Two New Iridoid Esters from the Root and Rhizome of *Valeriana jatamansi* JONES

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Two new iridoid esters, named valerjatadoids A and B (**1** and **2**, resp.), together with three known iridoid esters, jatamanin O (**3**), jatamanvaltrate P (**4**), and jatamanvaltrate Q (**5**), have been isolated from the root and rhizome of *Valeriana jatamansi* JONES. The structures of the two new compounds were elucidated by spectroscopic analyses, including 2D-NMR techniques.

Introduction. – The genus *Valeriana* (Valerianaceae), containing about 200 species, is distributed throughout the world. The root preparation of *Valeriana officinalis* L., which is commonly referred to as valerian, has been employed as a mild sedative in Europe for centuries [1]. *Valeriana jatamansi* JONES (Valeriana), is one of the crude drugs which has been used in China as a traditional folk medicine for treatment of various diseases including sleep disorder, nervous disorders, epilepsy, insanity, snake poisoning, and skin diseases [2][3]. Previous phytochemical studies on *V. jatamansi* revealed the presence of sesquiterpenoids [4], essential oil [5], and a series of iridoid esters [6–8]. In our effort to search for new bioactive natural products from this plant, two new iridoid esters, valerjatadoid A and valerjatadoid B (**1** and **2**, resp.), along with three known iridoid esters, jatamanin O (**3**), jatamanvaltrate P (**4**), and jatamanvaltrate Q (**5**) (*Fig. 1*), were isolated from the root and rhizome of *V. jatamansi*. Herein, we report the isolation and structure elucidation of the new compounds.

Results and Discussion. – Repeated normal phase and reversed phase column chromatography of the CH_2Cl_2-soluble fraction of the 95% EtOH extract from the root and rhizome of *Valeriana jatamansi* JONES. (DEVJ) afforded compounds **1**–**5**.

Compound **1** was obtained as yellow oil, its molecular formula $C_{24}H_{36}O_9$, was determined by HR-ESI-MS (491.2246 ($[M+Na]^+$, $C_{24}H_{36}NaO_9^+$; calc. 491.2257)). The IR spectrum indicated the presence of OH (3473 cm^{-1}) and ester CO groups (1738 cm^{-1}). From the ^{1}H-NMR spectrum, the presence of two isovaleroxy groups, one AcO group, and one EtO group was evident. The ^{13}C-NMR spectrum showed 24 C-atom signals. In addition to the 14 C-atom signals for the substituents (two isovaleroxy,

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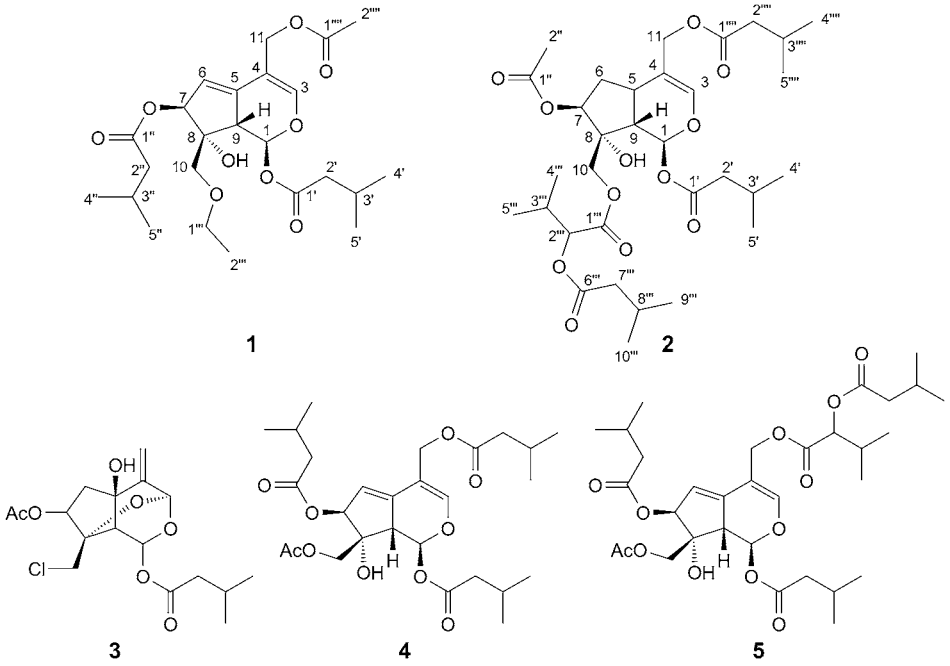

Fig. 1. *Chemical structures of* **1**–**5**

one AcO, and one EtO group), there were ten additional C-atoms composing the iridoid skeleton. The two C=C bonds in **1** were assigned to C(3)=C(4) (δ(H) 6.68 *s*, H–C(3); δ(C) 147.7 C(3) and 108.7 C(4)), and C(5)=C(6) (δ(H) 5.80 *s*, H–C(6); δ(C) 139.0 C(5) and 118.1 C(6)). After defining the iridoid skeleton and substituent moieties, an HMBC experiment was performed. The long-range correlations of H-atoms H–C(1), H–C(7), and CH_2(11) to C(1′) at δ(C) 170.7, C(1″) at δ(C) 170.9, and C(1‴) at δ(C) 171.9, respectively, of the acyloxy groups suggested that the two isovaleroxy groups and the AcO group were located at C(1), C(7), and C(11), respectively (*Fig. 2*). The EtO group was attached at C(10) based on the cross-peak of H-atoms CH_2(10) to the corresponding C-atom C(1‴) at δ(C) 67.1 in the HMBC spectrum. By analyzing the HSQC, HMBC, and ^{1}H,^{1}H-COSY spectra, all the H- and C-atom signals were assigned unambiguously. The relative configuration of **1** was elucidated by NOESY experiments. Since all the naturally occurring valepotriates exhibit an α-orientation for H–C(1) and β-orientation for H–C(9) [9][10], NOESY correlations were observed for CH_2(10)/H–C(9), but not for CH_2(10)/H–C(7) or H–C(9)/H–C(7), which implied that the isovaleroxy group at C(7) was β-oriented and the OH at C(8) α-oriented, which was consistent with the configurations of H–C(1), H–C(7), H–C(9), and CH_2(10) of natural iridoids reported in the literature. Thus, compound **1** was elucidated as (1*R**,6*R**,7*S**,7a*R**)-4-[(acetyloxy)methyl]-7-(ethoxymethyl)-1,6,7,7a-tetrahydro-7-hydroxy-7a-methylcyclopenta[*c*]pyran-1,6-diyl bis(3-methylbutanoate), and named valerjatadoid A.

Compound **2** was isolated as colorless oil and the molecular formula, $C_{32}H_{50}O_{12}$, was deduced from HR-ESI-MS (649.3189 ($[M+Na]^+$, $C_{32}H_{50}NaO_{12}^+$; calc. 649.3200)). The IR spectrum also indicated the presence of OH (3496 cm^{-1}) and ester CO groups (1740 cm^{-1}). Analysis of the ^{1}H- and ^{13}C-NMR spectroscopic data (*Tables 1* and *2*) indicated signals for a trisubstituted olefinic bond (δ(H) 6.47 *s*, H–C(3); δ(C) 141.0 C(3) and 112.9 C(4)), and an acetal (δ(H) 6.24 *d*, $J = 4.6$, H–C(1); δ(C) 89.2 C(1)). These data, and the presence of five ester CO groups at δ(C) 173.0, 170.2, 169.7, 173.3, and 173.0, led to the conclusion that compound **2** is a 5,6-dihydrovaltrate iridoid with acyl substituents. The four acyl substituents were readily assigned by comparison with the ^{1}H- and ^{13}C-NMR data from the reported valepotriates as two isovaleroxy, an AcO, and an α-(isovaleroxy)isovaleroxy group, respectively. The connectivities of the acyloxy substituents to the iridoid nucleus were fully assigned by a HMBC experiment. The linkage of an isovaleroxy group to C(1) was established by the HMBC from H–C(1) to the CO C-atom at δ(C) 173.0. The connection of the AcO group to C(7) was proposed on the basis of the HMBC from H–C(7) to the CO C-atom at δ(C) 170.2. Further, HMBC interactions between CH_2(10) and the CO C-atom at δ(C) 169.7 led to the assignment of α-(isovaleroxy)isovaleroxy group to C(10), leaving an isovaleroxy residue to be located at C(11) (*Fig. 2*). HMBC interactions were also observed between

Table 1. *^{1}H-NMR Data of* **1** *and* **2** (at 600 MHz, in $CDCl_3$; δ in ppm, J in Hz)

	Position	**1**	**2**
	1	6.29 (*d*, $J = 10.0$, 1 H)	6.24 (*d*, $J = 4.6$, 1 H)
	3	6.68 (*s*, 1 H)	6.47 (*s*, 1 H)
	5		2.91 (*dd*, $J = 16.0$, 7.8, 1 H)
	6	5.80 (*s*, 1 H)	2.12–2.14 (*m*, 2 H)
	7	5.47 (*d*, $J = 2.8$, 1 H)	5.01 (t, 4.5, 1 H)
	9	2.86 (*dd*, $J = 10.0$, 2.6, 1 H)	2.42 (*dd*, $J = 9.5$, 4.6, 1 H)
	10	3.57, 3.77 (2 *d*, $J = 9.1$, 2 H)	4.31, 4.37 (2 *d*, $J = 11.5$, 2 H)
	11	4.66, 4.72 (*d*, $J = 12.4$, 2 H)	4.44, 4.64 (2 *d*, $J = 12.4$, 2 H)
R^1	2′	2.31–2.32 (*m*, 2 H)	2.20–2.22 (*m*, 2 H)
	3′	2.09–2.13 (*m*, 1 H)	2.13–2.14 (*m*, 1 H)
	4′	1.02 (*d*, $J = 1.3$, 3 H)	1.02 (*d*, $J = 6.8$, 6 H)
	5′	1.03 (*d*, $J = 1.3$, 3 H)	
R^7	2″	2.18–2.19 (*m*, 2 H)	2.09 (*s*, 3 H)
	3″	2.09–2.13 (*m*, 1 H)	
	4″	0.97 (*d*, $J = 2.3$, 3 H)	
	5″	0.98 (*d*, $J = 2.3$, 3 H)	
R^{10}	1‴	3.53, 3.55 (2 *d*, $J = 7.0$, 2 H)	
	2‴	1.20 (*t*, $J = 7.0$, 3 H)	4.76 (*d*, $J = 4.7$, 1 H)
	3‴		1.28 (*s*, 1 H)
	4‴		1.01 (*s*, 6 H)
	7‴		2.25–2.26 (*m*, 2 H)
	8‴		2.14–2.15 (*m*, 1 H)
	9‴		1.00 (*d*, $J = 2.8$, 6 H)
R^{11}	2⁗	2.06 (*s*, 3 H)	2.31–2.32 (*m*, 2 H)
	3⁗		2.14–2.15 (*m*, 1 H)
	4⁗		0.98 (*d*, $J = 6.6$, 6 H)

Table 2. *^{13}C-NMR Data of* **1** *and* **2** (at 150 MHz, in $CDCl_3$; δ in ppm, *J* in Hz)

	Position	**1**	**2**
	1	92.7	89.2
	3	147.7	141.0
	4	108.7	112.9
	5	139.0	31.8
	6	118.1	34.9
	7	83.7	80.1
	8	80.2	80.9
	9	48.1	44.5
	10	71.1	66.8
	11	61.1	63.4
R^1	1′	170.7	173.0
	2′	43.3	43.5
	3′	25.7	25.7
	4′	22.3	22.4
	5′	22.3	22.4
R^7	1″	170.9	170.2
	2″	43.7	21.0
		25.8	
		22.4	
		22.5	
R^{10}	1‴	67.1	169.7
	2‴	15.0	76.9
	3‴		30.0
	4‴		18.7
	5‴		17.3
	6‴		173.3
	7‴		43.0
	8‴		25.6
	9‴		22.3
	10‴		22.3
R^{11}	1⁗	171.9	173.0
	2⁗	20.9	43.0
	3⁗		25.7
	4⁗		22.4
	5⁗		22.4

CH_2(11) and the CO C-atom at δ(C) 173.0, in full support of this assignment. Furthermore, all of the key NOESY correlations supporting the structure of **1** were also observed in the 2D-NMR spectrum of **2**, suggesting that the relative configuration of **2** was the same as **1**. Accordingly, **2** was identified as [(1*R**,6*R**,7*S**,7a*R**)-6-(acetyloxy)-1,4a,5,6,7,7a-hexahydro-7-hydroxy-1-[(3-methylbutanoyl)oxy]-4-{[(3-methylbutanoyl)oxy]methyl}cyclopenta[*c*]pyran-7-yl]methyl 3-methyl-2-[(3-methylbutanoyl)oxy]-butanoate and named valerjatadoid B.

The known compounds were identified as jatamanin O (**3**) [11], jatamanvaltrate P (**4**) [12], and jatamanvaltrate Q (**5**) [12] by comparing their ^{1}H- and ^{13}C-NMR data with those reported in the literature. All these three known compounds have been previously reported from *V. jatamansi*.

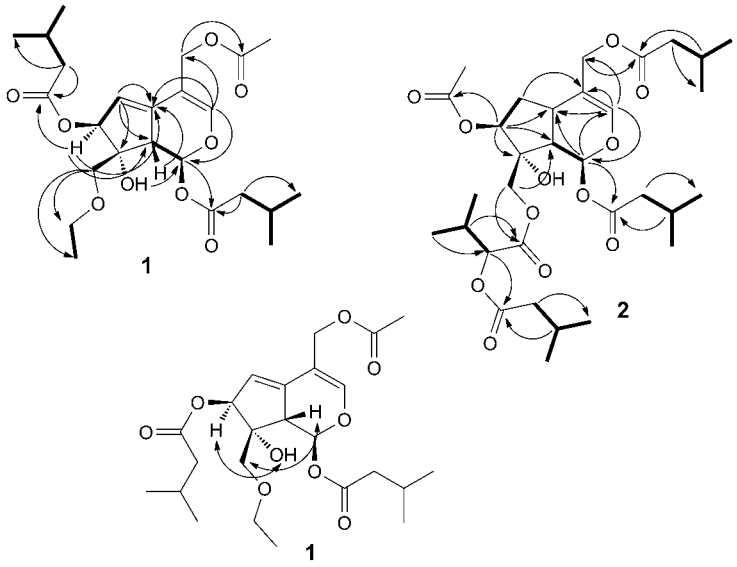

Fig. 2. *Key HMBCs* (arrows), *and COSY* (bold lines), *and NOESY* (double-headed arrows) *interactions of* **1** *and* **2**

Experimental Part

General. Anal. TLC: silica-gel plates (SiO_2; *Qingdao Ocean Chemical Co., Ltd.*), with petroleum ether (PE)/AcOEt; detection by spraying with *Epstahl* reagent [13], followed by heating. Column chromatography (CC): silica gel (SiO_2; 100–200 mesh; *Qingdao Ocean Chemical Co., Ltd.*). Medium pressure chromatograph was carried out using a *Büchi* system consisting of *C-605* pump and *C-615* pump manager. *ODS* (40–60 μm, *Daiso Co., Ltd.*, Japan). Semiprep. HPLC was carried out using a *Waters* system consisting of a *600* pump and a *2487* dual wavelength detector. The *Amethyst* C_{18} reversed-phase column (250 × 21.2 mm, 10 μm) for semiprep. HPLC was purchased from *Sepax Technologies* (USA). Optical rotations: *Autopol IV* spectropolarimeter. IR Spectra: *Bruker Tensor II* spectrophotometer (*Bruker*, Germany), compounds were dissolved using $CHCl_3$ to form liquid film; in cm^{-1}. ^1H- and ^{13}C-NMR spectra were measured on *Bruker Avance 600* spectrometer, in $CDCl_3$; δ in ppm, J in Hz. C-atom multiplicities were determined by DEPT-135 experiments. All 2D-NMR spectra were recorded using pulsed field gradients. One bond ^{13}C,^1H-correlations were observed in an HSQC experiment. Long-range ^{13}C,^1H-correlations were observed in HMBC. HR-ESI-MS: *Thermo Scientific Exactive*; in m/z.

Plant Material. The root and rhizome of *Valeriana jatamansi* JONES were collected in TongRen, Guizhou Province, P. R. China, in October of 2012, and identified by Professor *Ru-Song Zhang*, College of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Zhejiang, P. R. China. A voucher specimen (No. ZYGC-VJ-20121001) was deposited with the Laboratory of TCM Resources Engineering, Zhejiang Chinese Medical University.

Extraction and Isolation. The dried rhizome and root of *V. jatamansi* (2.0 kg) were crushed into coarse powder, and then the powder was soaked with 95% EtOH for 24 h, and extracted with 20-fold 95% EtOH at r.t. by percolation. The combined extract was concentrated *in vacuo* to give 148.4 g of crude extract, and the extract was suspended in 1 l of warm H_2O (30°) and partitioned with CH_2Cl_2 (3 × 1 l). After evaporation under reduced pressure, the CH_2Cl_2 extract (98.5 g) was subjected to CC (SiO_2) eluting with a gradient of PE/AcOEt (40:1, 20:1, 15:1, 12:1, 10:1, 8:1, 6:1, 4:1, 2:1, 1:1, 0:1) to give eleven fractions (*Frs. A–K*). *Fr. G* (5 g of 6.8 g) was separated by prep. MPLC using MeOH/H_2O as the mobile phase (60%, 70%, 75%, 80%, 90%, each 2 l), flow rate 50 ml/min, to obtain compound **1**

(255 mg), compound **3** (199 mg), and compound **4** (169.8 mg). Some of the fractions eluted with 90% MeOH (305 mg) were subjected to prep. HPLC (82% MeOH/H_2O, flow rate 13 ml/min) to obtain compound **2** (64 mg, $t_R = 42.2$ min) and compound **5** (106 mg, $t_R = 47.3$ min).

Valerjatadoid A (*=(1*R*,6*R*,7*S*,7a*R*)-4-[(Acetyloxy)methyl]-7-(ethoxymethyl)-1,6,7,7a-tetrahydro-7-hydroxy-7a-methylcyclopenta[*c*]pyran-1,6-diyl Bis(3-methylbutanoate)*; **1**). Yellow oil. $[\alpha]_D^{25} = 130.9$ ($c = 0.10$, MeOH). IR ($CHCl_3$): 3473, 2961, 2873, 1738, 1642, 1487, 1370, 1243, 1101, 1023. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 491.2246 ($[M+Na]^+$, $C_{24}H_{36}NaO_9^+$; calc. 491.2257).

Valerjatadoid B (*=[(1*R*,6*R*,7*S*,7a*R*)-6-(Acetyloxy)-1,4a,5,6,7,7a-hexahydro-7-hydroxy-1-[(3-methylbutanoyl)oxy]-4-{[(3-methylbutanoyl)oxy]methyl}cyclopenta[*c*]pyran-7-yl]methyl 3-Methyl-2-[(3-methylbutanoyl)oxy]butanoate*; **2**). Colorless oil. $[\alpha]_D^{25} = -2.4$ ($c = 0.10$, MeOH). IR ($CHCl_3$): 3496, 2962, 2874, 1740, 1467, 1372, 1327, 1032. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 649.3189 ($[M+Na]^+$, $C_{32}H_{50}NaO_{12}^+$; calc. 649.3200).

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