Alkaloids from *Aconitum barbatum* var. *puberulum*

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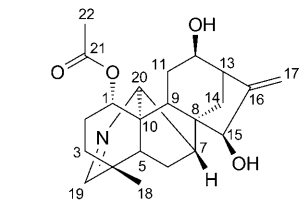
Three new diterpenoid alkaloids, puberulines A–C (**1**–**3**, resp.), and three new benzamide derivatives, puberulines D–F (**4**–**6**, resp.), as well as 16 known diterpenoid alkaloids were isolated from *Aconitum barbatum* var. *puberulum*. The structures of the new compounds were established on the basis of spectral data (IR, EI-MS, HR-ESI-MS, HR-EI-MS, ^{1}H- and ^{13}C-NMR, DEPT, NOE, HMQC, ^{1}H,^{1}H-COSY, HMBC, NOESY, and TOCSY).

Introduction. – *Aconitum barbatum* var. *puberulum* LEDEB. FL. ROSS. (Ranunculaceae), a traditional Chinese drug with a long history of use for the treatment of rheumatosis, rheumatoid arthritis, and some other inflammations, has been chemically investigated by an other group [1]. In continuation of our phytochemical investigation of traditional Chinese medicines, three new diterpenoid alkaloids, puberuline A–C (**1**–**3**, resp.), and three new benzamide derivatives, puberuline D–F (**4**–**6**, resp.), together with 16 known diterpenoid alkaloids were isolated from *Aconitum barbatum* var. *puberulum*. In this report, a detailed chemical study of the plant is described.

Results and Discussion. – Compound **1**, a white amorphous powder, had a molecular formula of $C_{22}H_{29}NO_4$, as determined by HR-ESI-MS ($[M+H]^+$ at m/z 372.2167, calc. 372.2169), with nine degrees of unsaturation. IR Absorptions at 3415, 1246, 1638, and 1732 cm^{-1} indicated the presence of a OH and a C=N group, an exocyclic C=C bond, and a CO group. The ^{1}H-NMR spectrum (*Table 1*) displayed a signal at δ(H) 4.96 (br. *s*) for an exocyclic CH_2 group, which correlated with the signals for a C=C bond observed at δ(C) 159.4 (*s*) and 108.2 (*t*) in the ^{13}C-NMR spectrum (*Table 1*). The ^{1}H-NMR spectrum also indicated the presence of an angular Me group at δ(H) 1.01 (*s*, Me(18)[1])), three O-bearing CH groups at δ(H) 3.20 (*dd*, J = 8.0, 2.4, H–C(12)), 3.90 (*s*, H–C(15)), and 4.86 (*dd*, J = 8.8, 6.4, H–C(1)). The absence of a MeO, EtN, as well as Me groups, and the presence of an exocyclic CH_2 group signal indicated that **1** is a C_{20}-diterpenoid alkaloid [2]. The ^{1}H,^{1}H-COSY and HSQC spectra revealed connectivity of four moieties: C(14)–C(13)–C(16)–(C(15))–C(17), C(11)–C(12), C(5)–C(6)–C(7)–C(20), and C(1)–C(2)–C(3), which corresponded to the spin systems given by the experiments of 1D-TOCSY. The connectivities of the first three fragments through C(12), C(8), and C(9) were suggested by the critical HMBC between H–C(9) and C(10), C(20), C(5), C(11), C(14), and C(15), and

[1]) Arbitrary atom numbering. For systematic names, see *Exper. Part.*



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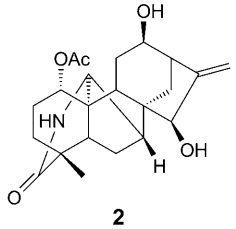

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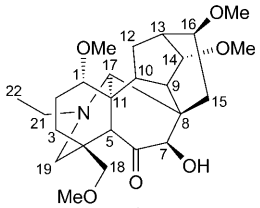

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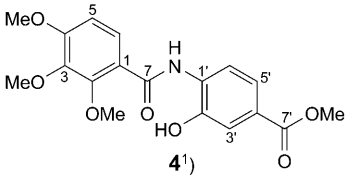

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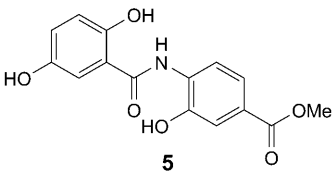

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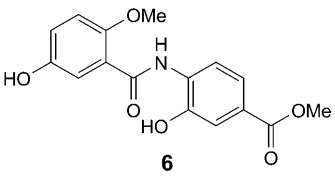

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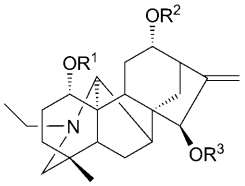
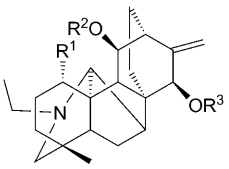
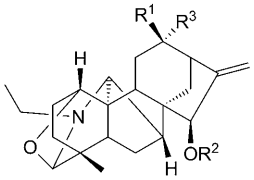


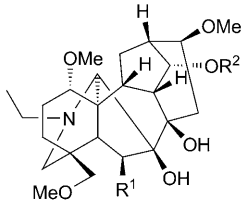
7 $R^1 = R^2 = R^3 = H$
8 $R^1 = R^2 = H$, $R^3 = Ac$
9 $R^1 = R^3 = H$, $R^2 = Ac$
10 $R^1 = Ac$, $R^2 = R^3 = H$



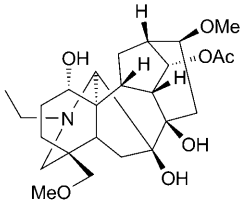
11 $R^1 = OH$, $R^2 = R^3 = H$
12 $R^1 = H$, $R^2 = R^3 = H$
13 $R^1 = OH$, $R^2 = Ac$, $R^3 = H$



14 $R^1 = OH$, $R^2 = R^3 = H$
15 $R^1 = AcO$, $R^2 = R^3 = H$
16 $R^1 = H$, $R^2 = Ac$, $R^3 = OH$



17 $R^1 = O$, $R^2 = MeO$
18 $R^1 = OH$, $R^2 = Me$
19 $R^1 = AcO$, $R^2 = Me$



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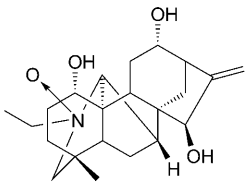

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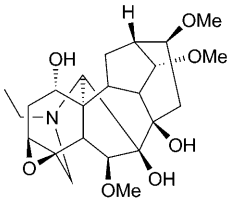

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further confirmed by correlations of H_a–C(14), H_a–C(11), and H_a–C(6) with C(8) and correlations of H_b–C(14), H_b–C(11), H–C(13), and H–C(15) with C(9). Similarly, the connectivity of the last two fragments through C(10) and C(4) was confirmed by the HMBC from H–C(1) to C(10), C(5), and C(20), and correlations from Me(18) to C(3), C(4), C(5), and C(19). Furthermore, the ^{1}H- and ^{13}C-NMR spectra showed signals for an AcO group (δ(H) 1.96 (*s*, 3 H); δ(C) 170.7 (*s*), 22.3 (*q*)). The AcO group could be located at C(1) from the HMBC between the H–C(1) and C(21). The ^{1}H-NMR spectrum revealed a broad *singlet* at δ(H) 3.90 (*s*), which was correlated with C(16), C(11), and C(8) in the HMBC, implying the presence of a OH group at C(15). The other OH group could be assigned at C(12) by the analysis of 2D-

NMR. The configuration of **1** was established on the basis of the NOESY and NOE experiments which showed the following relationships: correlations between H–C(13), H_a–C(11), and H–C(12), between H–C(1), H–C(9), H–C(12), and H_a–C(11), between H–C(9), H–C(15), H–C(20), and H–C(7), and between H–C(5), H_b–C(6), and Me(18). If H–C(20) was arbitrarily assigned to α-orientation, the relative configurations of H–C(1), H–C(9), H–C(12), and H–C(15) were determined to be β, α, α, and α, respectively. Meanwhile, the NOE difference spectrum gave the same configurational information. From this information, the structure of **1** was determined and named puberuline A.

Table 1. *NMR Data of Compounds* **1** *and* **2**[1]). δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	4.86 (*dd*, *J* = 8.8, 6.4)	74.0 (*d*)	4.92[a])	72.5 (*d*)
CH_2(2)	1.23–1.27 (*m*), 1.77–1.81 (*m*)	27.9 (*t*)	1.39[a]), 1.84 (br. *s*)	27.0 (*t*)
CH_2(3)	1.43–1.45 (*m*), 1.26–1.29 (*m*)	33.2 (*t*)	1.42 (br. *s*), 1.28–1.30 (*m*)	34.5 (*t*)
C(4)		45.4 (*s*)		40.2 (*s*)
H–C(5)	1.27–1.29 (*m*)	46.3 (*d*)	1.40[a])	47.6 (*d*)
CH_2(6)	1.02 (*dd*, *J* = 12.3, 5.4), 2.65 (*dd*, *J* = 12.3, 6.9)	24.8 (*t*)	1.10 (*d*, *J* = 8.0), 2.75 (*dd*, *J* = 8.0, 12.4)	24.9 (*t*)
H–C(7)	1.94–1.96 (*m*)	50.6 (*d*)	1.88 (br. *s*)	50.6 (*d*)
C(8)		50.2 (*s*)		48.8 (*s*)
H–C(9)	1.54 (*dd*, *J* = 9.9, 4.2)	38.9 (*d*)	1.58[a])	36.8 (*d*)
C(10)		49.5 (*s*)		48.6 (*s*)
CH_2(11)	1.26[a]), 1.35[a])	28.7 (*t*)	1.28[a]), 1.56[a])	29.2 (*t*)
H–C(12)	3.20 (*dd*, *J* = 8.0, 2.4)	74.8 (*d*)	3.19 (*dd*, *J* = 6.8, 8.4)	74.1 (*d*)
H–C(13)	2.24 (*d*, *J* = 3.6)	49.2 (*d*)	2.47 (br. *s*)	47.6 (*d*)
CH_2(14)	0.94[a]), 1.85 (*d*, *J* = 8.7)	30.1 (*t*)	0.97[a]), 1.71 (*d*, *J* = 12.4)	27.4 (*t*)
H–C(15)	3.90 (br. *s*)	76.3 (*d*)	3.91 (*s*)	75.5 (*d*)
C(16)		159.4 (*s*)		158.3 (*s*)
CH_2(17)	4.96 (br. *s*)	108.2 (*t*)	4.96 (br. *s*)	107.8 (*t*)
Me(18)	1.01 (*s*)	21.7 (*q*)	0.92 (*s*)	21.3 (*q*)
H–C(19)	7.12 (*s*)	169.1 (*d*)		174.7 (*d*)
H–C(20)	4.24 (br. *s*)	66.2 (*d*)	3.65 (br. *s*)	58.2 (*d*)
C(21)		170.7 (*s*)		170.0 (*s*)
Me(22)	1.96 (*s*)	22.3 (*q*)	1.98 (*s*)	21.6 (*q*)
NH			7.58 (br. *s*)	

[a]) Overlapped signals.

Compound **2** was obtained as a white amorphous powder. IR Absorptions at 3447, 1630, 1717, and 1625 cm^{-1} indicated the presence of OH and/or NH, N–C=O, and CO groups, and an exocyclic C=C bond. The HR-ESI-MS of alkaloid **2** showed a *quasi*-molecular-ion peak at *m*/*z* 388.2118 ($[M+H]^+$; calc. 388.2118), suggesting a molecular formula of $C_{22}H_{29}NO_5$ with nine degrees of unsaturation, and the EI-MS also displayed the molecular-ion peak at *m*/*z* 387 (M^+), 16 mass units more than that of compound **1**. Meanwhile, comparing the NMR data of **1** and **2** (*Table 1*), they exhibited similar features except for the presence of an NH–C=O group in **2** instead of an N=C group

in **1** at C(19). In addition, by the analysis of the ^{1}H,^{1}H-COSY and HMBC data (*Figure*) of compound **2**, the structure could be further determined unambiguously. The configuration of **2** was established by interpretation of the NOESY and NOE cross-peaks. The NOESY spectrum showed the following correlations: between H–C(13), H_a–C(11), HO–C(12), and H–C(12), between H–C(1), H–C(9), H–C(12), and H_a–C(11), between H_b–C(14) and H–C(15), between H–C(15), H_a–C(6), H–C(20), and H–C(7), between H_a–C(14) and H–C(20), and between H_a–C(6), H–C(5), and H_b–C(6). Therefore the relative configurations of H–C(1), H–C(12), and H–C(15) were assigned to be β, α, and α, respectively. The NOE difference spectrum also gave the same information. Therefore, the structure of **2** was confirmed and named puberuline B.

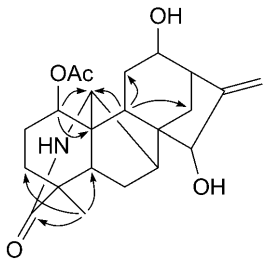

Figure. *HMBC data of compound* **2**

Compound **3** was obtained as a colorless solid. The HR-EI-MS peak at *m*/*z* 449.2767 (calc. 449.2772) indicated a molecular formula of $C_{25}H_{39}NO_6$. The IR spectrum indicated the presence of OH (3423 cm^{-1}) and C=O (1705 cm^{-1}) groups. The ^{1}H-NMR spectrum (*Table 2*) displayed signals for an EtN group at δ(H) 1.10 (*q*, *J* = 6.3, 3 H), 2.86–2.89 (*m*, 1 H), and 3.14–3.17 (*m*, 1 H), four MeO groups at δ(H) 3.25, 3.28, 3.30, and 3.35 (each 3 H, *s*). These characteristic data suggested that **3** was a C_{19}-norditerpenoid alkaloid, most of which have a OH or a MeO group at C(1), C(14), and C(16)[1]), respectively [3]. As no angular Me group was observed in the ^{1}H- and ^{13}C-NMR spectra (*Table 2*), C(18) was likely to be substituted with an oxygen functional group. Three moieties: C(1)–C(2)–C(3), C(9)–C(14)–C(13)–C(12)–C(10), and C(15)–C(16) were obtained from the ^{1}H,^{1}H-COSY and HSQC spectra. The HMBC experiments showed the following correlations: H_a–C(15)/C(16), C(7), C(17), C(8), C(9), and C(13); H–C(5)/C(18), C(7), C(17), C(19), C(11), C(3), and C(4); and H–C(1)/C(11), C(17), and C(5); H–C(10)/C(5) and C(11), which connected the three moieties. Thus the connectivity of **3** was determined: it was a rare rearranged-type C_{19}-norditerpenoid alkaloid. In the HMBC experiments, correlations between δ(H) 3.25, 3.28, 3.30, and 3.35 and C(18), C(16), C(1), and C(14), respectively, indicated that the four MeO groups were located at C(18), C(16), C(1), and C(14). The quaternary C-atom signal at δ(C) 213.0 (*s*) was attributed to C(6) from HMBC between the H–C(5), H–C(7), and C(6). The relative configuration of **3** was established by observing the NOESY experiments as follows: correlations between H–C(7), H_a–C(15), and H_b–C(21); between H–C(14) and H–C(13); between H–C(13), H–C(10), and H_b–C(12); between H–C(1) and H–C(10); between H_a–C(12), H–C(16), and H–C(19), and between H_a–C(15) and H–C(16).

Observing the molecular model of **3**, if H–C(19) was arbitrarily assigned to α-orientation, the relative configurations of H–C(1), H–C(7), H–C(14), and H–C(16) were determined to be β, α, β, and α, respectively. Therefore, the structure of **3** was established and named puberuline C.

Table 2. *NMR Spectroscopic Data for Compound* **3**[1]). δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.03 (*dd*, *J* = 8.4, 3.6)	83.3 (*d*)	H–C(14)	3.33[a])	83.0 (*d*)
CH_2(2)	1.98–1.99 (*m*),	25.1 (*t*)	CH_2(15)	2.12–2.14 (*m*),	28.3 (*t*)
	1.76–1.80 (*m*)			2.20–2.23 (*m*)	
CH_2(3)	1.46–1.50 (*m*),	34.6 (*t*)	H–C(16)	3.65 (*dd*, *J* = 11.7, 6.0)	83.8 (*d*)
	1.76–1.78 (*m*)		H–C(17)	3.62 (*s*)	63.5 (*d*)
C(4)		37.3 (*s*)	CH_2(18)	2.91–2.93 (*m*),	79.6 (*t*)
H–C(5)	2.39 (*s*)	58.8 (*d*)		2.78–2.83 (*m*)	
C(6)		213.0 (*s*)	CH_2(19)	2.82[a]), 2.76[a])	54.6 (*t*)
H–C(7)	4.23 (*s*)	81.0 (*d*)	CH_2(21)	2.86–2.89 (*m*),	50.4 (*t*)
C(8)		55.0 (*s*)		3.14–3.17 (*m*)	
H–C(9)	1.80–1.82 (*m*)	45.5 (*d*)	Me(22)	1.10 (*q*, *J* = 6.3)	14.5 (*q*)
H–C(10)	1.85–1.87 (*m*)	46.6 (*d*)	*Me*O–C(1)	3.30 (*s*)	57.5 (*q*)
C(11)		50.0 (*s*)	*Me*O–C(14)	3.35 (*s*)	57.4 (*q*)
CH_2(12)	2.43[a]), 1.92[a])	31.7 (*t*)	*Me*O–C(16)	3.28 (*s*)	57.0 (*q*)
H–C(13)	2.20–2.25 (*m*)	39.5 (*d*)	*Me*O–C(18)	3.25 (*s*)	59.4 (*q*)

[a]) Overlapped signals.

Compound **4** was isolated as a colorless solid. The HR-EI-MS peak at *m*/*z* 361.1158 (calc. 361.1156) corresponded to the molecular ion M^+, and the formula $C_{18}H_{19}NO_7$ with ten degrees of unsaturation was established. The characteristic base peak at *m*/*z* 195 in the EI-MS suggested the loss of $C_8H_8NO_3$ from M^+, which was also observed in the HR-EI-MS. The IR spectrum of **4** exhibited absorption bands at 3447 (NH), 3751 (OH), 1704 (ester CO), 1637 (amide CO), and 1460–1574 (aromatic) cm^{-1}. The ^{1}H-NMR spectrum showed signals of four MeO groups (δ(H) 4.06, 3.93, 3.93, 3.87, each 3 H, *s*), an *AX* spin system of two aromatic H-atoms (δ(H) 6.78 (*d*, *J* = 8.8) and 7.92 (*d*, *J* = 8.8)), and an *AMX* spin system of three aromatic H-atoms (δ(H) 7.06 (*dd*, *J* = 8.8, 2.8), 8.63 (*d*, *J* = 8.8), and 7.49 (*d*, *J* = 2.8)). The ^{13}C-NMR spectrum of **4** (*Table 3*), in combination with the DEPT spectrum, revealed the presence of four Me and five CH groups, as well as nine quaternary C-atoms, which was in agreement with the H-atom signals in the ^{1}H-NMR. In the HMBC spectrum, the correlations of H–C(5)[1]) with C(1), C(3) and C(4), and of H–C(6) with C(2), C(4), and C(7) suggested a 1,2,3,4-tetrasubstituted benzoyl moiety; the correlations of H–C(3′) with C(5′), H–C(5′) with C(1′), C(3′), and C(6′), and of H–C(6′) with C(2′) and C(4′) suggested a 1,2,4-trisubstituted benzoyl moiety. The connectivity of the two fragments through NH was suggested by analysis of the EI-MS and IR spectra. Four MeO groups could be located at C(2), C(3), C(4), and C(7′) from HMBC between the MeO–C(2), MeO–C(3), MeO–C(4), and MeO–C(7′) and the related C-atoms C(2), C(3), C(4), and C(7′), respectively. Consequently, the structure of **4** was deduced as puberuline D.

Table 3. *^{13}C-NMR Spectroscopic Data for Compounds* **4**, **5**, *and* **6**[1]). δ in ppm.

	4	**5**	**6**		**4**	**5**	**6**
C(1)	120.0 (*s*)	114.6 (*s*)	116.8 (*s*)	C(1′)	134.1 (*s*)	133.2 (*s*)	131.5 (*s*)
C(2)	152.7 (*s*)	155.3 (*s*)	152.6 (*s*)	C(2′)	151.0 (*s*)	153.3 (*s*)	153.9 (*s*)
C(3)	141.8 (*s*)	120.0 (*d*)	118.7 (*d*)	C(3′)	107.3 (*d*)	121.8 (*d*)	116.8 (*d*)
C(4)	156.8 (*s*)	123.0 (*d*)	121.0 (*d*)	C(4′)	119.0 (*s*)	117.9 (*s*)	119.0 (*s*)
C(5)	127.0 (*d*)	150.0 (*s*)	152.0 (*s*)	C(5′)	121.2 (*d*)	123.0 (*d*)	121.3 (*d*)
C(6)	124.4 (*d*)	111.6 (*d*)	113.9 (*d*)	C(6′)	116.8 (*d*)	116.8 (*d*)	125.4 (*d*)
C(7)	164.0 (*s*)	168.3 (*s*)	165.1 (*s*)	C(7′)	167.2 (*s*)	168.7 (*s*)	167.3 (*s*)
*Me*O–C(2)	62.0 (*q*)			*Me*O–C(6)			56.2 (*q*)
*Me*O–C(3)	61.1 (*q*)			*Me*O–C(7′)		52.6 (*q*)	52.6 (*q*)
*Me*O–C(4)	56.1 (*q*)						

Compound **5**, a colorless solid, showed a molecular formula of $C_{15}H_{13}NO_6$ as determined by HR-ESI-MS ($[M+Na]^+$ at m/z 326.0632; calc. 326.0635). IR Absorptions at 3561, 3366, 3256, 1613, and 1544 cm^{-1} indicated the presence of OH, NH, ester CO, and amide CO groups. The ^{1}H-NMR spectrum showed signals of one MeO group (δ(H) 3.98, *s*), two 1,2,4-trisubstituted aromatic moieties at δ(H) 7.29 (*d*, $J=2.7$), 7.06 (*dd*, $J=9.0, 2.7$), and 6.85 (*d*, $J=9.0$); δ(H) 7.56 (*d*, $J=3.0$), 7.20 (*dd*, $J=$ 9.6, 3.0), and 8.59 (*d*, $J=9.6$). The analysis of all spectra measured (1D-NMR, 2D-NMR, MS, and IR) revealed that the structure of **5** was very similar to **4** except for the location of the substitutes. Three OH groups could be located at C(5)[1]), C(2), and C(2′) by the HMBC of HO–C(5) with C(6) and C(5), HO–C(2) with C(2), C(3), and C(1), HO–C(2′) with C(3′) and C(2′). The MeO group could be located at C(7′) from the HMBC between the MeO group and C(7′). Accordingly, the structure of compound **5** was elucidated and named puberuline E.

The HR-ESI-MS of compound **6** exhibited a $[M+H]^+$ peak at 318.0971 (calc. 318.0972) indicating a molecular formula of $C_{16}H_{15}NO_6$ (14 mass units more than that of **5**). In fact, the signal pattern was rather similar to **5**, except for the presence of a MeO group in **6** instead of the OH group in **5**. The IR absorptions at 3427, 3412, 1655, and 1608 cm^{-1} indicated the presence of OH, NH, ester CO, and amide CO groups. The connectivity could be further determined unambiguously on the basis of NMR (^{1}H- and ^{13}C-NMR, DEPT, and HMBC). The MeO group was located at C(2)[1]) by the HMBC of the MeO group with C(2). Thus the structure of compound **6** was deduced and named puberuline F.

The structures of the known compounds luciculine (**7**) [4], lucidusculine (**8**) [4], 12-acetylluciculine (**9**) [5], 1-acetylluciculine (**10**) [4], lepenine (**11**) [6], denudatine (**12**) [6], 11-acetyllepenine (**13**) [7], 12-epidehydronapelline (**14**) [8], 11-acetyl-1,19-epoxydenudatine (**15**) [9], dehydroluciduscuiline (**16**) [10], dehydroacosanine (**17**) [11], demethylenedelcorine (**18**) [12], 6-*O*-acetyldemethylenedelcorine (**19**) [13], 14-*O*-acetylvirescenine (**20**) [14], flavamine (**21**) [14], and tuguaconitine (**22**) [15] were identified on the basis of physicochemical and spectroscopic methods.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Factory*), *Sephadex LH-20* (*Amersham Pharmacia Biotech*), *RP-18* silica gel (150–200 mesh, *Merck*). Thin-layer chromatography (TLC): silica gel GF_{254} (10–40 mm; *Qingdao Marine Chemical Factory*); detection under UV light and visualized by spraying with 5% H_2SO_4 in EtOH (*v*/*v*), followed by heating. M.p.: *X-4* melting-point apparatus (*Beijing TECH Instrument Co. Ltd.*, P. R. China); uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Nicolet Avatar 360* FT-IR spectrometer; in cm^{-1}. NMR Spectra: *Varian Mercury-300/400BB* spectrometer; δ in ppm, *J* in Hz, with Me_4Si as standard or residual solvent peak used for referencing. EI-MS: *HP-5988A GC/MS* instrument; in *m*/*z* (rel. %). HR-ESI-MS: *Bruker APEX-II* mass spectrometer.

Plant Material. Aconitum barbatum var. *puberulum* was collected in Jinzhong Country (Shanxi Province, P. R. China) in September 2005 and was identified by Dr. *Hu-Yuan Feng* (School of Life Science, Lanzhou University, Lanzhou, P. R. China). A voucher specimen (No. 20050901AB) is deposited with the Institute of Organic Chemistry, Lanzhou University, Lanzhou, P. R. China.

Extraction and Isolation. The air-dried and powdered herb (3.9 kg) was extracted with 95% EtOH (15 l) three times (each time for 7 d) at r.t. to give 520 g of a syrup, which was dissolved in H_2O and defatted with petroleum ether (PE). The defatted aq. extract was then extracted with AcOEt at two pH levels: pH 4–5 and 9–10, which were adjusted by the addition of H_2SO_4 (2%) and NaOH (2%) solns., resp. The basic fraction (pH 9–10, 39.0 g) was subjected to CC (6 × 100 cm) on SiO_2 eluting with $CHCl_3$/MeOH (99:1, 3 l; 50:1, 5 l; 30:1, 12 l; 10:1, 12 l; 5:1, 5 l) to afford 5 fractions (*Frs. 1–5*). *Fr. 1* (12.5 g) was chromatographed on a SiO_2 column (3 × 80 cm) using a PE/AcOEt (15:1, 1 l; 8:1, 1 l) gradient to give two fractions (*Frs. 1A* and *1B*). *Fr. 1A* (4.7 g) was further chromatographed on a SiO_2 column (2 × 50 cm) using a PE/Me_2CO (25:1, 200 ml; 20:1, 400 ml; 15:1, 400 ml; 10:1, 400 ml; 8:1 400 ml; 6:1, 200 ml) gradient to give compounds **4** (3 mg), **15** (12 mg), **16** (5 mg), and **17** (40 mg); *Fr. 1B* (3.8 g) was chromatographed on a SiO_2 column (1.5 × 35 cm) using PE/AcOEt (20:1, 500 ml) and further purified by prep. TLC (PE/$CHCl_3$/MeOH, 15:15:1 70 ml) to yield compounds **12** (3 mg, R_f 0.3), **6** (2 mg, R_f 0.5), and **20** (5 mg, R_f 0.6). *Fr. 2* (5.2 g) was recrystallized from PE/Me_2CO (4:1) to give **22** (60 mg), and the parent liquid was further chromatographed on SiO_2 CC (2 × 50 cm), eluted with a PE/AcOEt (16:1, 50 ml; 8:1, 100 ml; 2:1, 50 ml) gradient to give **19** (2 mg), **5** (2 mg), and **3** (2 mg). *Fr. 3* (8.3 g) was chromatographed on SiO_2 CC (3 × 80 cm), eluted with a PE/AcOEt (8:1, 1 l; 3:1, 1 l) gradient to give two fractions (*Frs. 2A* and *2B*); among these, *Fr. 2A* (3.6 g) was further separated on SiO_2 CC (2 × 50 cm) eluted with a PE/$CHCl_3$/MeOH (20:20:1, 150 ml; 15:15:1, 300 ml; 8:8:1, 300 ml; 4:4:1, 200 ml) gradient to afford **8** (5 mg), **10** (23 mg), **1** (25 mg), and **2** (15 mg), and *Fr. 2B* (2.9 g) was chromatographed on a SiO_2 column (1.5 × 50 cm) eluted with PE/Me_2CO (8:1, 300 ml; 6:1, 500 ml; 4:1, 500 ml; and 2:1, 200 ml) to give **13** (8 mg), **14** (56 mg), and **9** (14 mg). *Fr. 4* (4.2 g) was chromatographed on a SiO_2 column (2 × 50 cm), eluted with PE/$CHCl_3$/MeOH (8:8:1, 1 l; 3:3:1, 1 l) to give two fractions (*Frs. 3A* and *3B*), among which, *Fr. 3A* (0.8 g) gave **18** (26 mg) after purification by *Sephadex LH-20* CC (1.0 × 50 cm, $CHCl_3$/MeOH, 2:1, 70 ml), and *Fr. 3B* (2.4 g) was recrystallized from a $CHCl_3$/MeOH (5:1) soln. to give **11** (70 mg). *Fr. 5* (5.3 g) was further submitted to SiO_2 CC (3 × 80 cm), eluted with PE/AcOEt/MeOH (4:4:1, 200 ml; 3:3:1, 400 ml; 2:2:1, 400 ml; 1:1:1, 400 ml) to give **7** (23 mg) and **21** (10 mg).

Puberuline A (= *(1S*,4R*,7R*,8R*,10S*,13R*,16S*)-4,7-Dihydroxy-13-methyl-6-methylidene-11-azahexacyclo[7.7.2.1^{5,8}.0^{1,10}.0^{2,8}.0^{13,17}]nonadec-11-en-16-yl Acetate*; **1**). White amorphous powder. $[\alpha]_D^{20} = +38$ (*c* = 3.68, MeOH). IR (KBr): 3415, 1732, 1638, 1246. ^{1}H- ((D)$_6$DMSO, 300 MHz) and ^{13}C-NMR ((D)$_6$DMSO, 75 MHz): *Table 1*. EI-MS: 371 (M^+). HR-ESI-MS: 372.2167 ([M + H]$^+$, $C_{22}H_{30}NO_4^+$; calc. 372.2169).

Puberuline B (= *(1S*,4R*,7R*,8R*,10S*,13R*,16S*)-4,7-Dihydroxy-13-methyl-6-methylidene-12-oxo-11-azahexacyclo[7.7.2.1^{5,8}.0^{1,10}.0^{2,8}.0^{13,17}]nonadec-16-yl Acetate*; **2**). White amorphous powder. $[\alpha]_D^{20} = +10$ (*c* = 0.6, MeOH). IR (KBr): 3447, 1717, 1630, 1625. ^{1}H- ((D)$_6$DMSO, 400 MHz) and ^{13}C-NMR ((D)$_6$DMSO, 100 MHz): *Table 1*. EI-MS: 387 (M^+). HR-ESI-MS: 388.2118 ([M + H]$^+$, $C_{22}H_{30}NO_5^+$; calc. 388.2118).

Puberuline C (= *(1S*,2R*,5S*,8S*,12R*,14S*,16R*)-3-Ethyl-16-hydroxy-8,12,14-trimethoxy-5-(methoxymethyl)-3-azahexacyclo[7.6.3.1^{10,13}.0^{1,11}.0^{2,9}.0^{5,18}]nonadecan-17-one*; **3**). Colorless solid. $[\alpha]_D^{20} = +18$ ($c = 0.4$, MeOH); IR (KBr): 3423, 1705. ^{1}H- ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz): *Table 2*. EI-MS: 449 (M^+). HR-EI-MS: 449.2767 (M^+, $C_{25}H_{39}NO_6^+$; calc. 449.2772).

Puberuline D (= *Methyl 3-Hydroxy-4-{[(2,3,4-trimethoxyphenyl)carbonyl]amino}benzoate*; **4**). Colorless solid. $[\alpha]_D^{20} = -60$ ($c = 0.6$, MeOH). IR (KBr): 3751, 3447, 1704, 1637, 1574–1460. ^{1}H-NMR ($CDCl_3$, 400 MHz)[1]): 11.73 (br. *s*, HO–C(2′)); 9.05 (*s*, NH); 8.63 (*d*, *J* = 8.8, H–C(6′)); 7.92 (*d*, *J* = 8.8, H–C(6)); 7.49 (*d*, *J* = 2.8, H–C(3′)); 7.06 (*dd*, *J* = 8.8, 2.8, H–C(5′)); 6.78 (*d*, *J* = 8.8, H–C(5)); 4.06 (*s*, MeO–C(2)); 3.93 (*s*, MeO–C(4)), 3.93 (*s*, MeO–C(7′)); 3.87 (*s*, MeO–C(3)). ^{13}C-NMR ($CDCl_3$, 100 MHz): *Table 3*. EI-MS: 361 (9.7, M^+), 195 (100, $[M - C_8H_8NO_3]^+$). HR-EI-MS: 361.1158 (M^+, $C_{18}H_{19}NO_7^+$; calc. 361.1156).

Puberuline E (= *Methyl 4-{[(2,5-Dihydroxyphenyl)carbonyl]amino}-3-hydroxybenzoate*; **5**). Colorless solid. $[\alpha]_D^{20} = -25$ ($c = 0.4$, MeOH). IR (KBr): 3561, 3366, 3256, 1613, 1544. ^{1}H-NMR ((D$_6$)acetone, 300 MHz)[1]): 11.8 (*s*, NH); 11.60 (*s*, HO–C(2)); 8.73 (*s*, HO–C(2′)); 8.59 (*d*, *J* = 9.6, H–C(6′)); 8.40 (*s*, HO–C(5)); 7.56 (*d*, *J* = 3.0, H–C(3′)); 7.29 (*d*, *J* = 2.7, H–C(6)); 7.20 (*dd*, *J* = 9.6, 3.0, H–C(5′)); 7.06 (*dd*, *J* = 9.0, 2.7, H–C(4)); 6.85 (*d*, *J* = 9.0, H–C(3)); 3.98 (*s*, MeO–C(7′)). ^{13}C-NMR ((D$_6$)acetone, 75 MHz): *Table 3*. EI-MS: 303 (M^+). HR-ESI-MS: 326.0632 ($[M + Na]^+$, $C_{15}H_{13}NNaO_6^+$; calc. 326.0635).

Puberuline F (= *Methyl 3-Hydroxy-4-{[(5-hydroxy-2-methoxyphenyl)carbonyl]amino}benzoate*; **6**). Colorless solid. $[\alpha]_D^{20} = -12$ ($c = 0.2$, MeOH). IR (KBr): 3427, 3412, 1655, 1608. ^{1}H-NMR ((D)$_6$DMSO, 300 MHz)[1]): 11.60 (*s*, HO–C(2′)); 11.5 (*s*, NH); 9.76 (*s*, HO–C(5)); 8.21 (*d*, *J* = 9.6, H–C(6′)); 7.43 (*d*, *J* = 3.0, H–C(6)); 7.31 (*d*, *J* = 3.0, H–C(3′)); 7.03 (*dd*, *J* = 9.0, 3.0, H–C(4)); 7.00 (*dd*, *J* = 9.6, 3.0, H–C(5′)); 6.90 (*d*, *J* = 9.0, H–C(3)); 3.81 (*s*, MeO–C(7′)); 3.72 (*s*, MeO–C(2)). ^{13}C-NMR ((D)$_6$DMSO, 75 MHz): *Table 3*. EI-MS: 317 (M^+). HR-ESI-MS: 318.0971 ($[M + H]^+$, $C_{16}H_{16}NO_6^+$; calc. 318.0972).

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