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Terpenes from *Eupatorium adenophorum* and Their Allelopathic Effects on *Arabidopsis* Seeds Germination[†]

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The invasive plant *Eupatorium adenophorum* Spreng. (or *Ageratina adenophora* (Spreng.) King and Robinson) (Compositae) has caused great economic loss in China, especially the southwestern region, and is gravely threatening the native biodiversity. The aerial part of this plant was phytochemically investigated for its allelochemicals. Eleven terpenes (2 monoterpenes and 9 sesquiterpenes) were isolated and identified, which include a new monoterpene, (−)-(1*R**,2*S*,*4*R**,5*S**)-3,3-dimethyl-5-hydroxybicyclo[2,2,1]hept-2-ylmethanol (**1**), two new cadinane sesquiterpenes, (−)-(5*S**,6*S**,7*S**,9*R**,10*S**)-7-hydroxy-5,7-epidioxycadinan-3-ene-2-one (**2**) and (+)-(5*S**,6*R**,9*R**,10*S**)-5,6-dihydroxycadinan-3-ene-2,7-dione (**3**), and eight known terpene compounds (**4**, **6**−**12**). The new structures were established by spectroscopic studies such as 1D- and 2D-NMR and MS analyses. Meanwhile, the potential allelopathic effects of these compounds on the *Arabidopsis* seeds germination were tested. Compounds **3** and **7** retarded the *Arabidopsis* seeds germination at 0.5 mM and 1.0 mM concentrations, respectively, while other compounds showed no obvious inhibitory effects.

KEYWORDS: *Eupatorium adenophorum*; cadinane sesquiterpene; monoterpene; allelopathy; seed germination; *Arabidopsis*

INTRODUCTION

Eupatorium adenophorum Spreng. (or *Ageratina adenophora* (Spreng.) King and Robinson) (Compositae) is native to Mexico, but it is known in many other parts of the world as an introduced species and often a noxious weed. *E. adenophorum* was first introduced to Yunnan province of China around 1940. It is rapidly spreading in ecological terms as an invasive species and is one of the most economically destructive weeds in Southwest China and therefore is regarded as "Mexican Devil" in China. It has been proposed that the successful invasion of *E. adenophorum* is mostly due to its allelopathic strategy (*1, 2*), and an increasing number of studies have shown that *E. adenophorum* interferes with the growth and establishment of native plants via allelopathy. For example, Tripathi et al. thought that *E. adenophorum* might adversely affect other nearby vegetation by releasing inhibitors (*3*); Li et al. reported that aqueous extracts of the leaf, stem, and root of *E. adenophorum* had allelopathic effects on seedling growth of five species of the family Gesneriaceae (*4*); Zheng et al. found that aqueous extracts of the leaf of *E. adenopherum* also inhibited seed germination and seedling growth of another five herbaceous species (*5*). A study by Zhang et al. displayed that aqueous leachates from the root of *E. adenophorum* have an obvious allelopathic effect (*6*). However, so far the compounds involved in allelopathy are largely unknown. Different types of natural compounds such as terpenes (mono-, sesqui-, di-, and triterpenes), phenolics (flavonoids, phenylpropanoids, and coumarins), and alkaloids have been found in *E. adenophorum* (*7, 8*), which are potential allelochemicals of the plant. Recent studies revealed that the cadinane sesquiterpenes played a role in the allelopathy of *E. adenophorum* (*1, 2*). In our study of the allelochemicals of *E. adenophorum*, eleven terpenes (2 monoterpenes and 9 sesquiterpenes) were isolated and identified from its aerial part, which include a new monoterpene, (−)-(1*R**,2*S**,4*R**,5*S**)-3,3-dimethyl-5-hydroxybicyclo[2,2,1]hept-2-ylmethanol (**1**), two

[†] This paper is dedicated to Professor Han-Dong Sun on the occasion of his 70th birthday.

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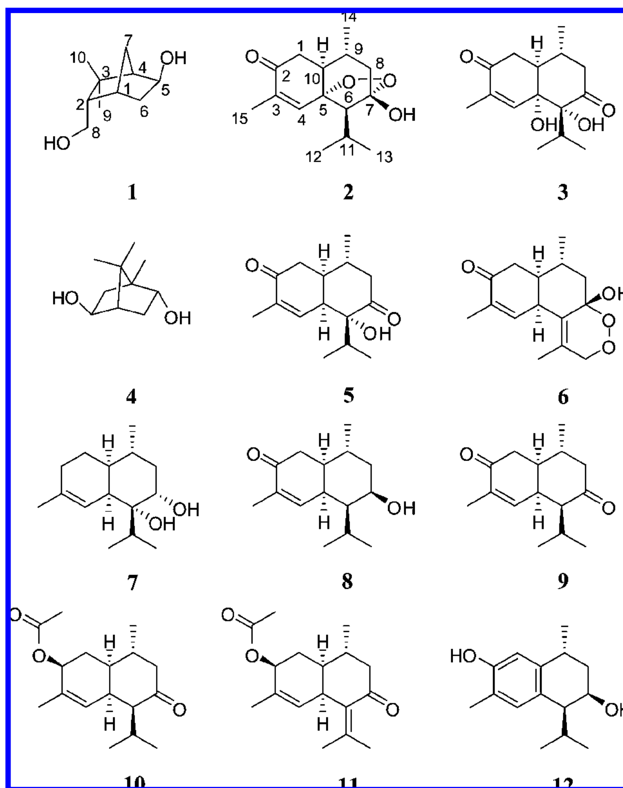
Table 1. ^{1}H and ^{13}C NMR Spectral Data of Compounds **1**−**3**[a]

	1[b]		**2**[c]		**3**[c]	
	δ_C	δ_H mult (*J*)	δ_C	δ_H mult (*J*)	δ_C	δ_H mult (*J*)
1a	39.8 d	2.23 m	38.4 t	2.60 dd (16.4, 4.0)	42.8 t	2.80 dd (15.2, 4.0)
1b				2.45 dd (16.4, 12.8)	197.2 s	2.32 dd (15.2, 14.0)
2	52.2 d	1.50 m	198.8 s			
3	36.1 s		141.3 s		141.3 s	
4	58.0 d	1.66 br s	134.7 d	6.63 d (1.2)	141.8 d	6.29 d (1.2)
5	69.7 d	4.02 d (6.4)	86.0 s		70.9 s	
6a	34.0 t	1.84 m	62.6 d	2.20 d (2.0)	72.5 s	
6b		1.06 m				
7a	33.4 t	1.62 br d (9.6)	108.8 s		207.2 s	
7b		1.44 br d (9.6)				
8a	60.6 t	3.47 dd (10.0, 7.6)	48.0 t	2.07 dd (12.8, 6.0)	43.3 t	2.89 dd (12.0, 13.2)
8b		3.44 dd (10.0, 8.8)		1.77 dd (12.8, 12.4)		2.01 dd (12.0, 4.4)
9	20.0 q	0.83 s	32.1 d	2.20 m	39.0 d	1.75 m
10	33.0 q	0.98 s	48.7 d	1.85 m	41.1 d	2.41 m
11			25.7 d	2.37 d spt (7.2, 2.0)	28.9 d	1.87 spt (7.2)
12			19.5 q	1.34 d (7.2)	17.1 q	1.21 d (7.2)
13			21.3 q	1.31 d (7.2)	18.4 q	1.19 d (7.2)
14			18.3 q	1.03 d (6.4)	21.1 q	1.07 d (6.8)
15			16.4 q	1.85 d (1.2)	15.9 q	1.87 d (1.2)

[a] Data were measured at 400 MHz for ^{1}H and 100 MHz for ^{13}C with reference to the solvent signals, δ in ppm and *J* in Hz. [b] Recorded in acetone-d_6. [c] Recorded in $CDCl_3$.

new cadinane sesquiterpene derivatives, (−)-(5*S**,6*S**,7*S**,9*R**,10*S**)-7-hydroxy-5,7-epidioxycadinan-3-ene-2-one (**2**) and (+)-(5*S**,6*R**,9*R**,10*S**)-5,6-dihydroxycadinan-3-ene-2,7-dione (**3**), and eight known terpene compounds (**4** and **6**−**12**). Here we report the isolation and structure elucidation of these terpenes and their potential allelopathic effects on the *Arabidopsis* seeds germination tested.



MATERIALS AND METHODS

General Experimental Procedures. Column chromatography was performed on a 200−300 mesh silica gel (Qingdao Marine Chemical Factory, P. R. China). Optical rotations were obtained on a Horiba-SEAP-300 spectropolarimeter. UV spectroscopic data were measured on a Shimadzu-210A double-beam spectrophotometer. IR spectra of samples in KBr discs were recorded on a Bruker-Tensor-27 spectrometer with KBr pellets. NMR spectra were carried out on either a Bruker AM-400 or a DRX-500 spectrometer with TMS as internal standard. MS were recorded on a VG-Auto-Spec-3000 spectrometer.

Plant Material. The aerial part of *E. adenophorum* was collected from the Botanic Garden of Kunming Institute of Botany, Chinese Academy of Sciences, in November 2007, and was identified by one of the authors (S.-H.L.). A voucher specimen (EA-07-11) was deposited in the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, P. R. China. The seeds of *Arabidopsis thaliana* Columbia ecotype were from Dr. Xuemin Wang's Laboratory (*9*).

Extraction and Isolation. The fresh aboveground part of *E. adenophorum* (10 kg) was cut into small pieces and immediately extracted with petroleum ether (3 × 25 L), which was evaporated to dryness to give 120 g of oily extract. The petroleum ether extract (120 g) was subjected to column chromatography over silica gel, eluting with a gradient of increasing $CHCl_3$ in petroleum ether (0−100%), to afford five fractions, Fr.B1 ($CHCl_3$−petroleum ether 10:90, 2 L), Fr.B2 ($CHCl_3$−petroleum ether 30:70, 2 L), Fr.B3 ($CHCl_3$−petroleum ether 50:50, 2 L), Fr.B4 ($CHCl_3$−petroleum ether 70:30, 2 L), and Fr.B5 ($CHCl_3$−petroleum ether 100:0, 2 L). Fr.B3 was repeatedly chromatographed on a silica gel column, eluting with $CHCl_3$−petroleum ether (1:1) to yield compounds **2** (6 mg), **3** (4 mg), **6** (10 mg), **7** (4 mg), **8** (4 mg), **9** (50 mg), **10** (50 mg), and **11** (40 mg). The plant residue was air-dried and further extracted with MeOH (3 × 25 L). The MeOH extract was concentrated and partitioned between H_2O and AcOEt (3 × 2.5 L). The AcOEt layer was dried to give 20 g of extract, which was subjected to column chromatography over silica gel, eluting with a gradient of increasing MeOH in $CHCl_3$ (0−100%), to afford seven fractions, Fr.A1 ($CHCl_3$−MeOH 5:95, 2 L), Fr.A2 ($CHCl_3$−MeOH 10:90, 2 L), Fr.A3 ($CHCl_3$−MeOH 15:85, 2 L), Fr.A4 ($CHCl_3$−MeOH 20:80, 2 L), Fr.A5 ($CHCl_3$−MeOH 30:70, 2 L), Fr.A6 ($CHCl_3$−MeOH 40:60, 2 L), and Fr.A7 ($CHCl_3$−MeOH 0:100, 1 L). Fr.A1 was further chromatographed on a silica gel column, eluting with $CHCl_3$−MeOH (2:98, 3 L) to yield four subfractions (Fr.A1−1−Fr.A1−4). Fr.A1−1 was rechromatographed over a silica gel column, eluting with $CHCl_3$−Me_2CO (7:1, 1 L) to yield compounds **1** (3 mg), **4** (8 mg), and **12** (2 mg).

(−)-(1R,2S*,4R*,5S*)-3,3-Dimethyl-5-hydroxybicyclo[2,2,1]hept-2-ylmethanol (***1***).* Colorless oil; $[\alpha]_D^{27}$ −16 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.75) nm; IR (KBr) ν_{max} 3331, 2961, 1465, 1041 cm^{-1}; ^{1}H NMR and ^{13}C NMR see **Table 1**; EIMS: *m/z* 152 (14), 137 (35), 109 (50), 108 (75), 95 (85), 93 (75), 83 (50), 69 (100), 67 (60); ESIMS: *m/z* 193 [M + Na]$^+$ (23), 169 [M − H]$^+$ (6), 153 [M − H_2O + H]$^+$

(38), 135 $[M - 2H_2O + H]^+$ (69), 107 (38), 93 (44), 88 (100), 74 (88); HRESIMS: *m/z* 193.1200 $[M + Na]^+$ (calcd for $C_{10}H_{18}O_2Na$, 193.1204).

(−)-(5S,6S*,7S*,9R*,10S*)-7-Hydroxy-5,7-epidioxycadinan-3-ene-2-one* (**2**). Colorless oil; $[\alpha]_D^{27}$ −85 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 284 (5.76), 227 (6.64) nm; IR (KBr) ν_{max} 3424, 2963, 2927, 1667, 1383, 1363, 1231 cm^{-1}; ^1H NMR and ^{13}C NMR see **Table 1**; EIMS: *m/z* 266 $[M]^+$ (20), 250 $[M - O]^+$ (5), 233 $[M - 2O - H]^+$ (15), 192 (20), 191 (100), 179 (30), 151 (30), 137 (35), 121 (30), 69 (34); ESIMS: *m/z* 289 $[M + Na]^+$, (100), 267 $[M + H]^+$ (55), 249 $[M - H_2O + H]^+$ (20), 231 $[M - 2H_2O + H]^+$ (5), 165 (15); HRESIMS: *m/z* 267.1594 $[M + H]^+$ (calcd for $C_{15}H_{23}O_4$, 267.1596).

(+)-(5S,6R*,9R*,10S*)-5,6-Dihydroxy-cadinan-3-ene-2,7-dione* (**3**). Colorless oil; $[\alpha]_D^{27}$ +173 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 293 (5.42), 247 (6.78) nm; IR (KBr) ν_{max} 3421, 2963, 2931, 1716, 1684, 1459, 1383 cm^{-1}; ^1H NMR and ^{13}C NMR see **Table 1**; EIMS: *m/z* 248 $[M - H_2O]^+$ (5), 206 (15), 205 (100), 177 (65), 149 (30), 135 (45), 71 (34), 57 (40); ESIMS: *m/z* 289 $[M + Na]^+$ (10), 271 $[M + Na - H_2O]^+$ (51), 267 $[M + H]^+$ (1), 229 (100), 139 (10); HRESIMS: *m/z* 289.1416 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_4Na$, 289.1415).

Seed Germination Bioassay. *Arabidopsis* seeds were washed with ethanol (70% v/v) for 2 min and surface sterilized using sodium hypochlorite (0.5% v/v) for 2 min, followed by three washes with sterile distilled water. After surface sterilization, seeds were stored in a refrigerator at 4 °C for 3 days before use. Three layers of filter paper were put in 6-cm-diameter glass Petri dishes, and the filter papers were impregnated with compounds dissolved in 3 mL of acetone. Compounds **1**−**3** were administered at 0.5 mM concentration, and other compounds were administered at 1.0 mM concentration. To avoid toxic effects of the organic solvent, filter paper treated with acetone solution was placed in a fume hood for 1 h to allow complete solvent evaporation (*10*). Subsequently, 3 mL of Hoagland solution (*11*) was added to each piece of filter paper in each Petri dish. Forty *Arabidopsis* seeds were evenly placed on the moist filter paper in each Petri dish. Two controls (filter paper treated with 3 mL of acetone and filter paper without any treatment) were set. Each treatment had three duplicates. Seeds were allowed to germinate under 12 h light and 12 h dark at 23 °C (day) and 18 °C (night). The light intensity in the growth chamber was 100 μmol m^{-2} s^{-1}. The number of germinated seeds was tracked day by day until most seeds (≥95%) in the control Petri dishes were germinated.

RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless oil. Its high resolution ESI mass spectrum displayed a $[M + Na]^+$ ion peak at *m/z* 193.1200, indicating a molecular formula of $C_{10}H_{18}O_2$. An absorption band at 3331 cm^{-1} in the IR spectrum was observed, suggesting the existence of hydroxyl groups. The ^1H NMR spectrum of compound **1** (**Table 1**), coupled with the $^1H-^1H$ COSY and HSQC spectra, showed two methyls at δ 0.83 and 0.98 (each 3H, Me-9 and 10), one oxymethine at δ 4.02 (1H, H-5), one oxymethylene at δ 3.47 (1H, H-8a) and δ 3.44 (1H, H-8b), three methines at δ 2.23 (1H, H-1), 1.66 (1H, H-4), and 1.50 (1H, H-2), and two methylenes at δ 1.62 (1H, H-7a), 1.44 (1H, H-7b) and δ 1.84 (1H, H-6a), 1.06 (1H, H-6b). In the ^{13}C NMR and DEPT spectra (**Table 1**), 10 carbons including two methyls, two methylenes, one oxy-methylene, three methines, one oxy-methine, and one quaternary carbon were displayed, which were in agreement with the above observations and suggested that compound **1** was a monoterpene. Careful comparison of the NMR data of **1** with those of a known monoterpene compound, *endo*-camphanol (*12, 13*), revealed that the two compounds were similar except that **1** had one more hydroxyl group than *endo*-camphanol. Analysis of correlations observed in the HMBC spectrum (**Figure 1**) allowed the position of the hydroxyl to be elucidated. The H-5 proton signal showed $^1H-^{13}C$ long-range correlations with C-1, C-3, and C-7, indicating that the additional hydroxyl group was assignable to C-5. The relative configuration of C-2 was determined as the *endo*-form by comparing the ^{13}C NMR data of Me-9 and Me-10 and the coupling pattern between H-1 and H-2 with those of the known *endo*-camphanol and *exo*-camphanol (*12, 13*). The ROESY correlation between H-5 and Me-9 established the relative stereochemistry of 5-OH as the *exo*-form. Compound **1** was therefore identified as (−)-(1*R**,2*S**,4*R**,5*S**)-3,3-dimethyl-5-hydroxybicyclo[2,2,1]hept-2-yl-methanol.

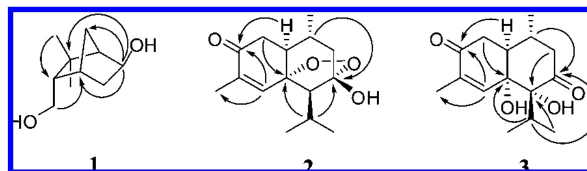


Figure 1. Significant correlations of compounds **1**−**3** in HMBC spectra (H→C).

Compound **2** was obtained as colorless oil and exhibited a purple fluorescence under 254 nm UV light. Accurate mass measurement of an $[M + H]^+$ ion peak at *m/z* 267.1594 in HR-ESI-MS allowed a molecular formula of $C_{15}H_{22}O_4$ to be assigned to compound **2**. IR absorption bands at 3424 cm^{-1} for the hydroxyl group and at 1667 cm^{-1} for the α,β-unsaturated carbonyl group were observed. The ^1H NMR spectrum of compound **2** (**Table 1**) displayed four methyls at δ 1.03 (3H, Me-14), 1.31 (3H, Me-13), 1.34 (3H, Me-12), and 1.85 (3H, Me-15), an olefinic proton at δ 6.63 (1H, H-4), and a number of aliphatic proton signals in the high field region (δ 1.7−2.7). In the ^{13}C NMR and DEPT spectra of **2** (**Table 1**), 15 carbons including four methyls, two methylenes, five methines (including an olefinic one at δ 134.7 d), an olefinic quaternary carbon (δ 141.3, s), a conjugated keto carbonyl carbon (δ 198.8, s), a ketalic carbon (δ 108.8, s), and an oxy-quaternary carbon (δ 86.0, s) were observed. This spectral evidence suggested a sesquiterpene skeleton for **2**. Careful comparison of the NMR data of **2** with those of 9-oxoageraphorone (**9**) (*14, 15*), a major cadinene-type sesquiterpene compound in *E. adenophorum* also isolated in this experiment, revealed that compound **2** was also a cadinene derivative similar to **9**, having an α,β-unsaturated carbonyl group in the A-ring. The major differences between **2** and **9** were at C-5 and C-7. Unlike the case for **9**, oxygenation occurred at C-5 in **2**, as revealed by the absence of the H-5 signal and the presence of the oxy-quaternary carbon (δ 86.0, s). A ketalic group at C-7 was evident for **2** instead of a keto group at the same position in **9**. The $^1H-^{13}C$ long-range correlations (**Figure 1**) from H_2-1 to C-5, from H-9 to C-7, and from H-11 to both C-5 and C-7 in the HMBC spectrum of **2** confirmed the above inference. The EI-MS of **2** showed ion peaks at *m/z* 266 $[M]^+$ (20), 250 $[M - O]^+$ (5), and 233 $[M - 2O - H]^+$ (15), suggesting the existence of a peroxide moiety in **2,** which should only be a peroxide bridge formed between C-5 and C-7. The relative stereochemistry of **2** was established by a 2D ROESY experiment measured in acetone-d_6. The ROESY interaction between 7-OH (δ 5.87, s) and H-9 indicated that 7-OH was β-oriented. Because the only natural C-5 oxygenated cadinene-type sesquiterpene so far isolated from *E. adenophorum*, 8aα-hydroxy-1-isopropyl-4,7-dimethyl-1,2,3,4,6,8a-hexahydronaphthalene-2,6-dione (*16*), was found to be 5α-hydroxylated, the oxygenated functionality at C-5 of **2** was proposed to be also α-oriented. Therefore, the peroxide bridge between C-5 and C-7 was assigned to be in the α-orientation. This was also supported by the absence of ROESY correlations between H-6 and H-10 and Me-14 (in both $CDCl_3$ and acetone-d_6), which was caused by the steric hindrance of the peroxide

bridge. Ultimately, compound **2** was determined as (−)-(5*S**,6*S**,7*S**,9*R**,10*S**)-7-hydroxy-5,7-epidioxycadinan-3-ene-2-one.

Recently, a naturally occurring peroxide cadinane analog, (+)-(5*R*,7*S*,9*R*,10*S*)-7-hydroxy-7,12-epidioxycadinan-3,6(11)-diene-2-one (**6**), was discovered from *E. adenophorum* (*8*), which was also obtained in our experiment. The isolation of new compound **2** implied that the biosynthesis of sesquiterpene peroxides in *E. adenophorum* may not be a coincidence.

Compound **3** was obtained as a colorless oil with a purple fluorescence under 254 nm UV light, possessing a molecular formula of $C_{15}H_{22}O_4$ by HR-ESI-MS (*m/z* 289.1416 $[M + Na]^+$). The IR spectrum indicated the presence of hydroxyl groups (3421 cm^{-1}) and carbonyl groups (1716 and 1684 cm^{-1}). In the ^1H NMR spectrum of compound **3** (**Table 1**), four methyls at δ 1.07 (3H, Me-14), 1.19 (3H, Me-13), 1.21 (3H, Me-12), and 1.87 (3H, Me-15) and an olefinic proton at δ 6.29 (1H, H-4) suggested that **3** was also a cadinene analog similar to **2**. A detailed analysis of its IR, MS, 1D NMR, and 2D NMR spectral data enabled the conclusion to be reached that the structure of **3** was closely comparable to that of **5**, a cadinene-type sesquiterpene isolated from *E. adenophorum* by Bohlmann et al. (*14*). The only difference between these two compounds was that a hydroxyl group could be located at the C-5α position in **3**, as evidenced from the missing characteristic H-5 signal and the observation of HMBC correlations from both H_2-1 and H-11 to C-5 (**Figure 1**). Unambiguous assignments of the ^1H and ^{13}C NMR data were achieved by analysis of all correlations in $^1H-^1H$ COSY, HSQC, and HMBC spectra. Thus, compound **3** was elucidated as (+)-(5*S**,6*R**,9*R**,10*S**)-5,6-dihydroxycadinan-3-ene-2,7-dione.

Eight known terpene compounds isolated from *E. adenophorum* were identified as 5-exohydroxyborneol (**4**) (*17*), 7-hydroxy-7,12-epidioxycadinan-3,6(11)-dien-2-one (**6**), cadinan-3-ene-6,7-diol (**7**) (*8*), 9*β*-hydroxyageraphorone (**8**), 9-oxoageraphorone (**9**) (*14, 18, 19*), 2-deoxo-2-(acetyloxy)-9-oxoageraphorone (**10**), 2-acetyloxy-3,4,6,11-tetradehydrocadinan (**11**) (*15, 20*), and calamenene-3,7-diol (**12**) (*21*), respectively, by comparison of their spectral data (^1H, ^{13}C NMR and MS) with those reported in the literature.

A number of recent studies have shown that invasive species interfere with the growth and establishment of native plants via allelopathy, and more and more allelochemicals such as catechin (*22*), juglone (*23*), apigenin (*24*), *m*-tyrosine (*10*), and gallic acid derivatives (*25*) have been discovered and their mechanisms were investigated. The allelopathic effects of the terpene compounds isolated from *E. adenophorum* were tested on the germination of *Arabidopsis* seeds, which is a model plant being used in our laboratory, using a method as described in the Materials and Methods section considering the limited amounts of substances, and seeds germination rates were recorded after 3 days and 6 days. Only compounds **3** and **7** retarded the *Arabidopsis* seeds germination while the other compounds showed no obvious inhibitory effects (**Figure 2**).

It has been reported that cadinene-type sesquiterpene compounds **8** and **9** inhibited the onion, radish, and cucumber seeds germination and growth (*1*). The allelopathic effect of compounds **9** on rice seedlings was also observed (*2*). These studies suggested that cadinene-type sesquiterpene compounds of *E. adenophorum* contribute at least partly to the inhibition of growth of neighboring plants. However, in our experiment, we did not observe any allelopathic effect for these two terpene compounds on the *Arabidopsis* seeds germination (**Figure 2**). The reason might be that organic solvent such as DMSO was adopted in the reported experiments to help to dissolve the allochemicals, while in our experiment organic solvents were removed completely by evaporation before adding Hoagland medium in order to mimic the natural settings, because water is very likely the only available medium to carry allochemicals from exuding plant to target plant in nature. In this case, the polarity or water solubility of an allochemical is closely related to its allelopathic effect. Therefore, it is not surprising that only compounds **3** and **7** showed certain allelopathic effects while other compounds were not active, because these two compounds are relatively more polar than the other isolated sesquiterpene compounds from a chemical structural point of view due to the availability of two hydroxyl groups. Monoterpenes **1** and **4**, however, are not allelochemicals of *E. adenophorum* although they also contain two hydroxyl groups (**Figure 2**).

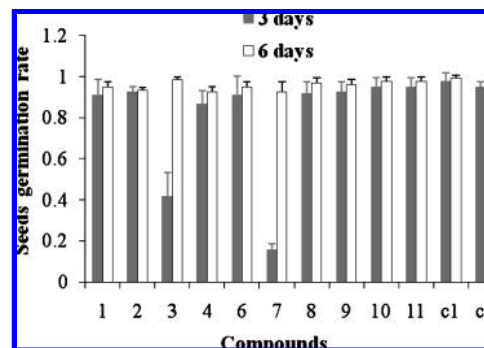


Figure 2. *Arabidopsis* seeds germination rates in Hoagland medium containing one of the isolated compounds **1**−**11** (**1**−**3**: 0.5 mM; **4**−**11**: 1.0 mM) or nothing (**c1**: control 1, filter paper treated with 3 mL of acetone; **c2**: control 2, filter paper without treatment) in 3 and 6 days. Values are means ± s.d. from three independent experiments (40 seeds per treatment). Seeds germination rate = (number of germinated seeds/40) × 100%.

It is worthwhile to note that allelochemicals released from plant species have to be available to exert an allelopathic effect in soil media. In our experiment, a bioassay was carried out with Petri dishes with filter paper instead of pot cultures with soil because of the sample amounts reason. However, we have attempted to explore the allelopathic mechanism of sesquiterpenes of *E. adenophorum* through HPLC analysis of their distribution in natural settings. In principle, there are four possible ways that leaf terpenes of *E. adenophorum* could go into the soil in natural ecosystems: (1) as volatiles through the air; (2) as leachates by rain bleaching; (3) as root exudates through stem transportation; and (4) in the old and dead leaves directly falling down. However, our preliminary experiments suggested that, although some sesquiterpenes we isolated did exist in leaf leachate, stem and root, and old and dead leaves, none of them are detectable in the rhizosphere of *E. adenophorum*. A plausible explanation might be that these sesquiterpenes are easily and rapidly decomposed or metabolized by the microorganisms in the soil. Therefore, terpene compounds of *E. adenophorum* seem not to act as the major allelochemicals of this plant during its invasion. In conclusion, our results suggest that cadinene-type sesquiterpenes are only part of the allelochemicals in *E. adenophorum*; further nonterpene substances with stronger allelopathic effects in this invasive plant should still be possible and worthy of further bioassay-directed investigation.

Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**−**3**. This information is available free of charge via the Internet at http://pubs.acs.org.

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