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STUDIES ON THE ALKALOIDS FROM *ACONITUM CONTORTUM* (I)

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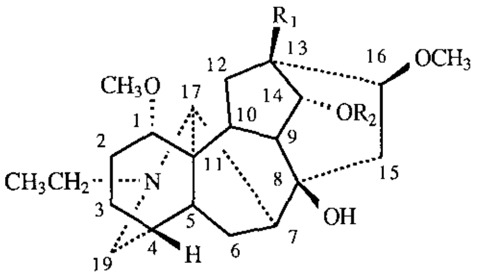
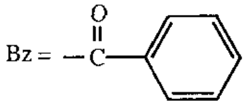
Abstracts - A new diterpenoid alkaloid, contortumine(**1**), and seven known diterpenoid alkaloids were isolated from the roots of *Aconitum contortum*. Structures of these alkaloids were determined on the basis of spectral and chemical data.

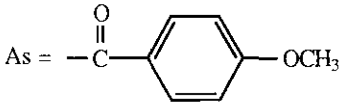
Aconitum contortum, chinese name "Hei-xin-jie", is one of the chinese *Aconitum* plants which has been used as an antidote for toxic *Aconitum* plants in China. We isolated a new alkaloid, contortumine(**1**), along with known alkaloids, delavaconitine(**2**),[1] delavaconine(**3**),[1] aconosine(**4**),[2] dolaconine(**5**),[2] delavaconitine C (**6**),[3] episcopalidine(**7**),[4] and cammaconine(**8**),[5] from the roots of *A. contortum*.

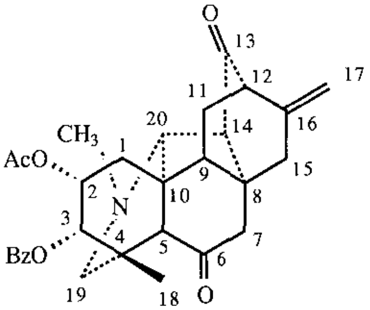
Contortumine(1) showed the molecular ion peak at m/z 527.2883 ($C_{30}H_{41}NO_7$) in its high resolution mass spectrum. The uv absorption (λ_{max}^{EtOH} 257 nm), ir absorption ($\nu_{max}^{CHCl_3}$ 1710 cm^{-1}) and ^{1}H-nmr spectrum [δ 3.86 (3H, s), 6.92 (2H, d, J=9.0 Hz), 7.99 (2H, d, J=9.0 Hz)] of **1** indicated the presence of anisoyl group. The ^{1}H-nmr spectrum of **1** revealed the presence of N-ethyl group [δ 1.09 (3H, t, J=7.1 Hz)] and two methoxyl groups [δ 3.29, 3.38 (each 3H, s)]. The appearance of the signal at δ 5.12 (1H, t, J=5.1 Hz) suggested



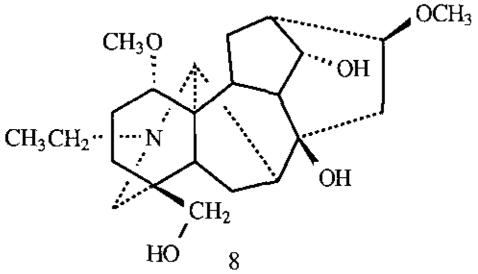
1 R_1 = OH, R_2 = As
2 R_1 = OH, R_2 = Bz
3 R_1 = OH, R_2 = H
4 R_1 = H, R_2 = H
5 R_1 = H, R_2 = Ac
6 R_1 = H, R_2 = Bz







7



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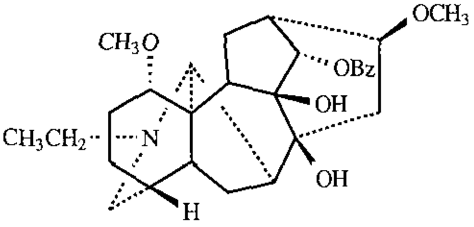

9

that the anisoyloxy group should be located at C-14 position.[1] The ^{13}C-nmr spectrum of 1 was similar to that of delavaconitine(2) except for signals of ester moiety. These spectral data indicated that 1 was assigned a 14-anisoyldelavaconine. *Furthermore, the structure of* 1 *was confirmed by* hydrolysis. Hydrolysis of 1 gave delavaconine(3).

The ir, uv, ^{1}H- and ^{13}C-nmr spectral data of a major alkaloid 2 were in good agreement with those of delavaconitine[1] and of reported episcopalisine(9)[4]. Delavaconitine differs from episcopalisine in the substitutional position of a tertiary hydroxyl group. Namely, delavaconitine has two tertiary hydroxyl

Table 1 ^{13}C-Nmr Data ($CDCl_3$) for the Alkaloids

carbon	1	2	3	4	5	6
1	85.9	86.0	86.3	86.7	86.1	86.1
2	26.4	26.4	26.0	26.2	26.5	26.6
3	29.8	29.9	29.8	30.1	30.0	30.0
4	36.5	36.5	36.4	36.6	36.7	36.7
5	44.9	44.9	44.9	45.6	45.5	45.4
6	29.3	29.3	29.0	29.0	29.5	29.6
7	46.9	47.0	45.9	46.1	46.5	46.9
8	74.0	74.1	73.4	73.2	74.0	74.2
9	46.9	46.9	49.1	47.2	45.5	45.5
10	42.5	42.6	42.5	45.9	45.0	45.3
11	48.7	48.7	48.6	48.8	48.9	48.9
12	36.1	36.1	35.6	27.8	28.8	28.8
13	76.5	76.5	77.2	38.1	36.0	36.9
14	80.3	80.6	79.8	75.7	77.0	77.1
15	41.7	41.7	39.9	39.2	41.5	41.4
16	83.5	83.6	84.8	82.3	82.0	82.0
17	63.0	63.0	63.4	63.3	62.6	62.7
19	50.2	50.2	50.4	50.4	50.4	50.4
N-CH_2	49.6	49.7	49.8	49.7	49.6	49.6
CH_3	13.5	13.6	13.6	13.7	13.5	13.6
1'	56.5*	56.6*	56.6*	56.4*	56.1*	56.0*
16'	58.2*	58.3*	57.8*	56.5*	56.4*	56.5*
C=O					170.7	
CH_3					21.4	
C=O	166.7	167.1				166.6
$C_6H_{4(5)}$	113.7	128.5				128.4
	122.6	129.8				129.6
	131.7	130.3				130.6
	163.4	133.0				132.7
OCH_3	55.4					

* The assignments may be interchanged in any vertical column.

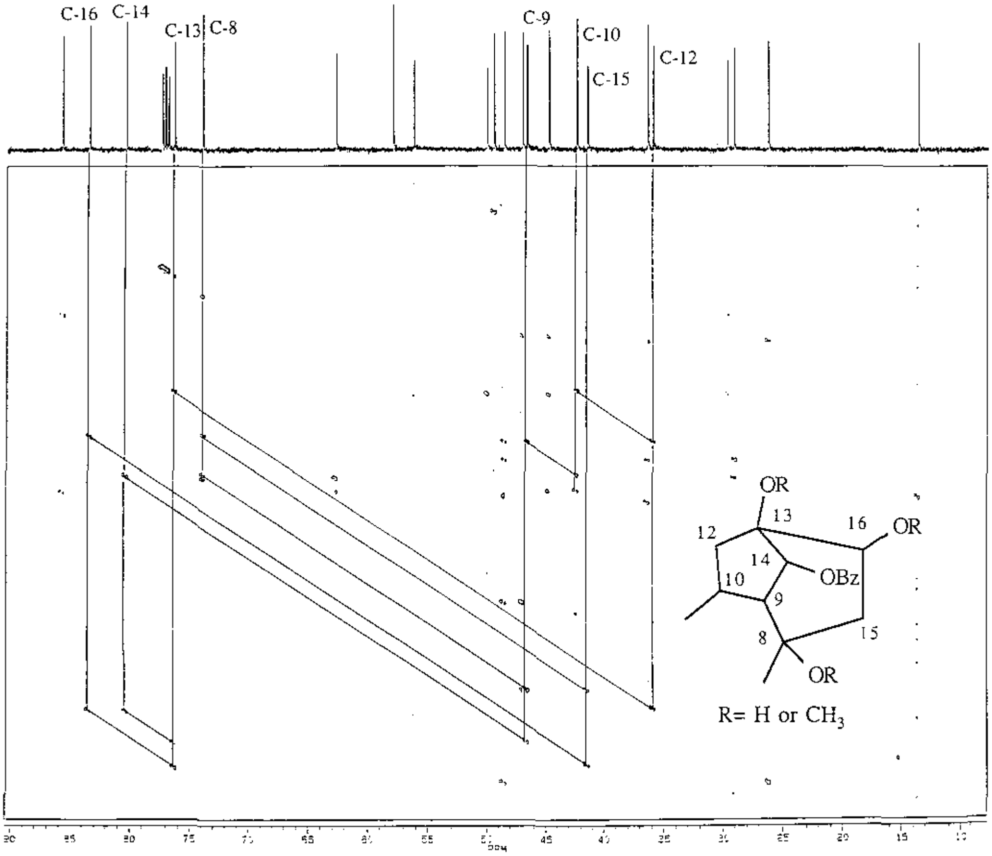

Figure 1. 2D-INADEQUATE spectrum of delavaconitine

groups at C-8 and C-13 positions, while episcopalisine has them at C-8 and C-9 positions. We determined the structure of 2 by 2D-INADEQUATE spectrum. The 2D-INADEQUATE spectrum of 2 (Figure 1) suggested that 2 had a partial structure shown in Figure 1. The C-14 carbon bearing a benzoyloxy group was assigned by ^{1}H,^{1}H- and ^{13}C,^{1}H-COSY spectra. The partial structure indicated that two tertiary hydroxyl groups linked to C-8 and C-13, not to C-9. From these nmr data 2 was determined as delavaconitine. Furthermore, the structure of 2 was confirmed by hydrolysis. Hydrolysis of 2 gave delavaconine(3).

EXPERIMENTAL

All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. Ir spectra in CHCl$_3$ solution were taken with a Hitachi 270-30 infrared spectrophotometer. Uv spectra in EtOH solution were measured with a Hitachi U-3200 spectrophotometer. Nmr spectra in $CDCl_3$ solution were recorded with Bruker AM-500 and JEOL FX-200 spectrometers. Mass spectra were determined with JEOL JMS-DX300 mass spectrometer. Prep. hplc was performed on C.I.G. column system (silica gel) manufactured by Kusano scientific Co., Tokyo.

Plant material --- The roots of *Aconitum contortum* were collected in Yunnan, China. This plant was identified by Professor Zhou Jun, Kunming Institute of Botany.

Extraction and Isolation of Alkaloids --- Dried and powdered roots of *A. contortum* (2.0 kg) were extracted with 85% EtOH (10 l x 4) at room temperature for several days. The EtOH extract (260 g) was dissolved in 2% HCl (2 l) and extracted with Et_2O (2 l x 5). The aqueous layer was basified with NH_4OH (pH 9) and extracted with $CHCl_3$ (2 l x 5) to afford the crude alkaloid (68 g). The crude alkaloid was chromatographed on alumina to give fractions 1 (25% EtOAc in hexane, 2.71 g), 2 (EtOAc, 14.2 g), and 3 (MeOH, 29.6 g). Fractions 1, 2, and 3 were purified on prep. hplc (cyclohexane - EtOAc - diethylamine, 100 : 10 : 1, 50 : 10 : 1, and 100 : 100 : 1, respectively). Delavaconitine C (**6**, 86 mg) was obtained from fraction 1. Contortumine (**1**, 570 mg), delavaconitine (**2**, 9.25 g), delavaconine (**3**, 142 mg), and dolaconine (**5**, 308 mg) were obtained from fraction 2. Aconosine (**4**, 1.81 g), episcopalidine (**7**, 2.18 g), and cammaconine (**8**, 237 mg) were obtained from fraction 3.

Contortumine (1) --- Amorphous. $[\alpha]_D$ -4.8° (c=0.97, EtOH). Hr-ms : calcd for $C_{30}H_{41}NO_7$ 527.2883, found 527.2893. Ms m/z : 527(M^+), 496(100%), 135. Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 - 3400, 1710, 1608, 1258. Uv λ_{max}^{EtOH} nm (log ε) : 257 (4.27). ^{1}H-Nmr ($CDCl_3$) δ : 1.09 (3H, t, J=7.1 Hz), 3.29, 3.38, 3.86 (each 3H, s), 5.12 (1H, d, J=5.1 Hz), 6.92 (2H, d, J=9.0 Hz), 7.99 (2H, d, J=9.0 Hz). ^{13}C-Nmr : shown in Table 1.

Hydrolysis of contortumine (1) --- Contortumine (46 mg) was stirred with 2 ml of 0.1N ethanolic NaOH at room temperature for 30 min. Ice water (10 ml) was added and the reaction mixture was concentrated under reduced pressure to remove ethanol. The solution was put on the Diaion HP20 column and eluted with water and methanol successively. The methanolic eluate was purified on prep. hplc (cyclohexane - EtOAc - diethylamine, 50 : 10 : 1) to afford 20 mg (59 %) of delavaconine (3). Crystallization of 3 from acetone gave colorless needles. mp 150 - 152°C. Ms m/z : 393 (M^+), 378, 362 (100%). Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 - 3300. ^{1}H-Nmr ($CDCl_3$) δ : 1.07 (3H, t, J=7.1 Hz), 3.26, 3.41 (each 3H, s), 4.03 (1H, d, J=4.4 Hz). The mp of 3 was not depressed by admixture with delavaconine.

Delavaconitine (2) --- Amorphous. $[\alpha]_D$ -14.4° (c=1.06, EtOH). Ms m/z : 497 (M^+), 466 (100%), 388, 105. Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 - 3400, 1716, 1602, 1276. Uv λ_{max}^{EtOH} nm (log ε) : 230 (4.15), 273 (3.02), 280 (2.94). ^{1}H-Nmr ($CDCl_3$) δ : 1.08 (3H, t, J=7.1 Hz), 3.28, 3.39 (each 3H, s), 5.14 (1H, d, J=5.1 Hz), 7.39 - 8.06 (5H, m). ^{13}C-Nmr : shown in Table 1.

Hydrolysis of delavaconitine (2) --- Delavaconitine (200 mg) was hydrolyzed in a similar manner as 1. The hydrolysate was crystallized from acetone to afford 103 mg (65 %) of delavaconine (3) as colorless needles. mp 151 - 152°C. $[\alpha]_D$ -7.2° (c=0.89, EtOH). Ms m/z : 393 (M^+), 378, 362 (100%). Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 3700 - 3300. ^{1}H-Nmr ($CDCl_3$) δ : 1.07 (3H, t, J=7.1 Hz), 3.26, 3.41 (each 3H, s), 4.02 (1H, d, J=5.1 Hz). ^{13}C-Nmr ($CDCl_3$) δ : 13.7 (q), 26.1 (t), 29.0 (t), 29.9 (t), 35.6 (t), 36.4 (d), 39.9 (t), 42.5 (d), 45.0 (d), 45.9 (d), 48.6 (s), 49.1 (d), 49.8 (t), 50.3 (t), 56.6 (q), 57.8 (q), 63.3 (d), 73.3 (s), 77.1 (s), 79.8 (d), 84.8 (d), 86.4 (d).

Delavaconine (3) --- Colorless needles from acetone. mp 148 - 149°C. $[\alpha]_D$ -5.0° (c=0.38, EtOH). Ms m/z : 393(M^+), 362(100%). Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 - 3300. ^{1}H-Nmr ($CDCl_3$) δ : 1.08 (3H, t, J=7.1 Hz), 3.26, 3.41 (each 3H, s), 4.02 (1H, dd, J=5.3, 1.5 Hz). ^{13}C-Nmr : shown in Table 1.

Aconosine (4) --- Colorless prisms from acetone. mp 149 - 152°C. $[\alpha]_D$

-24.4° (c=1.06, EtOH). Ms m/z : 377 (M^+), 346 (100%). Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 - 3300. ^{1}H-Nmr ($CDCl_3$) δ : 1.07 (3H, t, J=7.1 Hz), 3.27, 3.34 (each 3H, s), 4.15 (1H, dt, J=4.2, 4.8 Hz), 4.62 (1H, d, J=4.2 Hz, disappeared with D_2O). ^{13}C-Nmr : shown in Table 1.

Dolaconine (5) --- Amorphous. $[\alpha]_D$ -8.7° (c=0.40, EtOH). Ms m/z : 419 (M^+), 401, 388 (100%). Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 -3400, 1740, 1242. ^{1}H-Nmr ($CDCl_3$) δ : 1.07 (3H, t, J=7.1 Hz), 2.05 (3H, s), 3.23, 3.27 (each 3H, s), 4.83 (1H, t, J=4.9 Hz). ^{13}C-Nmr : shown in Table 1.

Delavaconitine C (6) --- Amorphous. $[\alpha]_D$ +10.4° (c=0.73, EtOH). Ms m/z : 481(M^+), 450(100%), 105. Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 -3300, 1714, 1604, 1272. Uv λ_{max}^{EtOH} nm (log ε) : 229 (4.03), 274 (3.52), 280 (3.51). ^{1}H-Nmr ($CDCl_3$) δ : 1.08 (3H, t, J=7.1 Hz), 3.19, 3.30 (each 3H,s), 5.15 (1H, t, J=4.9 Hz), 7.36 - 8.03 (5H, m). ^{13}C-Nmr : shown in Table 1.

Episcopalidine (7) --- Colorless prisms from acetone. mp 213 - 214°C. $[\alpha]_D$ -73.2° (c=1.12, $CHCl_3$). Ms m/z : 503(M^+), 382(100%), 322, 105. Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 1742, 1722, 1650, 1604, 1250. Uv λ_{max}^{EtOH} nm (log ε) : 230 (4.22), 273 (3.11), 281 (3.01). ^{1}H-Nmr ($CDCl_3$) δ : 1.58 (3H, s), 2.03 (3H,s), 2.30 (1H, d, J=18.6 Hz), 2.44 (3H, s), 2.75 (1H, d, J=18.6 Hz), 2.93 (1H, d, J=4.3 Hz), 3.30 (1H, d, J=11.8 Hz), 4.83 (1H, br s), 4.88 (1H, d, J=4.5 Hz), 4.99 (1H, br s), 5.53 (1H, ddd, J=4.5, 4.5, 2.3 Hz), 7.40 - 8.00 (5H, m). ^{13}C-Nmr ($CDCl_3$) δ : 21.2 (q), 22.8 (t), 25.6 (q), 34.5 (t), 34.6 (t), 41.7 (s), 41.8 (s), 43.2 (q), 44.3 (s), 49.6 (d), 50.2 (t), 52.7 (d), 56.3 (t), 58.1 (d), 63.0 (d), 67.4 (d), 70.6 (d), 76.1 (d), 110.9 (t), 128.5 (d), 129.5 (d), 129.7 (s), 133.3 (d), 141.8 (s), 165.5 (s), 169.3 (s), 200.6 (s), 211.4 (s).

Cammaconine (8) --- Colorless prisms from acetone. mp 134 - 135°C. $[\alpha]_D$ -8.4° (c=0.77, EtOH). Ms m/z : 407(M^+), 392, 376(100%). Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 - 3300. ^{1}H-Nmr ($CDCl_3$) δ : 1.07 (3H, t, J=7.1 Hz), 3.28, 3.35 (each 3H, s), 4.13 (1H, t, J=5.1 Hz). ^{13}C-Nmr ($CDCl_3$) δ : 13.7 (q), 24.6 (t), 25.8 (t), 27.8 (t), 32.2 (t), 37.6 (d), 38.4 (t), 39.1 (s), 45.6 (d), 45.8 (d), 45.9 (d), 46.9 (d), 48.8 (s), 49.5 (t), 53.1 (t), 56.3 (q), 56.5 (q), 62.9 (d), 68.7 (t), 73.0 (s), 75.6 (d), 82.3 (d), 86.3 (d).

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