Diterpenoid Alkaloids from *Delphinium majus*

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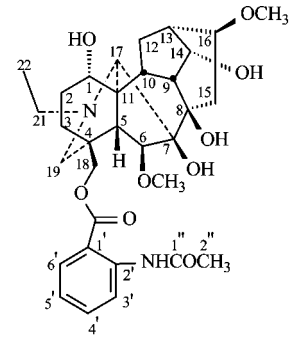
From the whole herbs of *Delphinium majus*, three new C_{19}-diterpenoid alkaloids, majusines A−C (**1**−**3**), and six new C_{20}-diterpenoid alkaloids, majusimines A−D (**4**−**7**) and majusidine A and B (**8** and **9**), have been isolated, together with 15 known compounds. The structures of compounds **1**−**9** were elucidated by spectroscopic data interpretation.

Delphinium species represent a large genus within the Ranunculaceae family. It is estimated that about 350 species of *Delphinium* exist in the world, mainly in the northern hemisphere, of which 173 are found in mainland China.[1] A large number of diterpenoid alkaloids have been isolated from various species of *Delphinium* and *Aconitum* and are classified according to their structures as C_{18}-, C_{19}-, and C_{20}-diterpenoid alkaloids.[2] Diterpenoid alkaloids have been the targets of considerable interest of medicinal chemists for a broad range of demonstrated pharmacological properties: analgesic, antiarrhythmic, anti-inflammatory, arrhythmogenic, curariform, hypotensive, local anesthetic, neurotropic, psychotropic, and spasmolytic.[3−8] These studies have shown that these alkaloids act as site 2 neurotoxins and can be subdivided into two main groups.[4,9] It is intriguing that these alkaloids exhibit different actions varying from poisonous (e.g., aconitine) to therapeutic (e.g., lappaconitine), even though they share a similar molecular skeleton. Interestingly, two reports on the antiproliferative activities of the diterpenoid alkaloids toward cancer cells have appeared in recent years.[10,11]

The plant *Delphinium majus* W. T. Wang is distributed mainly in southwest Sichuan and northwest Yunnan of mainland China, especially around the Jinsha River basin. To our knowledge, no previous work has been reported on the phytochemistry of this plant. As part of an ongoing research program to investigate the chemistry and analgesic activities of diterpenoid alkaloids from *Aconitum* and *Delphinium* plants, we have carried out a study on the whole plants of *D. majus*, which led to the isolation of three new C_{19}-diterpenoid alkaloids, majusines A−C (**1**−**3**), and six new C_{20}-diterpenoid alkaloids, majusimines A−D (**4**−**7**) and majusidines A and B (**8** and **9**), as well as 15 known compounds: acetydelgrandine,[12] ajacine,[13] blacknine,[14] browniine,[13] 14-dehydrobrowniine,[15] 14-dehydrodelcosine,[15] delcosine,[13] delgrandine,[12] delsemine A,[16] delsemine B,[16] deltaline,[16] isodelpheline,[17] lycoctonine,[16] methyllycaconitine,[16] and tatsiensine.[17] We describe herein the separation and structure elucidation of these new diterpenoid alkaloids.



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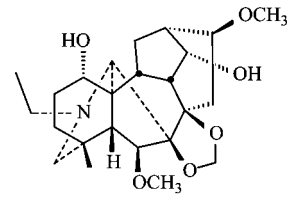

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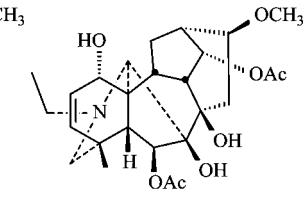

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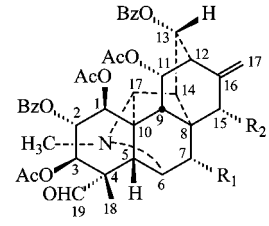
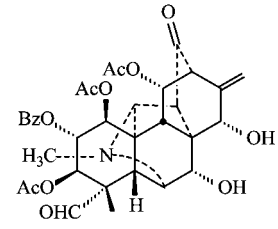


	R_1	R_2
4	OAc	OAc
5	OH	OAc
6	OH	OH

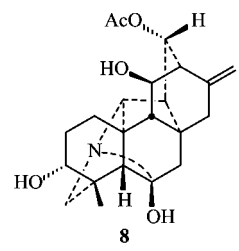


7

Results and Discussion

Three new C_{19}-diterpenoid alkaloids, designated as majusines A−C (**1**−**3**), were obtained as amorphous powders. Their molecular formulas, $C_{32}H_{44}N_2O_9$, $C_{24}H_{37}NO_6$, and $C_{26}H_{37}NO_6$, respectively, were inferred from their HRESIMS and NMR spectra, which showed that each contained a lycoctonine-type C_{19}-diterpenoid alkaloid skeleton.[18]

The NMR spectra of majusine A (**1**) exhibited signals at δ_H 1.14 (3H, t, J = 7.2 Hz), δ_C 13.5 (q), 50.2 (t), for a *N*-ethyl group, δ_H 3.30, 3.37 (each 3H, s), δ_C 57.8 (q), 56.2 (q), for two aliphatic methoxyl groups, and a *N*-acetyl anthranoyl group (δ_H 11.0, 1H, s, *N*H, 7.12−8.72, 4H, m, Ar-H; δ_C see Table 1). Along with the



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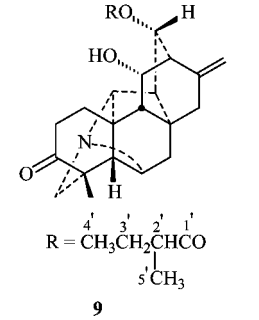

9

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Table 1. NMR Data of Majusines A−C (**1**−**3**)[a]

	1[b]		**2**[b]		**3**[b]	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.70 t (4.0)	72.3 d	3.74 t (3.2)	71.8 d	3.70 d (4.8)	70.7 d
2	1.63 m	29.1 t	1.59 m	29.5 t	5.78 dd (9.2, 4.8)	130.2 d
	1.63 m		1.59 m			
3	1.80 m	27.3 t	1.52 m	31.7 t	5.68 d (9.2)	136.8 d
	1.94 m		1.61 m			
4		36.7 s		32.5 s		35.2 s
5	1.93 s	45.6 d	1.37 s	51.7 d	1.74 s	55.3 d
6	4.03 s	90.4 d	3.65 s	89.2 d	5.28 s	81.6 d
7		87.9 s		92.1 s		87.2 s
8		77.9 s		83.2 s		77.4 s
9	2.96 m	43.9 d	3.56 t (5.6)	42.0 d	3.08 dd (6.8, 4.8)	42.1 d
10	2.07 m	45.2 d	2.05 dd (9.6, 5.6)	45.7 d	2.03 m	44.3 d
11		48.9 s		50.8 s		49.1 s
12	1.57 m	29.1 t	1.72 m	28.8 t	2.20 m	27.9 t
	1.62 m		2.03 m		2.23 m	
13	2.37 t (2.8)	39.3 d	2.35 (hidden)	38.6 d	2.58 m	37.4 d
14	4.12 dd (9.6, 4.8)	75.5 d	4.13 t (4.8)	74.6 d	4.87 t (4.8)	76.9 d
15	1.69 q (9.2)	34.3 t	1.88 dd (16.4, 5.2)	35.8 t	1.46 dd (14.8, 7.2)	38.3 t
	2.77 q (9.2)		2.55 dd (16.4, 5.2)		3.0 dd (14.8, 7.2)	
16	3.44 (hidden)	81.8 d	3.37 m	81.6 d	3.35 (hidden)	82.2 d
17	2.92 br s	65.9 d	3.01 s	65.1 d	2.87 br s	65.7 d
18	4.23 br s	69.3 t	1.02 s	27.1 q	1.03 s	23.8 q
19	2.67 (hidden)	56.7 t	2.30, 2.51	60.9 t	2.48, 2.55	56.1 t
	3.32 (hidden)		ABq (12.4)		ABq (12.0)	
21	2.88 m	50.2 t	2.67 m	49.8 t	2.93 m	50.2 t
	3.02 m		2.83 m		3.02 m	
22	1.14 t (7.2)	13.5 q	1.09 t (6.8)	13.4 q	1.06 t (7.2)	13.6 q
CH_3O-6	3.30 s	57.8 q	3.34 s	58.5 q		
CH_3O-16	3.37 s	56.2 q	3.30 s	56.1 q	3.31 s	56.0
7-CH_2-8			5.06 s, 5.09 s	93.8 t		
AcO-6					2.05 s	172.4 s
						21.3 q
AcO-14					2.05 s	170.6 s
						21.4 q
anthranoyl-18-COO4′5′6′		168.0 s				
1′		114.2 s				
2′		141.8 s				
3′	7.96 d (8)	120.5 d				
4′	7.59 t (8)	135.0 d				
5′	7.12 t (8)	122.5 d				
6′	8.72 d (8)	130.0 d				
-NH-	11.0 s					
1″		168.9 s				
2″	2.25 s	25.4 t				

[a] Recorded at 400 MHz for ^{1}H, 100Mz for ^{13}C, δ in ppm, J in Hz. [b] Recorded in $CDCl_3$.

above-mentioned signals, its ^{13}C NMR spectrum displayed seven oxygenated carbon signals (δ_C 69.3 t, 72.3 d, 75.5 d, 77.9 s, 81.8 d, 87.9 s, 90.4 d), suggesting that compound **1** possesses four hydroxyl groups in addition to two methoxyl groups and an *N*-acetyl anthranoyl ester group. All of this evidence, along with careful inspection of the NMR data (^{1}H, ^{13}C, DEPT, HMQC, and HMBC), indicated that majusine A is a lycoctonine-type C_{19}-diterpenoid alkaloid.[18,19] The two methoxyl groups could be assigned at C-6 and C-16, respectively, due to the correlations between OCH_3-6 (δ_H 3.30) and C-6 (δ_C 90.4), and between OCH_3-16 (δ_H 3.37) and C-16 (δ_C 81.8), in the HMBC spectrum. The *N*-acetyl anthranoyl group was located at C-18 on the basis of the correlation from H_2-18 to the ester carbonyl of the *N*-acetyl anthranoyl group (Figure 1). An α-oriented hydroxyl group at C-1 was evident from the presence of an oxymethine chemical shift at δ_C 72.3 in the ^{13}C NMR spectrum and a one-proton triplet signal of an associated methine at δ_H 3.70 (J = 4.0 Hz) in the ^{1}H NMR spectrum.[18] This was confirmed by the correlations between H-1 and H_2-2 in the COSY spectrum. An OH-14 α group was supported by the presence of a one-proton quartet signal at δ_H 4.12 (J = 4.8 Hz), which changed to a triplet on addition of D_2O.[18] These observations led to the assignment of the structure of majusine A as **1**.

The NMR spectra of majusine B (**2**) also exhibited characteristic features of a lycoctonine-type norditerpenoid alkaloid,[18,19] bearing

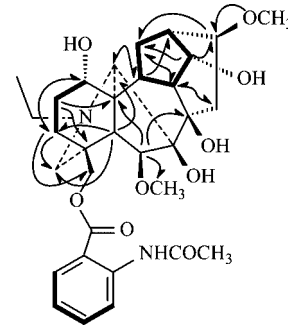

Figure 1. Key ^{1}H−^{1}H COSY (—) and HMBC (→).

a tertiary methyl (δ_H 1.02, 3H, s), an *N*-ethyl group (δ_H 1.09, 3H, t, J = 6.8 Hz), a methylenedioxy group (δ_H 5.06, 5.09, 2H, s), and two methoxyl groups (δ_H 3.34, 3.30, each 3H, s). A methylenedioxy group was readily located at C-7 and C-8, which was corroborated by the long-range correlations from a methylene group (δ_H 5.09, 5.06) to C-7 (δ_C 92.1) and C-8 (δ_C 83.2). The two methoxyl groups could be assigned at C-6 and C-16 on the basis of the related correlations in the HMBC spectrum (Figure S1, Supporting Information). A triplet signal (J = 4.8 Hz) at δ_H 4.13 in the ^{1}H NMR spectrum was assigned to H-14β, implying the presence of

Table 2. NMR Data of Majusimines A, C, and D (**4**, **6**, **7**) and Majusidines A and B (**8** and **9**)[a]

position	**4**[b]	**6**[c]	**7**[c]	**8**[c]	**9**[b]
1	6.01 d (3.6)	6.44 d (4.0)	6.20 t (4.0)	1.51 dd (6.4, 1.6) 2.66 dd (6.4, 1.6)	2.71d (16) 3.50d (16)
2	6.08 t (3.6)	6.77 t (4.0)	6.80 t (4.0)	1.47 (hidden) 2.20 (hidden)	2.20 (hidden) 2.35 (hidden)
3	5.18 d (3.6)	5.62 d (4.0)	5.63 d (4.0)	3.75 d (4.4)	
5	2.15 (hidden)	2.36 s	2.42 s	1.82 s	2.04 s
6	3.10 s	3.65 s	3.69 s		3.38 s
7	4.90 s	4.74 s	4.79 s	2.15, 2.24 ABq (20.8)	1.69,1.85 ABq (3.2)
9	2.72 d (9.6)	3.23 d (10)	3.69 (hidden)	1.64 d (1.6)	2.43 d (8.8)
11	5.61 d (9.6)	6.33 d (10)	6.40 d (5.2)	4.41 d (1.6)	4.28 d (8.8)
12	2.68 d (2.0)	2.99 br s	3.42 s	2.30 t (2.8)	2.41 (hidden)
13	5.22 dd (10.0, 2.0)	5.47 br d (10)		5.15 d (9.6)	5.15 d (10)
14	3.24 dd (10.0, 2.0)	3.68 d (10)	3.48 s	2.70 (hidden)	2.16 d (10)
15	5.66 s	5.20 s	5.32 s	2.02, 2.21 ABq (13.2)	2.10, 2.24 (hidden)
17	5.32 s 5.38 s	5.34 s 5.39 s	5.30 s 5.36 s	4.76 s 4.90 s	4.75 s 4.93 s
18	1.14 s	1.32 s	1.32 s	2.07 s	1.15 s
19	9.51 s	10.07 s	10.28 s	3.50, 3.96 ABq (12)	2.20, 2.74 (hidden)
20	3.91 s	4.40 s	4.47 s	4.06 s	2.72 s
N-CH_3	2.50 s	3.08 s	3.06 s		
AcO-1	1.89 s	1.88 s	2.09 s		
AcO-3	2.15 s	2.09 s	1.94 s	2.37 s	
AcO-7	2.12 s				
AcO-11	2.05 s	2.19 s	2.07 s		
AcO-15	2.11 s				
$OCOC_6H_5$-2					
2′,6′,	7.77 d (8)	7.98 d (8)	8.13 d (7.2)		
3′,5′	7.08 t (8)	7.14 t (8)	7.40 t (7.2)		
4′	7.34 t (8)	7.29 t (8)	7.50 t (7.2)		
$OCOC_6H_5$-13					
2′,6′	7.56 d (7.6)	8.06 d (7.6)			
3′,5′	7.29 t (7.6)	7.40 t (7.6)			
4′	7.52 t (7.6)	7.48 t (7.6)			
1′					
2′					2.57 m
3′					1.52, 1.73 m
4′					0.95 t (7.2)
5′					1.21 d (6.8)

[a] Recorded at 400 MHz. [b] Recorded in $CDCl_3$. [c] Recorded in C_5D_5N.

a OH-14 α functionality.[18,19] The OH-1*a* group was confirmed by a methine signal at δ_C 71.8 in the ^{13}C NMR spectrum and the correlations between H-1 and H_2-2 in the COSY spectrum (Figure S1, Supporting Information). The above findings led to the determination of the structure of majusine B as **2**.

The NMR spectroscopic data of **3** revealed signals at δ_H 1.06 (3H, t) and δ_C 13.6 (q), 50.2 (t) for a *N*-ethyl group, δ_H 1.03 (3H, s) and δ_C 23.8 (q) for a tertiary methyl, δ_H 3.31 (3H, s) and δ_C 56.1 (q) for a methoxyl group, δ_H 2.05, 2.05 (each 3H, s) and δ_C 172.4 (s), 21.3 (q); 170.6 (s), 21.4 (q), for two acetyl groups, and δ_H 5.78, 5.68 (each 1H) and δ_C 130.2, 136.8 (both d), for two unsaturated methines. The IR and ^{13}C NMR spectra (3510 cm^{-1}, δ_C 87.2 s, 77.4 s, and 70.7 d) of **3** afforded evidence of two tertiary hydroxyl groups and a secondary hydroxyl group. Correlations between OCH_3-16 and C-16, H-6 and the ester carbonyl (172.4 s), and H-14 and the ester carbonyl (170.6 s) in the HMBC spectrum (Figure S2, Supporting Information) suggested that the methoxyl group and two acetyl groups could be assigned at C-16, and C-6 and C-14, respectively. The occurrence of the OMe-16 group excluded the possibility of a $\Delta^{15(16)}$ functionality. A $\Delta^{2(3)}$ double bond was corroborated by the correlations from H-2 to C-4 and from H-19 to C-3 in the HMBC spectrum (Figure S2, Supporting Information). The secondary hydroxyl group was located at C-1 on the basis of the correlation with H-2 in the COSY spectrum, while the α-orientation of OH-1 was confirmed in the NOEDS spectrum by the coupling between the H-1β (3.70 d) and H-10β (2.03 m). The structure of majusine C was thus deduced as **3**.

Majusimines A−D (**4**−**7**) were isolated as amorphous powders and exhibited characteristic NMR features of vakognavine-type C_{20}-diterpenoid alkaloids.[2] Their molecular formulas were determined as $C_{45}H_{47}NO_{15}$, $C_{43}H_{45}NO_{14}$, $C_{41}H_{43}NO_{13}$, and $C_{34}H_{37}NO_{12}$, respectively, according to their HRESIMS and NMR spectra. The four compounds were found to possess a similar skeleton to that of vakognavine, for which the structure was established by X-ray crystallographic analysis.[20] The presence of an aldehyde in majusimines A−D (**4**−**7**) was apparent from singlet signals at δ_H 9.51 for **4**, 8.94 for **5**, 10.07 for **6**, and 10.28 for **7** and the methine signal at δ_C 193.3 for **4**, 193.0 for **5**, 194.0 for **6**, and 194.1 for **7**, respectively. The NMR spectra of **4** afforded evidence of a *N*-CH_3 group (δ_H 2.50, 3H, s; δ_C 34.0 q), a tertiary methyl group (δ_H 1.14, 3H, s; δ_C 23.2 q), an exocyclic double bond (δ_H 5.32, 5.38, each 1H, br s), five acetyl groups (δ_H 1.89, 2.15, 2.12, 2.05, 2.11, each 3H, s; δ_C see Table 3), and two benzoyl groups (δ_H 7.08−7.77, 10H, m) (Table 2). The five acetyl groups and two benzoyl groups could be assigned at C-1, C-2, C-3, C-7, C-11, C-13, and C-15, respectively, due to the correlations between H-1/OAc-1 (δ_C 169.6), H-2/OBz-2 (δ_C 164.0), H-3/OAc-3 (δ_C 169.4), H-7/OAc-7 (δ_C 170.1), H-11/OAc-11 (δ_C 170.7), H-13/OBz-13 (δ_C 165.6), and H-15/OAc-15 (δ_C 170.7), respectively (Figure 2). In addition, the relative configurations of these ester groups were evident from the NOESY correlations summarized in Figure 3. In the NOESY experiment, correlations between H-1α and H-20 and between H-3α and H-1α (Figure 3) indicated the α-orientation of H-1 and H-3. Similarly, correlations between H-2β and H-5β, H-7β and H-9β,

Table 3. NMR Data of Majusimines A−D (**4**−**7**) and Majusidines A and B (**8** and **9**)[a]

position	**4**[b]	**5**[b]	**6**[c]	**7**[c]	**8**[c]	**9**[b]
1	71.8 d	71.5 d	73.1 d	73.6 d	31.1 t	45.1 t
2	66.1 d	67.6 d	67.0 d	67.2 d	23.1 t	50.0 t
3	71.8 d	71.8 d	73.1 d	73.0 d	75.3 d	212.0 s
4	49.2 s	49.0 s	49.7 s	49.9 s	43.5 s	42.4 s
5	59.7 d	58.8 d	60.0 d	59.4 d	62.8 d	60.6 d
6	59.8 d	62.5 d	63.1 d	63.7 d	97.7 s	65.2 d
7	69.9 d	70.5 d	69.3 d	68.4 d	45.2 t	35.9 t
8	51.5 s	53.2 s	55.5 s	55.6 s	42.8 s	44.6 s
9	48.9 d	48.2 d	48.8 d	49.7 d	49.2 d	50.0 d
10	55.8 s	55.1 s	55.8 s	56.2 s	48.6 s	55.3 s
11	73.4 d	73.4 d	74.3 d	71.2 d	69.5 d	74.6 d
12	44.5 d	44.5 d	44.5 d	60.0 d	40.0 d	48.5 d
13	72.4 d	72.5 d	72.7 d	208.2 s	74.0 d	73.0 d
14	37.1 d	37.2 d	37.0 d	48.8 d	50.5 d	54.6 d
15	65.5 d	66.0 d	65.1 d	65.3 d	33.6 t	33.6 t
16	141.7 s	141.3 s	143.7 s	144.4 s	148.3 s	144.4 s
17	118.7 t	118.9 t	114.7 t	116.8 t	107.5 t	109.1 t
18	23.2 q	22.6 q	23.2 q	23.6q	28.1 q	28.7 q
19	193.3 s	193.0 s	194.0 s	194.1 s	58.4 t	64.6 t
20	63.3 d	63.3 d	64.9 d	67.7 d	68.3 d	70.7 d
N-CH_3	34.0 q	35.4 q	35.0 q	35.5 q		
AcO-1	169.6 s	170.2 s	170.3 s	170.1 s		
	20.4 q	20.5 q	20.3 q	20.7 q		
AcO-3	169.4 s	169.4 s	170.3 s	170.4 s	170.6 s	
	20.8 q	21.1 q	20.4 q	20.4 q	21.2 q	
AcO-7	170.1 s					
	21.0 q					
AcO-11	170.7 s	171.8 s	170.8 s	170.6 s		
	21.1 q	21.3 q	21.5 q	21.3 q		
AcO-15	170.7 s	170.9 s				
	21.0 q	21.4 q				
$OCOC_6H_5$-2	164.0 s	164.2 s	164.9 s	165.4 s		
1′	129.0 d	129.0 d	129.0 d	129.5 d		
2′,6′	129.4 d	129.6 d	129.4 d	129.8 d		
3′,5′	128.3 d	128.5 d	128.6 d	129.1 d		
4′	133.3 d	133.3 d	133.7 d	133.9 d		
$OCOC_6H_5$-13	165.6 s	165.7 s	165.9 s			
1′	129.0 d	129.0 d	129.0 d			
2′,6′	129.4 d	129.1 d	129.9 d			
3′,5′	128.3 d	128.4 d	128.9 d			
4′	133.3 d	133.3 d	133.3 d			
1′						176.0 s
2′						41.2 d
3′						26.5 t
4′						11.5 q
5′						16.4 q

[a] Recorded at 100 MHz, δ_c in ppm. [b] As in Table 1. [c] As in Table 2.

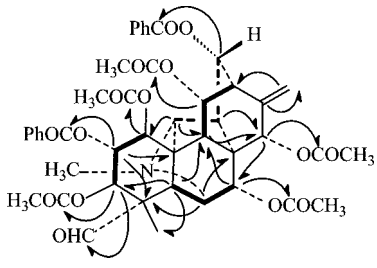

Figure 2. Key $^1H-^1H$ COSY (—) and HMBC (→).

H-1α and OAc-11α, H-13β and H-17, and H-15β and H-7β suggested the β-orientation of H-2, H-7, H-11, H-13, and H-15. Therefore, the structure of majusimine A (**4**) was assigned as shown.

Compounds **5** and **6** exhibited nearly identical ^1H and ^{13}C NMR resonances to those of **4**. Differences between the three sets of spectra were demonstrated by the absence of an acetyl group in **5** and the absence of two acetyl groups in **6**, thus validating the loss of 42 mass units in **5** and the loss of 84 mass units in **6**, as found by mass spectrometry. The one-proton signal of H-7 was shifted upfield from δ_H 4.90 in **4** to δ_H 3.67 in **5**, suggesting that the acetyl group at C-7 in **4** was replaced by a hydroxyl group in **5**. In the

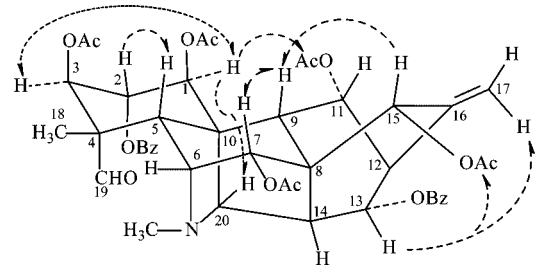

Figure 3. Key NOESY (→) correlations of **4**.

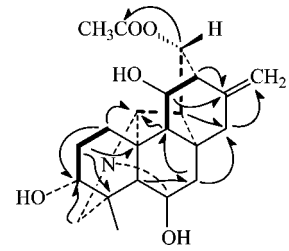

Figure 4. Key $^1H-^1H$ COSY (—) and HMBC (→).

HMBC spectrum of **6**, critical correlations for H-1/OAc (δ_C 170.3), H-2/OBz (δ_C 164.9), H-3/OAc (δ_C 170.3), H-11/OAc (δ_C 170.8), and H-13/OBz (δ_C 165.9) (Figure S3, Supporting Information) suggested the location of the ester groups at C-1, C-2, C-3, C-11, and C-13, respectively, implying that the two acetyl groups at C-7 and C-15 in **4** were replaced by two hydroxyl groups in **6**.

Compound **7** showed similar NMR spectroscopic patterns to those of **6**, except for H-1, H-2, H-9, H-12, H-14, and H-15 (Table 1) and C-11, C-12, C-13, C-14, C-17, and C-20 (Table 3), indicating that both compounds are of the same type of C_{20}-diterpenoid alkaloid. Comparison of the NMR data of **7** with those of **6** indicated the presence of an additional ketone carbonyl resonance (δ_C 208.2) in the ^{13}C NMR spectrum of compound **7**, in addition to the absence of resonances corresponding to a benzoyl group and a oxymethine. The additional ketone carbonyl group in **7** (δ_C 208.2) was assigned at C-13 on the basis of the correlations of C-13 with H-11 and H_2-17 in the HMBC spectrum. The structure of majusimine D was confirmed by the analysis of its 2D NMR data (Figure S4, Supporting Information).

Majusidine A (**8**) was isolated as an amorphous powder. Its positive-ion HRESIMS showed a quasimolecular ion peak at *m*/*z* 388.2120 $[M + H]^+$, corresponding to the molecular formula $C_{22}H_{29}N_5O$. The NMR and mass spectra indicated that it is a hetisine-subtype C_{20}-diterpenoid alkaloid with a *N*-C(6)-OH group.[2] The NMR spectra of **8** showed the presence of a tertiary methyl group (δ_H 2.07, 3H, s; δ_C 28.1 q), an exocyclic double bond (δ_H 4.76, 4.90, each 1H, br s; δ_C 107.5 t, 148.3 s), a quaternary carbon (δ_C 97.7 s), and an acetyl group (δ_H 2.37, 3H, s; δ_C 170.6 s, 21.2 q). Except for the above-mentioned signals, its NMR spectra displayed three oxymethine signals (δ_C 75.3 s; δ_H 3.75, 1H, d; δ_C 69.5 s; δ_H 4.41, 1H, d; δ_C 74.0 s; δ_H 5.15, 1H, d) and an oxygenated quarternary carbon (δ_C 97.7 s), showing that this compound possesses two hydroxyl groups in addition to an acetyl group and a *N*-C(6)-OH group. The HMBC experiment suggested that the ester group could be assigned to C-13 on the basis of the correlations from H-13 to the ester carbonyl carbon (Figure 4). The *N*-C(6)-OH group was also confirmed by the HMBC experiment that showed a correlation from H-19 to C-6. The other two hydroxyl groups were located at C-3 and C-11 on the basis of the correlations of H-19 to C-3 and H-1 to C-3, H-11 to C-16, and H-11 to C-20 in the HMBC spectrum. In the NOEDS spectrum (Figure S5, Supporting Information), correlations between H-3 and H-5 β or H-3 and H-18 β revealed the β-orientation of H-3. Similarly, correlations between H-20 and H-11, H-15, and H-13 suggested

the β-orientation of OH-11 and H-13. Accordingly, the structure of majusidine A was elucidated as **8**.

Positive-ion HRESIMS analysis of majusidine B (**9**) gave a pseudomolecular ion at *m/z* 412.2470 ([M + H]$^+$), which suggested a molecular formula of $C_{25}H_{33}NO_4$. This compound exhibited characteristic NMR features of an amine group hetisine-subtype C_{20}-diterpenoid alkaloid,[2] displaying a ketone carbonyl (δ_C 212.0), a 2-methylbutyryl (see Tables 2 and 3), and an exocyclic double bond (δ_H 4.75, 4.93, each 1H, br s; δ_C 109.1 t, 144.4 s). In addition, the presence of two oxymethines was also evident from the NMR data (δ_C 74.6 d; δ_H 4.28, 1H, d; δ_C 73.0 q; δ_H 5.15, 1H, d), implying that compound **9** possesses an additional hydroxyl group except for the ester group and ketone mentioned previously. The ketone carbonyl (δ_C 212.0) was correlated with H-18 (δ_H 1.15, 3H, s) and H-19 (δ_H 2.20, 1H, δ_H 2.74, 1H) in the HMBC spectrum, suggesting that the ketone carbonyl could be assigned to C-3. The hydroxyl group and ester group were assigned to C-11 and C-13, respectively, based on analysis of its HMQC and HMBC spectra (Figure S6, Supporting Information). The β-orientations of H-11 and H-13 were deduced by comparison of the coupling constants ($J_{\text{H-11 }\beta,\text{ H-9}} = 8.8$ Hz, $J_{\text{H-13 }\beta,\text{ H-14}} = 10$ Hz) with those of compounds **4** and **6** (Table 2). On the basis of the 1D and 2D NMR data (Figure S6, Supporting Information), the structure of majusidine B was assigned as **9**.

The known alkaloids were identified by comparison of their ^{1}H and ^{13}C NMR spectra with values reported in the literature.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were obtained on a Nicolet FT-IR 200 SXV spectrophotometer. ^{1}H and ^{13}C NMR spectra were taken on a Varian Unity INOVA 400/45 NMR spectrometer in $CDCl_3$ or C_5D_5N with TMS as the internal standard. The ESIMS and HRESIMS were recorded on a VG Auto Spec 3000 or a Finnigan-MAT 90 instrument. Silica gel H (Qingdao Sea Chemical Factory, Qingdao, People's Republic of China) was used for column chromatography. Zones on TLC (silica gel G) were detected using the modified Dragendorff's reagent.

Plant Material. *Delphinium majus* was collected in Miyi County, Sichuan Province, People's Republic of China, in July 2006. The plant was identified by Professor Q. E. Yang of the Beijing Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (No. 06-9-1) has been deposited.

Extraction and Isolation. Air-dried and powdered whole herbs (6.0 kg) were percolated with 0.1 M HCl (60 L). The acid aqueous solution was basified with 10% aqueous NH_4OH to pH 10 and then extracted with ethyl acetate (30 L × 3). Removal of the solvent under reduced pressure afforded the total crude alkaloids (31.4 g) as a yellowish, amorphous powder, which was chromatographed over a silica gel column, eluting with a cyclohexane−acetone (10:1 → 1:2) gradient system, to give fractions A (3.4 g), B (4.2 g), C (6.5 g), D (7.6 g), E (1.9 g), and F (3.6 g). Fraction A (3.4 g) was further chromatographed on a silica gel column employing cyclohexane−acetone (6:1 → 3:1) as eluent to afford fractions A-1 (89 mg), A-2 (1.7 g), A-3 (1.1 g), and A-4 (219 mg). Purification of fraction A-2 by silica gel column chromatography, eluting with cyclohexane−acetone (6:1), yielded majusine C (**3**, 57.7 mg), ajacine (308.9 mg), methyllycaconitine (380.6 mg), and isodelpheline (25.6 mg). Column chromatography of fraction B over a silica gel column using $CHCl_3$−CH_3OH (100:1 → 30:1) as eluent afforded fractions B-1 (174 mg), B-2 (1.9 g), B-3 1.2 g), B-4 (462 mg), and B-5 (156 mg). Further separation of fraction B-2 by silica gel column chromatography using cyclohexane−acetone (6:1 → 3:1) as eluent provided majusine B (**2**, 35.8 mg), 14-dehydrobrowniine (18.2 mg), deltaline (6.6 mg), delcosine (28.6 mg), blacknine (18.7 mg), and majusimine A (**4**, 18.7 mg). Fraction B-4 was separated over a silica gel H column, eluting with cyclohexane−acetone (4:1 → 1:1), to yield majusimine B (**5**, 2.8 mg), delsemine A (130.4 mg), delsemine B (41.6 mg), lycoctonine (58.3 mg), delgrandine (36.2 mg), and acetydelgrandine (35.4 mg). Fraction D was subjected to column chromatography over silica gel employing cyclohexane−acetone (6:1 → 1:1) as eluent to afford fractions D-1 (150 mg), D-2 (397 mg), D-3 (455 mg), D-4 (432 mg), and D-5 (1.3 g). Fraction D-3 was separated on a silica gel H column, eluting with cyclohexane−acetone (6:1 → 3:1), to yield majusine A (**1**, 55 mg). Separation of fraction E on a silica gel H column, employing cyclohexane−acetone (5:1 → 1:2) for elution, afforded fractions E-1 (464 mg), E-2 (150 mg), E-3 (307 mg), E-4 (938 mg), E-5 (596 mg), and E-6 (1.5 g). Further purification of fraction E-3 by silica gel column chromatography, with $CHCl_3$−CH_3OH (40:1 → 10:1) as eluent, yielded majusidine B (**9**, 19.6 mg), browniine (17.6 mg), tatsiensine (31.6 mg), and 14-dehydrodelcosine (23.6 mg). Fraction E-6 was separated by silica gel column chromatography, eluting with $CHCl_3$−CH_3OH (30:1 → 10:1), to yield majusimine D (**7**, 5.2 mg), majusimine C (**6**, 6.6 mg), and majusidine A (**8**, 7.3 mg).

Majusine A (1): white, amorphous powder; $[\alpha]_D^{20}$ +41.3 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3748, 3447, 2937, 1685, 1589, 1525, 1260 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; ESIMS *m/z* 601 [M + H]$^+$; HRESIMS *m/z* 601.3120 [M + H]$^+$ (calcd for $C_{32}H_{45}N_2O_9$, 601.3120).

Majusine B (2): white, amorphous powder; $[\alpha]_D^{20}$ +2.1 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3735, 2931, 1092 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; ESIMS *m/z* 458 [M + Na]$^+$; HRESIMS *m/z* 436.2682 [M + H]$^+$ (calcd for $C_{24}H_{38}NO_6$, 436.2694).

Majusine C (3): white, amorphous powder; $[\alpha]_D^{20}$ +27.9 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3510, 2924, 1737, 1250 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; ESIMS *m/z* 514 [M + Na]$^+$; HRESIMS *m/z* 492.2596 [M + H]$^+$(calcd for $C_{26}H_{38}NO_8$, 492.2592).

Majusimine A (4): white, amorphous powder; $[\alpha]_D^{20}$ −67.2 (*c* 1.0, $CHCl_3$); IR(KBr) ν_{max} 3735, 2936, 1745, 1540, 1230 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 3; ESIMS *m/z* 842 [M + H]$^+$; HRESIMS *m/z* 842.3003 [M + H]$^+$ (calcd for $C_{45}H_{48}NO_{15}$, 842.3018).

Majusimine B (5): white, amorphous powder; $[\alpha]_D^{20}$ −54.0 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3734, 2935, 1748, 1540, 1236 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.02 (1H, d, J = 4 Hz, H-1), 5.24 (1H, dt, J = 9.6, 2.4 Hz, H-14), 6.08 (1H, t, J = 4 Hz, H-2), 5.17 (1H, t, J = 4 Hz, H-3), 3.67 (1H, s, H-7), 5.60 (1H, br d, J = 9.6 Hz, H-11), 5.78 (1H, br s, H-15), 5.32, 5.38 (each 1H, s, H_2-17), 1.14 (3H, s, H-18), 3.91 (1H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$), see Table 3; ESIMS *m/z* 800 [M + H]$^+$; HRESIMS *m/z* 800.2875 [M + H]$^+$ (calcd for $C_{43}H_{46}NO_{14}$, 800.2913).

Majusimine C (6): white, amorphous powder; $[\alpha]_D^{20}$ −44.4 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3728, 3359, 2927, 1745, 1541, 1232 cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N), see Table 2; ^{13}C NMR (100 MHz, C_5D_5N), see Table 3; ESIMS *m/z* 758 [M + H]$^+$; HRESIMS *m/z* 758.2836 [M + H]$^+$ (calcd for $C_{41}H_{44}NO_{13}$, 758.2832).

Majusimine D (7): white, amorphous powder; $[\alpha]_D^{20}$ −24.7 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3736, 3360, 2928, 1745, 1541, 1232 cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N), see Table 2; ^{13}C NMR (100 MHz, C_5D_5N), see Table 3; ESIMS *m/z* 684 [M + Na]$^+$; HRESIMS *m/z* 652.2376 [M + H]$^+$ (calcd for $C_{34}H_{38}NO_{12}$, 652.2389).

Majusidine A (8): white, amorphous powder; $[\alpha]_D^{20}$ −38.0 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3736, 3360, 2934, 1729, 1239 cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N), see Table 2; ^{13}C NMR (100 MHz, C_5D_5N), see Table 3; ESIMS *m/z* 388 [M + H]$^+$; HRESIMS *m/z* 388.2120 [M + H]$^+$ (calcd for $C_{22}H_{30}NO_5$, 388.2118).

Majusidine B (9): white, amorphous powder; $[\alpha]_D^{20}$ +27.9 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3748, 3556, 2936, 1701, 1152 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 3; ESIMS *m/z* 412 [M + H]$^+$; HRESIMS *m/z* 412.2470 [M + H]$^+$ (calcd for $C_{25}H_{34}NO_4$, 412.2482).

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Supporting Information Available: Figures S1, S2, S3, S4, and S7 showing ^{1}H−^{1}H COSY and HMBC correlations of **2**, **3**, **6**, **7**, and **9**. Figures S5 and S6 showing key NOEDS correlations of **8** and **9**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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