

Defensive Sesquiterpenoids from Leaves of *Eupatorium adenophorum*[†]

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The invasive plant *Eupatorium adenophorum* Spreng has caused great economic loss in China, and is gravely threatening the native biodiversity and ecosystem. The plant has been phytochemically investigated for the defensive chemical substances in its leaves. Three active sesquiterpenoids were isolated and identified, which include a new sesquiterpenoid (**1**), and two known sesquiterpenoids (**2**, **3**). Their structures were established by spectroscopic studies such as 1D- and 2D-NMR and MS analyses. Meanwhile, the antifeedant activities of these compounds against two generalist plant-feeding insects, *Helicoverpa armigera* and *Spodoptera exigua*, were carried out. Compound **1** showed significant antifeedant activity against *S. exigua* with EC₅₀=7.46 µg/cm², while compounds **2** and **3** were more active against *H. armigera* (EC₅₀=2.57 and 3.04 µg/cm² respectively). These findings suggest a defensive role of sesquiterpenoids in *E. adenophorum* against herbivores.

Keywords *Eupatorium adenophorum*, leaves, chemical defense, sesquiterpenoids, antifeedant activity

Introduction

Being a perennial herbaceous invasive weed, *Eupatorium adenophorum* Spreng or *Ageratina adenophora* (Spreng), originally indigenous to Central America and mainly Mexico, has invaded around 30 countries in tropical and subtropical zones. Since 1950s, *E. adenophorum* has spread rapidly across southwest China, resulting in serious economic loss and threatening native biodiversity, ecosystem integrity, and even the health of humans and livestock.^[1] Therefore this plant is also called “Green Killer” or “Mexican Devil”. Studies of *E. adenophorum* mainly focused on its natural distribution, biological character, hazard, potential utilization and control.^[1,2] Phytochemically, various types of compounds have been isolated from *E. adenophorum*, including mono-, sesqui-, di-, and triterpenoids, sterols, flavonoids, phenylpropanoids, coumarins, alkaloids, and so on,^[3–8] and different biological activities of some of these constituents have also been reported.^[7–10] However, few studies related to the chemical defense of *E. adenophorum* have been carried out. We have investigated the defensive chemical substances in its leaves. As a result, a new sesquiterpenoid, 3,8-oxo-7 α -hydroxy-5,11(12)-tetradehydrocadinanene (**1**), together with two known sesquiterpenoids, 9-oxo-10,11-dehydroageraphorone (**2**)^[10] and muurol-4-en-3,8-dione (**3**)^[5] were

isolated. Their structures were determined by spectroscopic evidence. A dual-choice bioassay was carried out on *Helicoverpa armigera* and *Spodoptera exigua* to test their antifeedant activity.

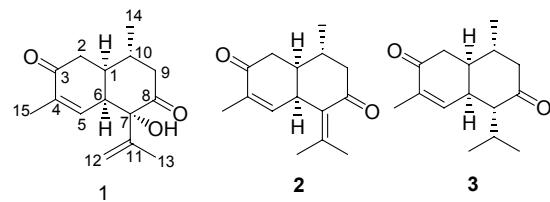


Figure 1 Chemical structures of compounds **1**–**3**.

Results and Discussion

Compound **1** was obtained as colorless oil. It exhibited a purple fluorescence under 254 nm UV light. Accurate mass measurement of an [M]⁺ ion peak at *m/z* 248.1407 (calcd 248.1412) in HR-EI-MS allowed a molecular formula of C₁₅H₂₀O₃ to be assigned to compound **1**. IR absorption bands at 3432 cm^{−1} for hydroxyl group, 1724 cm^{−1} for carbonyl group, and 1641 cm^{−1} for double bond were observed. The ¹H NMR spectrum of compound **1** (Table 1) displayed two tertiary methyls at δ 1.66 (s, 3H) and 1.83 (s, 3H), a secondary methyl at δ 1.01 (d, 3H), two olefinic methylene protons at δ 5.11

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(s) and 5.12 (s), an olefinic methine proton at δ 6.49 (s), and a number of aliphatic proton signals in the high field region (δ 1.95—3.36). In the ^{13}C NMR and DEPT spectra of **1** (Table 1), 15 carbons consisting of three methyls, three methylenes (including an olefinic one at δ 115.6), four methines (including an olefinic one at δ 142.3), an oxy-quaternary carbon at δ 81.7, two olefinic quaternary carbons at δ 136.8 and 145.2, and two keto carbonyl carbons at δ 197.5 and 211.4 were found. These spectral evidence suggested a sesquiterpenoid skeleton for **1**. Careful comparison of the NMR data of **1** with those of 9-oxo-10,11-dehydroageraphorone (**2**), a major cadinene-type sesquiterpenoid in *E. adenophorum*,^[10] which was also isolated in this experiment, revealed that compound **1** was also a cadinene derivative similar to **2**, having an α,β -unsaturated keto moiety. The major difference between **1** and **2** was that the tetra-substituted double bond between C-7 and C-11 in **2** was replaced by a di-substituted terminal double bond in **1**. In addition, **1** was one methyl group less than **2** while an oxygenated quaternary carbon more than **2**. Moreover, an isolated keto group in **1** took the place of one of conjugated keto groups in **2**. All these changes suggested that the $\Delta^{7,11}$ double bond in **2** shifted to $\Delta^{11,12}$ in **1** and C-7 was further hydroxylated, which were supported by HMBC correlations from 7-OH to C-6, C-7 and C-8, and from H₂-12 to C-7, C-11 and C-13 (Table 1). The relative stereochemistry of **1** was established by a ROESY experiment measured in acetone-*d*₆. The ROESY correlations of H-6 (δ 3.36, s) with H-1 and 7-OH, and Me-14 with H-1, together with the bio-

genetic consideration, suggested that H-1, H-6, 7-OH and Me-14 were all α -oriented. Therefore, compound **1** was determined as shown in Figure 1, and was named 3,8-oxo-7 α -hydroxy-5,11(12)-tetradehydrocadinanene.

Two known cadinene-type sesquiterpenoids were also isolated from the leaves of *E. adenophorum*. Through comparison of their MS, ^1H and ^{13}C NMR data with those reported in the literatures,^[5,10] they were identified as 9-oxo-10,11-dehydroageraphorone (**2**) and muurol-4-en-3,8-dione (**3**) respectively.

The antifeedant activity of all three compounds against two generalist plant-feeding insects, the cotton bollworm (*H. armigera*) and the beet armyworm (*S. exigua*), was assayed as described previously,^[22] with the exception of antifeedant activity for **1** against *H. armigera* due to insufficient sample. It was found (Table 2) that compound **1** showed significant antifeedant activity against *S. exigua*, with EC₅₀ of 7.46 $\mu\text{g}/\text{cm}^2$, and was more active than compounds **2** and **3** (EC₅₀=14.81 and 14.60 $\mu\text{g}/\text{cm}^2$ respectively). In addition, compounds **2** and **3** exhibited potent antifeedant activity against *H. armigera*, with EC₅₀ of 2.57 and 3.04 $\mu\text{g}/\text{cm}^2$, respectively.

E. adenophorum has been reported to be rich in sesquiterpenoids with various biological activities.^[5-17] However, most of these experiments were carried out at crude extract level,^[18,19] and the active compounds are basically not clear. A sesquiterpenoid, isolated from the leaves of *E. adenophorum*, (−)-(5*R*,6*R*,7*S*,9*R*,10*S*)-cadinan-3-ene-6,7-diol, was found to show *in vitro*

Table 1 ^1H and ^{13}C NMR spectral data of compound **1**^a

Position	δ_{H}	δ_{C}	$^1\text{H}-^1\text{H}$ COSY	HMBC	ROESY
1	2.64—2.68, m	40.2, d	H ₂ -2, H-6, H-10	C-2, C-6, C-10	H-6
2a	2.61, d, (4.0)	41.7, t	H-1, H-2b	C-1, C-3, C-4, C-6, C-10	
2b	2.57, d, (4.4)		H-1, H-2a	C-1, C-3, C-6, C-10	
3		197.5, s			
4		136.8, s			
5	6.49, s	142.3, d	Me-15		H ₂ -12
6	3.36, br s	48.8, d	H-1, Me-15		H-1, 7-OH
7		81.7, s			
8		211.4, s			
9a	2.85—2.89, m	45.7, t	H-9b, H-10	C-1, C-8, C-10, C-14	
9b	2.10—2.13, m		H-9a, H-10		
10	1.93—1.96, m	33.4, d	H-1, H ₂ -9, Me-14	C-1, C-14	
11		145.2, s			
12a	5.11, s				
12b	5.12, s	115.6, t	Me-13	C-7, C-11, C-13	H-6
13	1.83, s (3H)	20.2, q	H-12	C-7, C-11, C-12	
14	1.01, d (3H, 6.5)	20.1, q	H-10	C-1, C-9, C-10	H-1
15	1.66, s (3H)	15.9, q	H-5, H-6	C-3, C-4, C-5	
7-OH	4.83, s			C-6, C-7, C-8	H-6

^a Data were measured at 400 MHz for ^1H and 100 MHz for ^{13}C with reference to the solvent signals, J in Hz. Recorded in acetone-*d*₆.

Table 2 Antifeedant activity of compounds **1**–**3** from *E. adenophorum* ($EC_{50} = \mu\text{g}/\text{cm}^2$)

	Molecular formula	Molecular weight	<i>H. armigera</i>	<i>S. exigua</i>
1	$C_{15}H_{20}O_3$	248	—	7.46
2	$C_{15}H_{20}O_2$	232	2.57	14.81
3	$C_{15}H_{22}O_2$	234	3.04	14.60

cytotoxicity against the HCT-8, Bel-7402, and A2780 cancer cell lines.^[15] Some sesquiterpenoids, for example 9-oxo-ageraphorone and 9 β -hydroxy-ageraphorone, have been reported to be allelopathic substance of *E. adenophorum*.^[16,20,21] Our previous work showed that a few sesquiterpenoids isolated from *E. adenophorum* did exhibit slight allelopathic effect on *Arabidopsis* seeds germination, but the above-mentioned allelopathic sesquiterpenoids were not active at all.^[17] 9-Oxo-10,11-dehydroageraphorone, another sesquiterpenoid from *E. adenophorum*, has been showed to be toxic to animals,^[10] suggesting a defensive role of the compound in plant against herbivores. However, the actual natural function of the diversified sesquiterpenoids in *E. adenophorum* remained largely to be investigated. In the present study, purified sesquiterpenoids were employed to explore their biological activity against *H. armigera* and *S. exigua*, and all compounds exhibited significant antifeedant activity. These findings, together with those reported in literatures, allow us to conclude that sesquiterpenoids in *E. adenophorum* should serve as constitutive defense substances against herbivorous insects and animals, which will be helpful to disclose the invasion mechanism of *E. adenophorum*.

Experimental

General experimental details and plant material

Optical rotation was measured on a Horiba-SEAP-300 spectropolarimeter. MS was carried out on an AutoSpec Premier P776 spectrometer. UV spectral data were obtained on a Shimadzu-210A double-beam spectrophotometer. IR spectrum was recorded on a Bruker-Tensor-27 spectrometer with KBr pellet. NMR experiments were carried out on either a Bruker AV-400 or a DRX-500 spectrometer with TMS as internal standard. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Company, Qingdao, China) and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd); The TLC sheets were checked for spots spraying with 15% (*V/V*) H_2SO_4 : EtOH followed by heating.

Plant material Leaves of *E. adenophorum* were collected from plants growing in the Botanical Garden of Kunming Institute of Botany, Chinese Academy of Sciences, in June 2011.

Extraction and isolation 1 kg fresh leaves of *E. adenophorum* were immersed in petroleum ether (10 L) for 12 h at room temperature. Evaporation of the extract

gave a 12 g residue. The extract was subjected to column chromatography over silica gel, eluting successively with chloroform/acetone (from 10 : 0, 9 : 1, 8 : 2, 0 : 10, *V/V*) and finally methanol to give five fractions A–E. Fraction B (3.7 g) was subjected to silica gel column chromatography using solvents of petroleum ether/ethyl acetate (35 : 1, *V/V*) to afford five subfractions B1–B5. Subfraction B3 (1.2 g) was further chromatographed on a silica gel column using solvents of petroleum ether/ethyl acetate (15 : 1, *V/V*) and then purified by Sephadex LH-20 column eluted with acetone to yield compounds **2** (43 mg) and **3** (11 mg). Fraction C (0.7 g) was subjected to silica gel column chromatography using solvents of petroleum ether/acetone (35 : 1, *V/V*) to afford six subfractions C1–C6. Subfraction C4 (36 mg) was further chromatographed on a silica gel column eluted with petroleum ether/ethyl acetate (10 : 1, *V/V*) and then purified by Sephadex LH-20 column eluted with acetone to yield compound **1** (4 mg).

Compound **(1)**: colorless oil; $[\alpha]_D^{22} +4.5$ ($c=0.1$, MeOH); UV (MeOH) λ_{max} : (log ε): 202 (3.48), 224 (3.40) nm; IR (KBr) ν_{max} : 3432, 2923, 2852, 1724, 1641, 1457, 1380, 1167 cm^{-1} ; EI-MS m/z (%): 248 (4) [M]⁺, 247 (8) [M-H]⁺, 238 (29), 219 (15), 202 (45), 177 (30), 151 (45), 135 (31), 121 (30), 109 (42), 91 (45), 81 (36), 69 (100), 57 (76); HR-EI-MS m/z : 248.1407 [M]⁺ (m/z calcd for $[C_{15}H_{20}O_3]^+$ 248.1412).

Insects Larvae of *H. armigera* and *S. exigua* were purchased from Plot-scale Base of Bio-pesticides, Institute of Zoology, Chinese Academy of Sciences. The larvae were reared on an artificial diet in the laboratory under controlled photoperiod (light : dark, 12 : 8 h) and temperature (25 ± 2 °C). Larvae were starved for 4–5 h prior to each bioassay.

Larval antifeedant assay A dual-choice bioassay modified from previous methods^[23] was employed for antifeedant tests. The insects were second instar cotton bollworm (*H. armigera*) and beet armyworm (*S. exigua*). Fresh leaf discs were cut from *Brassica chinensis* by a cork borer (0.9 mm in diameter). Treated leaf discs were painted with 10 μL of acetone solution containing the testing compound, while control leaf discs were painted with the same amount of acetone. After air drying, two test leaf discs and two control ones were set in alternating positions in the same Petri dish (90 mm in diameter), with moistened filter paper at the bottom. Two second instars were placed at the center of the Petri dish. After feeding for 24 h, areas of leaf discs consumed were measured. The antifeedant index (AFI) was calculated according to the formula $AFI = [(C - T)/(C + T)] \times 100$, where C and T represent the control and treated leaf areas consumed by the insect. The insect antifeedant potency of test compound was evaluated in terms of the EC_{50} value (the effective dosage for 50% feeding reduction) which was determined by Probit analysis for each insect species. For each compound, five concentrations were tested with five replicates for each treatment.

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References

- [1] Qiang, S. *J. Wuhan Bot. Res.* **1998**, *16*, 366.
- [2] Lu, P.; Sang, W. G.; Ma, K. P. *J. Plant. Ecol.* **2006**, *29*, 1029.
- [3] Yan, Q. S.; Yang, J.; Li, H. M.; Cao, A. C.; Chen, Q. H.; Wen, Y. Q.; He, L. *J. Beijing Normal Univ.* **2006**, *42*, 70.
- [4] Li, Y. M.; Li, Z. Y.; Ye, M. *J. Yunnan Agric. Univ.* **2008**, *23*, 42.
- [5] Weyerstahl, P.; Marschall, H.; Seelmann, I.; Kaul, V. K. *Flav. Fragr. J.* **1997**, *12*, 387.
- [6] Padalia, R. C.; Bisht, D. S.; Joshi, S. C.; Mathela, C. S. *F. J. Essent. Oil Res.* **2009**, *21*, 522.
- [7] Adhikari, R.; Kraus, W. *J. Nepal Chem. Soc.* **1994**, *13*, 34.
- [8] Bardoli, M. J.; Shukla, V. S.; Sharma, R. P. *Tetrahedron Lett.* **1985**, *26*, 509.
- [9] Wang, Y. D.; Gao, P.; Zhang, Q. H.; Zheng, Y.; Liu, K.; Liu, S. G. *High. Tech. Lett.* **2002**, *12*, 21.
- [10] Bhardwaj, R.; Singh, A.; Sharma, O. P.; Dawra, R. K.; Kurade, N. P.; Mahato, S. B. *J. Biochem. Mol. Toxicol.* **2001**, *15*, 279.
- [11] Shukla, V. S.; Barua, N. C.; Chowdhury, P. K.; Sharma, R. P.; Bordoloi, M.; Rychlewska, U. *Tetrahedron* **1986**, *42*, 1157.
- [12] Bohlmann, F.; Gupta, R. K. *Phytochemistry* **1981**, *20*, 1432.
- [13] Yang, S. P.; Cheng, J. G.; Huo, J.; Jiang, H. L.; Chen, K. X.; Yue, J. M. *Chin. J. Chem.* **2005**, *23*, 1530.
- [14] He, L.; Yang, J.; Cao, A. C.; Liu, Y. M.; An, Y.; Shi, J. G. *Chin. J. Chem.* **2006**, *24*, 1375.
- [15] He, L.; Hou, J.; Gan, M. L.; Shi, J. G.; Chantrapromma, S.; Fun, H. K.; Williams, I. D.; Sung, H. H. Y. *J. Nat. Prod.* **2008**, *71*, 1485.
- [16] Baruah, N. C.; Sarma, J. C.; Sarma, S.; Sharma, R. P. *J. Chem. Ecol.* **1994**, *20*, 1885.
- [17] Zhao, X.; Zheng, G. W.; Niu, X. M.; Li, W. Q.; Wang, F. S.; Li, S. H. *J. Agric. Food Chem.* **2009**, *57*, 478.
- [18] Dey, S.; Sinha, B.; Kalita, J. *Microsc. Res. Techniq.* **2005**, *66*, 31.
- [19] Chakravarty, A. K.; Mazumder, T.; Chatterjee, S. N. *Evid-Based Compl. Alt.* **2011**, *2011*, 1.
- [20] Yang, G. Q.; Wan, F. H.; Liu, W. X.; Zhang, X. W. *Allelopathy J.* **2006**, *18*, 237.
- [21] Yang, G. Q.; Wan, F. H.; Liu, W. X.; Guo, J. Y. *Allelopathy J.* **2008**, *21*, 2.
- [22] Luo, S. H.; Luo, Q.; Niu, X. M.; Xie, M. J.; Zhao, X.; Schneider, B.; Gershenson, J.; Li, S. H. *Angew. Chem., Int. Ed.* **2010**, *49*, 4471.
- [23] Isman, M. B.; Koul, O.; Luczynski, A.; Kaminski, J. *J. Agric. Food Chem.* **1990**, *38*, 1406.

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