Diterpenoid Alkaloids from *Aconitum leucostomum*

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Two new norditerpenoid alkaloids, leucostines A (**1**) and B (**2**), and a new diterpenoid alkaloid, 11-acetyllepenine (**6**), have been isolated from the root of *Aconitum leucostomum*. Their structures were elucidated on the basis of spectral data and chemical evidence. Four known alkaloids, delsoline (**3**), delcosine (**4**), lepenine (**7**), and songorine (**8**), were also isolated and identified from this plant for the first time.

Aconitum leucostomum Vorosch. (Ranunculaceae) is a perennial herb distributed in the Gansu and Xinjing provinces of China. It has been used as a folk medicine to treat traumatic injury. The isolation of several alkaloids from this plant has been reported.[1−6] This paper describes the isolation and structure elucidation of three new diterpenoid alkaloids, named leucostines A (**1**) and B (**2**) and 11-acetyllepenine (**6**), as well as the isolation of four known alkaloids, delsoline (**3**), delcosine (**4**), lepenine (**7**), and songorine (**8**), from this plant. The latter were isolated from this plant for the first time.

Leucostine A (**1**), $C_{27}H_{43}NO_8$, is an amorphous powder. The IR spectrum indicated the presence of hydroxyl (3542 cm^{-1}) and ester (1735 cm^{-1}) groups. The ^1H NMR revealed the presence of four methoxy groups (3.43, 3.38, 3.31, and 3.28 ppm, each 3H, s), an acetyl group (2.21, 3H, s), and an ethylamine group (1.06, 3H, t, J = 7.1 Hz). In the ^{13}C NMR, 27 signals appeared, corresponding to 27 carbon atoms in the molecule. These spectral data suggested that the chemical formulation of this compound was a norditerpenoid alkaloid, $C_{19}H_{21}(OH)_2(OCH_3)_4(OAc)(NCH_2CH_3)$. Most alkaloids of this class have a hydroxyl or a methoxyl group on C-1, C-8, C-14, and C-16. As no angular methyl group was observed in the ^1H-NMR or ^{13}C-NMR spectrum, the C-18 most likely bears an oxygen function. The secondary carbon at δ 78.57 ppm indicates that the C-18 methylene carbon bears a methoxyl group. In the case of a hydroxyl group on C-18, the signal normally appears at about 66.5−68.5 ppm.[7,8] The tertiary carbons at δ 83.95 and 81.06 ppm indicate that both the C-1 and C-16 bear a methoxyl group. The signal at 3.74 ppm (1H, t, J = 5.3 Hz, C-14-β-H) suggests that C-13 and C-9 did not bear hydroxyl groups. It also appears that C-10 did not bear an oxygen function. This conclusion is supported by the quaternary carbon signal at δ 48.39 ppm assigned to C-11. In the case in which an oxygen group was located on the C-10, the C-11 signal appeared near δ 55−56 ppm[9] and the C-14-β-H signal was shifted more downfield.[10] Quaternary carbon signals at δ 88.87 and 76.33 ppm showed that C-7 and C-8 were connected by a hydroxyl group. The ^1H−NMR signal at δ 5.34 ppm did not disappear in the D_2O exchange experiment, suggesting that C-6 was connected with a β-OAc.[11−13] This conclusion was supported by the ^{13}C-NMR signal at δ 81.06 ppm assigned to C-6. The proton signal at δ 3.74 ppm (1H, t, J = 5.3 Hz) for C-14-β-H and the carbon signal at δ 84.31 ppm for C-14 indicated that C-14 bears an α-OMe. Comparison of the ^{13}C-NMR data of **1** with those of the known alkaloid delphatine (**5**)[17] showed that the chemical shifts of carbons were very similar except for the differences due to different substituents at C-6. The structure of leucostine A was thus assigned to be **1**.

Leucostine B (**2**), crystallized from *n*-hexane−Me_2CO as colorless needles, mp 263−264 °C, has the molecular formula $C_{24}H_{39}NO_8$ (HRMS). Its IR spectrum indicated the presence of hydroxyl groups (3477−3374 cm^{-1}). The presence of an ethylamine group was inferred by the proton resonances at δ 1.12 ppm (3H, t, J = 7.0 Hz) in the ^1H−NMR spectrum. The ^1H NMR also indicated the presence of three methoxy groups (3.40, 3.37, and 3.32, s, each 3H). The HRMS and NMR analyses suggested that the chemical formula of leucostine B (**2**) was $C_{19}H_{20}(OCH_3)_3(OH)_5(NCH_2CH_3)$, corresponding to that of a norditerpenoid alkaloid. The secondary carbon signal at δ 77.21 ppm indicated that the C-18 methylene carbon bore a methoxyl group. Quaternary carbon signals at δ 37.78 and 48.00 ppm was assigned to C-4 and C-11, respectively. The other two quaternary carbon signals at δ 77.34 and 87.90 ppm in the ^{13}C−NMR spectrum could be assigned to any of the carbons bearing hydroxyls, such as C-7, C-8, C-9, C-10, and C-13. The C-10 position can be excluded, as the C-11 signal appears at δ 48.00 ppm rather than near δ 55−56 ppm, as would be expected.[14] The C-14-β-H appeared as a quartet in the ^1H NMR, indicating that C-9 or C-13 did not bear a hydroxyl. Leucostine B (**2**) should, therefore, bear hydroxyls at C-7 and C-8, and these carbon signals are observed at δ 87.90 and 77.34 ppm, respectively. The tertiary signals at δ 89.93 and 78.91 ppm were, respectively, assigned to C-6 and C-16 bearing a β-OMe. The signal at δ 73.57 ppm indicated that an α-OH was located at C-1. The proton signal at 4.51 ppm (1H, q, J = 4.7 Hz) and the carbon signal at δ 75.69 ppm showed that an α-OH was located at C-14. The above spectral evidence leads to the partial structure of leucostine B, which is the same as delcosine (**4**). The remaining hydroxyl group can be at C-2, C-3, C-12, or C-15,

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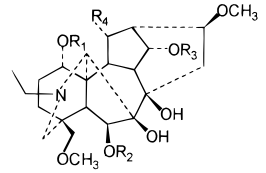
because the tertiary signal at δ 71.90 ppm can only be assigned to one of these carbons bearing a hydroxyl group. Comparison of the ^{13}C-NMR data of **2** with those of the known alkaloid delcosine (**4**) showed that the chemical shifts of carbons C-1, C-2, C-3, and C-4 of **2** were very similar to those of delcosine (**4**). Thus, location of a hydroxy group at C-2 or C-3 was ruled out. A hydroxy group at C-15 is unlikely because the chemical shifts of C-8 and C-15 are very close to those of delcosine (**4**). The C-16 (δ 78.91 ppm) of **2** compared with that of delcosine (**4**) (δ 81.97 ppm) was shifted upfield about 3 ppm. This is a shift opposite of that expected for a hydroxy group at C-15. The remaining hydroxyl group was thus definitely assigned to C-12. The C-13 (δ 55.59 ppm) and C-10 (δ 49.42 ppm) signals compared with those of delcosine (**4**) were shifted downfield about 10 ppm as a result of the deshielding of the C-12 hydroxyl group. The chemical shift of C-16 (δ 78.91 ppm) moved upfield about 3 ppm from that in delcosine (δ 81.97 ppm) as a result of the γ-gauche effect of the C-12 hydroxyl group, suggesting that the C-12-OH is α-oriented. If the C-12-OH were β-oriented, it would have a γ-gauche relation with C-14. The chemical shift of the C-14 (δ 75.51 ppm) of **2** is very close to that of delcosine (**4**) (δ 75.67 ppm), supporting the C-12-OH α-orientation. Structure **2** was thus assigned to leucostine B.

11-Acetyllepenine (**6**) was obtained as colorless prisms, mp 130−131 °C. Its formula was inferred as $C_{24}H_{35}NO_4$ by the ^{13}C NMR and EIMS. The IR spectrum showed the presence of hydroxyl (3387 cm^{-1}) and ester (1735 cm^{-1}) functions. Signals at δ 5.23 and 4.97 ppm (each 1H, d, J = 2.2 Hz, H-17), 1.05 (3H, t, J = 7.5 Hz, $-NCH_2CH_3$), and 0.70 ppm (3H, s, C-4-CH_3) in the ^{1}H NMR indicated that compound **6** was a diterpenoid alkaloid. Analysis of the ^{1}H- and ^{13}C-NMR spectra of compound **6** and lepenine (**7**) showed that both compounds possess the atisine skeleton and differ in the substituent at C-11. The quaternary carbon signals at δ 33.67, 43.53, and 50.99 ppm were assigned to C-4, C-8, and C-10, respectively. The tertiary carbon signals at δ 70.21 and 77.61 ppm were assigned to the C-1 and C-15 bearing a hydroxyl, respectively. The other tertiary carbon signal at δ 76.17 ppm was assigned to C-11 bearing an acetyl group. Comparison of the ^{1}H NMR of **6** with that of lepenine (**7**) showed that the ^{1}H-NMR signal of **6** at δ 5.52 ppm (1H, d, J = 9.1 Hz, C-11-α-H) was shifted upfield to 4.44 ppm (1H, d, J = 9.1 Hz, C-11-α-H) in the ^{1}H NMR of lepenine (**7**), further evidence that the acetyl was located at C-11. Thus, structure **6** was identified as 11-acetyllepenine. This conclusion was confirmed by hydrolysis of **6** with 5% KOH-aqueous MeOH to give lepenine (**7**).[15]

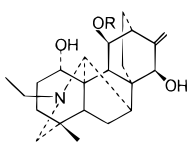
The other four known alkaloids isolated along with these new alkaloids were identified as delsoline (**3**),[16,17] delcosine (**4**),[17,18] lepenine (**7**),[15] and songorine (**8**)[7] on the basis of their physical constants and spectral data.

Experimental Section

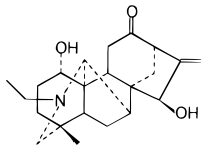
General Experimental Procedures. Melting points were obtained on a Kofler apparatus and are uncorrected. IR spectral data were measured on a FT-5DX instrument with KBr disks. EIMS data were obtained on a VGZAB-HS mass spectrometer. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker AM-400 instrument with TMS as an internal standard and $CDCl_3$ as solvent.



1: R_1=R_3=CH_3, R_2=Ac, R_4=H
2: R_1=R_3=H, R_2=CH_3, R_4=OH
3: R_1=R_4=H, R_2=R_3=CH_3
4: R_1=R_3=R_4=H, R_2=CH_3
5: R_1=R_2=R_3=CH_3, R_4=H



6: R=Ac
7: R=H



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Plant Material. *Aconitum leucostomum* was collected from Xinjiang Province, China, in September 1990. It was identified by Prof. Ru-Neng Zhao, Department of Pharmacy, Lanzhou Medical College. A voucher specimen has been deposited in the Department of Biology, Lanzhou University.

Extraction and Isolation. The powdered herb (2.5 kg) was extracted with 95% EtOH three times (each time for 4 days) at room temperature. After removal of EtOH under reduced pressure, 85 g of syrup remained. This was dissolved in 2% H_2SO_4 solution. The acidic solution, after extraction with CH_2Cl_2, was made alkaline with concentrated NH_4OH, adjusted to pH 11, and then extracted with $CHCl_3$ to give 12.5 g of crude alkaloid. The crude alkaloid (12.5 g) was chromatographed on a column of alumina (250 g) eluting with $CHCl_3$ and $CHCl_3$-CH_3OH (40:1, 30:1, 20:1, 10:1, and 5:1) to afford 21 fractions. Fractions 1 and 2 were combined and chromatographed on an alumina column eluting with petrol-Et_2O to give leucostine A (**1**) and 11-acetyllepenine (**6**). Fractions 4−6 were combined and crystallized from hexane-Me_2CO to give songorine (**8**). Fraction 7 was chromatographed on an alumina column eluting with petroleum-Me_2CO to afford delsoline (**3**). Fractions 8−13 were combined and chromatographed on an alumina column eluting with petroleum ether-Me_2CO to afford delcosine (**4**) and lepenine (**7**). Fractions 16−20 were combined and chromatographed on an alumina column with $CHCl_3$-MeOH (6:1) as eluent to yield leucostine B (**2**).

Conversion of 11-Acetyllepenine (6) to Lepenine (7). A mixture of 30 mg of **6** in 5 mL of KOH-MeOH solution was kept for 3 days at room temperature. After removal of MeOH under reduced pressure, a small amount of H_2O was added, and the mixture was extracted with $CHCl_3$. The extract was crystallized from hexane-Me_2CO to yield 20 mg of needle crystals whose physical constants and spectral data were in good agreement with those of lepenine.

Leucostine A (1): $C_{27}H_{43}NO_8$ (HRMS, M − OMe, found 478.2738, requires 478.2804), was isolated as an amorphous powder; UV (MeOH) λ max (nm) 248.5; IR ν max (KBr) cm^{-1} 3542 (−OH), 1735 (−COMe); ^{1}H NMR ($CDCl_3$ 400 MHz) δ (ppm) 5.34 (1H, s, C-6-α-H), 3.74 (1H, t, J = 5.3 Hz, C-14-β-H), 3.43, 3.38, 3.31, and 3.28 (each 3H, s, −OMe), 2.21 (3H, s, −COMe), 1.06 (3H, t,

Table 1. ^{13}C-NMR Data of Compounds **1**, **2**, and **6** ($CDCl_3$)[a]

carbons	1	2	6
1	83.95	73.57	70.21
2	25.96	28.64	30.95
3	31.79	29.49	38.52
4	38.56	37.78	33.67
5	43.24	45.80[b]	49.02
6	81.06	89.93	23.51
7	88.87	87.90	43.26
8	76.33	77.34	43.53
9	51.53	45.94[b]	51.96
10	37.27	49.42	50.99
11	48.39	48.00	76.17
12	28.73	71.90	42.02
13	45.60	55.60	23.97
14	84.31	75.51	37.15
15	37.93	34.76	77.61
16	82.12	78.91	153.78
17	66.08	66.96	109.57
18	78.57	77.21	25.91
19	52.67	56.17	56.59
20			67.59
NCH_2CH_3	51.17	50.59	50.73
	14.18	13.73	13.50
$COCH_3$	172.47		171.09
	21.54		21.51
C_1-OCH_3	55.64		
C_6-OCH_3		57.43	
C_{14}-OCH_3	57.67		
C_{16}-OCH_3	56.25	56.40	
C_{18}-OCH_3	59.41	59.08	

[a] Assignment are partly based on the DEPT techniques. [b] Assignments in the same column may be interchanged.

J = 7.1 Hz, $-NCH_2CH_3$); EIMS m/z 509 $[M]^+$ (5), 478 $[M - OMe]^+$ (100), 450 $[M - OAc]^+$ (30); ^{13}C NMR, see Table 1.

Leucostine B (2): $C_{24}H_{39}NO_8$ (HRMS, found 469.2793, requires 469.2776), obtained as colorless needles (MeOH): mp 263−264 °C; IR ν max (KBr) cm^{-1} 3374−3477 (−OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 4.51 (1H, q, J = 4.7 Hz, C-14-β-H), 4.11 (1H, d, J = 2.4 Hz, C-6-α-H), 3.40, 3.37, 3.32 (each 3H, s, −OMe), 1.12 (3H, t, J = 7.0 Hz, $-NCH_2CH_3$); EIMS m/z 469 $[M]^+$ (15), 4.54 $[M - CH_3]^+$ (100), 438 $[M - OMe]^+$ (70); ^{13}C NMR, see Table 1.

Delcosine (4) was obtained as colorless prisms (Me_2CO−*n*-hexane): mp 204−205 °C. IR ν max (KBr) cm^{-1} 3585, 3473, and 3325 (−OH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.33 (1H, d, J = 8.1 Hz, C-1-α-OH), 4.12 (1H, d, J = 4.8 Hz, C-14-β-H), 3.67 (1H, br d, J = 7.7 Hz, C-6-α-H), 3.41, 3.40, and 3.33 (each 3H, s, −OMe), 1.10 (3H, t, J = 7.2 Hz, $-NCH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 72.62 (C-1), 27.41 (C-2), 29.32 (C-3), 37.54 (C-4), 43.91 (C-5), 89.99 (C-6), 87.79 (C-7), 78.03 (C-8), 45.25 (C-9), 39.36 (C-10), 48.79 (C-11), 29.32 (C-12), 45.15 (C-13), 75.69 (C-14), 34.39 (C-15), 81.97 (C-16), 66.33 (C-17), 77.27 (C-18), 57.07 (C-19), 50.39, 13.54 (−NEt), 57.32 (C-6-OMe), 56.34 (C-16-OMe), and 59.06 (C-18-OMe); EIMS m/z 453 $[M]^+$ (13), 438 $[M - Me]^+$ (100), 422 $[M - OMe]^+$ (55).

11-Acetyllepenine (6), $C_{24}H_{35}NO_4$, was obtained as colorless prisms (Me_2CO−*n*-hexane): mp 130−131 °C; UV λ max (nm) 246.5; IR ν max (KBr) cm^{-1} 3387 (−OH), 1735 (−COMe), 1652 (−C═CH_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 5.52 (1H, d, C-11-α-H), 5.23, and 4.97 (each 1H, d, J = 2.2 Hz, C-17-H), 4.32 (1H, d, J = 2.2 Hz, C-15-α-H), 3.85 (1H, dd, J = 5.0 Hz, C-1-β-H), 2.08 (3H, s, −OCOMe), 1.05 (3H, t, J = 7.5 Hz, $-NCH_2CH_3$), 0.70 (3H, s, C-4-Me); EIMS m/z 401 $[M]^+$ (40), 384 $[M\text{-}OH]^+$ (10), 342 $[M\text{-}OAc]^+$ (87); ^{13}C NMR, see Table 1.

Lepenine (7) was obtained as colorless prisms (Me_2CO−*n*-hexane): mp 120−122 °C, IR ν max (KBr) cm^{-1} 3383 (−OH), 1655 (−C═CH_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 5.25 and 5.02 (each 1H, d, J = 2.2 Hz, C-17-H), 4.44 (1H, d, J = 9.1 Hz, C-11-α-H), 4.27 (1H, d, J = 2.2 Hz, C-15-α-H), 4.16 (1H, dd, J = 6.2 Hz, C-1-β-H), 1.06 (3H, t, J = 7.1 Hz, $-NCH_2CH_3$), 0.70 (3H, s, C-4-Me); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm) 70.64 (C-1), 31.10 (C-2), 38.65 (C-3), 33.71 (C-4), 53.82 (C-5), 23.06 (C-6), 46.85 (C-7), 43.68 (C-8), 52.31 (C-9), 50.98 (C-10), 72.95 (C-11), 42.29 (C-12), 34.61 (C-13), 27.37 (C-14), 77.87 (C-15), 154.37 (C-16), 109.40 (C-17), 25.96 (C-18), 57.02 (C-19), 67.72 (C-20), and 50.79, 13.54 (−NEt); EIMS m/z 359 $[M]^+$ (50), 342 $[M - OH]^+$ (81), and 43 (100).

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