# *Notes*

## New Norditerpenoid Alkaloids from *Aconitum hemsleyanum* var. *leueanthus*

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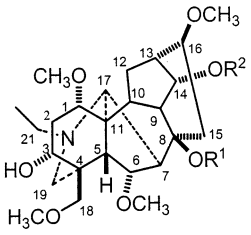
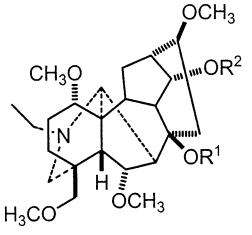
*Received July 31, 2002*

Four new norditerpenoid alkaloids, leueantines A (**1**), B (**2**), C (**3**), and D (**4**), were isolated from the roots of *Aconitum hemsleyanum* var. *leueanthus*. The structures of **1**−**4** were established by spectroscopic evidence.

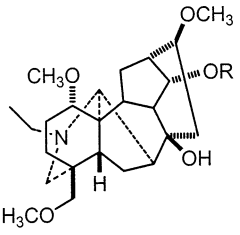
In the course of our comparative studies of diterpene alkaloids from *Aconitum* and *Delphinium* species,[1−8] we investigated the alkaloid contents of *Aconitum hemsleyanum* var. *leueanthus* P. Guo et M.R. Ji (Ranunculaceae).[9] The plant is endemic to the Sichuan Province of China, and its roots are occasionally used for the treatment of arthritic pain in folk medicine.[9] Apart from a short report on the isolation of several known compounds (indaconitine, 13,15-dideoxyaconitine, ezochasmanine, crassicaudine, franchetine, talatisamine, and chasmanine) from this plant species,[10] no other phytochemical information is available. In this paper we report the isolation and identification of four new norditerpenoid alkaloids, leueantines A (**1**), B (**2**), C (**3**), and D (**4**).



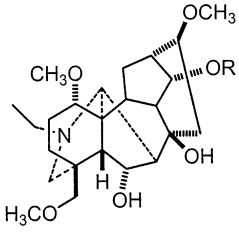
**1** $R^1$ = (*E*) Cinnamyol; $R^2$ = Ac
**5** $R^1$ = Ac; $R^2$ = (*E*) Cinnamoyl



**2** $R^1$ = (*E*) Cinnamoyl; $R^2$ = Ac
**6** $R^1$ = Ac; $R^2$ = Benzoyl



**3** R = (*E*) Cinnamoyl
**7** R = Ac



**4** R = (*E*) Cinnamoyl
**8** R = *p*-Methoxybenzoyl

Compound **1**, $C_{36}H_{49}NO_9$ (HREIMS), exhibited characteristic NMR features of an aconitine-type norditerpenoid alkaloid[11] bearing an *N*-ethyl ($\delta_H$ 1.08, 3H, t, $J$ = 7.2 Hz; $\delta_C$ 48.5, 13.2 q), four methoxyl ($\delta_H$ 3.44, 3.38, 3.30, 3.25), an acetyl ($\delta_H$ 1.81, 3H, s; $\delta_C$ 169.7 s, 22.3 q), and a cinnamoyl [$\delta_H$ 6.44 (1H, d, $J$ = 16.0 Hz), 7.69 (1H, d, $J$ = 16.0 Hz)] functional groups. The mass spectrum displayed a strong fragment signal at $m/z$ 608 ($M^+ - OCH_3$), suggesting the presence of a 1α-$OCH_3$ group.[12] On the other hand, a triplet signal at $\delta_H$ 4.94 ($J$ = 4.4 Hz) was attributed to H-14$\beta$,[11] implying the presence of an ester substituent at the C-14 position. A quaternary carbon at $\delta$ 42.9 was assigned to C-4 due to the $\beta$-effect of a 3-OH group.[13] Comparison of the NMR data of **1** (Table 1) and those of sungpanconitine (**5**)[14] indicated that the acetyl and cinnamoyl groups on these compounds were interchanged. Notably, the chemical shift value of the OAc group in **5** appears at higher field ($\delta_H$ 1.34). The assignment of 14-acetyl and 8-cinnamoyl functional groups in **1** was further supported by the NOE results (Figure 1). All available evidence indicated the structure of leueantine A to be as depicted (**1**).

Compound **2**, $C_{36}H_{49}NO_8$ (HREIMS), was also an aconitine-type alkaloid.[11] The NMR spectra displayed signals for *N*-ethyl, acetyl, cinnamoyl, and four methoxyl groups. In its $^1$H NMR spectrum, a triplet at $\delta_H$ 4.93 ($J$ = 4.8 Hz) could be assigned to the H-14$\beta$.[11] Comparison of the NMR data between **2** and crassicaudine (**6**)[2] revealed that they were similar. Notably, the acetyl signal in **2** appeared at $\delta_H$ 1.81 instead of $\delta_H$ 1.34 as in **6**.[2] The acetyl group was therefore attached to C-14 in **2**. This assignment was supported by the observation of an HMBC cross signal between H-14$\beta$ ($\delta_H$ 4.93) and the carbonyl carbon of the acetyl group ($\delta_C$ 169.6). The above findings led to assignment of 8-cinnamoyl and 14-acetyl substitutions as shown for leueantine B (**2**).

The molecular formulas of **3** ($C_{33}H_{45}NO_6$) and **4** ($C_{33}H_{45}NO_7$) were determined by HREIMS. The NMR data strongly suggested an aconitine-type structure for both compounds.[11] An *N*-ethyl group, three methoxyl groups, and a cinnamoyl group were present in each structure. In their $^1$H NMR spectra, a triplet ($J$ = 5 Hz) at $\delta_H$ 4.99 (**3**) and $\delta_H$ 4.97 (**4**) could be assigned to H-14$\beta$,[11] suggesting the presence of a 14-cinnamoyl functional group. Indeed, long-range coupling signals were observed between H-14$\beta$ and the cinnamoyl carbonyl carbon in the HMBC spectra of both compounds. Finally, comparisons of the $^{13}$C NMR data

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10.1021/np020350u CCC: $25.00 
Published on Web 01/01/2003

**Table 1.** $^{13}C$ NMR Data of Leueantines A (**1**), B (**2**), C (**3**), and D (**4**)

| carbon | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 83.6d | 84.8d | 85.7d | 85.2d |
| 2 | 33.2t | 26.3t | 26.1t | 26.1t |
| 3 | 71.7d | 34.7t | 32.6t | 35.1t |
| 4 | 42.9s | 38.9s | 38.5s | 38.8s |
| 5 | 48.4d | 48.9d | 36.0d | 50.3d |
| 6 | 82.2d | 82.7d | 24.9t | 72.3d |
| 7 | 48.5d | 44.7d | 45.9d | 56.8d |
| 8 | 85.8s | 85.5s | 73.7d | 74.7d |
| 9 | 43.6d | 48.9d | 46.3d | 46.9d |
| 10 | 39.0d | 39.2d | 45.4d | 45.0d |
| 11 | 50.4s | 50.2s | 48.7s | 50.6s |
| 12 | 28.4t | 28.9t | 28.5t | 29.2t |
| 13 | 44.7d | 43.9d | 45.1d | 37.0d |
| 14 | 75.4d | 75.4d | 76.8d | 76.7d |
| 15 | 38.0t | 37.7t | 40.9t | 41.0t |
| 16 | 82.6d | 83.4d | 81.7d | 81.9d |
| 17 | 61.2d | 61.4t | 61.2d | 61.9d |
| 18 | 77.1t | 80.2t | 79.5t | 80.9t |
| 19 | 47.5t | 53.7t | 53.1t | 54.3t |
| 21 | 48.5t | 49.0t | 49.3t | 49.0t |
| 22 | 13.2q | 13.3q | 13.5q | 13.5q |
| 1-$OCH_3$ | 56.6q | 56.5q | 56.2q | 56.0q |
| 6-$OCH_3$ | 57.8q | 57.8q | | |
| 16-$OCH_3$ | 55.6q | 55.9q | 56.0q | 56.0q |
| 18-$OCH_3$ | 59.0q | 59.0q | 59.4q | 59.1q |
| *C*$OCH_3$ | 169.7s | 169.6s | | |
| CO*C*$H_3$ | 22.3q | 22.3q | | |
| cinnamoyl | | | | |
| 1″ | 166.6s | 166.8s | 166.7s | 166.7s |
| 2″ | 145.1d | 144.9d | 144.9d | 145.0d |
| 3″ | 118.2d | 118.3d | 118.1d | 118.1d |
| 4″ | 134.2s | 134.2s | 134.3s | 134.3s |
| 5″, 9″ | 128.9d | 128.8d | 128.8d | 128.7d |
| 6″, 8″ | 128.0d | 127.9d | 128.1d | 128.3d |
| 7″ | 130.3d | 130.2d | 130.2d | 130.2d |

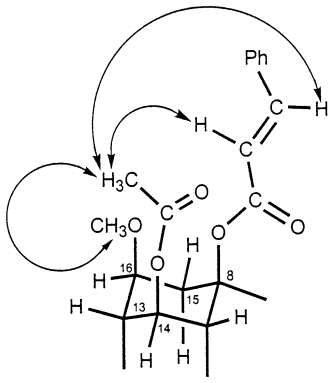


**Figure 1.** Key NOE correlations of **1**.

(Table 1) between **3** and 14-acetyltalatisamine (**7**),[15] as well as between **4** and geniconitine (**8**),[16] led to the structural assignment indicated for leueantines C (**3**) and D (**4**), respectively.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured using a Perkin-Elmer 241 polarimeter. $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker 200 spectrometer. All NMR experiments were run in $CDCl_3$ with TMS as internal standard. EIMS and HRMS were measured on a VG AutoSpec 3000 mass spectrometer. Polyvinyl sulfuric ion resin (H form, cross linking 1 × 3, Chemical Factory of Nan Kai University, China) was used in the extraction of total alkaloids.

**Plant Material**. *Aconitum hemsleyanum* var. *leueanthus* was collected by Wen-Jia Zhang from the An County of Sichuan Province, China, in August 1998. The plant was authenticated by Prof. Wen-Cai Wang of the Beijing Institute of Botany, Chinese Academy of Sciences, China. A voucher specimen (WFP-98) has been deposited in the West China College of Pharmacy, Sichuan University.

**Extraction and Isolation.** Dried roots of *A. hemsleyanum* var. *leueanthus* (9.3 kg) were milled and percolated with 0.15% HCl (100 L). Wet resin (dry wt 1 kg) was added to the percolates followed by washing repeatedly on a suction filter with deionized water. The air-dried resin was mixed with 10% aqueous $NH_4OH$ (1 L) and extracted sequentially in an extractor with $Et_2O$ and 95% EtOH under reflux until no alkaloid could be detected. Evaporation of the organic solvents afforded the total alkaloid fractions I (50.0 g) and II (0.7 g), respectively.

The total alkaloid fraction I (45.5 g) was acidified to pH 3 and extracted sequentially with petroleum (100 mL × 3), ether (100 mL × 3), $CH_2Cl_2$ (100 mL × 3), and $CHCl_3$ (100 mL × 3) to afford fractions A-1 (0.36 g), A-2 (0.46 g), A-3 (21.0 g), and A-4 (2.8 g). The acidic aqueous layer was then adjusted to pH 8 with concentrated $NH_4OH$ and extracted by ether (100 mL × 3) and $CHCl_3$ (100 mL × 3) to afford fractions B-1 (0.5 g), B-2 (3.0 g), and B-3 (14.0 g). Finally, the remaining aqueous layer was extracted with $CHCl_3$ (100 mL × 3) to give fraction C (1.2 g).

A portion of fraction A-3 (5.0 g) was chromatographed over silica gel H (100 g) eluting with $CHCl_3$−acetone−$NH_4OH$ (60:40:1 → 85:15:1) to provide six subfractions. Subfraction A-3-2 (2.7 g) was further separated on a Chromatotron (silica gel $GF_{254}$, petroleum−acetone, 6:1 → 8:2) to afford **1** (80 mg), **2** (60 mg), franchetine[10] (60 mg), and 13,15-dideoxyaconitine[10] (50 mg). Subfraction A-3-3 (0.9 g) was subjected to chromatography on a Chromatotron (silica gel $GF_{254}$, petroleum−acetone, 20:1 → 8:2) to yield 8-deacetylsungpanconitine[17] (60 mg). Separation of fraction A-3-5 over Chromatotron (silica gel $GF_{254}$, petroleum−acetone, 4:1→3:1) afforded **4** (55 mg). Another portion of fraction A-3 (16 g) was separated repeatedly by column chromatography over silica gel H, eluted with increasing polarity of petroleum−acetone mixtures to afford franchetine[10] (5 mg), crassicaudine[10] (28 mg), and **3** (28 mg). The known compounds were identified by comparison with literature values and known standards.

Fraction B-3 was chromatographed over Chromatotron (silica gel $GF_{254}$) eluting with increasing polarity of $CHCl_3$−MeOH mixtures to afford talatisamine[10] (1.8 g) and chasmanine[10] (90 mg). Finally, purification of fraction C over column chromatography (silica gel H, $CHCl_3$−MeOH, 97:3 → 85:15) yielded ezochasmanine[10] (35 mg).

**Leueantine A (1):** amorphous powder; $[\alpha]_D^{20}$ +13.4 (*c* 0.5, $CHCl_3$); $^1H$ NMR ($CHCl_3$, 200 MHz) $\delta$ 7.50−7.39 (5H, m, Ar-H), 7.69 (1H, d, $J$ = 16.0 Hz, H-3″), 6.44 (1H, d, $J$ = 16.0 Hz, H-2″), 4.94 (1H, d, $J$ = 4.4 Hz, H-14$\beta$), 4.12 (1H, d, $J$ = 16.0 Hz, H-6$\beta$), 3.79 (1H, dd, $J$ = 7.8, 4.4 Hz, H-3$\beta$), 3.44, 3.38, 3.30, 3.25 (each 3H, s, $OCH_3$), 1.81 (3H, s, OAc), 1.08 (3H, t, $J$ = 7.0 Hz, $NCH_2CH_3$); $^{13}C$ NMR ($CDCl_3$, 50 MHz), see Table 1; EIMS *m*/*z* (%) 639 ($M^+$, 6), 624 (7), 608 (74), 131 (100), 103 (57); HREIMS *m*/*z* 639.3417 (calcd for $C_{36}H_{49}NO_9$, 639.3407).

**Leueantine B (2):** amorphous powder; $[\alpha]_D^{20}$ +19 (*c* 0.5, $CHCl_3$); $^1H$ NMR ($CHCl_3$, 200 MHz) $\delta$ 7.52−7.35 (5H, m, Ar-H), 7.67 (1H, d, $J$ = 16.0 Hz, H-3″), 6.43 (1H, d, $J$ = 16.0 Hz, H-2″), 4.93 (1H, d, $J$ = 4.8 Hz, H-14$\beta$), 4.06 (1H, d, $J$ = 6.6 Hz, H-6$\beta$), 3.63 (1H, ABq, $J$ = 8.2 Hz, H-18), 3.61, 3.36, 3.27, 3.24 (each 3H, s, $OCH_3$), 1.81 (3H, s, OAc), 1.06 (3H, t, $J$ = 7.0 Hz, $NCH_2CH_3$); $^{13}C$ NMR ($CDCl_3$, 50 MHz), see Table 1; EIMS *m*/*z* (%) 623 ($M^+$, 4), 608 (5), 592 (100), 131 (79), 103 (33); HREIMS *m*/*z* 623.3470 (calcd for $C_{36}H_{49}NO_8$, 623.3458).

**Leueantine C (3):** amorphous powder; $[\alpha]_D^{20}$ +34.6 (*c* 0.5, $CHCl_3$); $^1H$ NMR ($CHCl_3$, 200 MHz) $\delta$ 7.55−7.36 (5H, m, Ar-H), 7.67 (1H, d, $J$ = 16.0 Hz, H-3″), 6.42 (1H, d, $J$ = 16.0 Hz, H-2″), 4.99 (1H, d, $J$ = 5.0 Hz, H-14$\beta$), 3.30, 3.28, 3.21 (each 3H, s, $OCH_3$), 1.06 (3H, t, $J$ = 7.0 Hz, $NCH_2CH_3$); $^{13}C$ NMR ($CDCl_3$, 50 MHz), see Table 1; EIMS *m*/*z* (%) 551($M^+$, 10), 520 (100), 131 (55), 103 (36); HREIMS *m*/*z* 551.3235 (calcd for $C_{33}H_{45}NO_6$, 551.3247).

**Leueantine D (4):** amorphous powder; $[\alpha]_D^{20}$ +34.0 (*c* 0.5, $CHCl_3$); $^1H$ NMR ($CHCl_3$, 200 MHz) $\delta$ 7.54−7.36 (5H, m, Ar-H), 7.68 (1H, d, $J$ = 16.0 Hz, H-3″), 6.45 (1H, d, $J$ = 16.0 Hz, H-2″), 4.97 (1H, d, $J$ = 5.0 Hz, H-14$\beta$), 4.78 (1H, d, $J$ = 6.8 Hz, H-6$\beta$), 3.76 (1H, ABq, $J$ = 8.6 Hz, H-18), 3.31, 3.26, 3.23 (each 3H, s, $OCH_3$), 1.09 (3H, t, $J$ = 7.0 Hz, $NCH_2CH_3$); $^{13}C$ NMR ($CDCl_3$, 50 MHz), see Table 1; EIMS *m*/*z* (%) 567 ($M^+$, 15), 536 (100), 131 (83), 103 (49); HREIMS *m*/*z* 567.3197 (calcd for $C_{33}H_{45}NO_7$, 567.3196).

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NP020350U