Three New Sesquiterpenoids from *Echinops ritro* L.

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From the whole plants of *E. ritro* L., the three new sesquiterpenoids (3α,4α,6α)-3,13-dihydroxyguaia-7(11),10(14)-dieno-12,6-lactone (**1**), (3α,4α,6α,11β)-3-hydroxyguai-1(10)-eno-12,6-lactone (**2**), and (11α)-11,13-dihydroarglanilic acid methyl ester (=(4β,6α,11α)-4,6-dihydroxy-1-oxoeudesm-2-en-12-oic acid methyl ester; **3**), together with eight known sesquiterpenoids, were isolated. Their structures were elucidated through analysis of spectroscopic data including extensive 2D-NMR.

Introduction. – The genus *Echinops* (Compositae) consists of *ca.* 100 species in the world. This genus is represented by 17 species mainly distributed in northwestern part of China [1]. In China, *E. ritro* L. is only found in Xinjiang Uyghur Autonomous Region and is used as a remedy for different ailments in *Uighur Pharmacopeia* instead of *E. grijissi* HANCE (commercial name: Yuzhou Loulu) [2]. Previous chemical investigation on the *Echinops* species demonstrated the presence of polyacetylene-containing thiophenes [3–5], alkaloids [6], sesquiterpenoids [7], and flavone and flavone glycosides [8]. Further chemical analysis of *Echinops* species from Xinjiang Uyghur Autonomous Region led to the isolation of three new sesquiterpenoids, (3α,4α,6α)-3,13-dihydroxyguaia-7(11),10(14)-dieno-12,6-lactone[1]) (**1**), (3α,4α,6α,11β)-3-hydroxyguai-1(10)-eno-12,6-lactone[1]) (**2**), and (11α)-11,13-dihydroarglanilic acid methyl ester[1]) (**3**), along with the eight known compounds **4**–**11** [9–15] from the AcOEt extract of the MeOH extract. This article describes the isolation and structure elucidation of these new compounds.

Results and Discussion. – The IR spectrum of compound **1** indicated the presence of an OH group (3442 cm^{-1}), an α,β-unsaturated γ-lactone group (1737cm^{-1}), and a C=C bond (1658 cm^{-1}). The molecular formula $C_{15}H_{20}O_4$ was determined by HR-ESI-MS experiments ($[M+Na]^+$ at *m*/*z* 287.1249), in combination with ^{1}H- and ^{13}C-NMR data (*Table 1*) indicating six degrees of unsaturation. Detailed analysis of the ^{1}H,^{1}H-COSY, HSQC, HMBC, and ROESY data established the structure of **1** as (3α,4α,6α)-3,13-dihydroxyguaia-7(11),10(14)-dieno-12,6-lactone[1]).

Analysis of the 1D-NMR data and HSQC spectra revealed that **1** contains one Me, three CH_2, and three CH groups, as well as an additional two CH groups and one CH_2

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

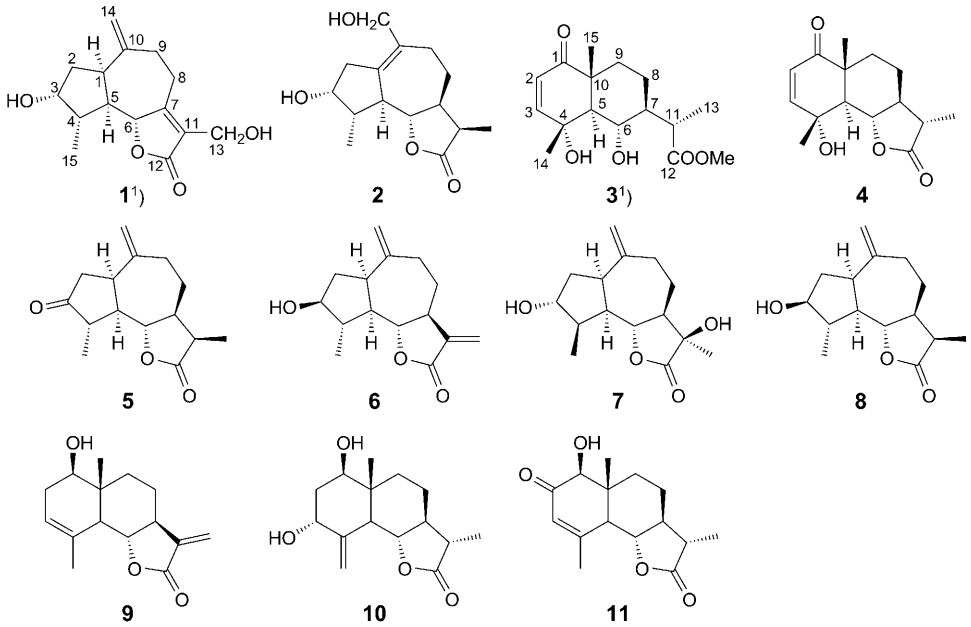

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; CD_3OD) *of* **1**[1]) *and* **2**[1]). δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	3.22–3.27 (*m*)	46.4		141.5
CH_2(2)	1.89–1.92 (*m*)	39.2	2.54–2.56 (*m*), 2.46–2.48 (*m*)	40.5
H–C(3)	4.14–4.15 (*m*)	75.4	4.04–4.06 (*m*)	75.4
H–C(4)	2.25 (*m*)	46.4	2.09–2.10 (*m*)	45.7
H–C(5)	1.76–1.81 (*m*)	50.8	2.47–2.49 (*m*)	53.2
H–C(6)	4.69 (*d*, J = 11.5)	86.9	3.91 (*t*, J = 10.5)	86.7
C(7) or H–C(7)		170.4	2.33–2.38 (*m*)	52.8
CH_2(8)	3.29–3.30 (*m*), 2.35–2.45 (*m*)	31.1	1.74–1.78 (*m*), 1.32–1.36 (*m*)	26.2
CH_2(9)	2.62–2.65 (*m*), 2.08–2.10 (*m*)	33.9	2.45–2.46 (*m*), 2.10–2.12 (*m*)	31.2
C(10)		150.4		136.5
C(11) or H–C(11)		126.1	2.64–2.67 (*m*)	41.5
C(12)		175.0		182.1
CH_2(13) or Me(13)	4.21–4.27 (*dd*, J = 18.5, 10.0)	53.7	1.11 (*d*, J = 7.5)	10.4
CH_2(14)	5.19 (*d*, J = 3.5), 4.90 (*d*, J = 3.5)	111.8	4.02	65.7
Me(15)	1.15 (*d*, J = 6.5)	16.0	1.19 (*d*, J = 6.5)	15.2

group attached to an O-function, an endocyclic C=C bond, an exocyclic CH_2= group, and one CO group (*Table 1*). The ^{1}H-NMR spectrum showed the exocyclic CH_2= group at δ(H) 5.19 and 4.90 (*d*, J = 3.5 Hz, 1 H, each), an O-bearing CH_2 group at δ(H) 4.21–4.27 (*dd*, J = 18.5, 10.0 Hz), and a Me group at δ(H) 1.15 (*d*, J = 6.5 Hz). These characteristic signals were in accordance with an guaiane-type sesquiterpene skeleton. The ^{13}C-NMR data of **1** were similar to those of (3β,4α,11β)-3-hydroxyguai-10(14)-

eno-12,6-lactone [13], except for C(7), C(11), C(13), and C(3), which suggested they have similar structures. In the ^{13}C-NMR spectrum, the signals of C(7) and C(11) were shifted downfield from δ(C) 55.7 and 74.5 in [13] to δ(C) 170.4 and 126.1 in **1**, which suggested that there is a C=C bond in **1** instead of single bond. The signal of C(13) was shifted downfield from δ(C) 21.6 in [13] to δ(C) 53.7 in **1**, meanwhile it was transformed into a CH_2 group from a Me group, which suggested that there is a CH_2OH group in **1** instead of a Me group. This was further confirmed by the HMBCs CH_2(2)/C(1), C(3), C(4), and C(5), H−C(4)/C(1), C(2), C(5), C(6), and C(15), H−C(5)/C(1), C(4), C(6), C(7), and C(15), CH_2(14)/C(1) and C(9), CH_3(15)/C(3), C(4) and C(5), and CH_2(13)/C(7), C(1l), and C(12), and the ^{1}H,^{1}H-COSY data confirmed the connectivity of C(1) to C(2) and C(5), of C(3) to C(2) and C(4), of C(5) to C(4) and C(6), and of C(8) to C(7) and C(9) (*Fig. 1*,*a*). The relative configuration of **1** was assigned by analysis of ^{1}H-NMR coupling constants (*Table 1*) and ROESY correlations. A *J* value of *ca.* 11.5 Hz for H−C(6) suggested a *trans* configuration of H−C(6), assumed to be β-oriented, and H−C(5). The ROESY plot of **1** showed correlations of H−C(5) to H−C(1) and Me(15), of H−C(6) to H_β−C(2) and H−C(4), and of H−C(3) to H_β-C(2) and H−C(4) (*Fig. 1*,*b*). These findings showed that the relative configuration of H−C(1), H−C(3), H−C(4), H−C(5), and H−C(6) in **1** was α, β, β, α, and β, respectively.

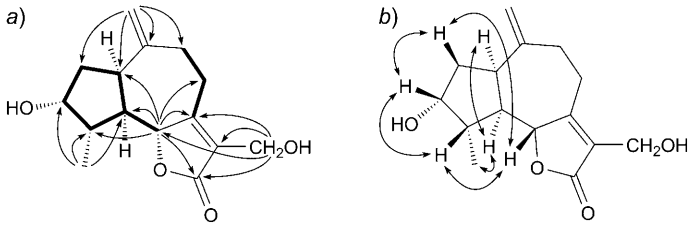

Fig. 1. a) *^{1}H,^{1}H-COSYs* (—) *and key HMBCs* (H → C) *of* **1**. b) *Key ROESY correlations* (↔) *of* **1**.

A molecular formula $C_{15}H_{22}O_4$ was established for compound **2** from the HR-ESI-MS ($[M+Na]^+$ at *m*/*z* 289.1425). The ^{1}H- and ^{13}C-NMR (*Table 1*) disclosed a guaiane-type skeleton [13]. The structure of **2** was established from the ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HSQC, HMBC, and ROESY data as (3α,4α,6α,11β)-3-hydroxyguai-1(10)-eno-12,6-lactone[1]).

The ^{13}C-NMR spectrum of **2** showed chemical-shift values similar to those of (3β,4α,11β)-3-hydroxyguai-10(14)-eno-12,6-lactone [13], except for C(1), C(10), and C(14). The chemical shifts of C(1) (δ(C) 141.5) and C(10) (δ(C) 136.5) indicated the presence of a C=C bond between C(1) and C(10) in **2**. The signal of C(14) was shifted upfield from δ(C) 111.2 in [13] to δ(C) 65.7 in **2**, which suggested that a CH_2OH group was present in **2** instead of a terminal CH_2=C moiety. The structure of **2** was further confirmed by the HMBCs H−C(2)/C(1) and C(10), H−C(4)/C(2), H−C(5)/C(1) and C(10), H−C(6)/C(1), CH_3(13)/C(7), and CH_2(14)/C(1), C(9), and C(10), and by the ^{1}H,^{1}H-COSYs H−C(2)/H−C(3), H−C(5)/H−C(4), and H−C(8)/H−C(9) (*Fig. 2*,*a*). Finally, the relative configuration of **2** was established by a ROESY experiment and H-atom coupling constants. The H−C(6) (*t*, *J* = 10.5 Hz) suggested a *trans* configuration of H−C(6) with respect to H−C(5) and H−C(7). When H−C(6) was assigned as β-

oriented, the ROESY correlations (*Fig. 2,b*) of H–C(6) to H–C(4), H_β–C(2), and Me(13) of H–C(5) to H–C(7) and Me(15), and of H–C(3) to H–C(4) and H_β–C(2) suggested the depicted relative configuration of **2**.

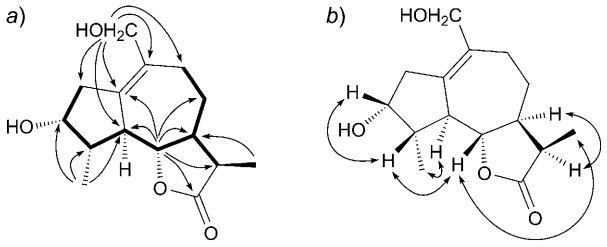

Fig. 2. a) *^{1}H,^{1}H-COSYs* (—) *and key HMBCs* (H → C) *of* **2**. b) *Key ROESY correlations* (↔) *of* **2**.

The HR-ESI-MS of compound **3** gave a *quasi*-molecular-ion peak at *m*/*z* 297.1709 ($[M+H]^+$), corresponding to the molecular formula $C_{16}H_{24}O_5$, requiring six degrees of unsaturation. The functional groups were also deduced from IR absorptions at 3312 (MeO), 1732 (α,β-unsaturated 6-ring ketone group), and 1658 (C=C bond) cm^{-1}. Finally, the structure of **3** was established by its ^{1}H,^{1}H-COSY, HSQC, HMBC, and ROESY data as (11α)-11,13-dihydroarglanilic acid methyl ester[1]).

The compound **3** probably originated from **4** through methanolysis during the plant extraction with MeOH. The ^{1}H- and ^{13}C-NMR of data (*Table 2*) were very close to that of vulgarin (**4**) [9]. It showed the presence of the same eudesmane sesquiterpenoid skeleton, except for the presence of an MeO group (δ(C) 51.9) in **3**. The functional groups were assigned on the basis of HMBC and ^{1}H,^{1}H-COSY studies (*Fig. 3,a*). The correlations of the MeO H-atoms to C(12) and C(11), and the correlations of H–C(6) to C(12) could not be found, which indicated that an acid ester was formed by opening

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; CD_3OD) *of* **3**[1]) *and* **4**[1]). δ in ppm, *J* in Hz.

	3			**4**	
	δ(H)	δ(C)		δ(H)	δ(C)
C(1)		205.5			201.6
H–C(2)	5.79 (*d*, *J* = 10)	125.2		5.97 (*d*, *J* = 10.5)	125.6
H–C(3)	6.61 (*d*, *J* = 10)	155.2		6.68 (*d*, *J* = 10.5)	151.7
C(4)		72.5			70.1
H–C(5)	2.03 (*d*, *J* = 10.5)	56.6		2.54 (*d*, *J* = 11.5)	55.0
H–C(6)	4.14 (*t*, *J* = 10.5)	70.3		3.46 (*t*, *J* = 11)	78.5
H–C(7)	1.62–1.63 (*m*)	50.9		2.10–2.13 (*m*)	48.2
CH_2(8)	1.68–1.70 (*m*), 1.36–1.37 (*m*)	22.3		1.89–1.92 (*m*), 1.60–1.64 (*m*)	20.0
CH_2(9)	1.88–1.90 (*m*), 1.36–1.37 (*m*)	35.3		2.10–2.13 (*m*), 1.67–1.71 (*m*)	34.2
C(10)		46.8			46.0
H–C(11)	2.94–2.97 (dq, *J* = 5.0, 6.0)	39.6		2.76–2.79 (*m*)	37.8
C(12)		177.4			178.8
Me(13)	1.18 (d, *J* = 6.0)	14.6	Me(13)	1.31 (*d*, *J* = 8.0)	9.7
Me(14)	1.51 (*s*)	23.2		1.80 (*s*)	23.9
Me(15)	1.08 (*s*)	20.0		1.28 (*s*)	19.7
MeO	3.67 (*s*)	51.9			

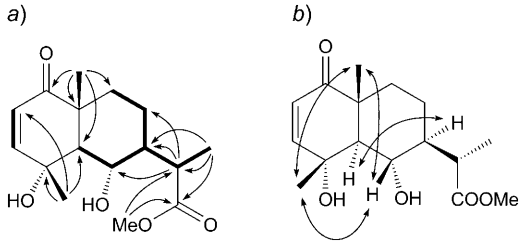

Fig. 3. a) *$^1H,^1H$-COSYs* (—) *and key HMBCs* (H → C) *of* **3**. b) *Key ROESY correlations* (↔) *of* **3**

the lactone ring of **4**. ROESY Correlations were observed between H–C(5) and H–C(7), and between H–C(6) and Me(14) and Me(15) (*Fig. 3,b*), which confirmed that Me(14), Me(15), and H–C(6) were *β*-oriented. We determined the relative configuration of the Me group at C(11) as (11*S*) by comparison with the data of vulgarin.

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Experimental Part

General. Colunm chromatography (CC) and TLC: silica gel (SiO_2; 200–300 mesh) from *Qingdao Marine Chemical Factory*, Qingdao, P. R. China. Optical rotations: *Jasco-DIP-370* digital polarimeter. M.p.: *YuHua-X-4* apparatus. IR: *Perkin-Elmer-577* spectrometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-AM-400* and *-DRX-500* spectrometers; Me_4Si as an internal standard. MS: *VG-Autospec-3000* mass spectrometer; in *m/z* (rel. %).

Plant Material. The whole plants of *E. ritro* L. were collected from Xinjiang Uyghur Autonomous Region, P. R. China, in August, 2006, and were identified by Prof. *Sun Hang* of the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen has been deposited with the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered sample (30 kg) was extracted exhaustively with MeOH (95%) under reflux and concentrated to give a crude extract which was suspended in H_2O and then extracted exhaustively with petroleum ether, AcOEt, and BuOH. The AcOEt fraction (160 g) was subjected to CC (SiO_2 (200–300 mesh; 1.5 kg), $CHCl_3$, then 99 → 90% $CHCl_3$/MeOH, then MeOH): *Fractions 1–6*. Compounds **1** (10 mg), **2** (19 mg), and **3** (35 mg) were obtained from *Fr. 3* after repeated CC (SiO_2, petroleum ether/acetone), and *RP-18*).

(3α,4α,6α)-3,13-Dihydroxyguaia-7(11),10(14)-dieno-12,6-lactone (= rel-*(6a*R,*8*R,*9*S,*9a*R,*9b*R*)-5,6,6a,7,8,9,9a,9b-Octahydro-8-hydroxy-3-(hydroxymethyl)-9-methyl-6-methyleneazuleno[4,5-*b*]furan-2(4*H*)-one*; **1**): Colorless crystals from MeOH. M.p. 172–174°. $[\alpha]_D^{21} = +20.9$ ($c = 0.00575$, MeOH). IR (KBr): 3442, 2933, 1737, 1658, 1641, 1016. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS (pos.): 287.1249 ($C_{15}H_{20}NaO_4^+$; calc. 287.1259).

(3α,4α,6α,11β)-3-Hydroxyguai-1(10)-eno-12,6-lactone (= rel-*(3*R,*3a*S,*8*R,*9*S,*9a*S,*9b*S*)-3a,4,5,7,8,9,9a,9b-Octahydro-8-hydroxy-6-(hydroxymethyl)-3,9-dimethylazuleno[4,5-*b*]furan-2(3*H*)-one*; **2**): Colorless crystals from MeOH. M.p. 167–169°. $[\alpha]_D^{21} = +4.1$ ($c = 0.0097$, MeOH). IR (KBr): 3440, 2933, 1767, 1749, 1219, 984. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 266 (20, M^+), 248 (100), 230 (30), 217 (35), 175 (60), 163 (90). HR-ESI-MS (pos.): 289.1425 ($C_{15}H_{22}NaO_4^+$; calc. 289.1415).

(11α)-11,13-Dihydroarglanilic Acid Methyl Ester (= rel-*(α*R,*1*R,*2*R,*4a*S,*8*S,*8a*R*)-1,2,3,4,4a,5,8,8a-Octahydro-1,8-dihydroxy-α,4a,8-trimethyl-5-oxonaphthalene-2-acetic Acid Methyl Ester*; **3**): Colorless

crystals from MeOH. M.p. 181–184°. $[\alpha]_D^{21} = -42.7$ ($c = 0.01475$, MeOH). IR (KBr): 3312, 2983, 2950, 1732, 1678, 1252. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 278 (4, $[M-H_2O]^+$), 264 (7), 249 (80), 203 (45), 172 (100), 149 (60). HR-ESI-MS (pos.): 297.1709 ($[M+H]^+$, $C_{16}H_{25}O_5^+$; calc. 297.1701).

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