibromide (81 mg, 0.25 mmol) in MeCN (7 ml); the mixture was stirred at 0 °C for 15 min. The resulting mixture was diluted with EtOAc, washed with H_2O, $Na_2S_2O_3$, and then dried (Na_2SO_4). After removal of the solvent, the residue was subjected to a silica gel cc to give **17** (29.1 mg, 36%).

3.1.10. 6,8-Dibromo-2H,3H,4H,5H-benzo[f]oxepin-7-ol (*7*)

A mixture of **17** (22.5 mg, 0.07 mmol) and $BF_3 \cdot OEt_2$ (0.026 ml, 0.21 mmol) in CH_2Cl_2 (0.3 ml) was stirred at ambient temp. for 3 h. The reaction mixture was diluted with $CHCl_3$, washed with H_2O, sat. aq. $NaHCO_3$ and brine, and dried (Na_2SO_4). After evaporation, the residue was purified by silica gel cc (hexane:EtOAc, 2/1) to give **7** (12.7 mg, 56%) as an oil. IR: δ_{max} 3487 cm^{-1}; ^1H NMR: δ 1.70 (2H, *m*), 1.96 (2H, *m*), 3.04 (2H, *t*, $J = 5.6$ Hz), 3.98 (2H, *t*, $J = 5.6$ Hz), 5.76 (1H, *s*), 7.17 (1H, *s*); ^{13}C NMR: δ 24.8, 31.8, 32.9, 74.3, 105.6, 111.8, 124.2,

136.4, 145.6, 154.2. Found: m/z 319.9044. Calc. for $C_{10}H_{10}O_2{}^{79}Br_2$: M^+, 319.9047.

3.1.11. 2-[4-(t-Butyldimethylsilyloxy)-3-methylphenyl]-6-methylhept-5-en-2-ol (19)

To a solution of **18** (19.5 g, 65 mmol) in THF (600 ml) at −78 °C was added *n*-BuLi (1.58 M solution in hexane, 41 ml) under Ar. After addition of 6-methylhept-5-en-2-one (21.2 g, 0.17 mol) in THF (500 ml), the mixture was stirred at the same temp. for 30 min. The resulting mixture was diluted with EtOAc, washed with H_2O, sat. aq. NH_4Cl and brine, and then dried (Na_2SO_4). After evaporation, the residue was subjected to a silica gel cc (hexane:EtOAc, 14/l) to give **19** (15.3 g, 68%) as an oil. IR: δ_{max} 3411 cm^{-1}; ^1H NMR: δ 0.21 (6H, *s*), 1.01 (9H, *s*), 1.49 (3H, *s*), 1.51 (3H, *s*), 1.65 (3H, *s*), 1.76-1.95 (4H, *m*), 2.21 (3H, *s*), 5.09 (1H, *m*), 6.71 (1H, *d*, $J = 8.3$ Hz), 7.08 (1H, *dd*, $J = 2$, 8.3 Hz), 7.18 (1H, *d*, $J = 2$ Hz); ^{13}C NMR: δ −4.1, 17.2, 17.7, 18.3, 23.1, 25.75, 25.83, 30.4, 43.8, 74.7, 117.9, 122.9, 124.2, 127.4, 128,2, 131,9, 140.1, 152.2. Found: m/z 348.2498. Calc. for $C_{21}H_{36}O_2Si$: M^+, 348.2483.

3.1.12. 2-[4-(t-Butyldimethylsilyloxy)-3-methylphenyl]-6-methylhept-5-ene (20)

To a solution of **19** (2.54 g, 7.3 mmol) in CH_2Cl_2 (73 ml) were added Et_3SiH (1.4 ml, 8.8 mmol) and $BF_3 \cdot OEt_2$ (0.9 ml, 7.2 mmol); the mixture was stirred at 0 °C for 15 min. The resulting mixture was washed with H_2O, sat. aq. $NaHCO_3$ and brine, and then dried (Na_2SO_4). After evaporation, the residue was applied to a silica gel column (hexane) to give an oily siloxy ether **20** (2.12 g, 87%). IR: δ_{max} 1595 cm^{-1}; ^1H NMR: δ 0.20 (6H, *s*), 1.01 (9H, *s*), 1.19 (3H, *d*, $J = 6.8$ Hz), 1.51 (3H, *s*), 1.55 (2H, *m*), 1.66 (3H, *s*), 1.86 (2H, *m*), 2.58 (1H, *m*), 5.09 (1H, *m*), 6.67 (1H, *d*, $J = 7.6$ Hz), 6.84 (1H, *dd*, $J = 1.4$, 7.6 Hz), 6.92 (1H, *d*, $J = 1.4$ Hz); ^{13}C NMR: δ-4.1, 17.1, 17.7, 18.3, 22.6, 25.8, 25.9, 26.3, 38.71, 38.75, 77.2, 118.1, 124.7, 124.8, 129.4, 131.1, 139.9, 151.6. Found: m/z 332.2514. Calc. for $C_{21}H_{36}OSi$: M^+, 332.2533.

3.1.13. 6-(4-Hydroxy-3-methylphenyl)-2-methylheptane-2,3-diol (21)

A mixture of **20** (1.51 g. 4.5 mmol), OsO_4 (0.04 M *t*-BuOH solution, 0.6 ml) and NMO (1.63 g, 14 mmol) in aq. acetone (45 ml) was stirred at ambient temp. for 18 h. The resultant mixture was diluted with EtOAc, washed with sat. aq. Na_2SO_3, H_2O and brine, and then dried (Na_2SO_4). After evaporation, a crude product was purified by silica gel cc (hexane:EtOAc, 2/1) to give a diol as a diastereomeric mixture (1.63 g, 98%) as an oil.

A mixture of the diol (2.33 g, 6.4 mmol) and *n*-Bu_4NF (1.0M THF solution, 7 ml) in THF (63 ml) was stirred at 0 °C for 75 min. The resulting mixture was diluted with EtOAc, washed with H_2O and brine, and then dried (Na_2SO_4). After evaporation, the residue was dissolved in $CHCl_3$ (127 ml), and Br_2 (1.25 g, 7.8 mmol) in $CHCl_3$ (85 ml) was added. After being stirred at ambient temperature for 15 min, the mixture was washed with H_2O, sat. aq. $Na_2S_2O_3$ and brine. After being dried (Na_2SO_4), the organic layer was evaporated and the residue was purified by silica gel cc (hexane:EtOAc, 2/1) to give diastereomeric **21** (2.07 g, 98%) as an oil. IR: δ_{max} 3392 cm^{-1}; ^1H NMR: δ 1.08 (1.5H, *s*), 1.11 (1.5H, *s*), 1.14 (3H. *s*), 1.19 (1.5H, *d*, $J = 3.9$ Hz), l.21 (1.5H, *d*, $J = 3.9$ Hz), 1.32 (2H, *m*), 1.56 (1H, *m*), 1.82 (1H, *m*), 2.26 (1.5H, *s*), 2.27 (1.5H, *s*), 2.59 (1H, *m*), 3.28 (0.5H, *dd*, $J = 2.7$, l0.0 Hz), 3.35 (0.5H, *dd*, $J = 2.0$, l0.3 Hz), 5.51 (1H, *s*), 6.88 (1H, *m*), 7.10 (1H, *m*); ^{13}C NMR: δ 16.9, 22.4, 22.8, 23.2, 26.6, 29.6, 29.9, 35.2, 35.6, 38.8, 39.2, 73.2, 78.3, 78.7, 109.9, 125.5, 127.3, 127.4, 128.88, 128.91, 140.1, 140.4, 148.3. Found: m/z 330.0798. Calc. for $C_{15}H_{23}O_3{}^{79}Br$: M^+, 330.0829.

3.1.14. 4-Bromo-8-(1-hydroxyisopropyl)-2,11-dimethyl-7-oxaspiro[5.5]undeca-1,4-dien-3-one (22)

A solution of **21** (74.8 mg, 0.23 mmol) in $MeNO_2$ (150 ml) in the presence of *n*-Bu_4NClO_4 (2.6 g, 7.7 mmol) was electrolyzed under CCE conditions (3.4–3.6 V vs SCE, 6.6 F/mol). The reaction mixture was evaporated and the residue was applied to a silica gel column (hexane:EtOAc, 1/1), followed by prep TLC (hexane:EtOAc, 2/1, 3 times development) to give diastereomeric **22** (46 mg, 62%) as an oil. IR: δ_{max} 3502, 2967, 1670 cm^{-1}; ^1H NMR: δ 0.69–0.72 (1.5H, *m*), 1.06–1.08 (1.5H, *m*), 1.17–1.18 (6H, *m*), 1.44–1.85 (5H, *m*), 1.96–1.99 (3H *m*), 2.38 (1H, *brs*), 3.53–3.69 (1H, *m*), 6.44 (0.25H, *dd*, $J = 1.6$, 2.8 Hz), 6.68 (0.25H, *dd*, $J = 1.6$, 2.8 Hz), 7.06 (0.25H, *dd*, $J = 1.2$, 2.8 Hz), 7.07 (0.25H, *d*, $J = 2.8$ Hz), 7.19 (0.25H, *dd*, $J = 1.6$, 2.4 Hz), 7.30 (0.25H, *d*, $J = 2.4$ Hz), 7.67 (0.25H, *d*, $J = 2.8$ Hz), 7.83 (0.25H, *d*, $J = 2.8$ Hz); ^{13}C NMR: δ 15.91, 15.93, 11.27, 16.33, 16.4, 16.5, 16.9, 17.1, 20.1, 20.3, 23.5, 23.6, 23.7, 23.8, 25.7, 25.8, 25,87, 25.93, 26.0, 26.1, 26.5, 26.6, 28.1, 28.2, 35.39, 35.45, 37.57, 37.62, 71.88, 71,89, 72.0, 72,1, 75.80, 75.82, 76.8, 77.5, 77.65, 77.69, 123.6, 124.4, 124.6, 126.3, 133.2, 133.3, 134.4, 135.8, 137.8, 142.3, 143.3, 145.6, 146.7, 147.2, 150.3, 151.1, 178.7, 178.8, 179.2. Found: m/z 328.0649. Calc. for $C_{15}H_{21}O_3{}^{79}Br$: M^+, 328.0672.

3.1.15. PIFA oxidation of (21)

Compound **21** (931 mg, 2.8 mmol) was oxidized with PIFA (3.73 g, 8.67 mmol) in MeCN (280 ml) essentially the same procedure as in the case of **17** to give **22** (250 mg, 27%).

3.1.16. 2-(1-Hydroxyisopropyl)-5,8-dimethyl-2H,3H,4H,5H-benzo[f]oxepin-7-ol (9) and 2-(1-hydroxyisopropyl)-5,6-dimethyl-2H,3H,4H,5H-benzo[f]oxepin-7-ol (10)

A mixture of **22** (117 mg, 0.36 mmol) and $BF_3 \cdot OEt_2$ (0.134 ml, 1.1 mmol) in CH_2Cl_2 (1.4 ml) was stirred at

ambient temp. for 1 h. The reaction mixture was diluted with $CHCl_3$, washed with H_2O, sat. aq. $NaHCO_3$ and brine, and then dried (Na_2SO_4). After evaporation, the residue was purified by silica gel cc (hexane:EtOAc, 4/1) to give a diastereomeric mixture **8** (90.6 mg, 77%) as an oil.

A solution of **8** (90.6 mg, 0.28 mmol) in MeOH (5 ml) in the presence of 10% Pd–C was stirred at ambient temp. for 30 min under a hydrogen atmosphere. After filtration, the filtrate was evaporated to give a residue, which was subjected to a silica gel cc (PhH:EtOAc 3/1) to give **9** (15.6 mg, 23%) and **10** (44.6 mg, 65%) as diastereomeric mixtures. **9**: IR: δ_{max} 3369, 1589 cm^{-1}; ^1H NMR: δ 1.22–1.39 (9H, *m*), 1.60–2.20 (4H, *m*), 2.22 (3H, *m*), 2.73 (1H, *brs*), 3.23–3.31 (1H, *m*) 3.35–3.45 (0.5H, *m*), 3.84 (0.5H, *dd*, $J = 5.4$, 11.3 Hz), 6.52 (1H, *d*, $J = 8.8$ Hz) 6.70 (1H, *d*, $J = 8.8$ Hz); ^{13}C NMR: δ 11.8, 12.0, 17.0, 19.8, 24.1, 24.5, 24.7, 25.8, 26.3, 26.9, 27.8, 31.2, 32.7, 33.0, 49.08, 49.14, 72.6, 88.1, 90.5, 112.8, 112.9, 117.8, 119.1, 122.1, 140.2, 149.8, 152.7. Found: *m/z* 250.1565. Calc. for $C_{15}H_{22}O_3$: M^+, 250.1567. **10**: IR: δ_{max} 3367, 1508 cm^{-1}; ^1H NMR: δ 1.25–1.31 (9H, *m*), 1.67–2.09 (5H, *m*), 2.16 (1.5H, *s*), 2.18 (1.5H, *s*), 2.73 (1H, *brs*), 2.85–3.04 (1H, *m*), 3.24 (0.5H, *dd*, $J = 3.2$, 10 Hz), 3.30 (0.5H, $J = 1.2$, 11 Hz), 4.77 (1H, broad *s*), 6.54 (0.5H, *s*), 6.61 (0.5H, *s*), 6.73 (0.5H, *s*), 6.76 (0.5H, *s*); ^{13}C NMR: δ 15.4, 18.7, 20.3, 24.42, 24.43, 24.5, 25.6, 26.2, 26.3, 31.0, 31.8, 34.3, 34.4, 38.5, 72.5, 72.7, 89.7, 90.4, 112.6, 115.7, 122.0, 122.8, 123.5, 138.1, 149.5, 151.5. Found: *m/z* 250.1564. Calc. for $C_{15}H_{22}O_3$: M^+, 250.1567.

3.2. Bioassay

Effects for coleoptile and root growth of oat (Avena sativa L.). Ten seeds of oat were put on a dish, in which 1 ml of a test solution was added. After incubation at 25 °C for 3 days in the dark, length of coleoptiles and roots of the seedlings was measured. Oat incubated without test samples during the same period were used as control.

Effects for hypocotyls and root growth of cress (Lepidium sativum L.). Ten seeds of cress in the presence of a 0.5 ml solution of a test sample were incubated at 23 °C in the dark. After 2 days, length of roots was measured and hypocotyls were measured after incubated one more day.

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