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Communication

New Sesquiterpenoids from *Ambrosia artemisiifolia* L.

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Abstract: A new pseudoguaianolide **1** and two new guaiane-type sesquiterpene glucosides **2** and **3**, were isolated from the aerial parts of *Ambrosia artemisiifolia* L together with two known sesquiterpene dilactones **4** and **5**. The new compounds were determined on the basis of spectroscopic and chemical methods to be 3β-acetoxy-4β-hydroxy-1α,7α,10β,11αH-pseudoguaia-12,8β-olide (**1**), 1β,7β,9β,10β,13αH-guaia-4(5)-en-12,6β-olide 9-*O*-β-D-glucoside (**2**) and 4β-hydroxy-1α,5α,7α,9αH-guaia-10(14),11(13)-dien-12-acid 9-*O*-β-D-glucoside (**3**). The isolated compounds were evaluated for cytotoxicity against human promyelocytic leukemia HL-60 cell lines *in vitro*, but were all inactive.

Keywords: *Ambrosia artemisiifolia* L.; sesquiterpenoid; sesquiterpene glucoside

1. Introduction

Ambrosia artemisiifolia L. (Asteraceae), an invasive alien plant species in China, is nowadays widespread in an area ranging from Guangdong Province in the south to Heilongjiang Province in the north [1]. This species is considered as a harmful weed capable of quickly colonizing both agricultural and urban areas with competitive seeds, and its pollen can induce serious allergic disorders during a certain period of its development [2]. However, many investigations into the composition and properties of *A. artemisiifolia* indicate that this species can serve a valuable source of biologically active substances. The extracts of this plant showed various bioactivities, such as molluscicidal [3], plant growth inhibitory [4], anti-inflammatory [5], antithrombin [6], antibacterial [7], insecticidal [8], hepatoprotective and hypolipemic activities [9]. On the other hand, the main chemical components of this species were identified as sesquiterpene lactones, which account for the antihelminthic, cardiotonic, antiinflammatory, analgesic, sedative (calming), antimalarial, anti-tumor, and other types of pharmacological activity [10,11]. As part of an ongoing search for novel bioactive compounds, we studied *Ambrosia artemisiifolia* L. growing in Hunan Province of China. Herein, this paper reports the isolation and identification of three new sesquiterpenoids from the aerial parts of the plant.

2. Results and Discussion

The dried aerial parts of *A. artemisiifolia* were extracted three times with MeOH at room temperature. The MeOH extract residue was suspended in water and then partitioned successively with petroleum ether and EtOAc. Column chromatography of the EtOAc-soluble fraction yielded three new compounds **1**–**3** and two known compounds, psilostachyin B (**4**) [12] and psilostachyin (**5**) [13] (Figure 1).

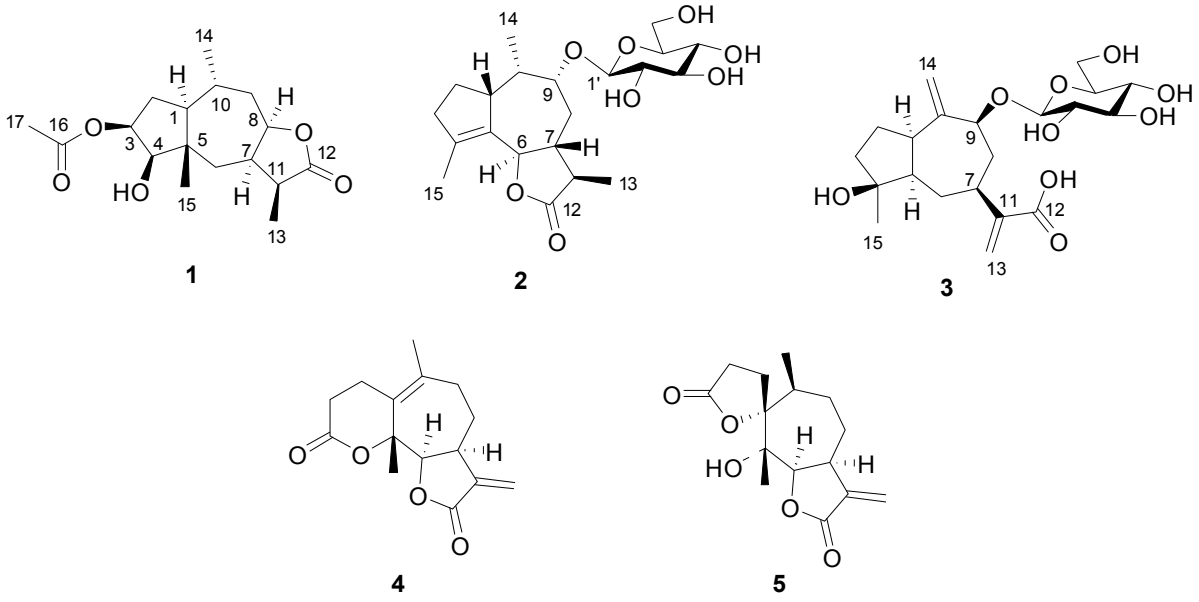

Figure 1. Structures of compounds **1**–**5** isolated from *A. artemisiifolia*.

Compound **1** was obtained as white amorphous powder, and the molecular formula was assigned as $C_{17}H_{26}O_5$ from its HRESIMS (*m/z* 311.1852 $[M+H]^+$) and NMR data. The ^{1}H-NMR spectrum

displayed readily recognizable signals for four methyl groups [δ_H 1.18 (3H, s, H-15), 2.04 (3H, s, H-17), 0.90 (3H, d, J = 7.0 Hz, H-14) and 1.02 (3H, d, J = 7.4 Hz, H-13)] (Table 1). The ^{13}C-NMR and DEPT spectra of **1** exhibited 17 carbons corresponding to four methyls, three methylenes, seven methines (including three oxy-methine carbons), a quaternary carbon, and two quaternary carbonyl groups (Table 1). The structure of **1** was deduced based on ^{1}H-^{1}H COSY, HSQC, and HMBC techniques using those methyl groups as starting points. The HMBC correlations from the H-atoms of the four methyl groups to corresponding C-atoms [H_3-17 (δ_H 2.04) to C-16; H_3-14 (δ_H 0.90) to C-1, C-9 and C-10; H_3-13 (δ_H 1.02) to C-7, C-11 and C-12; H_3-15 (δ_H 1.18) to C-1, C-4, C-5 and C-6) (Table 1)] established a typical acetyl moiety (δ_H 2.04, δ_C 169.8 and 20.3) and three fragments as $^{14}CH_3$-^{10}CH(^1CH)-^9CH-, $^{13}CH_3$-^{11}CH (^7CH)-$^{12}COOH$, and $^{15}CH_3$-^5C(^4CH/^1CH)-$^6CH_2$- (Figure 2). Those fragments were further linked to form a 5/7-membered fused-ring structure based on the analysis of the ^{1}H-^{1}H-COSY correlations (Figure 2). Considering the molecular formula $C_{17}H_{26}O_5$ and the presence of two C=O bonds, **1** was suggested to be an acetylated derivative of pseudoguaianolide [14]. The lactone ring was formed between C-12 and C-8, which deduced from the low-field chemical shift of C-12 (δ_C 177.8) and C-8 (δ_H 4.58 and δ_C 79.7) [15]. While the acetyl moiety was found to be attached to C-3 via an ester linkage from long-range correlation of H-3 [δ 5.45 (ddd, J = 8.1, 7.5, 5.0 Hz)] with C-16 (δ_C 169.8) observed in the HMBC spectrum (Figure 2). Thus the planar structure of **1** was deduced as 3-acetoxy-4-hydroxy-pseudoguaia-12,8-olide. The relative stereochemistry of **1** was assigned by analyses of the NOESY spectrum and the proton coupling patterns (Table 1). The key NOE correlation observed between H-7 and H-8, as well as the large coupling constant measured for H-8 (8.6 Hz), indicating the presence of a *cis*-fused lactone ring [16]. While the NOE correlation was absent between H-1 and H_3-15 suggesting a typical *trans*-fused 5/7-membered ring, a model of this molecule indicated that H-1 was α-orientation [14]. Meanwhile, the correlations of H-1 with H-3, H-4 and H-7, and H-7 with H-8 and H-11 revealed that all of these protons (H-1, H-3, H-4, H-7 and H-11) were cofacial and were arbitrarily assigned α-oriented. The β-orientation of H-10 was revealed by the coupling constant measured H-1 ($J_{H\text{-}1/H\text{-}10}$ = 13.6 Hz), and corroborated by an NOE between H-10 and H_3-15. Finally, compound **1** was established as 3β-acetoxy-4β-hydroxy-1α,7α,10β,11αH-pseudoguaia-12,8β-olide.

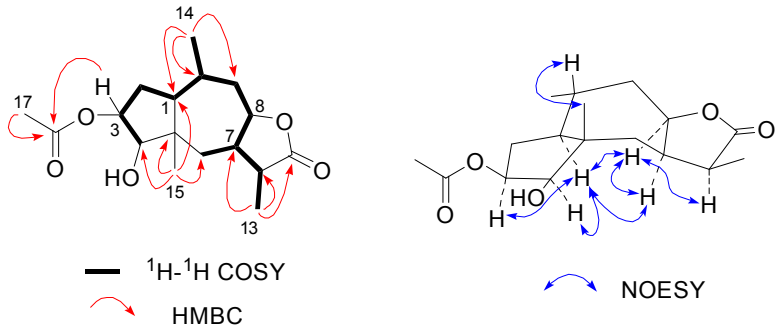

Figure 2. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of **1**.

Table 1. NMR assignments of **1** by DEPT, HSQC, HMBC, and NOESY experiments in C_5D_5N [a].

Position	δ_H (*J* in Hz)	δ_C, Mult.	HMBC	NOESY
1	1.51, ddd (13.6, 5.6, 5.6)	39.0 CH	C-2, C-4, C-5, C-10, C-14,C-15	H-3, H-4, H-7
2	Hα: 1.82, m	31.5 CH_2	C-1, C-3	
	Hβ: 2.01, m		C-1, C-3	
3	5.45, ddd (8.1, 7.5, 5.0)	71.0 CH	C-1, C-4, C-5, C-16	Hα-2, Hβ-2, H-4
4	3.64, d (7.5)	75.9 CH	C-3, C-5, C-6, C-15	H-1, H-3, H-7
5	–	43.3 qC		
6	Hα: 1.94, br d (14.3)	30.5 CH_2	C-5, C-7, C-15	H-4, H-7
	Hβ: 1.16, overlapped			Hβ-9
7	2.51, m	36.7 CH	C-5, C-6, C-11, C12	H-1, H-4, H-8, H-11
8	4.58, ddd (13.2, 8.6, 2.8)	79.7 CH	C-7	H-7, Hα-9, Hβ-9, H-11
9	Hα: 2.07, m	36.4 CH_2	C-1, C-7, C-8, C-10, C-14	
	Hβ: 1.66, br d (12.7)		C-1, C-7, C-8, C-10	Hβ-6, H-14, H-15,
10	1.80, m	28.5 CH	C-1, C-5, C-14	
11	2.94, m	37.5 CH	C-6, C-7, C-12, C-13	H-7, H-8, H-13
12	–	177.8 qC		
13	1.02, d (7.4)	9.7 CH_3	C-7, C-11, C-12	Hβ-6, H-7, H-11
14	0.90, d (7.0)	16.4 CH_3	C-1, C9, C-10	Hα-9
15	1.18, s	17.5 CH_3	C-1, C-4, C-5, C-6	Hβ-9, H-10
16	–	169.8 qC		
17	2.04, s	20.3 CH_3	C-16	H-3

Note: [a] ^1H (400 MHz) and ^{13}C (100 MHz) NMR; δ in ppm.

Compound **2** has the molecular formula $C_{21}H_{32}O_8$ according to its HRESIMS and ^{13}C-NMR data. ^1H-, ^{13}C-NMR and DEPT spectra revealed that **2** contained a typical β-D-glucopyranosyl [δ_H 4.38 (d, *J* = 7.8, H-1'); δ_C 102.5, 75.2, 77.9, 71.8, 78.2, 62.8], which was confirmed by acid hydrolysis and then co-chromatography with an authentic sample. Besides, the remaining 15 carbon signals, which belong to the aglycone, were attributable to three methyls, three methylenes, six methines (including two oxy-methines), two olefinic quaternary carbons, and a quaternary carbonyl group (Table 2). To deduce the structure of the aglycone and the glycosidic connection, a complete ^1H and ^{13}C-NMR spectral assignment was carried out using a combination of DEPT, HSQC, ^1H-^1H COSY, HMBC and NOESY experiments. The ^1H-^1H COSY spectrum of **2** revealed a fragment with eleven carbons [-$^3CH_2$-$^2CH_2$-^1CH-^{10}CH($^{14}CH_3$)-^9CH(OR)-$^8CH_2$-^7CH(^6CHOR)-^{11}CH-$^{13}CH_3$] which was corroborated by HMBC correlations (Figure 3). The fragment was further connected by HMBC spectrum, the key HMBC correlations from H-1 to C-4, C-5 and C-6, from H-6 to C-1, C-4 and C-5, from H-13 to C-11, C-12 and C-7, and from H-15 to C-3, C-4 and C-5 indicated a sesquiterpene skeleton with a 5/7-membered fused-ring, which belong to that kind of guaiane-type sesquiterpenoid (Figure 3) [17,18]. In addition, the oxy-methine carbon δ_C 82.6 (C-9) showed a long-range correlation with the anomeric proton of glucose at δ_H 4.38 (d, *J* = 7.8 Hz, H-1'), indicating that the hydroxyl group at C-9 is glucosylated. The low-field chemical shift of δ_C 181.2 (C-12) and δ_C 83.3 (C-6), as well as the molecular formula $C_{21}H_{32}O_8$ revealed that a lactone ring was formed between C-12 and C-6 [18,19]. Therefore, the planar structure of compound **2** was determined as guaia-4(5)-en-12,6-olide 9-*O*-β-D-glucoside. In the NOESY spectrum, there were no NOE correlations between H-6 and H-7, as well as the large values of

the constants of $J_{6,7}$ and $J_{7,11}$ (10–11 Hz), suggesting the presence of a *trans*-fused lactone ring [19]. Moreover, the correlations of H-7 with H-1 and H-9, and H-6 with H-11 and H_3-14 revealed that protons H-7, H-1 and H-9 were cofacial, while H-6, H-11 and H_3-14 were on the opposite side. Assume the usual α-configuration for the isopropyl at C-7 [19], the stereochemistry of **2** was established as 1β,7β,9β,10β,13αH-guaia-4(5)-en-12,6β-olide 9-*O*-β-D-glucoside.

Table 2. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectral data of compounds **2** and **3**.

Position	**2** [a]		**3** [b]	
	δ_H (*J* in Hz)	**δ_C, Mult.**	**δ_H (*J* in Hz)**	**δ_C, Mult.**
1	3.09, m	49.9 CH	3.24, m	45.4 CH
2	Hα: 1.56, m; Hβ: 2.10, overlapped	29.4 CH_2	1.96, m	27.0 CH_2
3	2.36, m	39.4 CH_2	Hβ: 1.77, m; Hα: 1.98, m	40.9 CH_2
4	–	131.6 qC	–	80.7 qC
5	–	142.5 qC	2.38, br t (10.8)	54.2 CH
6	4.80, d (11.5)	83.3 CH	Hβ: 1.39, ddd (12.2, 11.0, 10.8); Hα: 2.01, br d (12.2)	33.4 CH_2
7	1.89, ddd (11.5, 11.6, 11.6)	46.8 CH	2.92, br t (11.0)	39.8 CH
8	Hα: 1.67, m; Hβ: 2.14, overlapped	32.2 CH	Hα: 1.88, m; Hβ: 2.62, br d (11.8)	45.3 CH_2
9	3.88, m	82.6 CH	4.79, dd (11.0, 3.0)	79.5 CH
10	2.11, overlapped	41.3 CH	–	152.5 qC
11	2.34, m	42.5 CH	–	149.0 qC
12	–	181.2 qC	–	169.8 qC
13	1.18, d (6.9)	12.5 CH_3	5.55, br s; 6.42, br s	121.9 CH_2
14	0.80, d (7.0)	8.1 CH_3	5.27, br s; 6.18, br s	110.1 CH_2
15	1.81, s	15.5 CH_3	1.32, s	24.9 CH_3
Glc-1'	4.38, d (7.8)	102.5 CH	5.06, d (7.7)	101.1 CH
2'	3.15, dd (8.0, 7.8)	75.2 CH	4.11, dd (8.2, 7.7)	75.8 CH
3'	3.35, m	77.9 CH	4.23, m	78.7 CH
4'	3.31, m	71.8 CH	4.26, m	71.9 CH
5'	3.27, m	78.2 CH	3.88, m	78.8 CH
6'	3.66, dd (11.6, 5.2); 3.88, br d (11.6)	62.8 CH_2	4.36, dd (11.9, 5.2); 4.51, dd (11.9, 2.2)	62.9 CH_2

Notes: [a] measured in CD_3OD, [b] measured in C_5D_5N; δ in ppm.

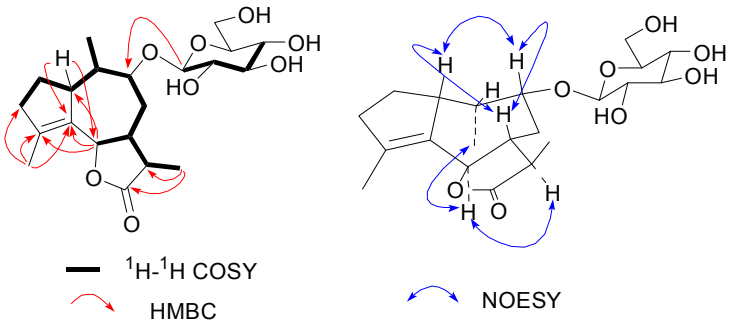

Figure 3. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of **2**.

Compound **3** was obtained as a colorless gum. The molecular formula was shown to be $C_{21}H_{32}O_9$ by its HRESIMS and ^{13}C-NMR data. The structure of **3** contained a β-D-glucopyranosyl, which was confirmed by methods previously described in compound **2**. Moreover, two pair of exomethylene singlets at δ_H 5.55 /6.42 (br s, H_2-13) and 5.27 /6.18 (br s, H_2-14), along with a methyl singlets δ_H 1.32 (s, H_3-15) was clearly observed in ^{1}H-NMR spectrum. The ^{13}C-NMR and DEPT spectra (Table 2) showed a total of 21 carbon signals, among which, 15 were ascribable to the aglycone. The structure of the aglycone and the glycosidic connection were carried out by 2D-NMR methods (HSQC, ^{1}H-^{1}H COSY, HMBC and NOESY experiments). The HMBC correlations from the H-atoms of the exomethylenes and methyl groups to corresponding C-atoms revealed three C_4 units as $^{13}CH_2$=^{11}C(^{7}CH)-^{12}COOH, $^{14}CH_2$=^{10}C(^{1}CH)-^{9}CH-, and $^{15}CH_3$-^4C($^3CH_2$)-^{5}CH-, respectively. Those C_4 units in combination with a C_8 unit -$^3CH_2$-$^2CH_2$-^{1}CH-^{5}CH-$^6CH_2$-^{7}CH-$^8CH_2$-^{9}CHOR deduced from ^{1}H-^{1}H COSY correlations (Figure 4), established the structure of the aglycone as 4,9-dihydroxyguaia-10(14),11(13)-dien-12-acid, which was good agree with the formula $C_{21}H_{32}O_9$. Moreover, an informative correlation was also observed between the anomeric proton signal at δ_H 5.06 (d, J = 7.7 Hz, H-1') and a methine carbon signal at δ_C 79.5 (C-9) in the HMBC spectrum (Figure 4), implying that the sugar moiety was linked at the C-9 position. Therefore, the planar structure of **3** was established as 4-hydroxyguaia-10(14),11(13)-dien-12-acid 9-*O*-β-D-glucoside. The key NOESY correlations of H-1 with H-5 and H-9, and H-5 with H-7, H-9 and H_3-15 showed that all of those protons (H-1, H-5, H-7, H-9 and H_3-15) were cofacial. Meanwhile, the coupling constants of H-9 (δ_H 4.79, dd, J = 11.0, 3.0 Hz) revealed its α-oriented position. Thus compound **3** was determined to be 4β-hydroxy-1α,5α,7α,9αH-guaia-10(14),11(13)-dien-12-acid 9-*O*-β-D-glucoside.

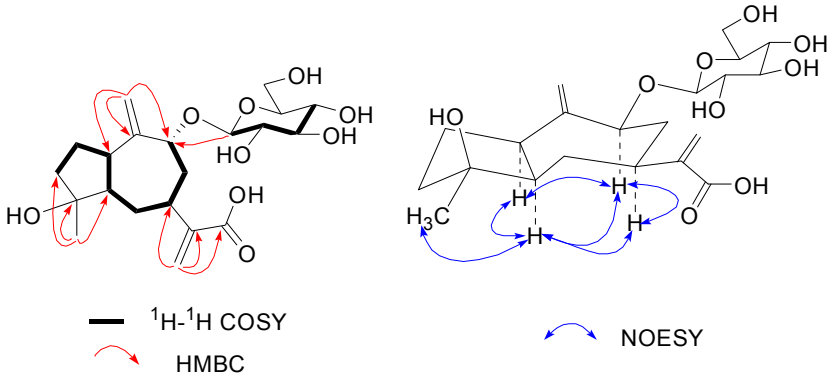

Figure 4. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of **3**.

The cytotoxicity of compounds **1**–**5** were tested against human promyelocytic leukemia HL-60 cell by the MTT method *in vitro*. The results revealed that all compounds were inactive (LC_{50} > 100 μM).

3. Experimental Section

3.1. General

Optical rotations were measured on WZZ2B automatic polarimeter (Precision Instrument Co., Shanghai, China); melting points were obtained on a SGW X-4 micromelting point apparatus (INESA Physico Optical Instrument Co., Ltd, Shanghai, China). The 1D- and 2D-NMR spectra were measured

with a Bruker DRX-400 instrument (Bruker BioS-pin GmbH Company, Rheinstetten, Germany) with TMS as internal standard. ESIMS data were recorded on an API QSTAR mass spectrometer (Applied Biosystem/MSD Sciex, Concord, ON, Canada). Column chromatography was performed on silica gel 60 (200–300 mesh, Qingdao Marine Chemical Ltd, Qingdao, China), Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) and Develosil ODS (50 μm, Nomura Chemical Co. Ltd., Osaka, Japan). Preparative HPLC was performed on a Waters 1525 Binary HPLC pump and a Waters 2414 refractive index detector (Waters Corp, Millipore, Milford, MA, USA) using a YMC-Pack ODS-A column (250 mm × 10 mm I.D.; S-5 μm, 12 nm).

3.2. Plant Material

The aerial parts of *A. artemisiifolia* were collected from Miluo, Hunan Province, P. R. China, in August 2012, and identified by Prof. Zhongshi Zhou, Institute of Plant Protection, Chinese Academy of Agricultural Sciences. The voucher specimen (No. 20120821) was deposited in Hunan Agricultural University.

3.3. Extraction and Isolation

Dried aerial parts of *A. artemisiifolia* (10.0 kg) were powdered and extracted three times with MeOH (95% v/v) at room temperature, then concentrated under reduced pressure to obtain a crude residue (0.5 kg). The residue was further suspended in H_2O (2 L) and extracted with petroleum ether (PE) and EtOAc successively, to yield a PE-soluble fraction (90.0 g), an EtOAc-soluble fraction (90.0 g). The EtOAc-soluble fraction was subjected to silica gel column chromatography (CC) (100–200 mesh) with elution of $CHCl_3$ – MeOH (100:0 → 60:40, v/v) to give six fractions (Fr. C_1–C_6). The Fr. C_2 was further separated by ODS-C_{18} CC (MeOH–H_2O 30:70 → 70:30, v/v) to produce seven sub-fractions (C_2-1–C_2-7), then sub-fraction C_2-3 was further purified by Sephadex LH-20 column (MeOH) and normal silica gel CC (petroleum ether–EtOAc, 9:1) to yield colourless crystal **4** (100.0 mg) and **5** (30.0 mg). Similarly, Fractions C_3 (6.0 g) and C_5 (8.3 g) were fractionated by an ODS-C_{18} column with elution of MeOH–H_2O (30:70 → 70:30, v/v), respectively. Compound **1** (60.0 mg) was obtained from sub-fraction C_3-5 by Sephadex LH-20 column (MeOH) and semi-preparative HPLC chromatography (MeOH-H_2O 48%, v/v, flow rate 3 mL/min, t_R = 23.6 min). Compounds **2** (8.0 mg) and **3** (6.0 mg) were obtained from sub-fraction C_5-2 which purified successively on Sephadex LH-20 column (MeOH) and semi-preparative HPLC chromatography (MeOH-H_2O 38%, v/v, flow rate 3 mL/min; t_R-**3** = 37.3 min, t_R-**2** = 50 min).

3.4. Characterization of Compounds **1**–**3**

Compound **1**: white powder, mp 290–293 °C, $[\alpha]_D^{25}$ −44.8 (*c* 0.9, MeOH); IR (KBr) υ_{max} 3342, 2951, 1769, 1740, 1727, 1704, 1660, 1176, 1035 cm^{-1}; ^{1}H-NMR (400 MHz, C_5D_5N) and ^{13}C-NMR (100 MHz, C_5D_5N) spectroscopic data, see Table 1; positive ion ESIMS *m/z*: 311 $[M+H]^+$, 333 $[M+Na]^+$; negative ESIMS *m/z*: 655 $[2M+Cl]^-$; HRESIMS *m/z*: 311.1852 $[M+H]^+$ (calcd for $C_{17}H_{27}O_5$, 311.1853).

Compound **2**: white powder, mp 305–307 °C, $[\alpha]_D^{25}$ +33.2 (*c* 0.5, MeOH); IR (KBr) υ_{max} 3392, 2921, 2850, 1754, 1688, 1458, 1384, 1229, 1176 cm^{-1}; ^{1}H-NMR (400 MHz, MeOD) and ^{13}C-NMR

(100 MHz, MeOD) spectroscopic data, see Table 2; positive ion ESIMS *m/z*: 413 $[M+H]^+$, 435 $[M+Na]^+$; negative ESIMS *m/z*: 411 $[M-H]^-$, 859 $[2M+Cl]^-$; HRESIMS *m/z*: 435.1971 $[M+Na]^+$ (calcd for $C_{21}H_{32}O_8Na$, 435.1989).

Compound **3**: yellowish syrup, $[\alpha]_D^{25}$ −37.7 (*c* 1.1, MeOH); IR (KBr) υ_{max} 3400, 2923, 2870, 1702, 1458, 1380, 1221 cm^{-1}; ^{1}H-NMR (400 MHz, C_5D_5N) and ^{13}C-NMR (100 MHz, C_5D_5N) spectroscopic data, see Table 2; positive ion ESIMS *m/z*: 451 $[M+Na]^+$, 879 $[2M+Na]^+$; negative ESIMS *m/z*: 427 $[M-H]^-$, 463 $[M+Cl]^-$; HRESIMS *m/z*: 451.1935 $[M+Na]^+$ (calcd for $C_{21}H_{32}O_9Na$, 451.1939).

3.5. Determination of the Configurations of Sugar Unit in **2** *and* **3**

Compounds **2** (2.0 mg) in 1 N HCl (5 mL, 1,4-dioxane–H_2O, 1:1) was heated under reflux for 8 h. After removal of the solvent, the residue was partitioned between Et_2O and H_2O. The water layer was neutralized with 5% NaOH and desalted (Sephadex LH-20, MeOH) to afford the sugar residue (1.0 mg). The sugar residue was derivatized with Sigma Sil-A for 35 min at 70 °C and analyzed by GC-MS [HP-5MS column (30 m × 0.25 mm, 0.25 mm); injection temperature: 250.0 °C; column flow: 1.33 mL/min; ion source temperature: 200.0 °C; interface temperature: 220.0 °C]. In the acid hydrolysate of **2**, D-Glucose was confirmed by comparison of the retention times of the aforementioned derivative with the authentic D-glucose derivative prepared in a similar way, both of which showed identical retention time of 12.17 min. By the same method, the sugar moiety of **3** was identified as D-glucose.

3.6. Cytotoxicity Assays

The cytotoxic activity of each compound against human promyelocytic leukemia HL-60 cell was examined *in vitro* at the Second Xiangya Hospital of Central South University, and was determined by the MTT assay [20,21].

4. Conclusions

The phytochemical investigation of the aerial parts of *A. artemisiifolia* afforded a new pseudoguaianolide (**1**), two new guaiane-type sesquiterpene glucosides (**2** and **3**), as well as two known sesquiterpene dilactones psilostachyin B (**4**) and psilostachyin (**5**). Although all the isolated compounds were inactive against human promyelocytic leukemia HL-60 cell, the discovery of these new compounds further expands our knowledge of the structural diversity of the sesquiterpenoids produced by the plant *A. artemisiifolia*.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/20/03/4450/s1.

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Author Contributions

In this paper, the extraction and isolation were accomplished by Rui Huang; Zhongshi Zhou was in charge of the plant material identification; Wenbing Ding carried out the structural elucidations and the manuscript writing; as corresponding author Youzhi Li organized the study and analyzed the results.

Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds **1**, **4** and **5** are available from the authors.