Two New Sesquiterpenes from the Roots of *Valeriana fauriei* BRIQ.

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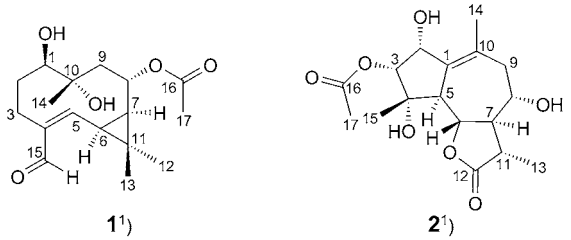
A phytochemical investigation of the MeOH extract of *Valeriana fauriei* BRIQ. roots resulted in the isolation of two new sesquiterpenes, isovalerianin A (=(1β,4*Z*,6β,8α)-8-(acetyloxy)-1,10-dihydroxy-6,11-cyclogermacr-4-en-15-al=*rel*-(1*R*,2*Z*,6*S*,7*R*,9*R*,10*S*)-9-(acetyloxy)-6,7-dihydroxy-7,11,11-trimethylbicyclo[8.1.0]undec-2-ene-3-carboxaldehyde; **1**) and valerianin C (=(2α,3α,6α,8α)-3-(acetyloxy)-2,4,8-trihydroxyguai-1(10)-ene-12,6-lactone=*rel*-(3*R*,3a*S*,4*R*,7*S*,8*S*,9*R*,9a*R*,9b*R*)-8-(acetyloxy)-3a,4,5,7,8,9,9a,9b-octahydro-4,7,9-trihydroxy-3,6,9-trimethylazuleno[4,5-*b*]furan-2(3*H*)-one; **2**), together with six known compounds, *i.e.*, camphor, methyl 4-hydroxybenzoate, 2-methoxybenzoic acid, benzoic acid, quercetin, and kaempferol. The structures of the compounds were established by detailed spectral analysis and comparison with previously reported data.

Introduction. – The genus *Valeriana* (Valerianaceae) consists of *ca*. 250 species with many more subspecies and has been widely used as a mild sedative and sleep aid in Europe, Asia, and North America [1][2]. *Valeriana officinalis* is the official species categorized as plant C extract in the European Pharmacopoeia and is commonly referred to as valerian, and the rhizomes and roots of this plant exhibit anxiolytic, antidepressant, antispasmodic, sedative, and anti-HIV activities [3][4]. *Valeriana fauriei* BRIQ., which belongs to the same genus plant as *V. officinalis* L. and is abundant in the northeast of China, South Korea, and Japan, has been used for hundreds of years in folk medicine. It is not yet clearly understood, which components of *V. fauriei* are responsible for its therapeutic properties [5–7].

Previous investigations on the roots of *V. fauriei* BRIQ. by our group have resulted in the isolation and characterization of phenols, flavones, and terpenoids. Some of the isolated compounds were evaluated for their antidepressant-activity potential based on recording the total duration of immobility of the forced-swim test on mice [8]. To clarify the activity ingredients of *V. fauriei* BRIQ., the hexane extract of *V. fauriei* BRIQ. was subjected to phytochemical investigation resulting in the isolation of a novel

germacrane-type sesquiterpene, **1**[1]), and a novel guaianolide-type sesquiterpene, **2**[1]) (*Fig. 1*), together with six known compounds. In this article, we describe the isolation and structural characterization of all the eight compounds by their physicochemical properties and spectral data, such as UV, IR, 1D- and 2D-NMR, and MS.



1[1]) **2**[1])

Fig. 1. *Sesquiterpenes* **1** *and* **2**, *isolated from* Valeriana fauriei BRIQ.

Results and Discussion. – Repeated column chromatography (CC) and semi-prep. HPLC of the hexane-soluble fraction from the MeOH extracts of the dried roots of *V. fauriei* BRIQ. yielded two new compounds, isovalerianin A (**1**) and valerianin C (**2**), and six known compounds.

Compound **1** was obtained as white powder, whose molecular formula was determined as $C_{17}H_{26}O_5$ by an EI-MS. A UV maximum was observed at 254 nm. The IR spectrum showed absorptions at 3433 (OH), 2924 (CH_2), 1739 (C=O), and 1668 (C=C) cm^{-1}. The ^{1}H-NMR spectrum (*Table*) established the presence of four Me groups at δ(H) 2.05, 1.24, 1.23, and 1.03 (4*s*), two H-atoms bearing an O-atom at δ(H) 4.59 (*td*, $J = 11$, 2.5 Hz) and 2.94 (*dd*, $J = 11$, 3 Hz), an olefinic H-atom at δ(H) 6.95 (*d*, $J =$ 9.5 Hz), and an aldehyde H-atom at δ(H) 9.40 (*s*). The ^{13}C-NMR spectrum of **1** (*Table*) revealed the presence of 17 C-atoms which were identified with the aid of a DEPT-135 experiment as four Me, three CH_2, and six CH groups, and four quaternary C-atoms. A detailed analysis of the ^{1}H- and ^{13}C-NMR spectra of **1** disclosed the characteristic features of a germacrane-type sesquiterpene skeleton (germacrane = 1,7-dimethyl-4-(1-methylethyl)cyclodecane). The planar formula of compound **1** was deduced from a ^{1}H,^{1}H-COSY and a HMBC experiment (Fig. 2). The ^{1}H,^{1}H-COSY plot revealed the correlations H–C(1)/CH_2(2), CH_2(2)/CH_2(3), CH_2(5)/CH_2(6), CH_2(6)/CH_2(7), CH_2(7)/H–C(8), as well as H–C(8)/CH_2(9), which confirmed the presence of a germacrane skeleton. Key correlations in the HMBC spectrum included a ^3J correlation between δ(H) 1.03 (*s*, Me(14)) and both the signal of a C-atom bearing an O-atom at δ(C) 62.4 (C(1)) and that of a CH_2 group at δ(C) 46.5 (C(9)), a ^3J correlation between δ(H) 1.23 and 1.24 (2*s*, Me(12), Me(13)) and the signals of both C(6) and C(7), a ^3J correlation between the signal of a H-atom bearing an O-atom at δ(H) 4.59 (*td*, $J = 11$, 2.5 Hz) and the C=O signal at δ(C) 170.0 (C(16)), and a ^2J correlation between the aldehyde signal at δ(H) 9.40 (H–C(15)) and the olefinic signal at δ(C) 144.4 (C(4)). The relative configuration of **1** was established by a NOESY experiment

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

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) *of* **1** *and* **2**. δ in ppm, J in Hz.

Position[1])	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1) or C(1)	2.94 (*dd*, *J* = 11.0, 3.0)	62.4		137.8
CH_2(2) or H–C(2)	1.21 – 1.25 (*m*, H_β), 2.21 – 2.27 (*m*, H_α)	27.2	4.19 (*d*, *J* = 4.0)	71.3
CH_2(3) or H–C(3)	2.79 (*td*, *J* = 13.5, 3.5, H_β), 2.39 (*td*, *J* = 13.5, 3.0, H_α)	20.4	4.85 (*d*, *J* = 4.0)	78.7
C(4)		144.4		80.0
H–C(5)	6.95 (*d*, *J* = 9.5)	151.8	2.82 (*d*, *J* = 10.0)	53.6
H–C(6)	1.93 (*t*-like, *J* = 9.5)	28.8	4.04 (*dd*, *J* = 11.0, 10.0)	75.9
H–C(7)	1.39 (*dd*, *J* = 10.0, 11.0)	40.2	2.24 (*dd*, *J* = 11.0, 10.0)	39.2
H–C(8)	4.59 (*td*, *J* = 11.0, 2.5)	69.8	4.71 (*dd*, *J* = 10.0, 4.0)	82.4
CH_2(9)	1.32 – 1.38 (*m*, H_β), 2.27 – 2.31 (*m*, H_α)	46.5	2.38 – 2.45 (*m*), 2.21 – 2.25 (*m*)	41.7
C(10)		57.7		131.3
C(11) or H–C(11)		24.3	2.64 (*dd*, *J* = 7.0, 10.0)	56.7
Me(12) or C(12)	1.23 (*s*)	17.8		178.1
Me(13)	1.24 (*s*)	28.1	1.18 (*d*, *J* = 7.0)	15.2
Me(14)	1.03 (*s*)	15.6	1.77 (*s*)	23.2
H–C(15) or Me(15)	9.40 (*s*)	193.5	1.33 (*s*)	21.3
C(16)		170.0		170.0
Me(17)	2.05 (*s*)	21.1	2.04 (*s*)	21.0

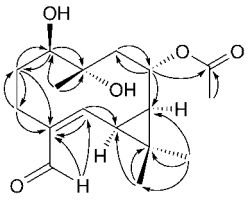


Fig. 2. *Key HMBCs* (H → C) *of compound* **1**

and the ^{1}H-NMR spectrum (chemical shift and coupling constants). The geometry of the C(4)=C(5) bond was assigned to be (*Z*) on the basis of the low-field olefinic-H-atom resonance at δ(H) 6.95 (H–C(5)) which is different from that of similar (*E*)-isomers (δ(H) 6.32 – 6.51 [8][9]). The large coupling constant between H–C(8) and H–C(7) (11.0 Hz) established their *trans* reslationship and β-configuration of H–C(8). Additionally, the generally observed *cis*-fusion of the two rings [10] was corroborated by the coupling constant between H–C(6) and H–C(7) (*J* = 9.5 Hz). Significant NOEs between H–C(8) and Me(13) indicated that these group, *i.e.*, H–C(8) and the *cis*-fused 3-membered ring, were positioned on the same side of the 10-membered ring (*Fig. 3*). A significant NOE between H–C(1) and H–C(7) also indicated their position on the same but opposite side of the avaraged ring plane. Therefore, the structure of **1** was identified as (1β,4*Z*,6β,8α)-8-(acetyloxy)-1,10-dihydroxyl-6,11-cyclogermacr-4-en-15-al, and given the trivial name isovalerianin A.

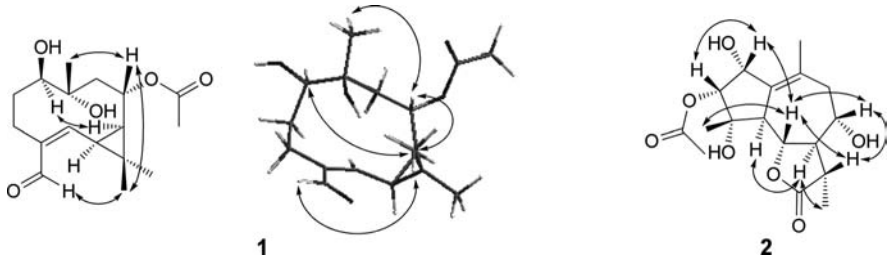


Fig. 3. *Key NOE correlations* (H ↔ H) *of compounds* **1** *and* **2**

Compound **2** was obtained as colorless needles. It has the molecular formula $C_{17}H_{24}O_7$, based on the EI-MS ($[M+Na]^+$ at m/z 363). The ^{13}C-NMR and DEPT spectra showed 17 signals, including four Me, one CH_2, and seven CH groups, and five quaternary C-atoms. The ^{1}H-NMR spectrum (*Table*) established the presence of four Me groups of which two were assigned to Me(14) (δ(H) 1.77 (*s*)) and Me(15) (δ(H) 1.33 (*s*)), one to the Me(13) in α-position at the γ-lactone ring (δ(H) 1.18 (*d*, $J = 7.0$ Hz) and one to Me(17) bearing a C=O group (δ(H) 2.04 (*s*)). The signal at δ(H) 4.04 (*dd*, $J = 11.0$, 10.0 Hz) was assigned to H–C(6) which was coupled both with H–C(5) (δ(H) 2.82 (*d*, $J = 10.0$ Hz)) and H–C(7) (δ(H) 2.24 (*dd*, $J = 11.0$, 10.0 Hz)) and thus established the *trans* relationship of these three H-atoms [11]. Since the naturally occurring guaianolides (guaiano-12,6-lactones) have an α-oriented H–C(7) [12], this meant that the orientation of H–C(5) was α and H–C(6) was β, respectively (guaiane = (1*S*,3a*S*,4*S*,7*R*,8a*S*)-decahydro-1,4-dimethyl-7-(1-methylethyl)azulene). C(5)/H–C(7) as well as H–C(6)/H–C(11). These results also indicated that Me(11) was α-oriented. Significant NOESY correlations were observed for H–C(2)/H–C(3), H–C(2)/H–C(6), H–C(6)/H–C(3), H–C(6)/Me(15), H–C(6)/H–C(8) and H–C(6)/H–C(11) indicating that they were all on the same β-side (*Fig. 3*). Based on the above evidence, **2** was identified as (2α,3α,6α,8α)-3-(acetyloxy)-2,4,8-trihydroxyguai-1(10)-ene-12,6-lactone, and given the trivial name valerianin C.

Further studies to clarify the pharmacologically active ingredients of *V. fauriei* BRIQ. are currently underway in our laboratory.

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Experimental Part

General. Solvents used were of anal. grade. Column chromatography (CC): silica gel (SiO_2; *Qingdao Factory of Marine Chemical Industry*). M.p.: microscope melting-point apparatus (*Yanaco*); uncorrected. Optical rotations: *Jasco-P-1020* spectrometer; cell length 100 mm; at 589 nm. UV Spectra: *Shimadzu-UV-260* spectrophotometer; in EtOH; λ_{max} (log ε) in nm. IR Spectra: *Bruker-IR-27G* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR and 2D-NMR: *Inova-500* spectrometer; in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *VG7070E* mass spectrometer; in m/z (rel. %).

Plant Material. The roots of *Valeriana fauriei* BRIQ. were collected in Liaoning, in October, 2009. A voucher specimen (LNBX 09108) has been deposited with the Plant Laboratory of the Liaoning University of Traditional Chinese Medicine, Shenyang, China.

Extraction and Isolation. The air-dried roots of *V. fauriei* BRIQ. (2.0 kg) were percolated with 20 times the volume of MeOH under N_2 and the resulting solution was concentrated to yield 200 g of MeOH

extract. This was suspended in H_2O and partitioned with hexane, $CHCl_3$, and AcOEt, resp., to give hexane extract (30 g), $CHCl_3$ extract (17.5 g), and AcOEt extract (13 g). The hexane extract was subjected to CC (SiO_2 (1 kg), stepwise elution with hexane/Et_2O 9:1 → 3:7); *Fractions 1–4*. *Fr. 2* (6 g) was repeatedly subjected to CC (SiO_2, hexane/$CHCl_2$ and hexane/AcOEt): **1** (18 mg), camphor (47 mg), methyl 4-hydroxybenzoate (24 mg), and 2-methoxybenzoic acid (27 mg). *Fr. 3* (5g) was subjected to CC (SiO_2, $CHCl_2$/MeOH 100:1 → 5:1) and further purified by CC (*Sephadex LH-20*): **2** (11 mg), benzoic acid (18 mg), quercetin (20 mg), and kaempferol (15 mg).

Isovalerianin A (= *(1β,4Z,6β,8α)-8-(Acetyloxy)-1,10-dihydroxy-6,11-cyclogermacr-4-en-15-al* = rel-*(1*R*,2Z,6*S*,7*R*,9*R*,10*S*)-9-(Acetyloxy)-6,7-dihydroxy-7,11,11-trimethylbicyclo[8.1.0]undec-2-ene-3-carboxaldehyde*; **1**): White powder. M.p. 120–122°. $[\alpha]_D^{25} = -35$ ($c = 0.1$, EtOH). UV: 254 (0.38). IR: 3433 (OH), 2934*m*, 1739*s* (br.), 1668*s*, 1626*w*, 1251*s*, 1021*m*, 607.4*w*. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 310 (M^+, 12), 43 (100).

Valerianin C (= *(2α,3α,6α,8α)-3-(Acetyloxy)-2,4,8-trihydroxyguai-1(10)-ene-12,6-lactone* = rel-*(3*R*,3a*S*,4*R*,7*S*,8*S*,9*R*,9a*R*,9b*R*)-8-(Acetyloxy)-3a,4,5,7,8,9,9a,9b-octahydro-4,7,9-trihydroxy-3,6,9-trimethylazuleno[4,5-*b*]furan-2(3*H*)-one*; **2**): Colorless needles. M.p. 248°. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 363.

Camphor: White solid. M.p. 177°. Spectral data: consistent with [13].

Methyl 4-Hydroxybenzoate: White solid. M.p. 275°. ^{13}C-NMR: 171.8 (C=O); 52.2 (MeO); 114.2 (C(1)); 132.4 (C(2)); 117.9 (C(3)); 168.1 (C(4)); 117.9 (C(5)); 132.0 (C(6)).

2-Methoxybenzoic Acid: White solid. M.p. 103°. ^{13}C-NMR: 165.0 (C=O); 57.1 (MeO); 118.0 (C(1)); 134.0 (C(2)); 122.0 (C(3)); 136.2 (C(4)); 111.0 (C(5)); 158.8 (C(6)).

Benzoic Acid: White solid. M.p. 122°. ^{13}C-NMR: 165.0 (C=O); 130.0 (C(1)); 130.0 (C(2)); 129.0 (C(3)); 133.2 (C(4)); 129.0 (C(5)); 130.8 (C(6)).

Quercetin: Yellow solid. M.p. 314°. Spectral data: consistent with [14].

Kaemferol: Yellow solid. M.p. 276–278°. Spectral data: consistent with [15].

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