Unusual Guaiane Sesquiterpenoids from *Artemisia rupestris*

by **Zhi-Yong Xie**, **Ting-Ting Lin**, **Mei-Cun Yao**, **Jin-Zhi Wan**, and **Sheng Yin***

School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510006, P. R. China (phone: +86-20-39943090; fax: +86-20-39943090; e-mail: yinsh2@mail.sysu.edu.cn)

Two new guaiane sesquiterpenoids, (1β,5β)-1-hydroxyguaia-4(15),11(13)-dieno-12,5-lactone (**1**) and 1,5-epoxy-4-hydroxyguai-11(13)-en-12-oic acid (**2**), together with five known compounds, rupestonic acid (**3**), strobilactone A (**4**), antiquorin (**5**), isosclerone (**6**), and 5-hydroxy-2′,3′,4′,7,8-pentamethoxyflavone (**7**), were isolated from a 95% EtOH extract of *Artemisia rupestris*. Compounds **1** and **2** are rare examples of guaiane sesquiterpenoids, incorporating a 12,5-lactone group or featuring a 1,5-epoxy ring, respectively. The structures of **1** and **2** were identified by various spectroscopic methods. Compounds **1**, **4**, and **5** exhibited moderate cytotoxic activities against the human lung cancer 95-D cell line with IC_{50} values of 11.3, 19.8, and 34.5 μM, respectively.

Introduction. – *Artemisia rupestris* L. (Asteraceae), a perennial herb widely distributed in Xinjiang Uygur Autonomous Region of P. R. China, Mongolia, Middle Asia, and northern Europe, has long been used as antiphlogistic and hemostatic agents in traditional Chinese medicine [1]. Previous phytochemical studies have demonstrated that guaiane sesquiterpenoids [2], guaipyridine sesquiterpene alkaloids [3], and flavonoids [4] are the main constituents of *A. rupestris*. A major sesquiterpenoid, rupestonic acid, has been structurally modified for the improvement of its antiviral activities [5]. In the current study, two new guaiane sesquiterpenoids, (1β,5β)-1-hydroxyguaia-4(15),11(13)-dieno-12,5-lactone (**1**) and 1,5-epoxy-4-hydroxyguai-11(13)-en-12-oic acid (**2**), togther with the five known compounds **3**–**7**, were isolated from a 95% EtOH extract of *A. rupestris* (*Fig. 1*). Their structures were identified by spectroscopic methods. All compounds were evaluated for cytotoxic activity against the human lung cancer 95-D cell line. We report herein the isolation, structure elucidation, and cytotoxicities of these compounds.

Results and Discussion. – Compound **1** was isolated as a white amorphous powder. A HR-ESI-MS pseudomolecular-ion peak at *m*/*z* 271.1306 ($[M+Na]^+$) allowed the determination of the molecular formula as $C_{15}H_{20}O_3$. In the IR spectrum, absorption bands at 3000–2500, 1691, and 1631 cm^{-1} indicated the presence of OH, α,β-unsaturated C=O, and C=C moieties [6]. The ^{1}H-NMR spectrum of **1** (*Table*) displayed signals for four olefinic H-atoms (δ(H) 6.24 and 5.58 (2*d*, each $J=1.4$ Hz), and 5.13 and 5.11 (2*t*, each $J=2.3$ Hz)), a Me *d* (δ(H) 1.00 ($J=7.3$ Hz)), and a large number of aliphatic CH_2 and CH *m*s (δ(H) 1.30–2.50). The ^{13}C-NMR spectrum, combined with a HSQC experiment, allowed to assign 15 C-atom signals arising from two terminal C=C bonds (δ(C) 155.7, 142.0, 126.3, and 110.5), a lactone C=O group (δ(C) 167.7), two O-bearing sp^3 quaternary C-atoms (δ(C) 96.1 and 85.7), and two sp^3

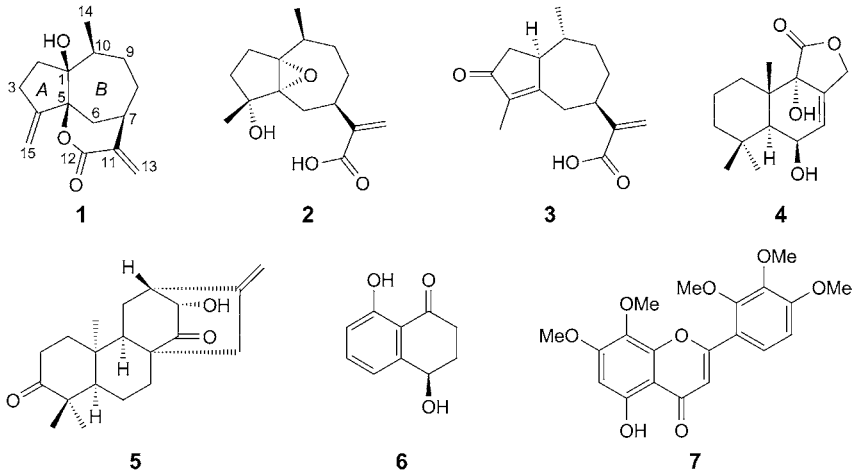

Fig. 1. *Compounds* **1**–**7**, *isolated from* Artemisia rupestris

CH, five sp^3 CH_2, and a Me group. The abovementioned data indicated that compound **1** was a guaiane-type sesquiterpenoid with a lactone ring and two terminal C=C bonds, closely related to the major component rupestonic acid (**3**) [7]. Based on the guaiane skeleton, the O-bearing quaternary C-atoms had to be located at the *A*/*B*-ring-fusion sites (C(1) and C(5)). In the HMBC spectrum (*Fig. 2*), the correlations CH_2(13)/C(11), C(12), and C(7), and CH_2(6)/C(11) and C(5) confirmed that the location of the lactone ring was at C(5). HMBC Cross-peaks Me(14)/C(10), C(1), and C(9) located an OH group at C(1). The CH_2(2) and CH_2(8) groups were distinguished by the key correlations CH_2(2)/C(5) and CH_2(8)/C(11), respectively. The relative configuration of **1** was determined by analysis of its NOESY data (*Fig. 2*). The key NOEs H_α–C(6)/H_α–C(2), H_α–C(6)/H_α–C(8), and H_α–C(2)/H–C(10) suggested that rings *A* and *B* were *cis*-fused, and that these H-atoms were α-oriented. The NOEs H_β–C(6)/H_b–C(15) and H_a–C(13)/H_β–C(8) showed the β-orientation of OH–C(1) and the lactone group at C(5), and the correlations Me(14)/H_β–C(2) and Me(14)/H_β–C(9) the β-orientation of Me(14). Therefore, the structure of compound **1** was identified as (1β,5β)-1-hydroxyguaia-4(15),11(13)-dieno-12,5-lactone. To the best of our knowledge, compound **1** represents only the second example of a guaiane sesquiterpenoid with a 12,5-lactone ring found so far, while the first example, pulicazine, was isolated from *Pulicaria laciniata* in 2009 [8].

Compound **2**, a white powder, displayed a pseudomolecular-ion peak in the HR-ESI-MS at *m*/*z* 289.1410 ($[M+Na]^+$), consistent with a molecular formula $C_{15}H_{22}O_4$, with five degrees of unsaturation. The IR absorptions at 3401, 1691, and 1627 cm^{-1} showed the presence of OH, α,β-unsaturated COOH, and C=C moieties, respectively [6]. Two olefinic H-atoms at δ(H) 6.03 and 5.47 (2*s*) in the ^{1}H-NMR spectrum showed HSQC cross-peaks to a C-atom at δ(C) 121.6, suggesting the presence of a terminal C=C bond. Two Me signals were observed at δ(H) 1.11 (*d*, $J = 7.1$ Hz) and 1.16 (*s*). The ^{13}C-NMR spectrum of **2** displayed 15 C-atom signals including those of a COOH group at δ(C) 173.0, a C=C bond at δ(C) 150.8 and 121.6, three O-bearing sp^3 C-atoms at δ(C)

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2** *in CD_3OD.* δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		85.7		74.1
CH_2(2)	2.24 (*ddd*, J = 12.8, 11.5, 9.0), 1.64 (*dd*, J = 12.8, 7.1)	34.8	1.64 – 1.72 (*m*), 1.89 (overlapped)	29.0
CH_2(3)	2.46 – 2.57 (*m*)	29.0	1.56 – 1.63 (*m*), 1.46 (overlapped)	35.9
C(4)		155.7		80.6
C(5)		96.1		73.6
CH_2(6)	2.56 (*dd*, J = 14.7, 3.2), 2.08 (*dd*, J = 14.7, 3.7)	29.8	2.33, 1.91 (both overlapped)	30.5
H–C(7)	3.02 – 3.06 (*m*)	37.5	2.65 – 2.76 (*m*)	38.7
CH_2(8)	2.15 – 2.20, 1.70 – 1.78 (2*m*)	32.3	1.53 (overlapped), 1.34 – 1.43 (*m*)	31.4
CH_2(9)	1.92 – 2.00, 1.28 – 1.37 (2*m*)	30.5	1.49, 1.84 (both overlapped)	32.1
H–C(10)	2.12 (overlapped)	42.9	2.29 (overlapped)	34.6
C(11)		142.0		150.8
C(12)		167.7		173.0
CH_2(13)	6.24, 5.58 (2*d*, J = 1.4)	126.3	6.03 (*s*), 5.47 (*s*)	121.6
Me(14)	1.00 (*d*, J = 7.3)	19.2	1.11 (*d*, J = 7.1)	16.3
CH_2(15) or Me(15)	5.13, 5.11 (2*t*, J = 2.3)	110.5	1.16 (*s*)	24.0

[a]) ^{1}H- and ^{13}C-NMR data were recorded at 500 and 125 MHz, resp. [b]) ^{1}H- and ^{13}C-NMR data were recorded at 400 and100 MHz, resp.

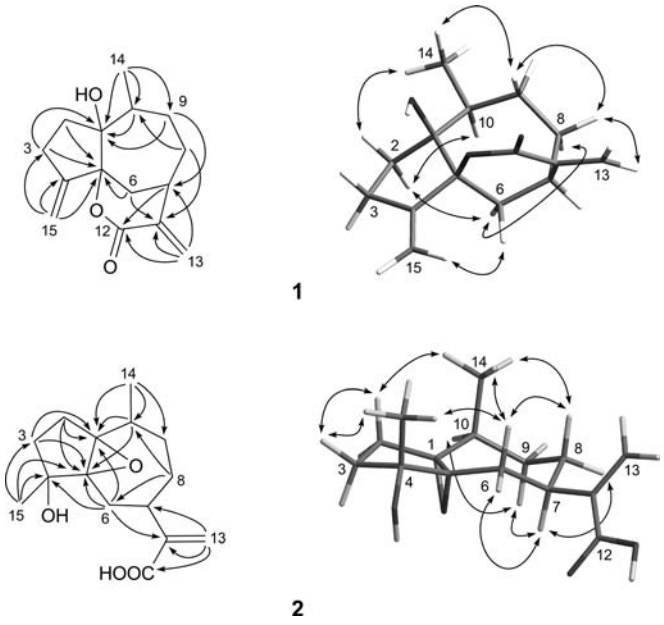

Fig. 2. *Key HMBCs* (H → C) *and NOESY correlations* (H ↔ H) *of compounds* **1** *and* **2**

80.6, 74.1, and 73.6, and nine aliphatic C-atoms. As a C=C bond and a COOH group consumed two of the five degrees of unsaturation, compound **2** had to be tricyclic. The aforementioned data resembled those of **1** indicating that **2** was also a guaiane sesquiterpenoid containing an additional ring. The relatively upfield-shifted O-bearing quaternary C-atoms at δ(C) 74.1 and 73.6 suggested the presence of an epoxy ring between C(1) and C(5), as in the case of 1,5 : 6,7-diepoxy-4-guaiol [9]. The above described deduction was confirmed by 2D-NMR experiments. The α,β-unsaturated COOH group was confirmed by HMBC features to be positioned between CH_2(13) and C(12), C(11), and C(7) (*Fig. 2*). HMBC Cross-peaks Me(15)/C(4), C(3), and C(5), and Me(14)/C(10), C(1), and C(9) placed Me(15) and Me(14) at C(4) and C(10), respectively. HMBCs from both CH_2(6) and CH_2(2) to C(1) and C(5) confirmed the location of the 1,5-epoxy ring. A NOESY experiment allowed the determination of the relative configuration of **2**. The NOEs H–C(7)/H_α–C(9), H_α–C(9)/H–C(10), and H–C(7)/H_α–C(6) established the α-orientation of these H-atoms. The β-orientation of Me(14) and Me(15) were determined by the NOEs Me(14)/H_β–C(8)/H_β–C(6) and Me(15)/H_β–C(6). The NOEs Me(14)/H_β–C(2), H_β–C(2)/H_β–C(3), H_β–C(3)/Me(15), Me(15)/H_β–C(6) revealed the α-orientation of the 1,5-epoxy ring. The structure of compound **2** was thus determined as 1,5-epoxy-4-hydroxyguai-11(13)-en-12-oic acid. Compound **2** is only the second guaiane sesquiterpenoid containing an 1,5-expoxy ring found so far, the first example being 1,5 : 6,7-diepoxy-4-guaiol, isolated before from the soft coral *Sinularia* sp. [9].

The structures of the known compounds were determined as rupestonic acid (**3**) [7], strobilactone A (**4**) [10], antiquorin (**5**) [11], isosclerone (**6**) [12], and 5-hydroxy-

2′,3′,4′,7,8-pentamethoxyflavone (**7**) [13], by their spectroscopic data and comparision with literature data.

All compounds were submitted to a cytotoxic sulforhodamine B (SRB) assay against the human lung cancer 95-D cell line. Compounds **1**, **4**, and **5** exhibited moderate cytotoxic effects with IC_{50} values of 11.3, 19.8, and 34.5 μM, respectively. *Taxol* ($IC_{50} = 11.6$ nM) was used as the positive control.

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Experimental Part

General. Column chromatography (CC): commercial silica gel for TLC (SiO_2; *Qingdao Hai Yang Chemical Group Co., Ltd.*); C_{18} column (*Phenomenex 00G-4324-N0*; 10 μm, 10 mm (i.d.) × 25 cm); *MCI* gel (*Mitsubishi*, Japan); chiral CD-Ph column (*Shiseido*, Japan). HPLC: *Agilent 1500*, American. TLC: precoated SiO_2 plates (*HSGF 254*; *Yantai Jiang You Silica Gel Development Co., Ltd.*). Optical rotation: *Jasco-P-1010* polarimeter. UV Spectra: *Beckman DU-600* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker-Vector-22* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian-Inova-400* and *Bruker-Avance-500* spectrometers: δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *Micromass-Quattro* triple-quadrupole mass spectrometer equipped with an ESI source (*Micromass*, Manchester, UK); in *m*/*z*.

Plant Material. The whole plant of *A. rupestris* was collected from the Xinjiang Uygur Autonomous Region, P. R. China, in 2009, and authenticated by Prof. *Shi-Man Huang*, Department of Biology, Hainan University, P. R. China. A voucher specimen (YZH-2009-I) was deposited with the School of Pharmaceutical Science, Sun Yat-sen University.

Extraction and Isolation. The air-dried and powdered whole plant of *A. rupestris* (10 kg) was percolated at r.t. with 95% EtOH (3 × 25 l, 5 d for each time) to afford 1480 g of crude extract. The extract was suspended in H_2O (1.5 l) and partitioned successively with petroleum ether, AcOEt, and BuOH. The AcOEt fraction (468 g) was applied to CC (*MCI* gel, 50 → 95% aq. EtOH: *Fractions A – F*. *Fr. D* (11 g) was subjected to CC (SiO_2, petroleum ether/AcOEt 50:1 → 1:1): *Frs. D1 – D4*. *Fr. D2* (580 mg) was subjected to CC (SiO_2, petroleum ether/acetone 10:1): **7** (87 mg). *Frs. E* (21 g) and *F* (28 g) were each subjected to CC (SiO_2, petroleum ether/AcOEt 20:1): *Frs. E1 – E4* and *Frs. F1 – F5*. *Fr. E1* was further purified by CC (*Sephadex LH-20*, CH_2Cl_2/MeOH 1:1): **6** (194 mg). *Frs. E3* and *E4* were each subjected to CC (SiO_2, cyclohexane/AcOEt 15:1): **3** (138 mg) and **5** (52 mg), resp. HPLC Purification of *Frs. F1*, *F3*, and *F4* (70% aq. MeOH) afforded pure **4** (8 mg), **2** (5 mg), and **1** (7 mg), resp.

(1β,5β)-1-Hydroxyguaia-4(15),11(13)-dieno-12,5-lactone (= rel-*(4*R,*7*S,*7a*S,*10a*S*)-Octahydro-7a-hydroxy-7-methyl-3,10-bis(methylene)-4,410a-methano-10a*H*-cyclopent[*b*]oxonin-2(3*H*)-one*; **1**): White amorphous powder. $[\alpha]_D^{20} = +8.5$ ($c = 0.50$, MeOH). IR (KBr): 3394, 2969, 2929, 1691, 1631, 1317, 1220, 1134, 937. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 249 ($[M+H]^+$), 231 ($[M+H-H_2O]^+$). HR-ESI-MS: 271.1306 ($[M+Na]^+$, $C_{15}H_{20}NaO_3^+$; calc. 271.1310).

1,5-Epoxy-4-hydroxyguai-11(13)-en-12-oic Acid (= rel-*(3*R,*3a*R,*5*R,*8*S,*8a*R*)-Hexahydro-3-hydroxy-3,8-dimethyl-α-methylene-1*H,*4*H-*3a,8a-epoxyazulene-5-acetic Acid*; **2**): White amorphous powder. $[\alpha]_D^{20} = +10.2$ ($c = 0.15$, MeOH). IR (KBr): 3401, 2965, 2926, 1691, 1627, 1364, 1221, 1172, 918. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 555 ($[2M+Na]^+$), 289 ($[M+Na]^+$). HR-ESI-MS: 289.1410 ($[M+Na]^+$, $C_{15}H_{22}NaO_4^+$; calc. 289.1416).

Cytotoxic Assay. Suspended human lung tumor 95-D cells were cultured in *RPMI 1640* (*Gibco*, Grand Island, NY, USA) and supplemented with 10% fetal bovine serum (*Sigma–Aldrich*), 2 nM L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml) at 37° in a humidified atmosphere with 5% CO_2. The logarithmic phase cells (80 μl) were seeded onto 96-well plates at the concentration of $5 \cdot 10^3$ cells per well. After 24 h, different concentrations of the sample, dissolved in DMSO, were added at

10 µl/well, and 3 parallel wells for each concentration were tested. Control cells were treated with DMSO alone and positive controls with taxol. The cells were cultivated for 72 h and then fixed with 10% trichloroacetic acid for 1 h and washed with dist. H_2O. SRB (sulforhodamine B) was dissolved at 4 mg/ml in PBS (phosphate-buffered saline). Then 100 µl of this soln. were added to each well, and the cells were stained for 20 min. The supernatant was then removed, and 100 µl of *Tris* buffer (10 mM) was added into each well. The absorbance (*A* value) at a wavelength of 515 nm was measured with a microplate reader (*Thermo*). The inhibition rates were calculated with *OD* mean values from the equation inhibition rate $= (OD_{control} - OD_{sample})/OD_{control}$.

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