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Diterpenoid alkaloids from aconitum episcopale

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Abstract

Three new diterpenoid alkaloids, liaconitine A (*N*-ethyl-1α,6α,16β,18-tetramethoxy-13β-ol-2,3-dehydroaconitane-8-acetate-14-anisoylate), B (*N*-ethyl-1α,6α,16β,18-tetramethoxy-13β-ol-2,3-dehydroaconitane-8,14-dianisoylate and C (*N*-ethyl-1α,6α,16β,18-tetramethoxy-8-ethoxy-13β-ol-2,3-dehydroaconitane-14-anisoylate) have been isolated from the roots of *Aconitum episcopale*. The structures of the new compounds were established by spectroscopic methods and reduction of liaconitine A to the known alkaloid, crassicauline A.

Keywords: Aconitum episcopale; Ranunculaeae; Roots; Diterpenoid alkaloids; Liaconitines A-C; Crassicauline A

1. Introduction

Aconitum species have been used as a traditional Chinese medicinal herb having analgesic activity. In our studies of the alkaloids of the genus *Aconitum*, three new C_{19} nordeterpenoid alkaloids, named liaconitine A (**1**), B (**2**) and C (**3**) have been isolated from the roots of *A. episcopale*, which was collected from Lijiang, Yunnan Province, P.R. China. These novel alkaloids all bear a double bond at C(2),C(3). This type of norditerpenoid alkaloid has not been reported from *Aconitum* species native to Yunnan Province. 2,3-Dehydrodelcosine was isolated earlier from *A. japonicum* var. *montanum* Nakai in 1988 (Takayama et al., 1988). The possibility that **3** is an artifact of the isolation process has been addressed.

2. Results and discussion

Three new alkaloids were isolated from a $CHCl_3$ extract by column chromatography over silica gel. Liaconitine A was isolated as colourless needles and its molecular formula $C_{35}H_{47}NO_{11}$ was derived from the HR mass spectrum (*m*/*z* 641.3188) and NMR spectral chemical shifts (Tables 1 and 2). The ^1H,^{13}C NMR and mass spectra showed that it is a C_{19}-type diterpenoid alkaloid with an *N*-ethyl group, an acetyl group, five methoxyl groups and an anisoyl group. Its spectral characters were similar to the known compound, crassicauline A (Wang & Fang, 1981). The main differences between liaconitine A and crassicauline A were ^{13}C NMR chemical shifts of C(2),C(3) at δ 125.1 and 137.5 that showed two methine carbons in place of two methylene carbons of crassicauline A. In the ^1H NMR spectrum of liaconitine A, two olefinic protons indicated a one proton doublet at δ 5.75 ($J = 10$ Hz) and a one proton double doublet at δ 6.08 ($J = 10$, 3.6 Hz). The latter is coupled to C(1)-βH (δ 3.27, 1H, d, $J = 3.6$ Hz). This suggested the presence of a double bond located at C(2),C(3) in liaconitine A. On the basis of spectral data, the structure of liaconitine A was assigned as **1** and confirmed by reduction of liaconitine A to crassicauline A, identical with an authentic sample by comparison of TLC behavior, IR and ^1H NMR spectral signals (Wang & Fang, 1981).

Similarly, by their spectral data (Tables 1 and 2), liaconitine B and C were determined to be C_{19}-type alkaloids and all bearing a double bond at C(2),C(3) as in **1**. Comparison of their NMR data indicated that the only difference among the three alkaloids was the C(8)

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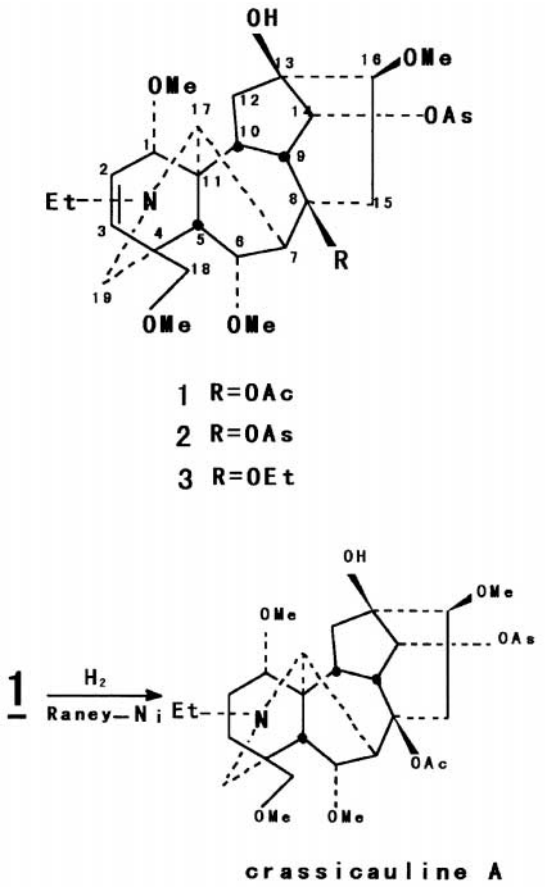

substituent group. Liaconitine B showed in the ^{1}H NMR the absence of acetyl group signals, the presence of signals at δ 6.86 (4H, d), 7.54(2H, d) and 7.55(2H, d) and, in the ^{13}C NMR, characteristic signals for two anisoyl groups. This was confirmed by mass spectral fragments at *m/z* 135 as base peak and *m/z* 733 as $[M]^{+}$. Thus, the structure of liaconitine B should be **2**. For liaconitine C, the methylene carbon at δ 56.0 and the methyl carbon at δ 15.3 indicated the presence of an ethoxyl group (Sun, Sha, Wang, Zhao, & Yuan, 1989). The ^{13}C NMR spectrum showed a quaternary carbon at δ 79.2 and a signal at C(8) for an ethoxyl group (δ 79.2) in **3** in place of an acetyl group (δ 85.8) in **1**. On the basis of these data, liaconitine C must possess an ethoxyl group at C(8) as in **3**.

Compound **3**, the aconitine-type alkaloid having an alkoxyl group at C(8) was probably an artifact of **1** formed in the extraction procedure with ethanol. In a previous paper, the acetoxyl group at C(8) of an aconitine-type alkaloid was found to be easily changed to an alkoxyl group by use of alcohols, such as methanol and ethanol, in the extraction procedure (Bando, Wada, Watanabe, Mori, & Amiya, 1985).

3. Experimental

All m.p.'s are uncorr. Optical rotations were measured in $CHCl_3$. NMR spectra were measured in

Table 1
^{1}H NMR data for compounds **1**–**4** (400 MHz, *δ*)

H	1	2	3	4
NEt	1.10 (t, 7.1)	1.11 (t, 7.1)	1.08 (t, 7.2)	1.07 (t, 7.1)
OMe	3.15	2.88	3.24	3.12
OMe	3.29	3.24	3.28	3.26
OMe	3.30 (s)	3.29 (s)	3.29 (s)	3.27 (s)
OMe	3.54	3.51	3.53	3.51
OMe	3.80	3.65	3.83	3.81
		3.71		
1-βH	3.27 (d, 3.6)	3.27 (d, 3.6)	3.25 (d, 3.5)	3.09 (m)
2-H	6.08 (dd, 10, 3.6)	6.01 (dd, 9.8, 3.5)	6.04 (dd, 9.8, 3.5)	–
3-H	5.78 (d, 10)	5.76 (d, 9.8)	5.78 (d, 9.8)	–
5-H	2.39 (d, 6.3)	2.39 (d, 6.2)	2.39 (d, 6.2)	2.39 (d, 5.8)
6-βH	3.98 (d, 6.4)	4.09 (d, 6.4)	4.01 (d, 6.2)	3.90 (d, 6.5)
14-βH	4.87 (d, 5.2)	4.92 (d, 5.2)	4.82 (d, 5.3)	4.84 (d, 5.2)
16-αH	3.42 (t)	3.51 (t)	3.44 (t)	3.31 (t)
17-H	3.86 (s)	3.92 (s)	3.88 (s)	3.78 (s)
18-H	3.68 (d, 8.4)	3.68 (d, 8.5)	3.68 (d, 8.3)	3.52 (d, 8.4)
OAc	1.33 (s)	–	–	1.24 (s)
OEt	–	–	0.57 (t, 7.0)	–
	7.97 (d, 8.8)	6.86 (d, 8.8)	7.98 (d, 6.8)	7.95 (d,7.0)
Ar-H		7.54 (d, 6.9)		
	6.89 (d, 8.8)	7.55 (d, 7.1)	6.90 (d, 6.8)	6.85 (d, 7.0)

Table 2
^{13}C NMR data for compounds **1**–**4** (400 MHz, δ)

Carbon	4	1	2	3	Carbon	4	1	2	3
1 (d)	84.2	83.4	83.6	84.0	OAc	169.6	169.9		
2 (d)	25.8 (t)	125.1	125.1	125.6		21.5	21.6		
3 (d)	35.7 (t)	137.5	137.6	137.6	14-OAs	163.4	163.3	163.1	163.4
4 (s)	38.9	40.7	40.8	40.9		122.6	122.6	122.6	122.4
5 (d)	48.5	47.7	48.0	47.9		131.6	131.3	131.6	131.7
6 (d)	82.8	81.1	81.2	81.6		113.7	113.7	113.2	113.5
7 (d)	44.9	44.5	44.9	45.9		165.9	165.7	166.3	166.1
8 (s)	85.2	85.8	86.1	79.2		55.3	55.3	55.1	55.3
9 (d)	48.5	46.1	46.4	46.1					
10 (d)	40.8	40.8	40.9	41.6	8-OAs			162.8	
11 (s)	50.2	48.1	48.8	49.2				122.3	
12 (t)	35.7	33.7	33.9	34.3				131.1	
13 (s)	74.7	74.7	74.9	74.9				113.0	
14 (d)	78.4	78.5	78.3	78.3				165.0	
15 (t)	39.3	40.0	40.2	38.0				55.1	
16 (d)	83.6	83.4	83.4	83.7	O–CH_2				56.0
17 (d)	61.6	59.2	59.2	58.5	CH_3				15.1
18 (t)	80.0	78.7	78.8	78.8					
19 (t)	53.9	52.7	52.7	52.7					
N–CH_2 (t)	49.0	47.4	47.6	47.5					
CH_3 (q)	12.8	12.5	12.6	12.7					
1′-OMe	55.8	56.1	56.1	56.3					
6′-OMe (q)	58.7	58.9	58.7	58.8					
16′-OMe	57.7	57.7	57.9	58.2					
18′-OMe	59.0	59.1	59.1	59.2					

$CDCl_3$ (TMS as int. standard) with a Bruker AM-400 spectrometer. MS were measured at 70 eV. CC was performed on silica gel (100–200, 200–300 mesh), TLC on silica gel GF_{254}. Visualization was made with Dragendorff's reagent.

3.1. Plant material

Roots of *A. episcopale* were collected from plants growing in Lijiang, Yunnan Province, P.R. China. Voucher specimens are deposited in our laboratory at the Department of Chemistry in Yunnan University.

3.2. Isolation

Dried and powdered roots (5 kg) were extracted (×3) with 80% EtOH (10 l) at room temp. After removing solvent under vacuum, the EtOH extract was washed with 5% HCl and treated with aq. NH_3 to pH 10, then extracted with $CHCl_3$ to give crude alkaloidal material (30 g). Crude alkaloids were eluted with a gradient of petrol, Et_2O and EtOAc to give frs A (15.0 g), B (3.5 g), C (2.8 g), D (1.7 g) and E (4.0 g).

Fr. C was dissolved in CH_2Cl_2 (15 ml) and adsorbed onto silica gel (100–200 mesh). The column was eluted with petrol–Et_2O–Et_3N (5:1:0.3) and the content of petrol was decreased gradually. Purification by repeated CC gave compound **1** (80 mg, colourless needles, recrystallized from MeOH) and **2** (50 mg, amorphous powder), respectively.

Fr. D was chromatographed on silica gel with petrol–Me_2CO–Et_3N (4:1:0.2) to afford compound **3** (30 mg) as an amorphous powder.

3.3. Liaconitine A (*1*)

Colourless needles, m.p. 146–147.3°C. $[\alpha]_D^{11}$ +53.85°C ($CHCl_3$; *c*, 0.26). HRMS (*m*/*z*): 641.3188 for $C_{35}H_{47}NO_{10}$. IR ν_{max}^{KBr} cm^{-1}: 3500 (-OH), 1640 (C=C), 1730, 1250 (C=O), 1610, 1510, 1460, 850 (-Ar), 1100. EI MS *m*/*z* (rel. int.) 641 $[M]^+$ (82), 626 $[M–CH_3]^+$ (70), 610 $[M–OMe]^+$ (57), 596 (45), 582 (87), 135 (100). ^1H NMR: Table 1. ^{13}C NMR: Table 2. (found: C, 65.5; H, 7.4. $C_{35}H_{47}NO_{10}$ requires: C, 65.6; H, 7.3%.).

3.4. Liaconitine B (*2*)

Amorphous powder. $C_{41}H_{51}NO_{11}$. $[\alpha]_D^{13}$ −2.26°C ($CHCl_3$; *c*, 0.77). IR ν_{max}^{KBr} cm^{-1} 3500 (OH), 1715, 1260 (C=O), 1632 (C=C), 1610, 1510, 1460, 850 (-Ar), 1100. EIMS *m*/*z* (rel.int.): 733 $[M]^+$ (18), 718 $[M–CH_3]^+$ (15), 715 $[MH_2O]^+$ (18), 702 $[M–OCH_3]^+$ (10), 582 $[M–OAs]^+$ (61), 135 (100). ^1H NMR: Table 1. ^{13}C NMR: Table 2. (found: C, 67.1; H, 7.0. $C_{41}H_{51}NO_{11}$ requires: C, 67.1; H, 7.0%.).

3.5. Liaconitine C (*3*)

Amorphous powder. $[\alpha]_D^{13}$ +45.96°C ($CHCl_3$; *c*, 0.36). HRMS *m/z* 627.3391 for $C_{35}H_{49}NO_9$. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 3010, 1720, 1250 (C=O), 1610, 1510, 1460, 850 (-Ar), 1100. EIMS *m/z* (rel.int.): 627 $[M]^+$ (22), 612 $[M–CH_3]^+$ (48), 596 $[M–OMe]^+$ (35), 582 (50), 135 (100). ^1H NMR: Table 1. ^{13}C NMR: Table 2. (found: C, 66.9; H, 7.8. $C_{35}H_{49}NO_9$ requires: C, 67.0; H, 7.8%.).

3.6. Reduction of liaconitine A (*1*)

Compound **1** (20 mg) and Raney-Ni (0.5 ml) were added to EtOH (10 ml). The mixt. was hydrogenated at room temp. for 1.5 h, filtered and the filtrate evapd to give a residue (17 mg). This was recrystallized from Et_2O to afford colourless crystals of **4** (14 mg). TLC and m.m.p. were identical to an authentic sample of crassicauline A.

3.7. Compound *4*

Colourless needles, m.p. 162.5–164.5°C, IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1730, 1250 (C=O), 1610, 1512, 1460, 850 (Ar-), 1100. $[M]^+$ *m/z* 643. ^1H NMR: Table 1. ^{13}C NMR: Table 2.

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References

Bando, H., Wada, K., Watanabe, M., Mori, T., & Amiya, T. (1985). *Chem. Pharm. Bull., 33,* 4717.

Sun, W. J., Sha, Z. F., Wang, A. X., Zhao, X. W., & Yuan, Z. Z. (1989). *Acta Pharm. Sinica, 24,* 71.

Takayama, H., Okazaki, T., Yamaguechi, K., Aimi, N., Haginiwa, J., & Sakai, S. (1988). *Chem. Pharm. Bull., 36,* 3210.

Wang, F. P., & Fang, Q. C. (1981). *Planta Med., 42,* 375.