Sesquiterpenoids and Lignans from the Roots of *Valeriana officinalis* L.

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Two new guaiane-type sesquiterpenoids, valerol A (**1**) and kessyl 3-acetate (**2**), together with nine known compounds, valeracetate (**3**), anismol A (**4**), orientalol C (**5**), spatulenol (**6**), 4α,10α-epoxyaromadendrane (**7**), (+)-8-hydroxypinoresinol (**8**), pinorespiol (**9**), pinoresinol 4-*O*-β-D-glucopyranoside (**10**), and 8-hydroxypinoresinol 4′-*O*-β-D-glucopyranoside (**11**) were isolated from the roots of *Valeriana officinalis*. The structures and relative configurations of **1** and **2** were elucidated on the basis of spectroscopic methods (1D- and 2D-NMR, MS, UV, and IR). These compounds were evaluated for inhibitory activity on acetylcholinesterase (AChE) and enhancing activity on nerve growth factor (NGF)-mediated neurite outgrowth in PC12 cells.

Introduction. – The genus *Valeriana* consists of *ca.* 200 species and belongs to the family of Valerianaceae, which is widely distributed throughout the world, and valerian has been a most commonly used herb medicine as a mild sedative and sleep aid in Europe, Asia, and North America for centuries [1][2]. *Valeriana officinalis* is the official species used in Europe and is commonly referred to as valerian. Previous phytochemical investigations of *V. officinalis* have resulted in identification of iridoids, sesquiterpenoids, flavone glycosides, lignans, and alkaloids [3–7]. In our previous work, a series of valerane-type sesquiterpenoids and iridoids were isolated from this plant [8]. In our further study of the chemical constituents of this plant, two new guaiane-type sesquiterpenoids, valerol A (**1**) and kessyl 3-acetate (**2**), together with five sesquiterpenoids, **3**–**7**, and four lignans, **8**–**11** (*Fig. 1*), have been obtained. In addition, the acetylcholinesterase (AChE) inhibitory activity and the nerve growth factor (NGF)-potentiating activity of these compounds were evaluated. Acetylcholine (Ach) is required for cholinergic neurotransmission in the central and peripheral nervous systems [9], and AChE activity has been used as a marker for cholinergic activity, which plays an important role in the learning and memory [10][11]. The NGF plays a crucial role in the differentiation and survival of nerve cells in the brain, which is theoretically and clinically important in the central nervous system. The substances possessing NGF-potentiating activities are expected to be useful for the medical treatment of *Alzheimer*'s disease [12].

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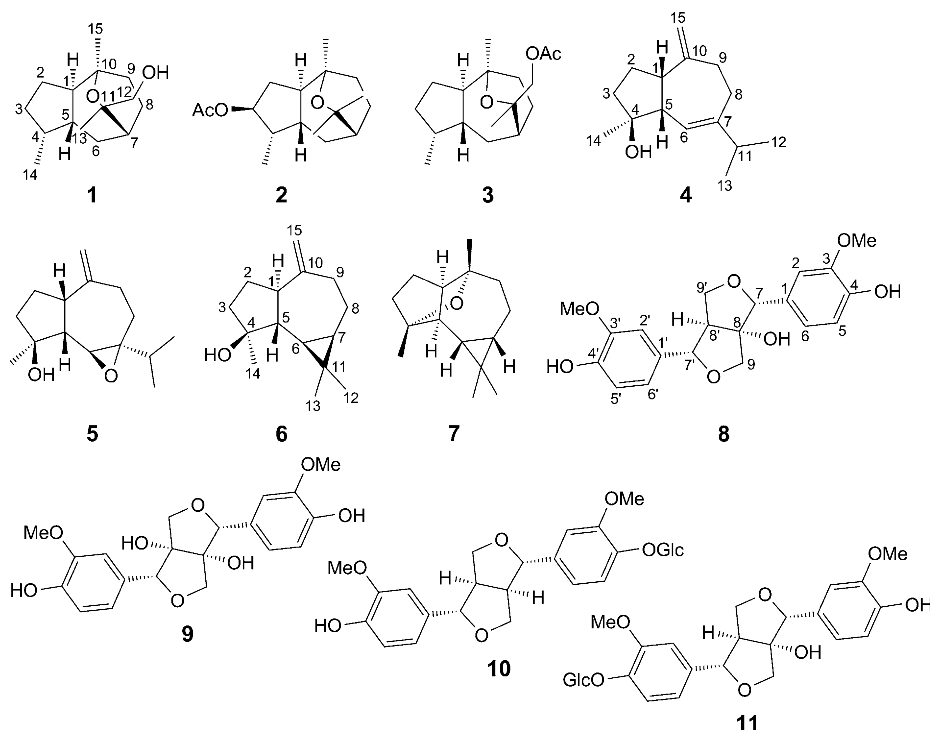

Fig. 1. *Chemical structures of compounds* **1**–**11**

Here, the isolation and structure elucidation of these new sesquiterpenoids are described, as well as the inhibitory activity of all the isolated compounds for AChE and their effects on NGF-mediated neurite outgrowth in PC12 cells.

Results and Discussion. – 1. *Isolation and Structure Elucidation*. The $CHCl_3$-soluble fraction obtained from the EtOH extract of the roots of *V. officinalis* was further fractionated by successive column chromatography to afford two new sesquiterpenoids, **1** and **2**, and nine known compounds, **3**–**11**.

Compound **1** was obtained as colorless oil. Its molecular formula was determined as $C_{15}H_{26}O_2$ by HR-ESI-MS (*m*/*z* 261.1827 $[M+Na]^+$; calc. 261.1830), with three degrees of unsaturation. The IR absorption bands at 3466 cm^{-1} indicated the presence of OH group. The ^{1}H-NMR spectrum of compound **1** (*Table*) showed two *singlet*s (δ(H) 1.23 (*s*, Me(13)), 1.09 (*s*, Me(15))) and a *doublet* (δ(H) 0.76 (*d*, $J = 6.8$, Me(14))) for Me groups. The ^{13}C-NMR and DEPT spectrum (*Table*) exhibited 15 C-atom resonances, including those for three Me groups, six CH_2 groups (containing an O-bearing one), four CH groups, and two O-bearing quaternary C-atoms. The ^{1}H- and ^{13}C-NMR data of compound **1** were similar to those of valeracetate [13], indicating of a guaiane-type sesquiterpenoid. The only difference of compound **1** from valeracetate was the absence of the AcO group at C(12) in valeracetate, which was supported by the molecular weight of **1** less than that of valeracetate by 42. In the HMBC spectrum (*Fig. 2*), the

Table. *^{1}H- and ^{13}C-NMR Data for Compounds* **1** *and* **2**[a]). δ in ppm, J in Hz.

	Valerol A (**1**)		Kessyl 3-acetate (**2**)	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	1.69–1.72 (*m*)	49.9 (*d*)	1.78–1.80 (*m*)	49.0 (*d*)
H_α–C(2)	1.39–1.41 (*m*)	28.2 (*t*)	1.46–1.49 (*m*)	35.5 (*t*)
H_β–C(2)	1.53–1.55 (*m*)		2.07–2.10 (*m*)	
H_α–C(3)	1.01–1.05 (*m*)	32.0 (*t*)	4.61 (*t*, J=6.8)	82.1 (*d*)
H_β–C(3)	2.00–2.02 (*m*)			
H–C(4)	2.09–2.11 (*m*)	32.8 (*d*)	2.03–2.04 (*m*)	41.1 (*d*)
H–C(5)	2.03–2.04 (*m*)	41.9 (*d*)	2.29–2.39 (*m*)	39.6 (*d*)
H_α–C(6)	1.33–1.36 (*m*)	33.2 (*t*)	1.30–1.31 (*m*)	32.9 (*t*)
H_β–C(6)	1.97–1.99 (*m*)		1.93–1.97 (*m*)	
H–C(7)	1.73–1.77 (*m*)	31.9 (*d*)	1.72–1.75 (*m*)	35.7 (*d*)
H_α–C(8)	2.05–2.07 (*m*)	24.0 (*t*)	1.49–1.52 (*m*)	24.1 (*t*)
H_β–C(8)	1.56–1.58 (*m*)		2.12–2.13 (*m*)	
H_α–C(9)	1.58–1.59 (*m*)	35.2 (*t*)	1.52–1.55 (*m*)	34.7 (*t*)
H_β–C(9)	1.64–1.67 (*m*)		1.84–1.87 (*m*)	
C(10)		74.6 (*s*)		73.5 (*s*)
C(11)		76.8 (*s*)		75.1 (*s*)
H_a–C(12)	3.20 (*d*, J=10.5)	69.3 (*t*)	1.27 (*s*)	28.3 (*q*)
H_b–C(12)	3.61 (*d*, J = 10.5			
Me(13)	1.23 (s)	23.1 (*q*)	1.26 (s)	31.1 (*q*)
Me(14)	0.76 (*d*, J=6.8)	18.5 (*q*)	0.91 (*d*, J=7.4)	14.4 (*q*)
Me(15)	1.09 (s)	28.0 (*q*)	1.10 (*s*)	28.4 (*q*)
C(16)				170.3 (*s*)
Me(17)			2.01 (*s*)	21.3 (*q*)

[a]) ^{1}H- and ^{13}C-NMR at 500 and 125 MHz, respectively, and multiplicities inferred from DEPT and HSQC experiments.

correlations from CH_2(12) (δ(H) 3.20, 3.61) to C(11) (δ(C) 76.8), C(13) (δ(C) 23.1), and C(7) (δ(C) 31.9) confirmed the location of the OH group was at C(12) (δ(C) 69.3), and the correlations from Me(15) (δ(H) 1.09) to C(1) (δ(C) 49.9), C(9) (δ(C) 35.2), and C(10) (δ(C) 74.6) suggested that the Me(15) group was connected to an O-bearing quaternary C-atom (C(10)), in accordance with the connections in valeracetate. The same relative configuration of **1** as valeracetate [13] was elucidated by comparison of NMR data and the further ROESY experiments. The ROESY cross-peaks H–C(1)/ H_α–C(2) and Me(14)/H_α–C(2) indicated the α-orientation H–C(1) and Me(14). The

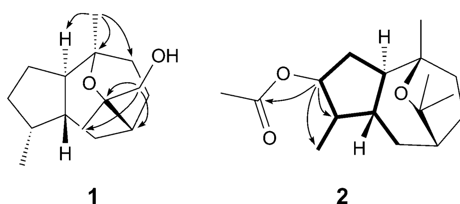

Fig. 2. *The Key HMBC* (→) *and ^{1}H,^{1}H-COSY* (—) *correlations of compounds* **1** *and* **2**

key NOE observed between H–C(5) and Me(13) in the ROESY spectra suggested the β-orientations of H–C(5) and C(11), which was in accordance with the α-orientation of Me(15). Therefore, the structure of compound **1** was established as shown and named valerol A.

Compound **2** was isolated as a colorless oil, and its molecular formula was established as $C_{17}H_{28}O_3$ by the HR-ESI-MS (*m/z* 303.1929 ($[M+Na]^+$; calc. 303.1936)), with four degrees of unsaturation. The IR spectrum showed the presence of OH (3433 cm^{-1}) and CO (1737 cm^{-1}) groups. Comparing the 1D- and 2D-NMR spectrum of **2** with those of α-kessyl acetate [14] indicated that compound **2** had a similar structure, and the two compounds possessed the same molecular formula. The key difference was the location of the AcO group, which was established at C(3) in compound **2** instead of at C(2) in α-kessyl acetate by downfield shift of C(3) ($\Delta = +49.9$ ppm) and upfield shift of C(2) ($\Delta = -41.8$ ppm) in **2**, and which was further confirmed by the key HMBCs (*Fig. 2*) from H–C(3) (δ(H) 4.61) to C(16) (δ(C) 170.3), C(4) (δ(C) 41.1), and Me(14) (δ(C) 14.4). In the ^{1}H,^{1}H-COSY spectrum (*Fig. 2*), the cross-peaks H–C(1)/CH_2((2), CH_2((2)/H–C(3), H–C(3)/H–C(4), H–C(4)/H–C(5), H–C(5)/H–C(6), H–C(1)/H–C(5), and H–C(4)/Me(14) also supported this conclusion. The other signals in the ^{1}H- and ^{13}C-NMR spectra were highly similar to those of α-kessyl acetate. The relative configuration of compound **2** was the same as compound **1** and other guaiane-type sesquiterpenoids isolated from this genus previously [13][14], exhibiting α-orientations of H–C(1), H–C(7), Me(14), and Me(15), and β-orientation of H–C(5). The key ROESY cross-peak H–C(3)/Me(14) indicated the α-orientation of H–C(3). Thus, the structure of compound **2** was established as shown and named as kessyl 3-acetate.

The known compounds were identified as valeracetate (**3**) [13], anismol A (**4**) [15], orientalol C (**5**) [15], spatulenol (**6**) [16], 4α,10α-epoxyaromadendrane (**7**) [17], (+)-8-hydroxypinoresinol (**8**) [7], pinorespiol (**9**) [7], pinoresinol-4-*O*-β-D-glucopyranoside (**10**) [18], and 8-hydroxypinoresinol-4′-*O*-β-D-glucopyranoside (**11**) [7] by comparison of their spectroscopic data with those reported in the literatures.

2. *Biological Studies. The Acetylcholinesterase* (AChE) *Inhibitory Activity.* The AChE inhibitory activity of the compounds **1** and **3**–**11** was assayed using the *Ellman* method [19]. Compound **6** showed strong inhibitory activity (inhibition percentage was 49.1%) at the concentration of 100 μM, and compound **4**, **8**, and **10** showed weak inhibitory activities (inhibit percentages were 20.2, 19.3, and 32.0%, resp.). Tacrine (0.33 μM) was used as the positive control (49.0% inhibition). The remaining compounds were inactive at 100 μM.

NGF-Promoting Activity. The propensity of compounds **1** and **3**–**11** to promote the effects of NGF (*R&D Systems Inc.*, $\geq$97%) to stimulate neurite outgrowth from PC12 cells was examined according to the assay reported in [20]. The percentages of neurite-bearing cells were 8.1 and 46.2% following incubation with 5 ng/ml NGF (negative control) and 50 ng/ml NGF (positive control) after 72 h, respectively, and the percentage was 11.9% with compound **6** at 50 μM and 5 ng/ml NGF. The proportion of the NGF (5 ng/ml)-induced neurite-bearing cells was enhanced by compound **6** at 50 μM, but not by the other compounds.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qindao Haiyang Chem. Ind. Co. Ltd.*, P. R. China), *Lichroprep RP-18* gel (40–63 μm; *Merck*), and *Sephadex LH-20* (*Amersham*). TLC: *GF254* Plates (*Qindao Haiyang Chem. Ind. Co. Ltd.*, P. R. China); detected by UV light (254 and 365 nm) and heating silica-gel plates sprayed with 10% H_2SO_4 in EtOH. Optical rotations: *Horiba SEAP-300* polarimeter, in MeOH or $CHCl_3$. IR Spectra: *Bio-Rad FTS-135* spectrometer with KBr pellets, $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-400* or *DRX-500* NMR spectrometer (D-Karlsruhe), in $CDCl_3$; δ in ppm rel. to solvent signals, J in Hz. MS: *VG Auto Spec-3000* mass spectrometer (*VG*, Manchester).

Plant Material. The plants, cultivated from the seeds of *V. officinalis* (purchased in Germany) at Songhuaba in Kunming, Yunnan Province, P. R. China, in March 2007, was collected in January 2008 and identified as *V. officinalis* L. by Prof. *Hu-Biao Chen*, School of Pharmaceutical Sciences, Peking University, P. R. China. A voucher specimen (KIB-XC0701) was deposited with Kunming Institute of Botany, the Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The air-dried roots powder of *V. officinalis* (5 kg) was extracted with 95% EtOH at r.t. to give a residue (1 kg) after removal of solvent under reduced pressure. The EtOH extract was suspended in H_2O (3 l) and then partitioned successively with petroleum ether (PE; 3 × 2 l), AcOEt (3 × 2 l), and BuOH (3 × 2 l). The PE extract (106 g) was subjected to CC (SiO_2, PE/acetone 100 : 1 → 1 : 1) to afford four fractions: *Frs. A–H*. *Fr. A* (15 g) was subjected to CC (SiO_2, PE/AcOEt 100 : 1 → 5 : 1) to give five fractions: *Frs. Aa–Ae*. *Fr. Aa* (5 g) was subjected to CC (SiO_2; PE/AcOEt 50 : 1 → 5 : 1) and purified over a *Sephadex LH-20* column with $CHCl_3$/MeOH 1 : 1 to provide compounds **3** (50 mg) and **6** (35 mg). *Fr. Ab* (2 g) was submitted to CC (*RP-18*; MeOH/H_2O 40–80%) to afford **4** (50 mg) and **5** (8 mg). *Fr. Ad* (3 g) was subjected to CC (SiO_2; PE/AcOEt 50 : 1 → 5 : 1; and *RP-18*; MeOH/H_2O 40–70%) to afford compounds **1** (10 mg) and **2** (5 mg). Compound **7** (10 mg) was obtained from *Fr. Ae* (3 g) by repeated CC (SiO_2; $CHCl_3$/CH_3OH 100 : 1 → 10 : 1) and then purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1). *Fr. C* (10 g) was subjected to CC (SiO_2; PE/AcOEt 10 : 1 → 0 : 1; and *Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1) to yield compounds **8** (25 mg) and **9** (30 mg). Compounds **10** (20 mg) and **11** (25 mg) were obtained from the BuOH extract by repeated CC ($CHCl_3$/MeOH 50 : 1 → 1 : 1; PE/AcOEt 10 : 1 → 0 : 1).

Valerol A (= *[*rel*-(1*R*,3a*R*,4*R*,7*S*,8a*R*)-Decahydro-1,4,9-trimethyl-4,7-(epoxymethano)azulen-9-yl]methanol*; **1**). Colorless oil. $[\alpha]_D^{23} = -9.04$ ($c = 0.2$, MeOH). IR (KBr): 3466 (OH), 2932, 2884, 1460, 1381, 1227, 1037. ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 261 ($[M+Na]^+$). HR-ESI-MS: 261.1827 ($[M+Na]^+$; $C_{15}H_{26}NaO_2^+$, calc. 261.1830).

Kessyl 3-Acetate (= rel*-(1*S*,2*S*,3a*R*,4*R*,7*S*,8a*R*)-Decahydro-1,4,9,9-tetramethyl-4,7-(epoxymethano)azulen-2-yl Acetate*; **2**). Colorless oil. $[\alpha]_D^{28} = +8.03$ ($c = 0.2$, $CHCl_3$). IR (KBr): 2931, 2871, 1737 (CO), 1453, 1378, 1070. ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 303 ($[M+Na]^+$). HR-ESI-MS: 303.1929 ($[M+Na]^+$, $C_{17}H_{28}NaO_3^+$; calc. 303.1936).

Acetylcholinesterase (AChE) *Inhibitory Activity*. AChE Inhibitory activities of the compounds isolated were assayed by the spectrophotometric method developed by *Ellman et al.* [19]. Acetylthiocholine iodide (*Sigma*) was used as substrate in the assay. Compounds were dissolved in DMSO. The mixture contained 1100 μl phosphate buffer (pH 8.0), 10 μl of test compound soln. (100 μM), and 40 μl AChE soln. (0.04 U/100 μl), and the mixture was incubated for 20 min (30°). The reaction was initiated by the addition of 20 μl of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB; 6.25 mM) and 20 μl of acetylthiocholine. The hydrolysis of acetylthiocholine was monitored at 405 nm after 30 min. Tacrine was used as positive control. All reactions were performed in triplicate. The percentage inhibition was calculated as follows: % inhibition = $(E - S)/E \times 100$ (E is the activity of the enzyme without test compound, and S is the activity of enzyme with test compound).

Cell Culture and Bioassay for Neurite Outgrowth-Promoting Activity. The neurotrophic activities of the test compounds were examined according to an assay using PC12 cells as reported in [20]. Briefly, PC12 cells were maintained in F12 supplemented with 12.5% horse serum (HS), and 2.5% fetal bovine serum (FBS), medium in a sat. atmosphere of 5% CO_2 and were incubated at 37°. Test compounds were dissolved in DMSO. For the neurite outgrowth-promoting activity bioassay, PC12 cells were seeded at a density of 2×10^4 cells/ml in 48-well plate coated with poly-l-lysine. After 24 h, the medium was changed to that containing various concentrations of NGF (50 ng/ml for the positive control, and 5 ng/ml for the negative control and compound group), 10% HS, 5% FBS, then 50 μM of each test compounds was added. The final concentration of DMSO was 0.05%, and the same concentration of DMSO was added into the negative control. After 72-h incubation, the neurite outgrowth was assessed under a phase-contrast microscope. Neurite processes with a length equal to or greater than the diameter of the neuron cell body were scored as neurite bearing cells. The ratio of the neurite-bearing cells to total cells (with at least 100 cells examined/view area; 5 viewing area/well) was determined and expressed as a percentage.

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