New Alkaloids from *Aconitum vaginatum*

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Two new amide alkaloids, vaginatunine A and B (**1** and **2**, resp.), and a new C_{18}-diterpenoid alkaloid vaginatunine C (**3**), together with four known alkaloids, were isolated from the tubers of *Aconitum vaginatum*. Their structures were determined by means of spectroscopic analyses and comparison of the data with those reported previously.

Introduction. – *Aconitum* L. is a large genus of the Ranunculaceae family, which consists of over 400 species distributed all over the world. *Aconitum* species produce highly toxic norditerpenoid alkaloids that have attracted considerable interest because of their complex structures, interesting chemistry, and remarkable physiological effects [1]. *Aconitum vaginatum* PRITZ. is distributed in Shennongjia mountainous area of Hubei Province in China [2], and it has been used as a folk medicine for the treatment of rheumatoid arthritis, cancer, and various types of pains for a long time. To the best of our knowledge, no scientific study on this plant has hitherto been reported. Herein, we report the isolation and structure elucidation of the three new compounds vaginatunines A – C (**1** – **3**, resp.; *Fig. 1*).

Results and Discussion. – Compound **1** was isolated as colorless prisms. It gave a positive reaction with *Dragendorff*'s reagent. Its molecular formula $C_{19}H_{20}N_2O_5$ was established by the HR-EI-MS (*m*/*z* 356.3729 (M^+, calc. 356.3726)), indicating six degrees of unsaturation. The IR spectrum showed absorption bands for OH and NH groups (3438 and 3272 cm^{-1}, resp.), and ester and amide C=O groups (1691 and 1637 cm^{-1}, resp.), as well as for aromatic groups (1453 – 1591 cm^{-1}). The main fragment-ion peaks at *m*/*z* 151, 119, 92, and 65 observed in the EI-mass spectrum indicated the presence of a methyl 2-(carbonylamino)benzoate moiety [3]. The ^{1}H-NMR spectrum (*Table 1*) of compound **1** indicated the presence of two Me groups (δ(H) 1.59 (*s*, 6 H)), a MeO group (δ(H) 3.98 (*s*, 3 H)), two amide H-atoms (δ(H) 12.03, 11.96 (*s*, each 1 H)), as well as eight aromatic H-atoms (δ(H) 8.83, 8.12, 8.74, 7.91 (*d*, J = 8.0, each 1 H) and 7.63, 7.18, 7.57, 7.26 (*t*, J = 8.0, each 1 H)). The ^{13}C-NMR and DEPT spectra exhibited 19 C-atom signals for twelve aromatic C-atoms, three C=O C-atoms, two Me C-atoms, one MeO C-atom and one oxygenated C-atom (*Table 1*). The HMBC spectrum exhibited long-range correlations (*Fig. 2*) from MeO–C(C=O), H–C(5), H–C(6) to C(1), from H–C(3), H–C(4), H–C(6) to C(2), from H–C(4),

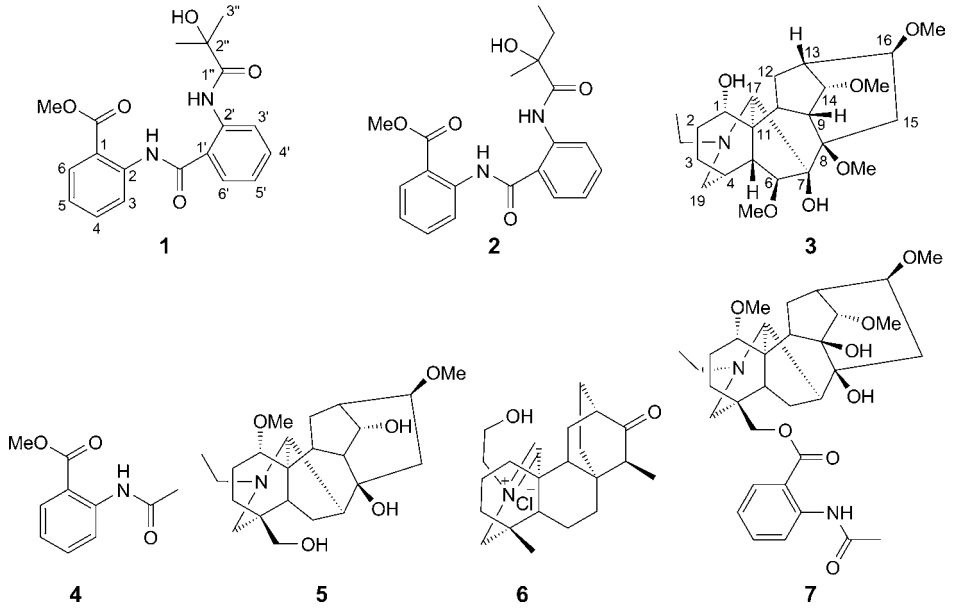


Fig. 1. *The chemical structures of the isolated compounds* **1**–**7**

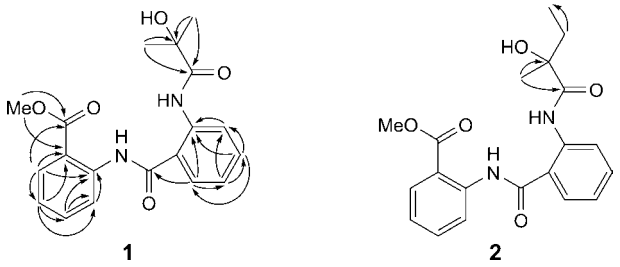


Fig. 2. *HMBCs* (H → C) *of compounds* **1** *and* **2**

H–C(5) to C(3), from H–C(3), H–C(5) to C(4), from H–C(6) to C(5), from H–C(3′), H–C(4′), H–C(5′), H–C(6′) to C(2′), from H–C(4′), H–C(5′), H–C(6′) to C(3′), from H–C(3′), H–C(5′) to C(4′), from H–C(6′) to C(5′), and from H–C(4′), H–C(5′) to C(6′) indicated two sets of *ortho*-disubstituted benzene ring systems. Correlations from MeO, H–C(6) to *C*(=O)–C(1), from H–C(6′) to *C*(=O)–C(1′), and from H–C(3″), Me–C(2″) to C(1″) and C(2″) evidenced the locations of three substituents. On the basis of the above data, the structure of **1** was identified as depicted in *Fig. 1*, and named vaginatunine A.

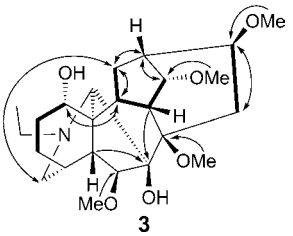
Compound **2** was isolated as colorless needles. It gave a positive reaction with *Dragendorff*'s reagent. Its molecular formula of $C_{20}H_{22}N_2O_5$ was deduced from the HR-EI-MS (*m*/*z* 370.3989 (M^+, calc. 370.3991)) with six degrees of unsaturation. The IR, NMR, and mass spectra of **2** were very similar to those of **1**, except for additional signals arising from a CH_2 group at δ(H) 1.78–1.81, 1.95–1.98 (2*m*, each 1 H) and δ(C) 33.5 (C(3″)) observed in the ^{1}H- and ^{13}C-NMR spectra of **2**, respectively. The HMBC

Table 1. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; $CDCl_3$) *of* **1** *and* **2**. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
MeO	3.98 (*s*)	52.6	3.98 (*s*)	52.6
C(=O)–C(1)		169.0		169.1
1		115.7		115.6
2		141.2		141.2
3	8.12 (*d*, *J* = 8.0)	131.1	8.11 (*d*, *J* = 8.0)	131.1
4	7.63 (*t*, *J* = 8.0)	134.8	7.64 (*t*, *J* = 8.0)	134.7
5	7.18 (*t*, *J* = 8.0)	123.2	7.18 (*t*, *J* = 8.0)	123.2
6	8.83 (*d*, *J* = 8.0)	120.7	8.84 (*d*, *J* = 8.0)	120.7
1′		121.2		121.1
2′		139.9		139.8
3′	7.91 (*d*, *J* = 8.0)	127.3	7.91 (*d*, *J* = 8.0)	127.3
4′	7.57 (*t*, *J* = 8.0)	133.1	7.57 (*t*, *J* = 8.0)	133.1
5′	7.26 (*t*, *J* = 8.0)	123.5	7.27 (*t*, *J* = 8.0)	123.5
6′	8.74 (*d*, *J* = 8.0)	121.3	8.75 (*d*, *J* = 8.0)	121.3
C(=O)–C(1′)		167.7		167.6
1″		175.7		175.4
2″		73.6		76.0
3″	1.59 (*s*)	27.9	1.78 – 1.81 (*m*), 1.95 – 1.98 (*m*)	33.5
4″			0.97 (*t*, *J* = 8.0)	7.9
Me–C(2″)	1.59 (*s*)	27.9	1.55 (*s*)	26.3
NH–C(2)	11.96 (*s*)		11.91 (*s*)	
NH–C(2′)	12.03 (*s*)		12.10 (*s*)	

spectrum exhibited long range correlations (*Fig. 2*) from H–C(3″) to C(4″), and from Me–C(2″) to C(1″) and C(2″), indicating the side chain of **2** to be (2-hydroxy-2-methylbutanoyl)amino group. Thus, the structure of **2** was determined as shown in *Fig. 1* and named vaginatunine B.

Compound **3** was isolated as a white amorphous powder, and its molecular formula $C_{24}H_{39}NO_6$ was derived from the HR-ESI-MS (*m*/*z* 438.5758 ($[M+H]^+$; calc. 438.5760)) and ^{13}C-NMR data. Its NMR spectra exhibited signals of an *N*-Et group (δ(H) 1.10 (*t*, *J* = 7.6, 3 H), 2.78 – 2.81 (*m*, 2 H); δ(C) 13.5 (*q*), 50.8 (*t*)) and of four MeO groups (δ(H) 3.34, 3.36, 3.37, 3.42 (*s*, each 3 H); δ(C) 56.3 (*q*), 59.1 (*q*), 57.2 (*q*), 57.3 (*q*)). The single non-oxygenated quaternary C-atom signal (δ(C) 49.3 (*s*)) suggested that compound **3** was a C_{18}-norditerpenoid alkaloid on the basis of combined NMR data and biogenetic considerations [4]. The MS displayed a base peak at *m*/*z* 420 ($[M-OH]^+$), indicating the presence of a 1α-OH group [5]. In the HMBC experiments (*Fig. 3*), correlations from the signals at δ(H) 3.34, 3.36, 3.37, and 3.42 to those of C(8), C(16), C(6), and C(14), respectively, indicated that the four MeO groups were located at C(8), C(16), C(6), and C(14), respectively. In addition, a OH group was located at C(7) based on the correlations from H–C(9), H–C(5) to C(7) in the HMBC of **3**. The ^{13}C-NMR signals at δ(C) 72.0 (*d*) and 82.9 indicated that the alkaloid bears an α-OH group at C(1) [6], and a β-MeO group at C(16) [7][8], respectively. The MeO group at C(6) had a β-orientation due to the *singlet*



3

Fig. 3. *Significant* $^1H,^1H$*-COSY* (—), *HMB* (H → C), *and NOESY* (↔) *correlations of* **3**

corresponding to H_α–C(6) in the ^{1}H-NMR spectrum [4], and the 1-H *triplet* at δ(H) 3.64 (*t*, $J = 4.8$ Hz) was attributed to H_β–C(14) based on the multiplicity and the coupling constant [9]. The relative configuration of **3** was established by the following NOESY correlations (*Fig. 3*): H–C(14)/H–C(13); H–C(13)/H–C(10) and H–C(12); H–C(1)/H–C(10); H_α–C(12)/H–C(16) and H–C(19); and H–C(15)/H–C(16). Therefore, the structure of **3** was established as depicted in *Fig. 1* and named vaginatunine C.

The known compounds were identified by comparison of their spectroscopic data with those in the literature. These include methyl 2-(acetylamino)benzoate (**4**) [3], cammaconine (**5**) [10], atisinium chloride (**6**) [11], and lappaconitine (**7**) [11].

The present study led to the isolation of three amide alkaloids, **1**, **2**, and **4**, two C_{19}-diterpenoid alkaloids, **5** and **7**, one C_{18}-diterpenoid alkaloid, **3**, and one C_{20}-diterpenoid alkaloid, **6**, for the first time from the tubers of *Aconitum vaginatum*. Four types of alkaloids were found in *Aconitum vaginatum*, a rare finding in nature from a chemotaxonomic point of view.

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Experimental Part

General. TLC: Silica gel (SiO_2; *Qingdao Marine Chemistry Ltd.*, P. R. China). Column chromatography (CC): silica gel (SiO_2, 100–200, 200–300 mesh; *Qingdao Marine Chemistry Ltd.*, P. R. China), *Lichroprep RP-18* gel (40–63 μm; *Merck*, DE-Darmstadt), and *MCI* gel (75–150 μm; *Mitsubishi Chemical Corporation*, Tokyo, Japan). Medium-pressure liquid chromatography (MPLC): silica gel *H* (SiO_2; *Qingdao Marine Chemistry Ltd.*, P. R. China); fractions were monitored by TLC, and spots were visualized by spraying with *Dragendorff*'s reagent. 1D- and 2D-NMR spectra: *Bruker AM-400 MHz* spectrometers; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Xevo TQ-S* Mass spectrometer; in *m*/*z*. HR-ESI-MS: *API QSTAR* time-of-flight (TOF) spectrometer; in *m*/*z*.

Plant Material. The tubers of *A. vaginatum* PRITZ were collected in Shennongjia mountainous area of Hubei Province in China and authenticated by Prof. *Shigui Shi* (Shennongjia Institute for Food and Drug Control, P. R. China). A voucher specimen (2010080103) has been deposited with the Faculty of Pharmaceutical Sciences, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, P. R. China.

Extraction and Isolation. The air-dried tubers of *A. vaginatum* (5.5 kg) were powdered and extracted with 95% boiling EtOH three times. After removal of the solvent under reduced pressure, the EtOH extract (630 g) was dissolved in 2% HCl. Then, the acidic soln. was alkalized with NH_4OH to pH 11 and extracted with $CHCl_3$ to give crude alkaloids (80 g) after evaporation of the solvent under vacuum. After decoloration over *MCI* gel, the crude alkaloids were applied to CC (SiO_2, petroleum ether (PE)/acetone/Et_2NH 10:1:0.01–1:10:0.01) to give six fractions (*A*–*F*). *Fr. F* (320 mg) was purified by CC (SiO_2;

Table 2. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; $CDCl_3$) *of* **3**. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	3.30 (t, J = 8.4)	72.0	13	2.36–2.40 (m)	37.6
2	1.80–1.83 (m), 2.31–2.35 (m)	29.3	14	3.64 (t, J = 4.8)	84.5
3	1.48–1.51 (m), 2.02–2.05 (m)	30.5	15	1.96–1.99 (m), 2.06–2.09 (m)	33.5
4	2.38–2.41 (m)	37.5	16	3.38–3.41 (m)	82.9
5	1.88 (d, J = 1.6)	44.9	17	2.90–2.92 (m)	66.0
6	4.01 (br. s)	90.4	19	3.06–3.09 (m), 2.90–2.94 (m)	57.2
7		87.7	$MeCH_2$–N	1.10 (t, J = 7.6)	13.5
8		78.5	$MeCH_2$–N	2.78–2.81 (m)	50.8
9	2.61 (dd, J = 4.8, 6.6)	43.3	MeO–C(6)	3.37 (s)	57.2
10	2.37–2.40 (m)	43.8	MeO–C(8)	3.34 (s)	56.3
11		49.3	MeO–C(14)	3.42 (s)	57.3
12	1.45–1.47 (m), 1.82–1.85 (m)	27.2	MeO–C(16)	3.36 (s)	59.1

$CHCl_3$/MeOH/Et_2NH (100 : 1 : 0.01–10 : 1 : 0.01) to afford compound **6** (8.0 mg). *Fr. B* (8.5 g) was subjected to CC (SiO_2, eluted with PE/acetone/Et_2NH 3 : 1 : 0.01–1 : 1 : 0.01) to afford several subfractions and pure compounds **1** (8.0 mg), **2** (2.0 mg), and **4** (45.0 mg). *Fr. D* was subjected to CC (*RP-18*; 10–100% gradient MeOH/H_2O) to give *Subfr. D-1–D-5*. The subfraction *D-1* (1.3 g) was further submitted CC to (SiO_2; $CHCl_3$/acetone/Et_2NH 30 : 1 : 0.01, 20 : 1 : 0.01, and 10 : 1 : 0.01) to provide compound **3** (7.1 mg), **5** (110.0 mg), and **7** (3.4 mg).

Vaginatunine A (= *Methyl 2-({2-[(2-Hydroxy-2-methylpropanoyl)amino]benzoyl}amino)benzoate*; **1**). Colorless prism (acetone). M.p. 210.7–211.8°. $[\alpha]_D^{20} = -23$ (c = 0.4, MeOH). IR (KBr): 3438, 3272, 1691, 1637, 1453–1591. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 356, 297, 265, 151, 119, 92, 65. HR-ESI-MS: 356.3729 (M^+, $C_{19}H_{20}N_2O_5^+$; calc. 356.3726).

Vaginatunine B (= *Methyl 2-({2-[(2-Hydroxy-2-methylbutanoyl)amino]benzoyl}amino)benzoate*; **2**). Colorless needles (acetone). M.p. 202.9–203.1°. $[\alpha]_D^{20} = -47$ (c = 0.5, MeOH). IR (KBr): 3458, 3324, 1688, 1613, 1544. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 370, 297, 265, 151, 119, 92, 65. HR-ESI-MS: 370.3989 (M^+, $C_{20}H_{22}N_2O_5^+$; calc. 370.3991).

Vaginatunine C (= *(1α,6β,7β,14α,16β)-20-Ethyl-6,8,14,16-tetramethoxyaconitane-1,7-diol*; **3**). White amorphous powder, M.p. 138.7–139.8°. $[\alpha]_D^{20} = +38$ (c = 0.5, $CDCl_3$). IR (KBr): 3446, 3357, 1100. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 438.5758 (([M + H]$^+$, $C_{24}H_{39}NO_6$; calc. 438.5760).

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