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Puberunine and Puberudine, Two New C_{18}-Diterpenoid Alkaloids from *Aconitum barbatum* var. *puberulum*

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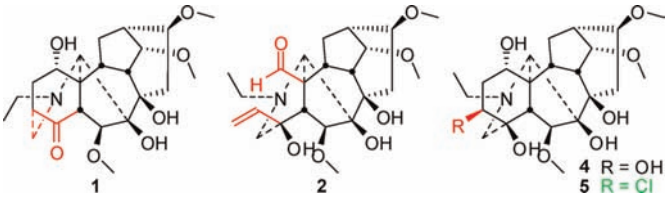
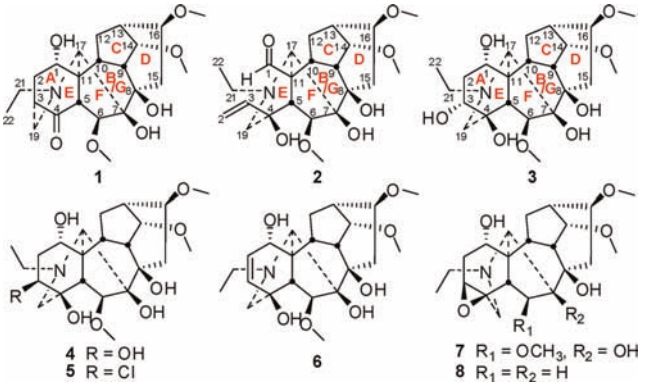
ABSTRACT



Two C_{18}-diterpenoid alkaloids, puberunine (1) and puberudine (2), together with four other new alkaloids, including the first examples having β-oriented substitution at C-3 and a rare chloro-substituent were isolated from *Aconitum barbatum* var. *puberulum*. Their structures were elucidated by spectroscopic methods. Puberunine and puberudine, which possess a unique rearranged E ring and an opened A ring, respectively, represent new subtypes of the C_{18}-diterpenoid alkaloids. A plausible biosynthetic pathway of 1 and 2 was proposed.

Aconitum barbatum Pers. var. *puberulum* Ledeb. (Subgen *Lycoctonum*), a herb distributed in the northern part of China, as well as in the Siberia region of Mongolia and Russia,[1] has been used over a long period of time in China as a folk medicine to treat rheumatism and pain.[2] Previous investigations of this herb have led to the isolation of some C_{18}-, C_{19}-, and C_{20}-diterpenoid alkaloids, along with several anthranilamides.[3,4]

In a current study of *A. barbatum* var. *puberulum* roots, we isolated six new C_{18}-diterpenoid alkaloids (**1**–**6**) along with two known alkaloids tuguaconitine (**7**) and monticamine (**8**). Puberunine (**1**) possesses an unusual rearranged E ring, and puberudine (**2**) contains an opened A ring. Interestingly, these structural features are unprecedented in the field of the diterpenoid alkaloids. In addition, puberumines B (**4**) and C (**5**) represent the first examples of naturally occurring diterpenoid alkaloids that have β-oriented C-3 substitution and a rare chloro-substituent, respectively.



Puberunine (**1**), isolated as a colorless amorphous solid, was shown by HRESITOFMS (*m*/*z* 438.2496 $[M + H]^+$,

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calcd. 438.2486) to have the molecular formula $C_{23}H_{35}NO_7$. Absorption bands in the IR spectrum indicate the presence of hydroxyl (3600−3000 cm^{-1}) and carbonyl (1712 cm^{-1}) groups. Analysis of the ^{13}C NMR spectrum along with DEPT135 and HSQC spectra shows that puberunine (**1**) contains 23 carbons, including four methyl groups (three oxygenated), five methylenes, ten methines (four oxygenated), and four quaternary carbons (one ketone, two oxygenated). Furthermore, the presence of four methyl groups is supported by ^1H NMR results that show a methyl of an *N*-ethyl group (δ_H 1.19, 3H, t, J = 7.2 Hz) and three aliphatic methoxyl groups (δ_H 3.50, 3.43, and 3.37, each 3H, s). The carbonyl group, the sole multiple bond in **1**, accounts for one of its seven degrees of unsaturation, indicating the presence of six rings in **1**. The data summarized above, in combination with biogenetic considerations, suggest that **1** might be a C_{18}-diterpenoid alkaloid.

Detailed analysis of the COSY and HMBC spectra of **1** (Figure 1a and Supporting Information (SI) S2) demonstrates that it is a new C_{18}-diterpenoid alkaloid containing a rearranged E ring, in which C-19 of the complex polycyclic framework is bonded to C-3 instead of to typically C-4, leaving a carbonyl group at C-4, which is confirmed by the key consecutive COSY correlation chain H-1/-H-2(α, β)/H-3/H-19(a, b) and HMBC correlation networks from H-2β, H-5, H-6, and H-19(a, b) to C-4 and from H-17 and H-21 to C-19. Owing to the rigidity of ring system, C-19 needs to be α-oriented at C-3, in a cofacial manner with respect to H-6. The existence of this feature gains support from ROESY correlations of H-19b/H-6 and H-6/H-21. ROESY correlations of H-14/H-10, H-14/H-12β, and H-10/H-1 indicate that H-1, H-10, and H-14 are β-oriented, while correlations of H-16/H-12α and H-16/H-17 demonstrate the α orientation of H-16 (Figure 1b).

Puberudine (**2**), isolated as a colorless amorphous solid, has the molecular formula $C_{23}H_{35}NO_7$ (by HRESI-TOFMS at m/z 438.2493 $[M + H]^+$, calcd. 438.2486), which corresponds to seven degrees of unsaturation. Analysis of the IR spectrum shows the existence of hydroxy (3600−3200 cm^{-1}) and carbonyl (1705 cm^{-1}) groups. The ^{13}C NMR spectrum of **2**, fortified by the results of DEPT135 and HSQC experiments, contains 23 carbon signals corresponding to four methyls (three oxygenated), five methylenes (one olefinic), ten methines (one aldehyde, one olefinic, and three oxygenated), and four quaternary carbons (three oxygenated). The ^1H NMR spectroscopic measurements show that puberudine (**2**) possesses an aldehyde group (δ_H 9.88, 1H, s), a terminal double bond (δ_H 5.33, 1H, d, J = 17.5 Hz; δ_H 5.19, 1H, d, J = 10.7 Hz; δ_H 5.79, 1H, dd, J = 17.4, 10.6 Hz), three aliphatic methoxyl groups (δ_H 3.43, 3.40, and 3.33, each 3H, s), and a methyl of an *N*-ethyl group (δ_H 1.12, 3H, t, J = 7.2 Hz). The aldehyde group and the double bond account for two of the seven degrees of unsaturation in **2**, and the remaining ones are comprised of five rings. The combined data suggest that **2** is an unusual C_{18}-diterpenoid alkaloid containing one less ring.

The structure of **2** was definitively assigned by extensive analysis of the COSY and HMBC spectroscopic data (Figure 1c and SI S3). The data reveal, in particular, that the aldehyde group is attached to C-11 (based on the HMBC correlations from H-1 to C-11 and C-17) and that the terminal double bond is linked to C-4 (based on HMBC correlations of H-2(a, b)/C-4, H-3/C-4 and C-19, and H-5/C-3). ROESY correlations from H-19b and H-21 to H-6 suggest that H-6 is α-oriented. The correlations of H-14/H-10 and H-14/H-12β indicate that H-10 and H-14 are β-oriented, and correlations of H-16/H-12α and H-16/H-17 show the α-orientation of H-16 (Figure 1d).

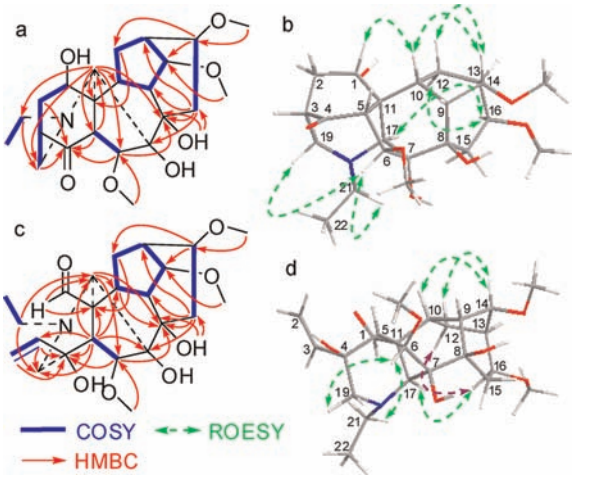

Figure 1. Selected COSY, HMBC, and ROESY correlations of **1** (a, b) and **2** (c, d).

The structures of the other new alkaloids, designated as puberumines A−D (**3**−**6**), were elucidated by extensive analysis of their spectroscopic data (Table 1). Especially informative in this regard are the 2D NMR data (SI S4). In addition, two known alkaloids, tuguaconitine (**7**)[5,6] and monticamine (**8**),[5] were also isolated (SI S5).

Puberunine (**1**) possesses an unprecedented skeleton containing a rearranged seven-membered E ring (consisting of C-3, C-4, C-5, C-11, C-17, *N*, and C-19), in which the C19−C4 bond typically present in C_{18}- and C_{19}-diterpenoid alkaloids is missing and a new C19−C3 bond exists. Puberudine (**2**) is also a unique 1,2-seco diterpenoid alkaloid. By using Wang's system to classify C_{18}-diterpenoid alkaloids,[7] both **1** and **2** cannot be assigned to any of the known subtypes, including the aconosine and lappaconine subtypes of the lappacontitine type, and leuconine and ranaconitine subtypes of ranaconitine type. Consequently, **1** and **2** represent two new subtypes of ranaconitine type, which we have named as the rearranged subtype and 1,2-seco subtype, respectively.

It is plausible to trace the biogenetic origin of the new alkaloids **1**−**6** to tuguaconitine (**7**). We propose that

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Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Data for **1**–**6** in $CDCl_3$ (*J* in Hz)[a]

	1		**2**		**3**		**4**		**5**		**6**	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.67 (br. d, 8.1)	69.5	9.88 (s)	207.4	3.57 (t, 7.6)	71.7	3.64 (t, 4.1)	70.9	3.69 (t, 8.3)	69.7	3.76 (d, 4.7)	70.9
2α	2.53 (m)	39.3	5.33 (d, 17.5)	116.2	1.75	41.8	1.73	39.5	1.98 (m)	40.8	5.80 (dd, 9.6, 4.7)	131.2
2*β*	2.03 (dt, 15.4, 1.7)		5.19 (d, 10.7)		2.49		2.14 (m)		2.49			
3	2.65	48.8	5.79 (dd, 17.4, 10.6)	144.1	3.71 (dd, 10.0, 6.1)	72.7	3.95 (dd, 9.2, 5.8)	71.0	4.07	75.0	5.91 (d, 9.6)	136.1
4		213.7		71.4		72.8		70.0		78.0		70.2
5	2.35 (m)	60.8	2.28	58.11	1.65 (br. s)	53.2	1.89 (s)	50.5	2.50	50.3	1.91 (br. s)	56.21
6	4.14 (d, 4.1)	92.9	4.46 (s)	89.9	4.25 (s)	89.5	4.22 (s)	90.0	4.21 (s)	91.8	4.12 (s)	90.2
7		87.7		87.7		87.4		87.5		86.9		87.1
8		79.2		78.3		78.2		78.4		78.1		78.6
9	2.63	44.2	3.04 (m)	43.3	2.97 (dd, 6.9, 4.9)	43.6	2.92 (br. t, 5.5)	43.2	3.05 (dd, 7.1, 5.0)	43.5	2.97 (dd, 7.0, 5.1)	43.6
10	2.10 (m)	48.0	2.43 (m)	39.4	1.98 (m)	44.8	1.95	43.7	2.12 (m)	45.0	2.02 (m)	44.5
11		53.0		58.16		50.7		49.77		52.5		50.4
12α	2.27 (dd, 15.0, 4.8)	28.0	0.83 (dd, 14.5, 4.8)	31.9	2.29 (dd, 14.2, 4.4)	28.5	1.96	29.8	2.51	28.4	2.14 (dd, 13.3, 3.8)	28.9
12*β*	1.92 (m)		1.82		1.86 (m)		1.67		1.84 (m)		1.96 (m)	
13	2.45 (dd, 7.5, 4.4)	39.0	2.29	38.4	2.38 (dd, 7.0, 4.4)	38.4	2.38 (dd, 6.3, 4.2)	37.9	2.38 (dd, 7.1, 4.4)	38.3	2.40 (dd, 6.4, 4.7)	38.4
14	3.70 (t, 4.6)	83.6	3.58 (t, 4.4)	83.6	3.64 (t, 4.4)	84.1	3.61 (t, 4.4)	84.2	3.64 (t, 4.3)	84.1	3.65 (t, 4.5)	84.4
15α	1.75 (br. dd, 14.3, 7.9)	34.3	2.64 (dd, 14.7, 8.5)	33.3	1.73	33.5	1.71	33.3	1.75 (dd, 14.6, 7.8)	33.7	1.74 (dd, 14.2, 5.5)	33.6
15*β*	2.70 (dd, 15.2, 8.9)		1.75 (m)		2.62 (dd, 14.8, 8.7)		2.60 (dd, 14.7, 8.6)		2.60 (dd, 15.0, 8.7)		2.64	
16	3.23	83.1	3.13	83.0	3.22 (t, 8.5)	82.9	3.22 (t, 8.4)	82.9	3.21 (t, 8.4)	82.8	3.29	82.9
17	3.00 (br. s)	65.4	2.87	63.3	2.82 (d, 1.6)	63.7	2.76	64.7	2.89 (br. s)	63.6	2.80 (d, 1.7)	64.5
19a	3.25	51.2	3.15	54.6	3.33 (d, 9.2)	52.2	2.80	58.6	3.10	57.4	2.70 (d, 11.2)	56.16
19b	3.07 (dd, 13.7, 7.1)		2.85		2.43		2.72		3.10		2.65	
21a	3.21	50.4	2.99 (m)	50.5	3.04 (m)	50.2	3.02 (m)	49.81	3.08	50.4	3.06 (m)	49.8
21b	2.96 (m)		2.92 (m)		2.86 (m)		2.84		2.90 (br. s)		2.88 (m)	
22	1.19 (t, 7.2)	14.5	1.12 (3H, t, 7.2)	13.9	1.12 (3H, t, 7.2)	14.0	1.07 (3H, d, 7.2)	13.4	1.12 (3H, t, 7.2)	13.9	1.08 (3H, t, 7.3)	13.6
6′	3.50 (3H, s)	59.3	3.43 (3H, s)	58.21	3.43 (3H, s)	58.3	3.38 (3H, s)	58.0	3.50 (3H, s)	58.7	3.41 (3H, s)	58.1
8-OH	4.27 (d, 1.0)		4.06 (s)		4.18 (s)		4.19 (s)		4.08 (s)		4.06 (s)	
14′	3.43 (3H, s)	57.9	3.40 (3H, s)	57.8	3.42 (3H, s)	57.8	3.39 (3H, s)	57.6	3.43 (3H, s)	57.8	3.43 (3H, s)	57.7
16′	3.37 (3H, s)	56.3	3.33 (3H, s)	56.3	3.35 (3H, s)	56.3	3.33 (3H, s)	56.2	3.35 (3H, s)	56.3	3.37 (3H, s)	56.3

[a] Overlapped signals are reported without designating multiplicity.

puberunine (**1**) and puberudine (**2**) originate through the pathway shown in Scheme 1. The biogenesis sequence for **1** involves epimerization at C-3 by Grob fragmentation of **3** (Scheme 1, step a) and retro-Grob fragmentation (b) to produce **4** and **5**, two key intermediates bearing a *β*-oriented substituent at C-3 to form **1** via Wagnner–Meerwein rearrangement (c). In the boat conformation adopted by **4** and **5** having a C-1α hydroxyl group,[8,9] the C19–C4 bond and C-3*β* substituent were *trans*-coplanar, which facilitated the shift of the C-19 alkyl from C-4 to C-3.

Scheme 1. A Plausible Biogenetic Pathway for **1** and **2**

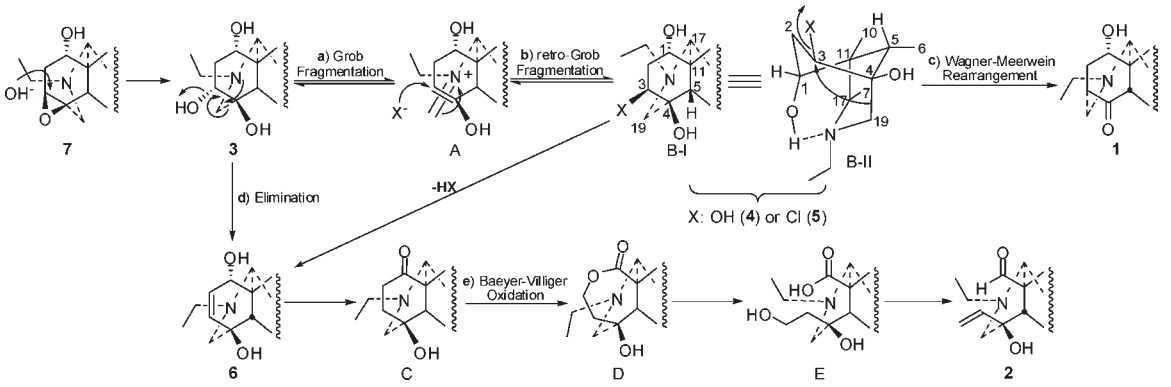
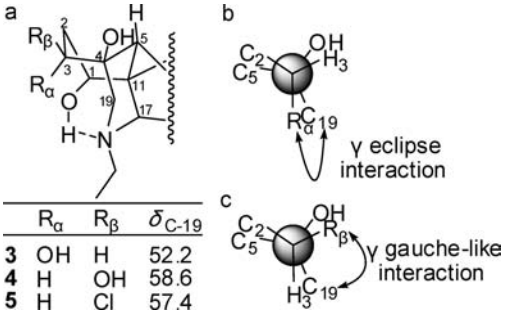


Table 1 and Figure 2 show how the orientation of the C-3 substituent influences the chemical shifts of C-19 and C-5 through steric interactions. With an α-hydroxyl group at C-1, the A rings in **3**, **4**, and **5** exist in boat conformations (Figure 2a).[8,9] Thus, C-19 experiences a γ-eclipse effect of the C-3α group instead of a γ-gauche-like effect of the C-3β group. This effect results in a larger upshield shift of C-19 to 52.2 ppm for **3** and a smaller shift to 58.6 ppm for **4** or to 57.4 ppm for **5** (Figure 2). On the other hand, the presence of a β-oriented group at C-3 introduces a γ-gauche-like interaction to C-5, while a C-3α group is too remote from H-5 to produce a comparative effect (Figure 2a).[10] Consequently, the chemical shift of C-5 for **3** is larger than that for **4** and **5** (Table 1). Furthermore, this analysis suggests a potential method to deduce the orientation of C-3 substituents based on the chemical shift of C-19 in the cases of alkaloids whose A rings adopt a boat conformation (C-1α hydroxyl group). Specifically, a larger chemical shift of C-19 (*ca.* 57 ppm) designates a β-disposition of the substiuent or that no substitution is present at C-3, while a smaller shift (*ca.* 53 ppm) designates the presence of an α-oriented substituent.

Compounds **1**–**8** were tested for their cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human cancer cell lines by the MTT method. However, none of them exhibited significant activity (IC_{50} > 40 μM).



	R_α	R_β	$\delta_{C\text{-}19}$
3	OH	H	52.2
4	H	OH	58.6
5	H	Cl	57.4

Figure 2. Steric interactions between substituents at C-3 and C-19/C-5 for **3** (a, b), **4** (a, c), and **5** (a, c).

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Supporting Information Available. Experimental section, tabulated 1D and 2D NMR data of **1** and **2**, structural elucidation of **3**–**6**, physico-chemical data of **1**–**8**, UPLC-HRESIMS analysis of **1**–**7** in the extract prepared under ultrasonic conditions, and a listing of IR, ESI-MS, HRESITOFMS, and NMR spectra for **1**–**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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