Germacrane-Type Sesquiterpenoids from the Roots of *Valeriana officinalis* var. *latifolia*

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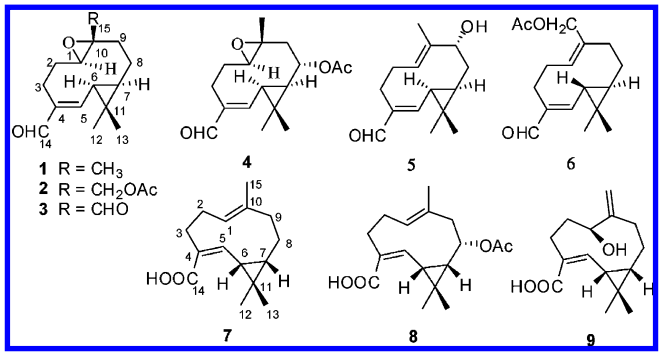
Eight new germacrane-type sesquiterpenoids, volvalerenals A−E (**2**−**6**) and volvalerenic acids A−C (**7**−**9**), along with four known compounds, were isolated from a chloroform extract of the roots of *Valeriana officinalis* var. *latifolia*. The structures and relative configurations of **2**−**9** were elucidated on the basis of spectroscopic data interpretation. The effects of all compounds isolated on acetylcholinesterase were evaluated.

Valerian (Valerianacae) is a perennial herb native to Europe, Asia, and North America and has been used widely as a mild sedative and sleep aid for centuries.[1−3] The species of *Valeriana* used most in Western preparations is *Valeriana officinalis* L., and the rhizomes and roots of this plant exhibit anxiolytic, antidepressant, antispasmodic, sedative, and anti-HIV activities.[4−8] Previous phytochemical investigations of *V. officinalis* have resulted in the identification of iridoids, sesquiterpenoids, flavone glycosides, lignans, and alkaloids.[9−13] *V. officinalis* is still the subject of considerable research aimed at establishing the chemical basis for its observed biological activities.[14]

Valeriana officinalis var. *latifolia* has been used as an alternative species for *V. officinalis* in mainland China. Our previous studies on the chemical constituents of the roots of *V. officinalis* have led to the isolation of several iridoids and sesquiterpenoids.[15] In continuing studies on the chemical constituents of the genus *Valeriana*, the chemical constituents in *V. officinalis* var. *latifolia* have been investigated. As a result, 12 germacrane-type sesquiterpenoids, including eight new substances, volvalerenals A−E (**2**−**6**) and volvalerenic acids A−C (**7**−**9**), along with four known compounds, have been obtained. The known compounds were identified as madolin A (**1**),[16] isobicyclogermacrenal,[17] kissoone B,[18] and kissoone C[18] by comparison of their spectroscopic data with those reported in the literature. In this paper, the isolation and structural elucidation of these new sesquiterpenoids are described, as well as the inhibitory activity of the isolated sesquiterpenoids for acetylcholinesterase (AChE).



Results and Discussion

Compound **2** was obtained as white, amorphous solid, which was assigned the molecular formula $C_{17}H_{24}O_4$ from its HREIMS (*m*/*z* 292.1666 $[M]^+$, calcd for $C_{17}H_{24}O_4$, 292.1675) and NMR data (Tables 1 and 2). The IR spectrum displayed the presence of carbonyl (1738 cm^{-1}), α,β-unsaturated aldehyde (1686 cm^{-1}), and double-bond (1627 cm^{-1}) absorptions. The ^1H NMR spectrum of compound **2** (Table 1) displayed three methyl singlets [δ_H 1.19 (3H, s, H-12), 1.24 (3H, s, H-13), 2.07 (3H, s, H-17)] and signals for an olefinic proton [δ_H 6.54 (1H, d, J = 9.5 Hz, H-5)], an oxygenated methine proton [δ_H 3.08 (1H, dd, J = 2.9, 11.3 Hz, H-1)], and an aldehydic proton [δ_H 9.40 (1H, s, H-14)]. Analysis of its ^{13}C NMR and DEPT spectra (Table 2) showed 17 carbon resonances, including three methyls, five methylenes (one oxygenated), three methines (one oxygenated), an oxygenated quaternary carbon, a trisubstituted double bond [δ_C 143.9 (s, C-4), 154.7 (d, C-5)], a carbonyl carbon [δ_C 170.8 (s, C-16)], and an aldehydic carbon [δ_C 193.3 (d, C-14)]. The ^1H and ^{13}C NMR data of **2** were similar to those of madolin A (**1**),[16] a germacrane-type sesquiterpenoid, except for the additional signals at δ_C 170.8 (s, C-16), 20.8 (q, C-17), and 62.5 (t, C-15) and the absence of the methyl signal at δ_C 17.2 (C-15), indicating that the methyl at C-15 in madolin A is replaced by a $-CH_2OOCCH_3$ moiety in **2**. This was in accordance with the HMBC correlations (Figure 1) from H-15 [δ_H 3.30 (1H, d, J = 12.1 Hz), 4.35 (1H, d, J = 12.1 Hz)] to C-9 (δ_C 34.0), C-10 (δ_C 60.2), and C-16. The other correlations in the HMBC and $^1H-^1H$ COSY spectra as shown in Figure 1 also confirmed the connectivities in compound **2**.

The relative configurations at C-1, C-6, C-7, and C-10 in **2** were deduced by a ROESY NMR experiment (Figure 2) to be the same as those of madolin A, for which the relative configuration was confirmed by X-ray crystallographic analysis.[16] The α-orientations of H-1, H-6, and H-7 in **2** were established by the correlations of H-1/H-6, H-1/H-7, and H-6/H-7, and the β-orientation of C-15 was deduced by the correlations of H-7/H-8b (α-H), H-15b/H-8a (β-H), and H-15a/H-2a. The α-orientation of CH_3-12 and the β-orientation of CH_3-13 were assigned by the correlations of CH_3-12/H-6 and H-5/CH_3-13.[17] The geometry of the double bond between C-4 and C-5 was determined to be *E* by the ROESY correlation of H-14/H-5. Thus, the structure of compound **2** was assigned as shown, and this compound was named volvalerenal A.

Compound **3** was isolated as a white, amorphous solid, and its molecular formula was determined to be $C_{15}H_{20}O_3$ by HRESIMS (*m*/*z* 271.1306 $[M + Na]^+$, calcd for $C_{15}H_{20}O_3Na$, 271.1310), requiring six degrees of unsaturation. Its IR spectrum indicated the presence of carbonyl (1718, 1667 cm^{-1}) and double-bond (1626 cm^{-1}) groups. The ^1H NMR spectra of compound **2** (Table 1) displayed resonances for two methyl groups [δ_H 1.18 (3H, s, H-12),

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Table 1. ^{1}H NMR Spectroscopic Data for Compounds **2**−**9** in $CDCl_3$

position	**2**[a]	**3**[b]	**4**[a]	**5**[b]	**6**[a]	**7**[b]	**8**[b]	**9**[b]
1	3.08, dd (2.9, 11.3)	3.37, dd (3.2, 11.6)	2.92, dd (3.2, 11.2)	5.46, dd (5.2, 11.4)	5.27, dd (5.2, 9.6)	4.93, dd (5.2, 9.8)	5.20, dd (5.6, 11.0)	3.77, d (11.2)
2a	1.35, m (β-H)	1.62, m	1.25, m	1.72, m	1.98, m	2.12, m	2.12, m	1.76, m
2b	2.38, m (α-H)	2.45, m	2.23, m	2.11, m	2.55, m	2.17, m	2.20, m	2.37, m
3a	2.39, m (α-H)	2.43, m	2.36, td (3.2, 13.6)	2.24, m	1.99, m (β-H)	1.70, m	1.80, m	2.05, m
3b	2.79, m (β-H)	2.79, m	2.80, dt (3.2, 13.6)	2.33, m	2.77, m	2.95, dt (3.0, 12.5)	2.92, dt (4.0, 16.0)	2.70, m
5	6.54, d (9.5)	6.41, d (9.6)	6.48, d (9.6)	6.47, d (3.4)	6.36, d (10.2)	5.28, d (12.5)	5.43, d (12.4)	5.63, d (11.9)
6	1.62, m	1.66, m	1.89, t (9.6)	1.24, m	1.18, dd (4.1, 9.3)	2.55, dd (9.0, 12.5)	2.80, m	2.49, m
7	1.15, m	1.09, m	1.36, m	0.96, t (10.1)	0.57, m	0.92, td (12.0, 2.5)	1.14, m	0.94, m
8a	1.19, m (β-H)	1.57, m (β-H)	4.60, td (2.2, 11.2)	1.60, m (β-H)	1.02, 2H, m	1.25, m (α-H)	4.82, m	1.45, m (α-H)
8b	1.92, dd (5.2, 14.0) (α-H)	1.98, m, (α-H)		2.07, m, (α-H)		2.03, m (β-H)		1.80, m (β-H)
9a	0.96, t (13.2), (α-H)	0.97, m (α-H)	1.37, m (α-H)	4.22, dd (3.0, 12.0)	1.97, m	1.72, m (β-H)	1.85, m (α-H)	2.10, m (β-H)
9b	2.40, m (β-H)	2.53, dd (4.0, 13.5),	2.28, m (β-H)		2.50, m	2.46, m (α-H)	2.74, dd (5.0, 12.4) (β-H)	2.53, m (α-H)
12	1.19, s	1.18, s	1.25, s	1.13, s	1.21, s	1.17, s	1.19, s	1.07, s
13	1.24, s	1.22, s	1.24, s	1.04, s	1.09. s	0.95, s	1.14, s	1.10, s
14	9.40, s	9.21,s	9.38, d (4.1)	9.48, s	9.31, s			
15a	3.30, d (12.1)	8.98, s	1.03, s	1.70, s	4.60, d (12.0)	1.42, s	1.47, s	4.87, s
15b	4.35, d (12.1)				4.62, d (12.0)			4.91, s
17	2.07, s		2.06, s		2.06, s		2.06, s	

[a] Recorded at 400 MHz. [b] Recorded at 500 MHz.

Table 2. ^{13}C NMR Spectroscopic Data for Compounds **2**−**9** in $CDCl_3$

position	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[a]	**7**[b]	**8**[a]	**9**[a]
1	62.5, CH	63.4, CH	62.2, CH	128.1, CH	133.5, CH	124.1, CH	127.2, CH	71.0, CH
2	27.6, CH_2	26.3, CH_2	27.3, CH_2	29.6, CH_2	26.7, CH_2	27.2, CH_2	26.2, CH_2	31.7, CH_2
3	20.3, CH_2	19.9, CH_2	20.4, CH_2	22.0, CH_2	24.3, CH_2	36.9, CH_2	42.4, CH_2	33.7, CH_2
4	143.9, qC	142.5, qC	144.5, qC	146.6, qC	140.3, qC	121.2, qC	122.9, qC	129.3, qC
5	154.7, CH	156.3, CH	151.7, CH	151.8, CH	157.8, CH	151.1, CH	148.5, CH	147.1, CH
6	28.3, CH	29.1, CH	28.8, CH	24.7, CH	35.0, CH	29.0, CH	29.8, CH	28.0, CH
7	38.0, CH	37.9, CH	40.2, CH	32.4, CH	37.3, CH	34.0, CH	35.7, CH	35.5, CH
8	21.5, CH_2	21.8, CH_2	69.8, CH	26.2, CH_2	24.3, CH_2	27.0, CH_2	74.8, CH	21.9, CH_2
9	34.0, CH_2	32.2, CH_2	46.6, CH_2	68.3, CH	35.7, CH_2	36.6, CH_2	36.2, CH_2	36.5, CH_2
10	60.2, qC	62.1, qC	57.7, qC	134.8, qC	130.6, qC	141.3, qC	137.5, qC	150.1, qC
11	23.8, qC	24.1, qC	24.3, qC	18.8, qC	23.6, qC	23.3, qC	23.9, qC	23.0, qC
12	29.0, CH_3	28.3, CH_3	28.1, CH_3	27.4, CH_3	22.3, CH_3	15.7, CH_3	15.5, CH_3	15.3, CH_3
13	15.5, CH_3	15.3, CH_3	15.7, CH_3	15.8, CH_3	21.3, CH_3	29.0, CH_3	28.6, CH_3	28.6, CH_3
14	193.3, CH	193.5, CH	193.5, CH	195.3, CH	194.0, CH	174.5, qC	172.5, qC	170.3, qC
15	62.5, CH_2	200.0, CH	17.9, CH_3	16.4, CH_3	61.3, CH_2	20.2, CH_3	19.8, CH_3	111.7, CH_2
16	170.8, qC		170.0, qC		171.1, qC		170.2, qC	
17	20.8, CH_3		21.2, CH_3		21.0, CH_3		21.4, CH_3	

[a] Recorded at 100 MHz. [b] Recorded at 125 MHz; multiplicities inferred from DEPT and HSQC experiments.

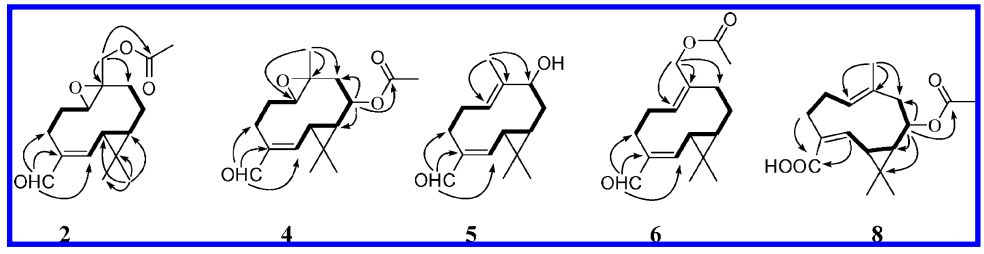

Figure 1. Key HMBC (→) and ^{1}H−^{1}H COSY (−) correlations of compounds **2**, **4**−**6**, and **8**.

1.22 (3H, s, H-13)], an olefinic proton [δ_H 6.41 (1H, d, J = 9.6 Hz, H-5)], and two aldehydic protons [δ_H 9.21 (1H, s, H-14), 8.98 (1H, s, H-15)]. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of compound **3** were also similar to those of madolin A (**1**).[16] The key difference was that the methyl signal at C-15 (δ_C 17.2) in **1** was replaced by an aldehyde group at δ_C 200.0 (C-15) in **3**, which was confirmed by the HMBC correlations from H-15 to C-9 (δ_C 32.2) and C-10 (δ_C 62.1). The relative configurations at C-1, C-6, C-7, and C-10 in compound **3** were established as being the same as those in **2** by a ROESY experiment. Therefore, the structure of compound **3** (volvalerenal B) was established as shown.

Compound **4** was found to possess the same molecular formula, $C_{17}H_{24}O_4$ (*m*/*z* 315.1577 [M + Na]$^+$, calcd for $C_{17}H_{24}O_4Na$, 315.1572), as compound **2**. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **4** were similar to those of **2**. The major difference was the presence of an oxygenated methine signal at δ_C 69.8 (d, C-8) in **4** instead of the oxygenated methylene resonance at δ_C 62.5 (t, C-15) in **2**, which indicated that the acetate group connected to C-15 in **2** is attached to C-8 in **4**. The key HMBC correlations (Figure 1) from H-8 (δ_H 4.60) to C-16 (δ_C 170.0), C-7 (δ_C 40.2), and C-9 (δ_C 46.6) and the ^{1}H−^{1}H COSY cross-peaks (Figure 1) of H-7/H-8 and H-8/H-9 (a, b) also supported this assignment. The relative configurations at C-1, C-6, and C-7 in **4** were established as being the same as those of **2** by the ROESY correlations of H-1/H-6, H-1/H-7, and H-6/H-7. The β-orientations of H-8 and CH_3-15 were established by the correlations of H-8/CH_3-13 (β-orientation as in compound **2**) and CH_3-15/H-8 in the ROESY spectrum (Figure 2). Thus, the structure of volvalerenal C (**4**) was determined as shown.

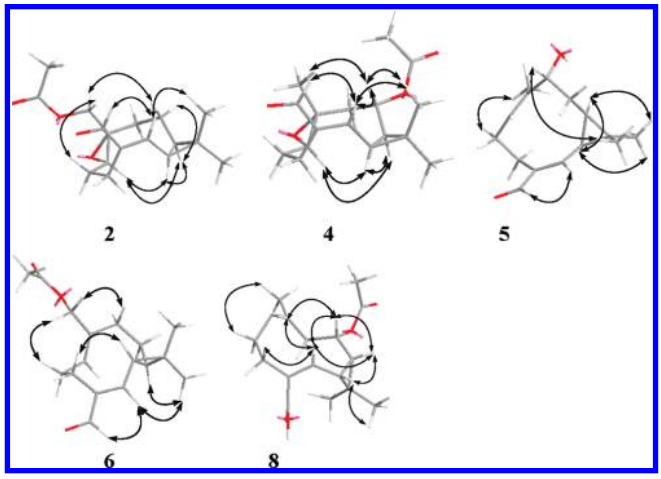

Figure 2. Key ROESY correlations of compounds **2**, **4**−**6**, and **8**.

Compound **5** gave a molecular formula of $C_{15}H_{22}O_2$ by HRESIMS (*m/z* 257.1513 $[M + Na]^+$, calcd for $C_{15}H_{22}O_2Na$, 257.1517), with five degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **5** were similar to those of kissoone B,[18] a compound that possesses the same molecular formula. The only differences found were the absence of the oxygenated methylene signals at δ_C 58.1 (t, C-15) and 34.3 (C-9) in kisoone B and the appearance of an oxygenated methine at δ_C 68.3 (d, C-9) and a methyl signal at δ_C 16.4 (s, C-15) in **5**, indicating that the hydroxy group at C-15 in kissoone B is linked to C-9 in compound **5**. This was confirmed by the HMBC correlations (Figure 1) from H-15 (δ_H 1.70) to C-1 (δ_C 128.1), C-10 (δ_C 134.8), and C-9, and the $^1H-^1H$ COSY cross-peak (Figure 1) of H-8 (δ_H 1.60, 2.07) with H-9 (δ_H 4.22). The other 2D NMR data (including HMBC and $^1H-^1H$ COSY) (Figure 1) also supported the same linkages and configurations of **5** as in kissoone B.[18] The relative configuration of **5** was assigned by a ROESY experiment (Figure 2) and molecular modeling, as well as by comparison of its NMR data with another related compound, isobicyclogermacrenal.[17] The chemical shifts of C-6, C-7, C-12, and C-13 in compounds with *syn* and *trans* orientations of H-6 and H-7 are variable.[17] H-6 and H-7 were assigned as *syn*- and α-oriented from the correlation of H-6/H-7 and the similar NMR data [δ_C 24.7 (C-6), 32.4 (C-7), 15.8 (C-12), 27.4 (C-13)] to those of kisoone B and isobicyclogermacrenal.[17,18] The α-orientation of CH_3-12 was deduced from the correlations of CH_3-12/H-6 and CH_3-12/H-7. The H-9 proton was determined to be *β*-oriented from the correlation of H-9/CH_3-13. Accordingly, the structure of compound **5** (volvalerenal D) was established as shown.

Compound **6** was assigned the molecular formula $C_{17}H_{24}O_3$, as established by HRESIMS (*m/z* 299.1616 $[M + Na]^+$, calcd for $C_{17}H_{24}O_3Na$, 299.1623). Comparison of the ^1H and ^{13}C NMR data (Tables 1 and 2) of **6** with those of lepidozenal[17] showed that they possess a similar skeleton, except that the methyl group at δ_C 15.6 (C-15) in lepidozenal is replaced by a $-CH_2OOCCH_3$ group [δ_H 4.60 (1H, d, $J = 12.0$ Hz, H-15a), 4.62 (1H, d, $J = 12.0$ Hz, H-15b), 2.06 (3H, s, H-17); δ_C 61.3 (t, C-15), 171.1 (s, C-16), 21.0 (q, C-17)] in **6**. This was confirmed by the HMBC correlations (Figure 1) from H-15 (a, b) to C-16, C-1 (δ_C 133.5), C-10 (δ_C 130.6), and C-9 (δ_C 35.7). The relative configuration of **6** was also assigned by a ROESY experiment and comparison to the NMR data of the model compound lepidozenal.[17] The *β*-orientation of H-6 and CH_3-13 and the α-orientation of H-7 and CH_3-12 were established on the basis of the similar NMR data of **6** [δ_C 35.0 (C-6), 37.3 (C-7), 22.3 (C-12), 21.3 (C-13)] to those of lepidozenal.[17] This was also confirmed by the NOE correlations of H-6/CH_3-13, H-7/CH_3-12, and H-5/CH_3-12, as shown in Figure 2. The correlations of H-5/H-14 and H-2 (a, b)/H-15 (a, b) indicated $\Delta^{4,5}$ and $\Delta^{1,10}$ to be *E*- and *Z*-configured, respectively. Therefore, the structure of compound **6** (volvalerenal E) was established as shown.

The molecular formula of compound **7** was determined as $C_{15}H_{22}O_2$ by HRESIMS (*m/z* 257.1512 $[M + Na]^+$, calcd $C_{15}H_{22}O_2Na$, 257.1517), with five degrees of unsaturation. The IR spectrum indicated the presence of an α,*β*-unsaturated carboxylic moiety (2500−3500, 1666 cm^{-1}) and double bonds (1601 cm^{-1}). The ^{13}C NMR and DEPT spectra showed 15 carbon signals, including three methyls, four methylenes, four methines, and four quaternary carbons. The NMR signals confirmed the presence of the α,*β*-unsaturated carboxylic moiety [δ_C 121.2 (C-4), 151.1 (C-5), 174.5 (C-14)] and also showed evidence for a dimethylcyclopropane ring [δ_H 2.55 (1H, dd, $J = 9.2$ Hz, H-6), 0.92 (1H, m, H-7), 0.95 (3H, s, H-12), 1.17 (3H, s, H-13); δ_C 23.3 (s, C-11), 15.7 (q, C-12), 29.0 (q, C-13)]. On comparing the ^1H and ^{13}C NMR spectra of **7** (Tables 1 and 2) with those of madolin P,[19] it was evident that they have similar structures. The major differences observed were the upfield shift of C-4 (δ_C 121.2, −8.6 ppm) and the downfield shifts of C-5 (δ_C 151.5, +4.5 ppm), C-3 (δ_C 36.9, +10.3 ppm), and C-14 (δ_C 174.5, +2.5 ppm), which were inferred as being due to the opposite geometry of the C=C double bond between C-4 and C-5. The relative configuration of **7** was assigned by a ROESY experiment and its ^1H NMR coupling constants. The coupling constant of 9.2 Hz and the NOE correlations between H-6 and H-7 suggested s *syn* configuration of the cyclopropane moiety,[19] with H-6 and H-7 on the same side and *β*-oriented. The NOE correlations of H-5/H-3a and H-2 (a, b)/H-15 indicated $\Delta^{4,5}$ and $\Delta^{1,10}$ to be *Z*- and *E*-configured, respectively, which was confirmed by a key correlation of H-1 with H-5. Therefore, the structure of compound **7** was established as shown, and this compound was named volvalerenic acid A.

Compound **8** was assigned the molecular formula $C_{17}H_{24}O_4$ (HRESIMS *m/z* 315.1572 $[M + Na]^+$, calcd for $C_{17}H_{24}O_4Na$, 315.1572), with six degrees of unsaturation. The 1D NMR spectroscopic data (Tables 1 and 2) of **8** were similar to those of **7**, except for an additional acetate group [δ_C 170.2 (s, C-16), 21.4 (q, C-17)] in compound **8**. The acetate group was determined to be located at C-8 by the HMBC correlations (Figure 1) from H-8 (δ_H 4.82) to C-16, C-11 (δ_C 23.9), C-7 (δ_C 35.7), and C-9 (δ_C 36.2), which was confirmed by the $^1H-^1H$ COSY correlations (Figure 1) of H-7/H-8 and H-8/H-9 (a, b). The NOE correlations (Figure 2) between H-6/H-7, H-6/CH_3-13, H-5/H-8, H-7/H-8, H-1/H-8, H-15/H-2b, H-1/H-5, and H-5/H-3a indicated that compound **8** has the same configuration and geometry as **7**, with the *β*-orientation of H-6, H-7, and H-8 and 4(5)-*Z*, 1(10)-*E* configurations of the double bonds. From the above evidence, the structure of **8** (volvalerenic acid B) was determined as shown.

Compound **9** gave a molecular formula of $C_{15}H_{22}O_3$, as deduced by HRESIMS (*m/z* 273.1460 $[M + Na]^+$, calcd for $C_{15}H_{22}O_3Na$, 273.1466), indicating five degrees of unsaturation. Compound **9** could be assigned a similar structure to **7** by comparison of their ^1H and ^{13}C NMR spectra (Tables 1 and 2). The major differences observed were the absence of the trisubstituted C=C double bond between C-1 and C-10 present in **7** and the appearance of an exocylic double bond between C-10 (δ_C 150.1) and C-15 (δ_C 111.7) and an additional oxygenated methine signal at δ_C 71.0 (C-1) in **9**. These were confirmed by the HMBC correlations from H-15 (δ_H 4.87, 4.91) to C-1, C-9 (δ_C 36.5), and C-10. On the basis of the ROESY correlations, the relative configuration of **9** was determined to be the same as that of **7**, with H-1 assigned as α-oriented by the NOE correlations of H-7/H-9a (*β*-H) and H-1/H-9b (α-H). The NOE correlations of H-3a/H-5 confirmed the *Z*-configuration of the double bond between C-4 and C-5. Thus, the structure of compound **9** (volvalerenic acid C) was assigned as shown.

The acetylcholinesterase (AChE) inhibitory activities of the 12 sesquiterpenoids isolated from *V. officinalis* var. *latifolia* were assayed using the Ellman method.[20] Compound **1** showed weak

inhibitory activity (25.9%) at a concentration of 100 μM. Tacrine (0.33 μM) was used as the positive control (49.0% inhibition). The other compounds were inactive at 100 μM.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a Horiba SEAP-300 polarimeter. UV spectra were obtained on a Hitachi UV 210A spectrophotometer. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker AM-400 or DRX-500 NMR spectrometer (Karlsruhe, Germany). Mass spectra were obtained on a VG Auto Spec-3000 mass spectrometer (VG, Manchester, UK). Column chromatography was performed on silica gel (200−300 mesh; Qindao Marine Chemical Inc., Qingdao, People's Republic of China) and RP-18 gel (LiChroprep, 40−63 μm; Merck, Darmstadt, Germany). Sephadex LH-20 for chromatography was purchased from Amersham Biosciences. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The plants of *V. officinalis* var. *latifolia* were collected in Badong County, Hubei Province, People's Republic of China, in October 2008. The plant was identified by Professor You-Wei Wang, School of Pharmaceutical Sciences, Wuhan University, People's Republic of China. A voucher specimen (KIB-XC0810) was preserved at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The dried root powder of *V. officinalis* var. *latifolia* (14 kg) was extracted with 95% EtOH at room temperature to give a residue (3 kg) after removal of solvent under reduced pressure. The EtOH extract was suspended in H_2O and then partitioned successively with $CHCl_3$ (3 × 4 L). The $CHCl_3$ extract (800 g) was subjected to silica gel column chromatography, eluted with petroleum ether−acetone (from 100:1 to 1:1), to afford fractions A−D. Fraction B (200 g) was subjected to column chromatography over silica gel (200−300 mesh), eluted with petroleum ether−EtOAc (from 100:1 to 1:1), to give nine fractions, B1−B9. Fraction B1 (80 g) was chromatographed over silica gel, eluted with petroleum ether−acetone (from 100:1 to 1:1), to afford five fractions, B1a−B1e. Compound **7** (3 g) was isolated from fraction B1a by repeated column chromatography over silica gel, eluted with ether−EtOAc (50:1 to 10:1). Fraction B1b was subjected to column chromatography over silica gel eluted with petroleum ether−EtOAc (50:1 to 5:1), to obtain isobicyclogermacrenal (3 g). Compounds **4** (13 mg), **6** (10 mg), and kissoone C (4 g) were obtained from fraction B8 by column chromatography over silica gel, by elution with petroleum ether−acetone (from 30:1 to 1:1), and purified over a Sephadex LH-20 column, eluted with $CHCl_3$−MeOH (1:1). Fraction B9 (20 g) was subjected to column chromatography over silica gel, eluted with petroleum ether−EtOAc (50:1 to 1:1), to afford four fractions, B9a−B9d. Fraction B9a (3 g) was subjected to column chromatography over silica gel, eluted with petroleum ether−acetone (from 20:1 to 1:1), and was chromatographed further over an RP-18 column, eluted with a MeOH−H_2O gradient system (40%−80%), to afford compounds **8** (15 mg), **2** (18 mg), and kissoone B (10 mg). Madolin A (**1**) (30 mg) was isolated from fraction B9b by repeated column chromatography over silica gel, eluted with ether−EtOAc (30:1 to 5:1). Fraction B9c (2 g) was subjected to column chromatography over silica gel, eluted with petroleum ether−EtOAc (from 20:1 to 1:1), and purified by Sephadex LH-20 column chromatography, eluted with $CHCl_3$−MeOH (1:1), to afford compound **3** (18 mg). Fraction B9d (5 g) was subjected to column chromatography over silica gel eluted with petroleum ether−EtOAc (from 10:1 to 1:1) and purified over a Sephadex LH-20 column, eluted with $CHCl_3$−MeOH (1:1), to afford compounds **5** (18 mg) and **9** (10 mg).

Volvalerenal A (2): white, amorphous solid; $[\alpha]_D^{13}$ +180.9 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 264 (4.2) nm; IR (KBr) ν_{max} 2942, 2866, 2816, 2709, 1738, 1686, 1627, 1453, 1375, 1239, 1182, 1033 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; EIMS *m/z* 292 $[M]^+$; HREIMS *m/z* 292.1666 $[M]^+$ (calcd for $C_{17}H_{24}O_4$, 292.1675).

Volvalerenal B (3): white, amorphous solid; $[\alpha]_D^{23}$ +254.4 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 264 (4.2) nm; IR (KBr) ν_{max} 2944, 2866, 2728, 1718, 1667, 1626, 1443, 1189, 1064 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; positive mode ESIMS *m/z* 271 $[M + Na]^+$; HRESIMS *m/z* 271.1306 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_3Na$, 271.1310).

Volvalerenal C (4): white, amorphous solid; $[\alpha]_D^{13}$ +190.5 (*c* 0.36, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 258 (4.1) nm; IR (KBr) ν_{max} 2955, 2855, 2713, 1732, 1679, 1630, 1460, 1450, 1374, 1243, 1135, 1019 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; positive mode ESIMS *m/z* 315 $[M + Na]^+$; HRESIMS *m/z* 315.1577 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_4Na$, 315.1572).

Volvalerenal D (5): white, amorphous solid; $[\alpha]_D^{25}$ +266.0 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 247 (3.9) nm; IR (KBr) ν_{max} 3294, 2941, 2814, 2709, 1686, 1627, 1454, 1375, 1183, 1001 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; EIMS *m/z* 234 $[M]^+$; HRESIMS *m/z* 257.1513 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_2Na$, 257.1517).

Volvalerenal E (6): white, amorphous solid; $[\alpha]_D^{21}$ +88.7 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 263 (3.9) nm; IR (KBr) ν_{max} 2930, 2869, 1739, 1676, 1623, 1451, 1372,1235, 1031 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; positive mode ESIMS *m/z* 299 $[M + Na]^+$; HRESIMS *m/z* 299.1616 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_3Na$, 299.1623).

Volvalerenic Acid A (7): white, amorphous solid; $[\alpha]_D^{23}$ +116.8 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 262 (4.1), 202 (4.0) nm; IR (KBr) ν_{max} 2500−3500 (br), 1666, 1601, 1447, 1380, 1272, 1160, 1070 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 1 and 2; positive mode ESIMS *m/z* 235 $[M + H]^+$; HRESIMS *m/z* 257.1512 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_2Na$, 257.1517).

Volvalerenic Acid B (8): white, amorphous solid; $[\alpha]_D^{23}$ +82.3 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 254 (4.0), 202 (3.9) nm; IR (KBr) ν_{max} 2500−3500 (br), 1731, 1673, 1610, 1449, 1370, 1242, 1164, 1016 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; positive mode ESIMS *m/z* 315 $[M + Na]^+$; HRESIMS *m/z* 315.1572 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_4Na$, 315.1572).

Volvalerenic Acid C (9): white, amorphous solid; $[\alpha]_D^{22}$ −27.4 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 263 (4.0), 203 (3.9) nm; IR (KBr) ν_{max} 2500−3500 (br), 1684, 1631, 1441, 1384, 1264, 1190 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; EIMS *m/z* 250 $[M]^+$; HRESIMS *m/z* 273.1460 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1466).

Acetylcholinesterase Inhibitory Activity. Acetylcholinesterase inhibitory activity of the compounds isolated was assayed by the spectrophotometric method developed by Ellman et al.[20] Acetylthiocholine iodide (Sigma) was used as substrate in the assay. Compounds were dissolved in DMSO. The reaction mixture contained 1100 μL of phosphate buffer (pH 8.0), 10 μL of test compound solution (100 μM), and 40 μL of acetyl cholinesterase solution (0.04 U/100 μL), and the mixture was incubated for 20 min (30 °C). The reaction was initiated by the addition of 20 μL of 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 the 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 the activity of enzyme with test compound).

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Supporting Information Available: 1D and 2D NMR spectra of compounds **2**−**9** are supplied, and this material is available free of charge via the Internet at http://pubs.acs.org.

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