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Identification of Diverse Sesquiterpenoids from *Eupatorium adenophorum*

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Abstract: Ten sesquiterpenoids (**1**-**10**), including two new compounds **1** and **2**, were isolated with the use of several chromatographic methods from the above-ground tissues of *Eupatorium adenophorum* Spreng. Comprehensive spectroscopic studies, such as single-crystal X-ray diffraction, 1D, 2D-NMR, and HRMS, were used to characterize their structures. The cytotoxic and anti-inflammatory actions and properties of the new compounds **1** and **2** were examined and briefly discussed.

Keywords: Chemical structures; compositae; sesquiterpenoids; *eupatorium adenophorum*.

1. Introduction

Sesquiterpenoids. are the most abundant and diverse compounds within the terpenoid compound class, and, as a result, they are the focus of research regarding the metabolic and chemical composition of plants due to their rich diversity in chemical structures and biological functions [1-2]. Sesquiterpenoids are the main constituents of volatile oils while oxygen-containing derivatives have a strong odor and biological activity and are important raw materials used in the food, cosmetics, and pharmaceutical industries [3-5]. *Eupatorium adenophorum* Spreng. (*E. adenophorum*) is synonymous with *Ageratina adenophora* (Spreng.) R. M. King & H. Rob., a perennial and herbaceous invasive plant, that is ubiquitous worldwide [6]. Although invasive, it is traditionally used as a medicinal remedy for treating wounds, inflammation, fever, diabetes, dysentery, and other ailments. Many studies have been published on the characterization of its chemical compositions, especially its sesquiterpenoid content and diversity [7-11]. In our continuous research on *E. adenophorum* phytochemistry, we have previously analyzed petroleum ether extracts from above-ground tissues, where we identified a series of novel sesquiterpenoids with unusual carbon skeletons [12-13].

Guided by our prior studies' findings, we continue to uncover novel sesquiterpenoids from the petroleum ether extracts of *E. adenophorum*. In the present study, we identified and characterized ten sesquiterpenoids, including eight known and, more importantly, two previously undescribed sesquiterpenoids (Figure 1). The anti-inflammatory and cytotoxic properties of compounds **1** and **2** against five cell lines from human cancer, HL-60, A-549, SMMC-7721, MDA-MB-231, and SW480, were examined by measuring their inhibitory ability on LPS-triggered NO generation in RAW264.7 macrophages.

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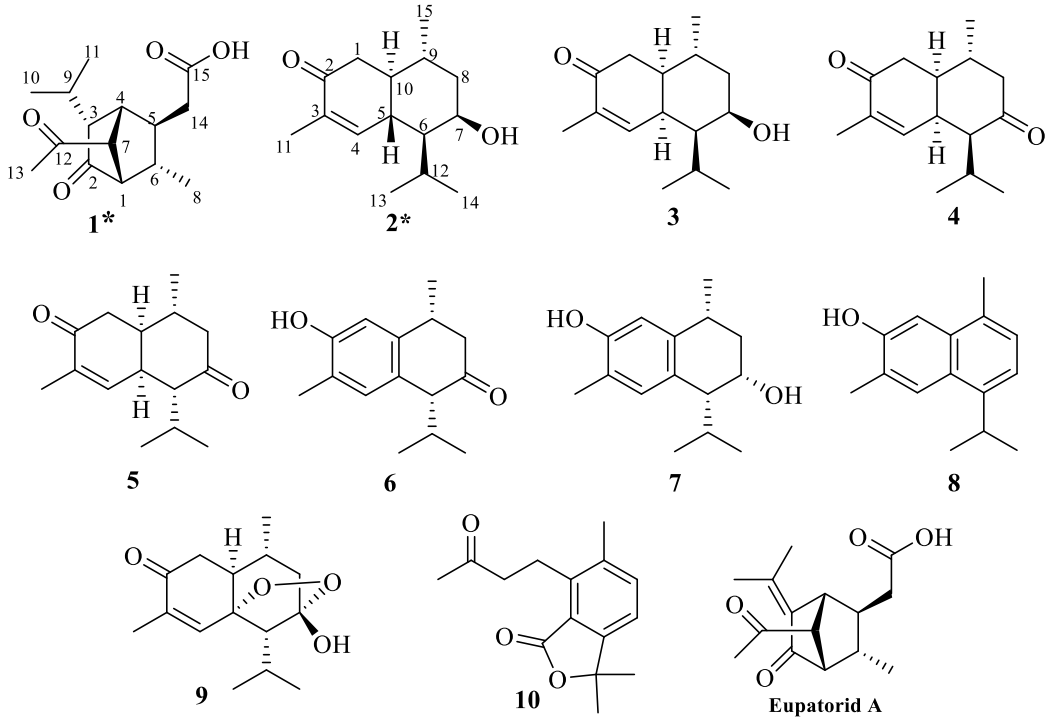

Figure 1. Chemical structures of compounds **1**-**10** and eupatorid A

2. Materials and Methods

2.1. General Protocols for Experiments

Sephadex LH-20 (20-100 μm, Amersham Pharmacia Biotech, Sweden) and silica gel (200-300 mesh, Qingdao Marine Chemical Factory, Qingdao, P. R. China) were used for column chromatography (CC). Subsequently, silica gel (GF254, 10-40 μm, Qingdao Marine Chemical Factory) was subjected to classical thin-layer chromatography (TLC). The spots were visualized on the TLC plates under UV light by spraying with 5% H_2SO_4 in EtOH (v/v) and heating. A Ruihe HPLC system was used for preparative HPLC, and a Zorbax SB-C18 column (5 μm, 9.4 × 150 mm, Agilent, America) was run at a flow rate of 4.0 mL per minute. TMS served as the internal standard for the NMR tests conducted on a Bruker AV-600 spectrometer. The Agilent G6230 spectrometer was utilized to obtain the mass spectra. The KBr pellets were employed to record IR spectra on a Bruker-Tensor-27 spectrometer. The UV spectrum was obtained on a Shimadzu 2700 or 2401 PC double-beam spectrophotometer. Utilizing a Horiba SEPA-300 polarimeter, the optical rotation was measured. A circular dichroism spectrometer from Agilent Applied Photophysics was used to carry out the ECD experiment. The Bruker SMART APEX CCD crystallography equipment was used for the X-ray diffraction analysis.

2.2. Plant Material

In July 2021, the aerial parts tissues of *Eupatorium adenophorum*, which belongs to the Compositae family, were collected during the wet season from Xichang, which is located in Sichuan province, China, at a GPS coordinate of 30°52′N/104°44′E). Prof. Qing-Shan Yang (Anhui University of Chinese Medicine) confirmed the plant's identity. A verified sample designated as EA20210708 was preserved at the Laboratory of Research and Development of Medicinal Plants in the Panxi Region.

2.3. Isolation and Extraction

Above-ground *E. adenophorum* tissues that had been air-dried and pulverized (12 kg) were milled and soaked in petroleum ether (35 L) at room temperature for 48 h. Three separate runs of the extraction were performed under identical conditions. To obtain an oily residue (162 g), the extracts were mixed and then dried by evaporation under reduced pressure. Silica gel CC was then performed, followed by elution with PE/ethyl acetate in a stepwise-gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 0:10, *v/v*), and four fractions (Frs. I- IV) were obtained.

Fr. II (46g) was fractionated on a silica gel CC with PE/acetone (from 10:1 to 2:1, v/v) as the eluent to give three sub-fractions (Frs. IIA-IIC). Fr. IIB (6.4 g) was passed through a Sephadex LH-20 column eluted with acetone to get three additional sub-fractions (Fr. IIB1-Fr. IIB2). After separating Fr. IIB1 (800 mg) on a silica gel CC eluted with PE/acetone (8:1, v/v) and loading it onto an acetone-eluted Sephadex LH-20 column, it was subjected to semi-preparative RP-HPLC with MeOH-H_2O (75:25, v/v) serving as the mobile phase to yield **1** (t_R 22.8 min, 8.6 mg).

Fr. IV (26 g) was fractionated on silica gel CC with PE/acetone (from 10:1 to 4:1, v/v) as the eluent, resulting in two sub-fractions (Frs. IVA- IVB). Fr. IVB (9.2 g) was passed through a Sephadex LH-20 column eluted with acetone, with four additional sub-fractions obtained (Fr. IVB1-Fr. IVB4). The next step involved separating Fr. IVB2 (900 mg) on a silica gel CC eluted with PE/acetone (8:1, v/v) before loading it onto an acetone-eluted Sephadex LH-20 column and subjecting it to semi-preparative RP-HPLC, with a mobile phase of MeOH-H_2O (75:30, v/v) to yield **2** (t_R 29.6 min, 4.8 mg), **6** (t_R 35.2 min, 4.2 mg), and **7** (t_R 38.5 min, 2.8 mg). A similar approach was adopted for Fr. IVB3 (1.1 g) to obtain **3** (t_R 30 min, 4.6 mg), **4** (t_R 34 min, 114.8 mg), **5** (t_R 40 min, 39.2 mg), and **8** (t_R 43.2 min, 3.5 mg). The same approach was also followed for Fr. IVB4 (300 mg) to yield **9** (t_R 31.5 min, 1.4 mg), and **10** (t_R 34.5 min, 6.8 mg).

2.4. Spectral Characteristics of The Novel Compounds

*Compound (**1**)*: Colorless needle crystal, [α] D^{20} 48 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.42), 283 (2.68) nm; IR ν_{max} (KBr) 3232, 2944, 1734, 1682, 1416, 1384, 1140, 997, 777 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 197 (-11.97), 225 (+1.58), 286 (+9.98), 320 (-1.38) nm; HRESIMS *m/z* 267.1589 $[M+H]^+$ (calc. for $C_{15}H_{23}O_4$, *m/z* 267.1591); Table 1 displays the ^1H and ^{13}C NMR data. X-ray crystallographic diffraction data of **1** (CCDC: 2305359): $C_{15}H_{22}O_4$, M = 266.32, a = 8.2605(3) Å, b = 9.2695(3) Å, c = 17.7639(6) Å, α = 90°, β = 90°, γ = 90°, V = 1360.19(8) $Å^3$, T = 100.0 K, space group $P212121$, Z = 4, μ(Cu Kα) = 0.64 mm^{-1}, 17275 reflections measured, and 2772 independent reflections (R_{int} = 0.0789). Additionally, 0.0322 ($I > 2\sigma(I)$) was determined to be the final R_1 value. The final $wR(F^2)$ value was calculated as 0.0800 ($I > 2\sigma(I)$). A final R_1 value of 0.0329 was obtained (all data). Moreover, 0.0806 was the final $wR(F^2)$ value for all data. F^2 exhibited a goodness-of-fit value of 1.042. Flack parameter = 0.09(9).

*Compound (**2**)*: Needle crystal without color, [α] D^{20} -103.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 252 (3.12) nm; IR ν_{max} (KBr) 3411, 2954, 2919, 1625, 1425, 1185, 1015, 906 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 204 (+56.20), 242 (-51.33), 331 (+6.45) nm; HRESIMS *m/z* 237.1848$[M+H]^+$ (calc. for $C_{15}H_{25}O_2$, *m/z* 237.1849); Table 1 displays the ^1H and ^{13}C NMR data. Crystallographic information from X-rays of **2** (CCDC: 2305360): $C_{15}H_{24}O_2$, M = 236.34, a = 5.2663(10) Å, b = 16.0934(4) Å, c = 8.5946(2) Å, α = 90°, β = 106.750(2)°, γ = 90°, V = 697.51(3) $Å^3$, T = 100.0 K, space group $P21$, Z = 2, μ(Cu Kα) = 0.64 mm^{-1}, 16736 reflections measured, and 2811 independent reflections (R_{int} = 0.0685) (R_{int} = 0.0685). The final R_1 values were calculated as 0.0403 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were calculated to be 0.1048 ($I > 2\sigma(I)$). For all data, 0.0416 was determined to be the final R_1 value. The final $wR(F^2)$ values were 0.1068 (all data). F^2 exhibited a goodness-of-fit value of 1.071. Flack parameter = -0.03(14).

2.5. In Vitro Assay of Tumor Growth Inhibition

The MTS [3-(4,5-imethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetrazolium] assay was utilized to examine the identified *E. adenophorum* compounds for their cytotoxicity in vitro [12]. Five human cancer cell lines (HL-60, A-549, SMMC-7721, MDA-MB-231, and SW480) were selected for the assay. After seeding the cell lines under evaluation onto 96-well plates, they were incubated in an incubator with 5% CO_2 and maintained at 37 °C for 24 hours. Afterward, 40 μM of the assessed compounds were introduced. The culture medium was supplemented with MTS 72 hours later, and an absorbance measurement was performed at 490 nm utilizing a microplate reader (SpectraMax plus 384, MD, USA). Triplicates of each experimental procedure were performed. The positive control was cisplatin (DDP).

2.6. Measurement of Nitric Oxide (NO) Generation

The NO assay kit (Beyotime Institute of Biotechnology, Haimen, China) was utilized to measure the amount of NO produced in supernatants of cultured RAW264.7 cells. Before stimulation with LPS (1 μg/ml) for 24 hours, cells were plated in 96 well plates, followed by pretreatment using the investigated compounds at a concentration of 50 μM. Thereafter, the supernatant was mixed with an equivalent volume of Griess reagent, which consisted of 2.5% phosphoric acid, 0.1% naphthylethylenediamine dihydrochloride, and 1% sulfanilamide before incubating it at RT for 24hrs The absorbance at 570 nm was used to calculate the nitric oxide concentration. The positive control used was NG-monomethyl-L-arginine, monoacetate salt (L-NMMA) [12].

3. Results and Discussion

3.1. Structure Elucidation

The extracted Compound **1** was in the form of a colorless needle crystal. As a result of the HRESIMS ion peak occurring at *m/z* 267.1589 $[M+H]^+$ (calcd 267.1591), the chemical formula of this compound was determined to be $C_{15}H_{22}O_4$, which indicates that it is unsaturated to a degree of five. The ^{13}C NMR and HSQC spectra of **1** revealed 15 carbons including four methyl groups present, including three bimodal methyl groups, seven methines, one methylene, and three quaternary carbons (including one carboxyl and two ketone carbonyls). Three double bond equivalents (DBEs) were attributed to three carbonyls, and the presence of the other two carbonyls suggested that compound **1** was a bicyclic natural compound.

Careful observation revealed that the ^1H NMR and ^{13}C NMR data (Table 1) were reminiscent of eupatorid A, having a similar 5/5 bicyclic carbon skeleton isolated from this plant in our previous work [12]. Notably, compound **1** lacked one C (sp^2)-C (sp^2) double bond. This difference was further confirmed by 2D-NMR (Figure 2). The key HMBC correlations between Me-10 (δ_H 1.16) with C-3/C-9/C-11, Me-11 (δ_H 0.93) with C-3/C-9/C-10, H-3 (δ_H 1.66) with C-1/C-2/C-5/C-7/C-10/C-11, H-4 (δ_H 1.93) with C-1/C-2/C-6/C-9/C-14 indicated a reduction in the C (sp^2)-C (sp^2) double bond between C-3 and C-9 in eupatorid A to the C (sp^3)-C (sp^3) single bond in compound **1**. The apparent ^1H-^1H COSY correlations (Figure 2) of H-3 with H-4/H-9 further determined the planar structure of compound **1**. The single-crystal structure was utilized to ascertain the absolute configuration of **1** as the ROSEY spectra demonstrated poor correlations. A solvent-volatilized needle crystal of compound **1** was produced from methanol and water (10:1 *v/v*). Using Cu Kα radiation, X-ray diffraction unambiguously established its structure, including the absolute configuration (1*S*, 3*R*, 4*R*, 5*S*, 6*S*, 7*R*) (Figure 3). As observed in Figure 1, compound **1** was subsequently identified and named 3, 9-dihyroeupatorid A.

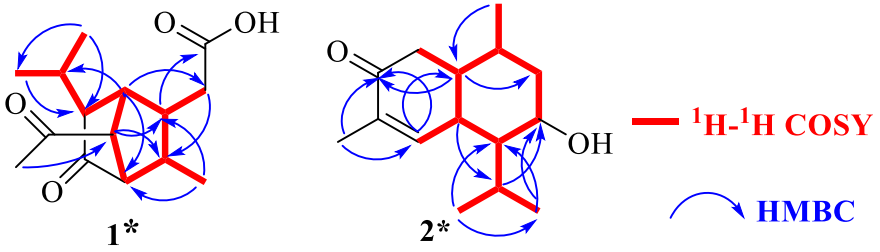

Figure 2. ^{1}H-^{1}H COSY and key HMBC correlations of compounds **1** and **2**

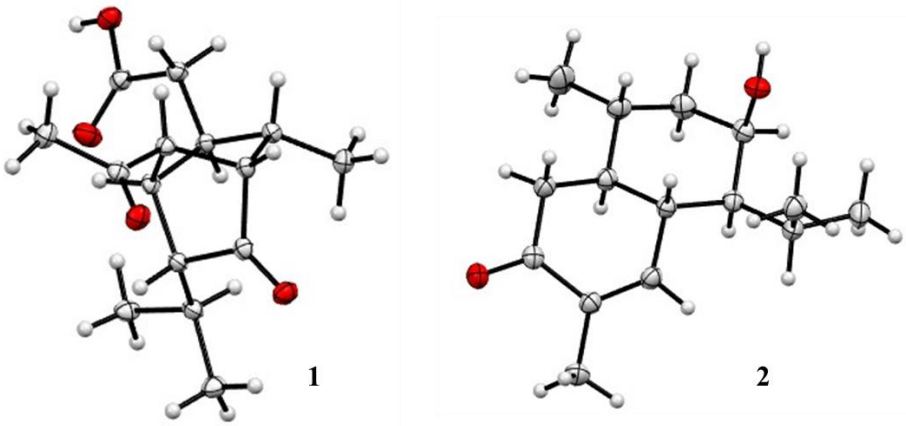

Figure 3. X-ray crystallographic structure of compounds **1** and **2**

Table 1. ^{1}H and ^{13}C NMR Data of compounds **1** and **2**

No.	**1**[a]		**2**[a]	
	δ_H (*J* in Hz)	**δ_C, type**	**δ_H (*J* in Hz)**	**δ_C, type**
1a	2.51 (m)	58.2, d	2.60 (m)	43.1, t
1b			2.06 (overlapped)	
2		213.4, s		199.2, s
3	1.66 (m)	55.1, d		136.0, s
4	1.93 (m)	39.0, d	6.93 (brs)	148.1, d
5	2.85 (brs)	45.5, d	2.68 (m)	38.6, d
6	1.87 (m)	39.4 d	1.23 (overlapped)	48.4, 6
7	3.31 (brs)	58.0, d	4.21 (brs)	67.0, d
8	0.92 (d, 7.0)	16.0, q	1.78 (overlapped)	44.4, t
9	1.73 (m)	25.4, d	1.22 (overlapped)	30.6, d
10	1.16 (d, 6.2)	21.4, q	1.38 (m)	49.3, d
11	0.93 (d, 6.2)	21.1, q	1.73 (s)	16.1, q
12		207.5, s	2.25 (m)	28.1, d
13	2.19 (s)	28.9, q	1.09 (d, 6.0)	21.0, q
14a	2.62 (dd, 14.8, 4.8)	38.9, t	1.07 (d, 6.0)	18.5, q
14b	2.52 (dd, 14.8, 4.8)			
15		172.8, s	0.86 (d, 6.5)	19.3, q

a): The ^{1}H and ^{13}C NMR data of all compounds were recorded at 600 MHz and 150 MHz, with $(CD_3)_2CO$ as solvent, respectively.

$C_{15}H_{24}O_2$ was the molecular formula assigned to Compound **2**, which was isolated as a needle crystal without color. This detection was based on the HRESIMS ion peak at *m/z* 237.1848 $[M+H]^+$ (calcd 237.1849), which highlighted the presence of four double-bond equivalents. Notably, the ^1H NMR and ^{13}C NMR spectroscopic data of **2** (Table 1) were highly similar to that of 9*β*-hydroxy-ageraphorone (**3**). However, through meticulous examination of their ^{13}C-NMR data, we were surprised to find that C-3, C-6, and C-10 around C-5 in compound **2** exhibited an upfield or downfield shift compared to compound **3**. Therefore, it could reasonably be inferred that the C-5 configuration in compound **2** was reversed. To confirm our hypothesis, its structure and absolute configuration (5*S*, 6*S*, 7*R*, 9*R*, 10*S*) (Figure 3) were also validated by Cu Kα radiation X-ray diffraction. Figure 1 illustrates its structure, which was subsequently named (5*S*, 6*S*, 7*R*, 9*R*, 10*S*)-7-hydroxyageraphorone.

Through comparison of their spectroscopic data with those reported in the literature, the other eight known compounds **3**-**10** were identified as 9*β*-hydroxy-ageraphorone (**3**) [10], 9-oxo-ageraphorone (**3**) [10], 9-oxo-ageraphorone (**4**) [10], muurol-4-en-3,8-dione (**5**) [10], (1*S*, 4*R*)-7-hydroxycalamenen-3-one (**6**) [14], (1*R*, 3*S*, 4*R*)-calamenene-3,7-diol (**7**) [14], 7-hydroxycadalin (**8**) [15], (5*S*, 6*S*, 7*S*, 9*R*, 10*S*)-7-hydroxy-5,7-epidioxycadinan-3-ene-2-one (**9**) [8], and phacadinane E(**10**) [16], respectively.

*3.2. Brief Overview of Compounds **1**, **2** and Their Bioactivity Tests*

Compounds **1** and **2** were examined to determine their anti-inflammatory and tumor growth inhibitory activities in vitro to learn more about their biological characteristics. Five different human cell lines were utilized to evaluate the inhibitory impact on tumor growth: HL-60, A-549, SMMC-7721, MDA-MB-231, and SW480. Compounds **1** and **2**, as demonstrated in Table 2, exhibited cytotoxic effects on all five tumor cell lines. At a dosage of 40 μM, the tumor growth inhibitory rates varied between 3.85 and 28.84 %. Subsequently, the anti-inflammatory properties of Compounds **1** and **2** were ascertained by measuring their inhibitory effects on LPS-induced NO production in RAW264.7 lipopolysaccharide (LPS)-activated macrophages. At a dosage of 50 μM, compounds **1** and **2** did not exhibit a statistically significant anti-inflammatory activity, and their inhibition rate on NO production was 16.62 and 37.26 %, respectively (Table 3).

Table 2. The cell inhibition of compounds **1**-**3** and eupatorid A against five human tumor cell lines

compound	Cell Inhibition (%) in 40 μM				
	HL-60	A-549	SMMC-7721	MDA-MB-231	SW480
1	17.25±2.85	7.21±1.34	10.39±0.48	28.84±2.51	11.77±1.64
Eupatorid A [12]	14.12±1.27	2.06±0.58	4.46±1.78	16.35±3.36	8.75±3.93
2	15.60±2.09	19.90±1.27	3.85±1.48	14.14±1.25	5.21±1.99
3	12.69±2.20	5.70±2.85	2.95±0.53	0±1.58	1.03±1.89
DDP[a]	56.23±1.56	91.30±1.23	64.40±2.31	62.28±1.86	72.56±2.34

As compound **1** is highly similar in structure to eupatorid A, and compound **2** is similar to **3,** we performed the same activity tests on eupatorid A and **3** to examine their structure-activity relationship (SAR). Based on the results, eupatorid A and **3** inhibition rates were lower than compounds **1** and **2**, respectively, and overall, they did not exhibit any significant inhibitory activities (Table 2 and 3). This may also indicate that differences in the functional groups and their configuration affect their activities and properties.

Table 3. Inhibitory rate of LPS-induced NO production in RAW246.7 cells treated with compounds **1-3** and eupatorid A

Compound	Concentration (μM)	NO Inhibition rate (%)
1	50	16.62±2.26
Eupatorid A [12]	50	6.62±2.26
2	50	37.26±2.83
3	50	9.75±2.38
L-NMMA	50	56.98±0.90

4. Conclusion

To summarize, ten sesquiterpenoids, including two compounds **1-2** that had not been characterized before, were isolated by phytochemical examination of the above-ground tissues of *E. adenophorum* Spreng. Extensive spectroscopic characterization and X-ray single-crystal diffraction were performed to determine their structures. The novel compounds **1-2** were examined in vitro for their SAR, anti-inflammatory, and tumor growth inhibitory activities. Although not all compounds possessed significant inhibitory anti-inflammatory properties, the findings add to our knowledge of *E. adenophorum*'s chemical compositions while laying the groundwork for future research into the ecological and biological effects of this invasive plant.

Acknowledgments

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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References

[1] B. M. Fraga (2012). Natural sesquiterpenoids, *Nat. Prod. Rep*. **29**, 1334-1366.
[2] Q. Q. Shi, J. J. Tang and J. M. Gao (2022). Picrotoxane sesquiterpenoids: chemistry, chemo-and bio-syntheses and biological activities, *Nat. Prod. Rep.* **39**, 2096-2131.
[3] R. A. Dixon (2001). Natural products and plant disease resistance, *Nature*. **411**, 843-847.
[4] A. A. Elkordy, R. R. Haj-Ahmad, A. S. Awaad and R. M. Zaki (2021). An overview on natural product drug formulations from conventional medicines to nanomedicines: past, present and future, *J. Drug Deliv. Sci. Tec*. **63**, 10.1016/j.jddst.2021.102459.
[5] J. Gershenzon and N. Dudareva (2007). The function of terpene natural products in the natural world, *Nat. Chem. Biol.* **3**, 408-414.

[6] G. Simran, S. Ranabir, P. Paramita, N. Gouranga and K. D （2022. Tarun. An updated review on *Eupatorium adenophorum* Spreng. [*Ageratina adenophora* (Spreng.)]: traditional uses, phytochemistry, pharmacological activities and toxicity, *Pharmacol. Res.Mod. Chin. Med*. **2**, 100068.

[7] L. He, J. Hou, M. Gan, J. Shi, S. Chantrapromma, H. K. Fun, I. D. Williams and H. H. Y. Sung (2008). Cadinane sesquiterpenes from the leaves of *Eupatorium adenophorum*, *J. Nat. Prod.* **71**, 1485-1488.

[8] X. Zhao, G. W. Zheng, X. M. Niu, W. Q. Li, F. S. Wang and S. H. Li （2009). Terpenes from *Eupatorium adenophorum* and their allelopathic effects on Arabidopsis seeds germination, *J. Agri. Food Chem.* **57**, 478-482.

[9] W. Shi, S. H. Luo and S. H. Li (2012）. Defensive sesquiterpenoids from leaves of *Eupatorium adenophorum*, *Chin. J. Chem*. **30**, 1331-1334.

[10] Y. Liu, S. H. Luo, J. Hua, D. S. Li, Y. Ling, Q. Luo and S. H. Li (2021). Characterization of defensive cadinenes and a novel sesquiterpene synthase responsible for their biosynthesis from the invasive *Eupatorium adenophorum*, *New Phytol*. **229**, 1740-1754.

[11] B. Yin, X.H. Li, Z. X. Li, X. X. Zhu, L. Zhang, X. L. Zhou, J. B. Xu, F. Z. Chen, P. Tang and F. Gao (2023). Adenophorone, an unprecedented sesquiterpene from *Eupatorium adenophorum*: Structural elucidation, bioinspired total synthesis and neuroprotective activity evaluation, *Angew. Chem. Int. Ed.* **62**, e202306326.

[12] H. Geng, J. H. Luo, W. J. Gu, J. J. Zhang, Y. X. Yang and Y. Yu (2023). Unusual 5/5 fused bicyclosesquiterpenoids from *Eupatorium adenophorum*, *Fitoterapia*, **170**, 105643.

[13] H. Geng, W. J. Gu, J. H. Luo, Y. X. Yang and Y. Yu. (2023). Eupatorione A, an unusual sesquiterpenoid from the aerial parts of *Eupatorium adenophorum*, *Rec. Nat. Prod*. **17**, 1064-1068.

[14] B.F. Bowden, J.C. Coll, L.M. Engelhardt, D.M. Tapiolas and A.H. White（1986). Studies of Australian soft corals. XXXVI* The isolation and structure determination of 11 calamenene-based sesquiterpenes from *Lemnalia cervicornis* (Coelenterata, Octocorallia, Alcyonacea), *Aust. J. Chem.* **39**, 103-21.

[15] I. Kubo, H. Muroi, A. Kubo, Swapan K. Chaudhuri, Y. Sanchez and T. Ogura (1994). Antimicrobial agents from *Heterotheca inuloides*, *Planta Med.* **60**, 218-221.

[16] J. Xu, H. L. Zhu, J. Zhang, T. Du, E. Y. Guo, W.-Y. Liu, J. G. Luo, F. Ye, F. Feng and W. Qu （2018). Sesquiterpenoids from *Chloranthus anhuiensis* with neuroprotective effects in PC12 cells, *J. Nat. Prod*. **81**, 1391-1398.