ALKALOIDS OF SOME MONGOLIAN RANUNCULACEAE SPECIES: DETAILED ^{1}H AND ^{13}C-NMR STUDIES OF DENUDATINE AND LEPENINE

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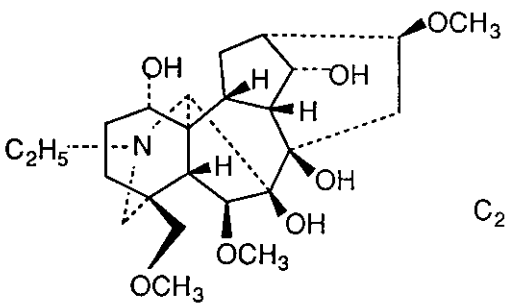
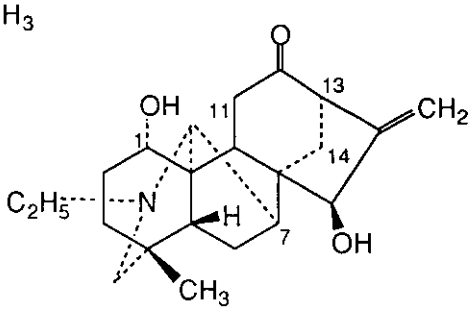
This paper is dedicated to Dr. Bernhard Witkop.

Abstract - We have investigated the alkloidal constituents of *Aconitum barbatum, A. kusnezoffii, A. volubile*, and *Delphinium cheilanthum* collected in Mongolia. Seven norditerpenoid and seven diterpenoid alkaloids were isolated and identified from these plants. ^{1}H Decoupled ^{13}C, 2D ^{1}H phase sensitive COSY, ROESY, TOCSY, HMQC-TOCSY and HMBC NMR spectral studies were carried out on denudatine and lepenine to make definitive assignments for the carbon atoms of these alkaloids.

The seeds of *Aconitum barbatum* Pers., the roots of *A. kusnezoffii* Reichb., aerial parts of *A. volubile* Pall., and *Delphinium cheilanthum* Fisch. were collected by one of the authors (D. Batsuren)



1 Delcosine



2 Songorine

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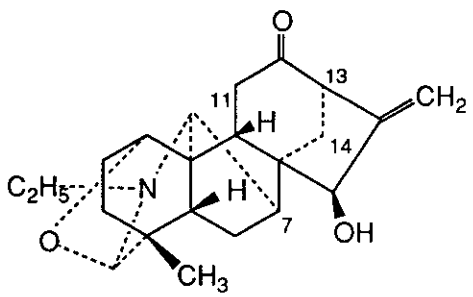
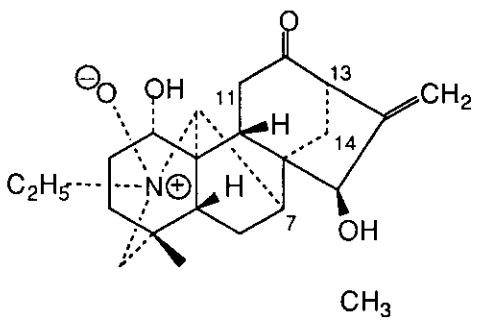
in Mongolia. The epigeal part of *A. barbatum* was investigated earlier[1] and the isolation of delcosine, lycoctonine, songorine, and senbusine A[2] (bataconine)[1] was reported. From the seeds of this plant, we have isolated the norditerpenoid alkaloid delcosine (**1**), and the diterpenoid alkaloids songorine (**2**), songoramine (**3**), songorine-*N*-oxide (**4**) and lepenine (**5**).

The roots of *Aconitum kusnezoffii* Reichb. collected near the river "Chono" of Shuchbaatar, afforded two diterpenoid alkaloids lepenine (**5**) and denudatine (**6**). In an earlier investigation, the norditerpenoid alkaloids aconitine, beiwutine, 3-deoxyaconitine, hypaconitine and mesaconitine were reported to be present in the roots[3] and the diterpenoid alkaloids (**5**) and (**6**) were isolated from the aerial parts.[4]

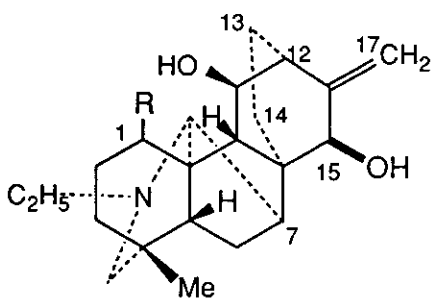


3 Songoramine

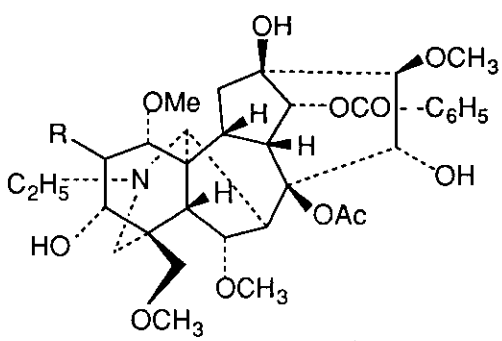


4 Songorine-*N*-oxide

Lepenine is reported to have been first isolated from *Aconitum pseudohuilience*.[5,6] The ^{13}C and ^{1}H NMR spectra of **5** and **6** appeared in a later publication.[4] Denudatine was isolated from the seeds of *Delphinium denudatum* and its structure (**6**) was established on the basis of an X-Ray crystallographic analysis.[7-10]



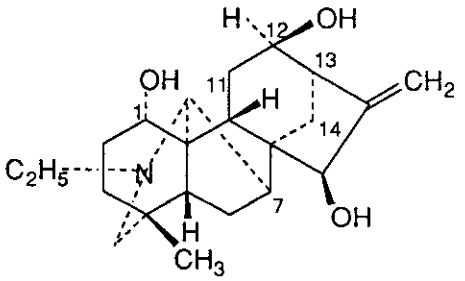
5 Lepenine R = α-OH
6 Denudatine R = H



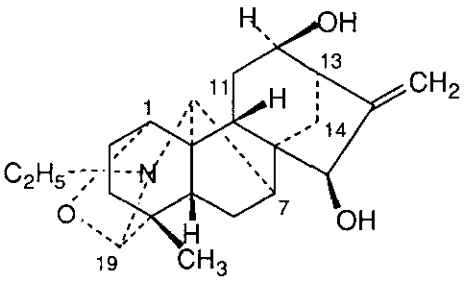
7 Altaconitine R = α-OH,
8 Aconitine R = H

To our knowledge no publications have appeared on the chemical investigations of *A. volubile*. We have investigated the aerial parts with flowers collected in Bajanhongor. By chromatograhic separation of the total alkaloidal mixture, altaconitine (**7**), aconitine (**8**), 12-*epi*-napelline (**9**), 12-*epi*-

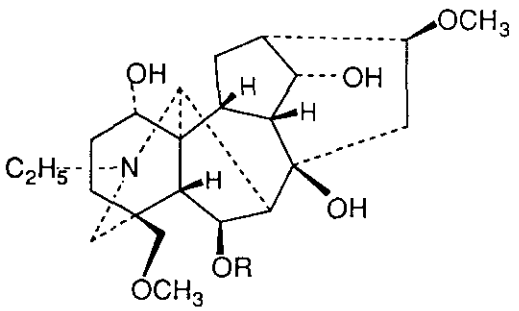
1,19-dehydronapelline (**10**), senbusine A (**11**), songorine (**2**) and neoline (**12**) were isolated. The structures of all the alkaloids were confirmed by the usual methods. Alkaloid **10** was isolated earlier from *A. napellus* L. subsp. *castellanum* J. Molero *et.* C. Blanche[11] and *A. lianghanium* W. Z. Wang.[12] The crude alkaloid mixture from *Delphinium cheilanthum* Fisch. afforded by purification on a Chromatotron, the norditerpenoid alkaloids deltaline (**13**) and methyllycaconitine (**14**).



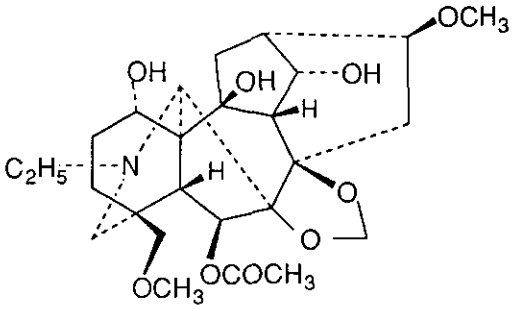
9 12-*epi*-Napelline



10 1,19-Dehydro-12-*epi*-napelline



11 Senbusine A R = H
12 Neoline R = OCH_3



13 Deltaline

NMR Spectral Assignments of Iepenine (5).

Assignments of ^1H and ^{13}C chemical shifts, shown in Table 1, were accomplished using a two-stage method.[13] In the first stage, the segments were established by the spin systems identified on the basis of scalar coupling in TOCSY and HMQC-TOCSY spectra. The sequence-specific assignments of ^1H and ^{13}C within a segment were obtained by the ^1H-^1H primary connectivities in COSY spectrum and the ^1H-^{13}C correlations in the HMQC spectrum. Then, the overall structure was obtained by the long-range ^1H-^{13}C coupling between the segments in the HMBC spectrum and the inter-segment ^1H-^1H NOE connectivities in the ROESY spectrum, summarized in Table 2.

Twenty-two carbons were observed using the 1D ^1H-decoupled ^{13}C, DEPT, and 2D HMQC spectra, which were determined by their characteristic ^1H and ^{13}C chemical shift patterns as two methyl, eight methylene, eight methine and four quaternary carbons. Inspection of the HMQC-TOCSY spectrum revealed six segments (Figure 1), one isolated methylene, one isolated methyl and three hydroxyl groups. Further inspection of the TOCSY spectrum located a weak crosspeak along the

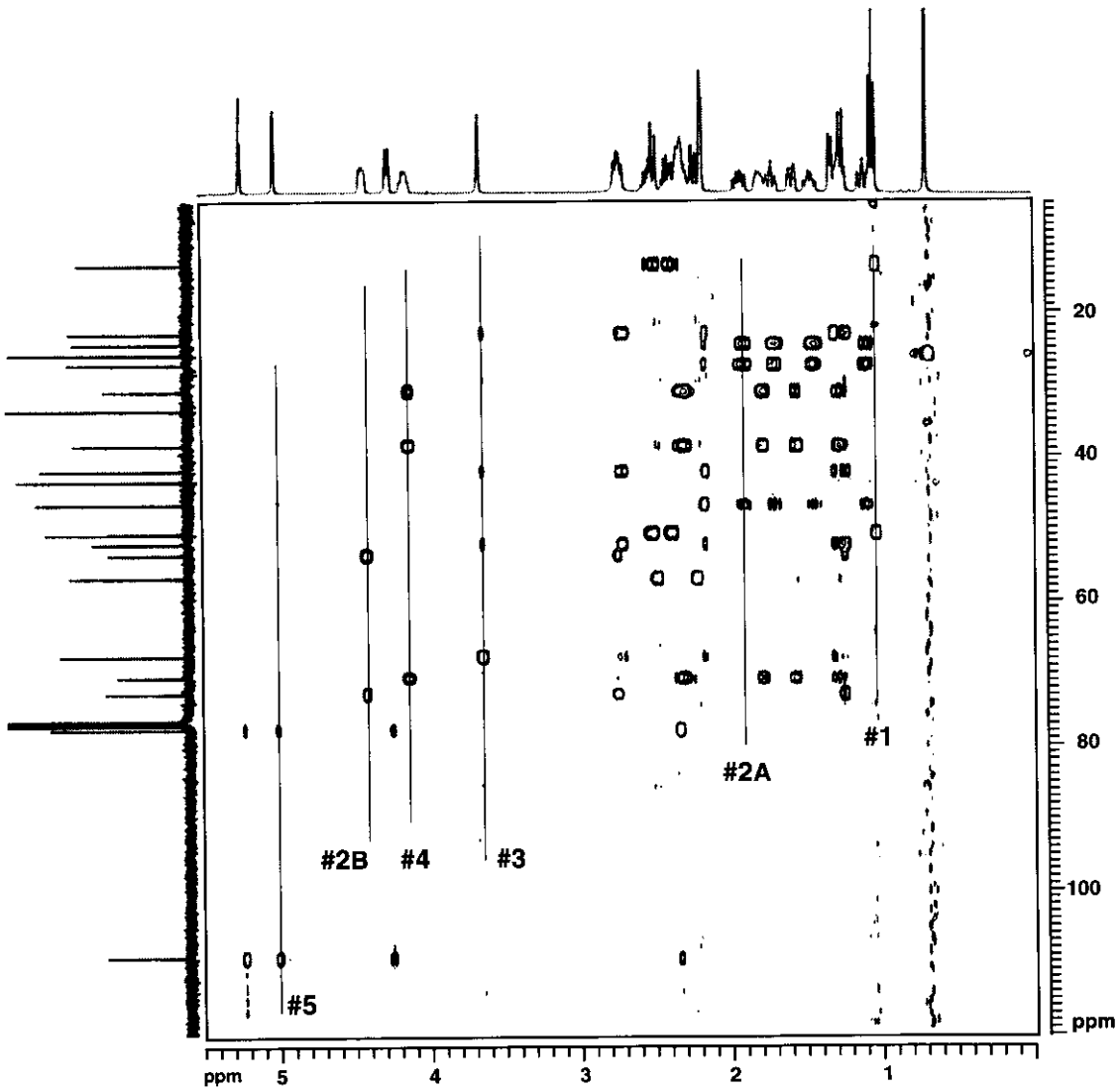


Figure 1. HMQC-clean TOCSY spectrum of lepenine (**5**) with 1D ^{1}H spectrum on top. The identified spin systems have been labeled by vertical lines.

Table 1. ^{13}C and ^{1}H NMR Chemical Shifts and Assignments of Lepenine (**5**), in $CDCl_3$ (ppm)

C #	^{13}C		H #	^{1}H		J = Hz
1	70.7	CH	1_β	4.15	(d,d)	$J_{1\beta,2\alpha}$ = 6.7; $J_{1\beta,2\beta}$ = 10.2
			OH-1_α	2.73		
2	31.1	CH_2	2_β	1.79	(m)	
			2_α	2.32	(m)	
3	38.6	CH_2	3_α	1.58	(d,d)	$J_{3\beta,2\beta}$ = 2.8; $J_{3\beta,2\alpha}$ = 4.3
			3_β	1.28	(d)	$J_{3\alpha,3\beta}$ = 13.4
4	33.7	C	4	—		
5	52.3	CH	5	1.32	(d)	$J_{5,6b}$ = 8.6
6	23.1	CH_2	6_a	1.25	(m)	
			6_b	2.73	(d,d)	$J_{6b,5}$ = 8.6; $J_{6a,6b}$ = 13.7
7	42.2	CH	7	2.17	(d)	$J_{7,6}$ = 7.0
8	43.6	C	8	—		
9	53.8	CH	9	1.26	(d,d)	$J_{9,11}$ = 9.0, $J_{9,15}$ = 7.8
10	50.9	C	10	—		
11	72.9	CH	11	4.43	(d,d)	$J_{11,OH}$ = 4.0; $J_{11,9}$ = 9.0
			OH-11	2.73		
12	46.2	CH	12	2.18	(m)	
13	24.5	CH_2	13_a	1.46	(m)	
			13_b	1.72	(m)	
14	27.4	CH_2	14_a	1.11	(m)	
			14_b	1.93	(m)	
15	77.9	CH	15	4.27	(d,d)	$J_{15,9}$ = 7.8; $J_{15,OH}$ = 6.2
			OH-15	2.33		$J_{OH,15}$ = 6.2
16	154.3	C	16	—		
17	109.5	CH_2	17_a	5.02	(s)	
			17_b	5.24	(s)	
18	26.0	CH_3	CH_3-18	0.69	(s)	
19	57.0	CH_2	19_a	2.49	(AB)	J_{gem} = 11.0
			19_b	2.23	(AB)	J_{gem} = 11.0
20	67.8	CH	20	3.66	(s)	
21	50.8	CH_2	21_a	2.40	(d,d)	J = 7.1; J_{gem} = 14.0
			21_b	2.52	(d,d)	J = 7.1; J_{gem} = 14.0
22	13.6	CH_3	22	1.04	(t)	J = 7.1

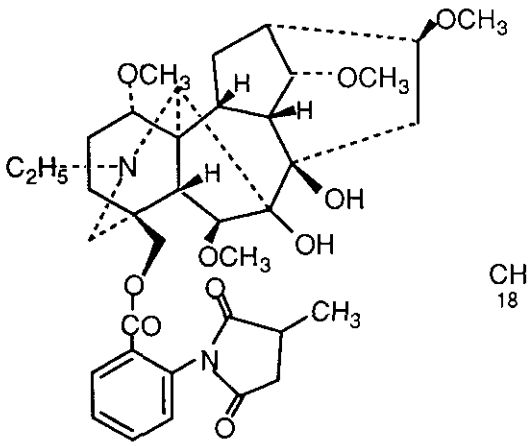
(*J* Constants are measured using ^{1}H traces of HMQC spectrum for overlapped ^{1}H resonances)

Table 2. Summary of HMBC, ROESY and COSY Correlation Data of Lepenine (**5**)

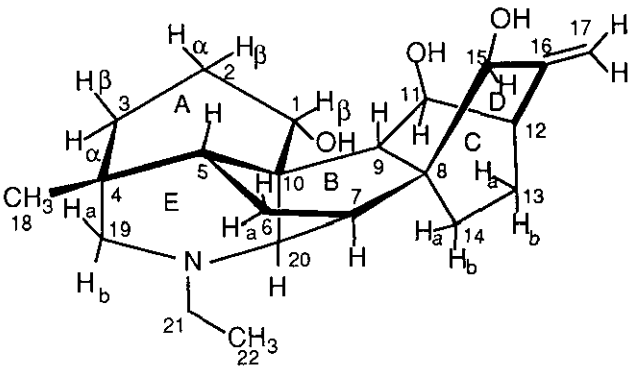
Obs. ^{1}H	HMBC	ROESY	COSY
H-1	C-20	OH-1, H-2α, H-2β, H-5, H-9, H-11, H-20	H-2α, H-2β
H-2α	C-1, C-3	H-1, H-2β, H-3α	H-1, H-2β, H-3α, H-3β
H-2β	C-3, C-10	H-2α, H-3α, H-3β	H-1, H-2α, H-3α, H-3β
H-3α	C-2, C-4, C-5	H-2α, H-3β, H-18, H-19$_a$	H-2α, H-2β, H-3β
H-3β	C-4, C-5, C-18, C-19	H-2β, H-3α, H-18	H-2α, H-2β, H-3α
H-5	C-4, C-7, C-9, C-10, C-18, C-19, C-20	H-1, H-6$_a$, H-18	H-6$_a$
H-6$_a$	C-5, C-8, C-9, C-10, C-20	H-6$_a$, H-7, H-19$_b$	H-6$_a$, H-7
H-6$_b$	C-4, C-7, C-8, C-20	H-5, H-6$_b$, H-7, H-15, OH-15	H-5, H-6$_b$
H-7	C-6, C-9, C-10, C-14	H-6$_a$, H-6$_b$, H-14$_a$, H-14$_b$, H-22	H-6$_b$, H-20
H-9	C-5, C-11, C-15, C-20	H-6$_b$, H-11, OH-15	H-11
H-11	C-5, C-9, C-13, C-16	H-9, OH-11, H-12, H-13$_a$, H-14$_a$, H-20	H-9, OH-11
H-12	C-11, C-14, C-15	H-11, H-13$_a$, H-13$_b$, H-17$_a$	H-13$_a$, H-13$_b$
H-13$_a$	C-16	H-11, H-12, H-13$_b$, H-14$_a$	H-12, H-13$_b$, H-14$_a$, H-14$_b$
H-13$_b$	C-11, C-12, C-14	H-12, H-13$_a$, H-14$_b$, H-15	H-12, H-13$_a$, H-14$_a$, H-14$_b$
H-14$_a$	C-9, C-13, C-15	H-7, H-11, H-13$_a$, H-14$_b$, H-20	H-13$_a$, H-13$_b$, H-14$_b$
H-14$_b$	C-8, C-13, C-15	H-7, H-13$_b$, H-14$_a$, H-15	H-13$_a$, H-13$_b$, H-14$_a$
H-15	C-9, C-14, C-16	H-12, H-13$_b$, H-14$_b$, OH-15, H-17$_b$	OH-15, H-17$_a$, H-17$_b$
H-17$_a$	C-12, C-15	H-12	H-15, H-17$_b$
H-17$_b$	C-12, C-15, C-16	H-15, OH-15	H-15, H-17$_a$
H-18	C-3, C-4, C-5, C-19	H-3α, H-3β, H-5, H-19$_a$, H-19$_b$	—
H-19$_a$	C-3, C-4, C-5, C-20	H-3α, H-18, H-19$_b$	H-19$_b$
H-19$_b$	C-3, C-4, C-18, C-21	H-6$_a$, H-18, H-19$_a$	H-19$_a$
H-20	C-5, C-6, C-19, C-21	H-7, H-11, H-14$_a$, H-21$_a$, H-21$_b$, H-22	H-5, H-6$_a$, H-7
H-21$_a$	C-19, C-20, C-22	H-20, H-21$_b$	H-21$_b$, H-22
H-21$_b$	C-19, C-20, C-22	H-21$_a$	H-21$_a$, H-22
H-22	C-21	H-7, H-20, H-21$_a$, H-21$_b$	H-21$_a$, H-21$_b$

resolved proton at 4.43 ppm, which connects two of the six segments. The weak intensity of this cross peak can be traced to the very small *J*-coupling constant, owing to a near perpendicular orientation between the two protons. The primary ^1H-^1H and ^1H-^{13}C correlations within the above five spin systems were obtained using the COSY and HMQC spectra, respectively. The assignments for ^1H signals were initiated at the resolved ^1H resonances such as CH protons at 3.66, 4.15, 4.27 and 4.43 ppm, CH_2 protons at (1.25, 2,73), (1.46, 1.72), (1.11, 1.93) and (2.23, 2.49) ppm and the CH_3 at 0.69, 1.04 ppm.

Starting with the methyl resonance frequency at 1.04 ppm, TOCSY and HMQC-TOCSY spectra showed that this methyl triplet is scalar-coupled to a pair of CH_2 protons at 2.40, 2.52 ppm. Thus, the two carbons (13.6, 50.8 ppm) were assigned to C(22) and C(21), respectively. The H(21) methylene protons showed ROESY connectivity to the CH proton at 3.66 ppm and to the isolated CH_2 protons at 2.23, 2.49 ppm, and ^1H-^{13}C couplings in the HMBC spectrum to their carbons at 67.8 and 57.0 ppm, respectively. The CH proton at 3.66 ppm was unambiguously assigned to



14 Methyllycaconitine



Stereostructure of lepenine **5**

H(20) while the CH_2 protons were assigned to H(19a) and H(19b). H(19) protons have ^1H-^{13}C couplings in the HMBC spectrum to several carbons including the methylene carbon at 38.6 ppm of segment #4, methine carbon at 52.3 ppm which is assigned as C(5), and the methine carbon at 67.8 ppm assigned to C(20). H(19a) also showed coupling with the C(18) carbon at 26.0 ppm and the C(21) in the HMBC spectrum and a ROESY crosspeak with H(3_α) at 1.58 ppm. The sequence of segment #4 was determined as CH (^1H, 4.15 ppm, ^{13}C, 70.7 ppm)-CH_2 (^1H, 1.79, 2.32 ppm, ^{13}C, 31.1 ppm)-CH_2 (^1H, 1.28, 1.58 ppm, ^{13}C, 38.6 ppm) by the ^1H-^1H and ^1H-^{13}C correlations in COSY and HMQC spectra, respectively. HMQC-TOCSY and TOCSY spectra indicated the presence of a hydroxyl group at 2.73 ppm in the segment, which is attached to C(1) carbon. The HMBC crosspeaks of H(19) protons to the CH_2 carbons of the segment, combined with the ROESY crosspeak of H(20) to the CH proton of the segment confirmed the assignments for this segment as C1-C2-C3 between the two quaternary carbons C(4) and C(10). C(10) was assigned by the HMBC

coupling of H(3) proton and H(14) *vide infra* to this carbon, while C(4) was assigned by the coupling of H(18) protons to this quaternary carbon. The methyl carbon at 26.0 ppm was assigned to C(18) on the basis of the ^{1}H-^{13}C couplings of H(19) to this carbon and its CH_3 protons to C(3) and C(4) carbons. Several other ^{1}H-^{13}C couplings were also observed between the proton of the segment to nearby carbons, such as the couplings of H(3) to C(4), C(5) and C(10) and H(2) to C(3).

The COSY correlation pattern indicated that segment #3 contains a sequence of CH (1.32 ppm)-CH_2 (1.25, 2.73 ppm)-CH (2.17 ppm)-CH (3.66 ppm). The HMBC ^{1}H-^{13}C couplings of CH (1.32 ppm) to C(4), C(18), C(19) and C(20), the ROESY connectivities of this CH proton to H(1) and H(18) determined the positions of the segment as H(5) (1.32 ppm), H(6) (1.25, 2.73 ppm), H(7) (2.17 ppm). Several ^{1}H-^{13}C couplings were observed for H(20) proton to C(5), C(6), and C(19) carbons.

The assignment of one of the segments #2 in the HMQC-TOCSY spectrum, was initiated at the resolved CH proton of 4.43 ppm and CH_2 protons of 1.11, 1.93 ppm. The HMQC-TOCSY spectrum showed that the 4.43 ppm proton correlates a CH carbon and its own carbon at 72.9 ppm correlates a hydroxyl proton, while the TOCSY spectrum showed a weak crosspeak of the proton to another proton at 2.18 ppm in addition to the correlations with the CH and OH protons. The sequence of this segment was identified by the COSY correlations of H(11) to H(9), H(12) to H(13) and H(13) to H(14). The COSY correlation between H(11) and H(12) protons is not observed, but the connectivity of H(11) to H(12) is supported by the TOCSY crosspeak between the two protons and ^{1}H-^{13}C couplings of H(11) to C(13), H(12) to C(11), H(13) to C(11) and H(14) to C(9) in the HMBC spectrum. The assignment for this spin system was also confirmed by the ROESY connectivities (Table 2) from the above protons in the segment to the surrounding protons. The couplings of H(6), H(7), and H(11) located the quaternary carbon C(8).

The hydroxyl proton at 2.33 ppm in segment #5 was assigned to C(15) by the COSY correlation of hydroxyl proton to H(15). The assignment of H(15) was made on the basis of the couplings of H(9), H(12), H(13), H(14) protons to C(15) in the HMBC spectrum. The CH_2 protons at 5.02 and 5.25 ppm were assigned to the exocyclic methylene H(17) protons by their characteristic ^{1}H and ^{13}C chemical shifts. The quaternary carbon at 154.3 ppm was assigned to C(16) using HMBC couplings of H(11), H(13), H(15) and H(17) to this carbon, which bonds to the C(12), C(15) and C(17) carbons. The weak correlation crosspeaks observed along H(17) resonances in the COSY spectrum are due to four-bond coupling between H(17) and H(15) through the double bond. The ROESY crosspeaks listed in Table 2 among the protons in this structural region also supported the assignment. The ^{1}H-^{13}C couplings of H(11) to C(5) and to C(13), both H(12) and H(13) to C(11), H(14) to C(9) and H(15) to C(9) and C(14) in the HMBC spectrum were then used to complete the assignments of the segment in the structure. The ^{1}H and ^{13}C NMR spectra taken in $CDCl_3$ differ in their chemical shifts from the spectra determined in CD_3SOCD_3.[4] The signals assigned earlier[4] for C(7) and C(12) need to be interchanged.

Table 3. ^{13}C and ^{1}H NMR Chemical Shifts and Assignments of Denudatine (**6**), in $CDCl_3$ + CD_3SOCD_3 (ppm)

C #	^{13}C		H #	^{1}H		J = Hz
1	26.1	CH_2	1_α	1.13	(d,t)	$J_{1\alpha,1\beta}$ = 13.6; $J_{1\alpha,2\alpha}$ = 4.0; $J_{1\alpha,2\beta}$= 4.0
			1_β	0.98	(d,t)	$J_{1\beta,1\alpha}$ = 13.6; $J_{1\beta,2\alpha}$ = 13.6; $J_{1\beta,2\beta}$= 5.5
2	20.3	CH_2	2_α	0.59	(m)	—
			2_β	1.56	(m)	—
3	40.0	CH_2	3_α	0.75	(br d)	$J_{3\alpha,3\beta}$ = 13.8; $J_{3\alpha,2\alpha}$ ~ 4.0; $J_{3\alpha,2\beta}$ = 3.8
			3_β	0.39	(d,t)	$J_{3\beta,3\alpha}$ = 13.8; $J_{3\beta,2\beta}$ = 3.5; $J_{3\beta,2\alpha}$ = 13.8
4	33.5	C	—	—	(—)	—
5	51.9	CH	5	0.37	(m)	—
6	22.5	CH_2	6_a	0.37	(m)	—
			6_b	1.98	(d,d)	$J_{6,7}$ = 7.9; $J_{6a,6b}$ = 13.8
7	41.9	CH	7	1.23	(d)	$J_{7,6}$ = 7.9
8	43.2	C	—	—	(—)	—
9	53.0	CH	9	0.53	(d)	$J_{9,11}$ = 9.6
10	45.0	C	—	—	(—)	—
11	71.4	CH	11	2.87	(d,d)	$J_{11,9}$ = 9.6; $J_{11,OH}$ = 4.9
			OH-11	3.18	(d)	$J_{OH,11}$ = 4.9
12	46.8	CH	12	1.30	(br s)	J = <1.0
13	24.2	CH_2	13_a	0.61	(m)	—
			13_b	0.84	(t)	$J_{13a,13b}$ = 12.0; $J_{13a,14}$= 12.0
14	27.6	CH_2	14_a	0.25	(m)	—
			14_b	1.03	(m)	—
15	77.0	CH	15	3.35	(d)	$J_{15,OH}$ = 6.1
			OH-15	3.88	(d)	$J_{OH,15}$ = 6.1
16	153.8	C	—	—	(—)	—
17	108.9	CH_2	17_a	4.09	(d,d)	$J_{17,15}$ = 2.2; $J_{17a,17b}$~ 3.4
			17_b	4.37	(d,d)	$J_{17,15}$ = 2.2; $J_{17b,17a}$~ 3.4
18	26.3	CH_3	18	0.09	(s)	—
19	57.1	CH_2	19_a	1.42	(AB)	J_{gem} = 11.1
			19_b	1.66	(AB)	J_{gem} = 11.1
20	71.7	CH	20	2.51	(s)	—
21	50.3	CH_2	21_a	1.58	(m)	J_{gem} = 15.7; $J_{21,22}$= 7.2
			21_b	1.69	(m)	J_{gem} = 15.2; $J_{21,22}$= 7.2
22	13.3	CH_3	22	0.23	(t)	J = 7.2

(*J* Constants are measured using ^{1}H traces of HMQC spectrum for overlapped ^{1}H resonances)

Table 4. Summary of HMBC, ROESY and COSY Correlation Data of Denudatine (**6**)

Obs. ^{1}H	HMBC	ROESY	COSY
H-1$_\alpha$	C-2, C-3, C-5, C-10, C-20	H-11	H-2$_\alpha$
H-1$_\beta$	C-2, C-3, C-5, C-10, C-11	H-2$_\beta$, OH-11	H-2$_\alpha$, H-2$_\beta$
H-2$_\alpha$	—	H-1$_\alpha$, H-2$_\alpha$, H-2$_\beta$, H-21	H-1$_\alpha$, H-1$_\beta$, H-2$_\beta$
H-2$_\beta$	—	H-20	H-1$_\beta$, H-2$_\alpha$
H-3$_\alpha$	C-1, C-2, C-4, C-5	H-19	H-3$_\beta$
H-3$_\beta$	C-1, C-4, C-5, C-19	H-2$_\beta$, H-3$_\alpha$	H-2$_\beta$, H-3$_\alpha$
H-5	C-1, C-4, C-7, C-9, C-19, C-20	—	H-6$_b$, H-7, H-20
H-6$_a$	C-4, C-5, C-7,C-10, C-11, C-20	H-7, H-9, H-19	H-6$_b$, H-7, H-20
H-6$_b$	C-3, C-4, C-8, C-20	H-6$_a$, H-9, OH-15	H-6$_a$, H-7, H-20
H-7	C-9, C-10, C-14, C-20	H-6$_b$, H-15, OH-15, H-20, H-21	H-6$_a$, H-6$_b$, H-20
H-9	C-5, C-7, C-10, C-11, C-14, C-15	H-6$_b$, H-11, OH-11, OH-15, H-17$_a$, H-17$_b$	H-11
OH-11	C-11, C-19	—	—
H-11	C-13, C-16	OH-11	H-9, OH-11
H-12	C-9, C-11, C-14, C-15	H-11, OH-11, H-17$_a$	—
H-13$_a$	C-11, C-12, C-14	H-11, H-12	H-12, H-13$_b$, H-14$_a$, H-14$_b$
H-13$_b$	—	H-12, H-13$_a$, H-14, H-15	H-12, H-13$_a$, H-14$_a$, H-14$_b$
H-14$_a$	—	H-11, H-20	H-13$_a$, H-13$_b$
H-14$_b$	C-7	H-7, H-13, H-14$_a$	H-13$_a$, H-13$_b$
H-15	C-9, C-14, C-16	OH-15, H-17$_b$	OH-15, H-17$_a$, H-17$_b$
OH-15	C-15, C-16	H-17$_b$	H-15
H-17$_a$	C-12, C-15, C-16	H-17$_b$	H-15, OH-15
H-17$_b$	C-12, C-15	H-17$_a$	H-15, OH-15
H-18	C-3, C-4, C-5, C-19	H-3$_a$, H-3$_b$, H-6$_a$, H-19$_a$, H-19$_b$	—
H-19$_a$	C-3, C-4, C-18, C-21	H-6$_a$, H-19$_b$	—
H-19$_b$	C-3, C-4, C-5, C-20	H-19$_a$, H-20	—
H-20	—	—	H-6$_a$, H-6$_b$, H-7
H-21$_a$	C-19, C-20, C-22	H-20	—
H-21$_b$	C-19, C-20, C-22	H-20	—
H-22	C-3, C-21	H-19, H-21$_a$, H-21$_b$	—

Stereochemistry of the compound was determined using the ROESY crosspeaks from the protons of asymmetric carbons to other protons nearby in distance. The ROESY crosspeak between H(3) at 1.58 ppm and H(19) at 2.49 ppm indicates that the two protons are H(3_α) and H(19a), respectively. The H(19_a) and H(19_b) indicate the pseudo-equitorial and pseudo-axial protons of the ring E (twist chair) formed by C(4), C(5), C(10), C(20), N and C(19). H(2_α) and H(2_β) were assigned by the ROESY crosspeaks of H(3_β) to both H(2) protons and H(3_α) to the H(2_α) proton. These ROESY correlations also suggest that this six member ring adopts a boat conformation. The α-orientation of the C(1) hydroxyl group enables hydrogen bonding of the OH group to the nitrogen when the A ring adopts a boat conformation.[30] H(1) has a β (equatorial) configuration because it is in the region close to H(5) and H(9) as evidenced by the ROESY crosspeaks of H(1) to the two protons. The NOEs of H(1) to both H(2) protons in the ROESY spectrum also support H(1_β) configuration. H(13) and H(14) protons are below the twist boat conformation of the ring defined by C(11), C(12), C(16), C(15), C(8) and C(9) because of the presence of ROESY connectivity between the H(20) and H(14a) protons. The hydroxyl groups of both C(11) and C(15) have an axial (β) configuration, which is determined by the NOEs of H(14a) and H(13a) to H(11) and of H(14b) and H(13b) to H(15), respectively. H(6_a) and H(6_b) were distinguished by the NOE of H(19b) to H(6a) in the ROESY spectrum.

The ^1H and ^{13}C NMR spectral data of denudatine (**6**) are given in Tables 3 and 4. The stereochemistry of the A ring is in a chair conformation as is evident from the coupling constants of the protons of C(1), C(2), and C(3).

EXPERIMENTAL

General Experimental Procedures. – Melting points are corrected and were determined on a Thomas-Koffler hot stage equipped with a microscope and a polarizer. Optical rotations were measured on Perkin-Elmer Model 141 polarimeter in $CHCl_3$. IR spectra were recorded in $CHCl_3$ on Perkin-Elmer Model 983 spectrophotometer. MS and HRMS were determined on a VG Zap Spec instrument and Autospec mass spectrometers. Chromatographic separations on a Chromatotron were carried out on rotors coated with 1 mm thick layers of Merck Al_2O_3 60 PF 254, 365 (EM 1104). Thin layer chromatograms were run using the solvent system toluene : acetone : MeOH : NH_4OH, 49.5 : 41.5 : 8 : 5.

All NMR data were acquired at 25°C on a Bruker AMX400 spectrometer (400.13 MHz, ^1H) using 20 mg of denudatine (**6**) in $CDCl_3$ and CD_3SOCD_3 and 36 mg of lepenine (**5**) dissolved in 0.5 mL $CDCl_3$. ^1H and ^{13}C chemical shifts at 25°C were referenced to TMS, *via* the $CDCl_3$ resonance frequency at 7.27 and 77.0 ppm, respectively. The 2D ^1H phase sensitive COSY[14-20] and clean-TOCSY[15] spectra were obtained using a spectral width of 2.0 kHz for both dimensions, while 2D ROESY[16] experiments were acquired with a spectral width of 4.0 kHz. For 2D TOCSY experiments, a spin lock field of 8 kHz was used during spin lock time of 60 ms, which includes 2 ms trim pulses.

ROESY spectra were recorded with a spin lock field of 1.8 KHz during the 500-ms mixing time. For 2D ^{1}H-^{13}C heteronuclear experiments, a spectral width of 10 KHz was used in the ^{13}C dimension for HMQC[17], HMQC-clean-TOCSY[18] and HMBC[19]. ^{1}H-^{13}C coupling constants of 150 Hz and 9 Hz were used in HMQC and HMBC experiments, respectively. HMQC data was acquired with a BIRD[20] sequence to suppress the signals from protons bound to ^{12}C and with GARP[21] decoupling during acquisition. Quadrature detection in the indirectly observed dimensions was obtained using TPPI[22] (time proportional phase increment) method for all 2D experiments. Typically, the data were acquired with acquisition time of 500 ms, 16 scans for each of 512 FID's in the homonuclear experiments, and 32 scans for each of 256 FID's in the heteronuclear experiments.

The 2D ^{1}H data were processed with 60°-shifted sine-bell-squared functions for ROESY and TOCSY spectra and with 5°-shifted sine-bell-squared functions for the phase sensitive COSY spectrum. The data sets were zero-filled to a final matrix size of 2048 X 2048 real points prior to Fourier transformation. All homonuclear spectra were plotted without symmetrization. The 2D ^{1}H-^{13}C HMQC and HMQC-clean-TOCSY were processed with 45°-shifted sine-bell-squared functions in ^{1}H dimension and 90°-shifted sine-bell-squared functions in ^{13}C dimension, while HMBC was processed with 0°-shifted sine-bell-squared functions in both dimensions. The data sets were zero-filled to 2048 x 1024 real data points prior to Fourier transformation. The HMBC was presented in magnitude mode after Fourier transform to gain maximum sensitivity of the spectrum.

Purification of the Alkaloidal Mixture of *Aconitum barbatum:* Isolation of delcosine (1), songorine (2), songoramine (3), songorine-*N*-oxide (4) and lepenine (5). The crude alkaloidal mixture was chromatographed by VLC[23] on an Al_2O_3 column. The eluting solvent was a gradient of hexane, $CHCl_3$ and MeOH and twenty fractions (100 mL each) were collected. Fraction 12 (elution with 1% MeOH) on further separation on a SiO_2 rotor of a Chromatotron gave delcosine (**1**, 20 mg) identified by comparison of the ^{1}H, ^{13}C NMR spectra and TLC with those of an authentic sample.[24] Fraction 8 (elution with hexane : 80% $CHCl_3$) afforded colorless crystals mp 210-212°C identified as songorine (Bullatine G, Shimoburo Base I, Napellonine) (**2**, 34 mg), by comparision of the ^{1}H, ^{13}C NMR spectra and TLC with those of an authentic sample.[25] Fractions 5 and 6 (454 mg; elution with hexane : 55% $CHCl_3$) gave after VLC separaion on SiO_2 (elution with $CHCl_3$: 2% MeOH), songoramine (**3**, 36 mg)[26] identified by comparison of the ^{1}H, ^{13}C NMR spectra. Fractions 7-10 (elution with hexane : 70-90% $CHCl_3$) afforded songorine-*N*-oxide (4,18 mg)[27] identified by ^{1}H, ^{13}C NMR and mass spectra. Fraction 11 (elution with $CHCl_3$: 0.5 % MeOH) on further VLC on SiO_2 (elution with $CHCl_3$: 40% MeOH) gave lepenine (**5**).[4]

Purification of the Alkaloidal Mixture of *Aconitum kusnezoffii:* Isolation of denudatine (6), and lepenine (5). The crude alkaloidal fraction (2.7 g) was purified by VLC on a column of Al_2O_3 (90 g) by gradient elution with hexane, $CHCl_3$ and MeOH. Twenty fractions were collected (100 mL each). Fraction 6 (elution with hexane : 60% $CHCl_3$) afforded colorless crystals mp

242-243°C from CH_2Cl_2, identified as denudatine (**6**, 28 mg).[9] (For ^1H, and ^{13}C NMR spectra see Tables 3 and 4). Fraction 10 (elution with $CHCl_3$: MeOH 0.5%) gave lepenine (**5**) as colorless needles mp 203-205°C (136 mg).[4] (For ^1H, and ^{13}C NMR spectra see Tables 1 and 2).

Purification of the Alkaloidal Mixture of *Aconitum volubile:* Isolation of altaconitine (7), aconitine (8), 12-*epi*-napelline (9) 1,19-dehydro-12-*epi*-napelline (10) senbusine A (11) and neoline (12). The crude alkaloidal mixture (18.5 g) was chromatographed by VLC on Al_2O_3 (140 g), gradient eluted with hexane, $CHCl_3$, MeOH and twenty-one fractions (100 mL each) were collected. Fraction 7 (1.5 g, elution with hexane : $CHCl_3$ 60%), on addition of hexane gave 116 mg of a crude crystalline mixture. This was purified on an Al_2O_3 rotor of a Chromatotron to afford colorless crystals (**7**; 25 mg). The alkaloid was identified as altaconitine by comparision of the ^1H, ^{13}C NMR spectra and TLC with those of an authentic sample.[28] Another fraction from the Chromatotron, gave aconitine (**8**; 6 mg)[24], identified by comparison of its TLC, mp 203-204°C and spectra with those of an authentic sample. The hexane insoluble portion (156 mg) of VLC Fraction 11 (319 mg), was purified on a SiO_2 rotor of a Chromatotron to give a homogeneous compound (53 mg; elution with $CHCl_3$, 5% MeOH). This was identified as 12-*epi*-napelline (**9**) by comparison of the NMR spectral data.[11, 29] VLC fraction 8 (350 mg, elution with hexane : $CHCl_3$ 60%), was purified on a SiO_2 rotor of a Chromatotron to afford after preparative TLC, a homogeneous compound (22 mg) identified as 1,19-dehydro-12-*epi*-napelline (**10**).[11] VLC fraction 15 (326 mg, elution with hexane : $CHCl_3$ 60%), was separated on an Al_2O_3 rotor to afford senbusine A (**11**; 70 mg) identified from the spectral data.[24] VLC fraction 9 (350 mg, elution with hexane : $CHCl_3$ 80%), was purified on a SiO_2 rotor of a Chromatotron to afford after preparative TLC, a homogeneous compound (32 mg) identified as neoline (**12**).[24]

Purification of the Alkaloidal Mixture of *Delphinium cheilanthum:* Isolation of deltaline (13) and methyllycaconitine (14). The crude alkaloidal fraction (5.7 g) was purified by VLC on a column of Al_2O_3 by gradient elution with hexane, $CHCl_3$ and MeOH. Forty-two fractions were collected (100 mL each) and fraction 4 (350 mg, elution with hexane : $CHCl_3$ 30%) was further purified on a SiO_2 rotor to give a homogeneous fraction (17-24; elution with hexane : $CHCl_3$ 40%) which was identified as deltaline (**13**, 84 mg)[24] by comparison of the TLC, mp 183-184°C, and NMR spectral data with those of an authentic sample. VLC fraction 6 (300 mg, elution with hexane : $CHCl_3$ 50%), was separated on a SiO_2 rotor to afford methyllycaconitine (**14**; 21 mg)[24] identified from the spectral data.

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