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

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Abstracts – Three new diterpenoid alkaloids, contorine (1), contortine (2) and contorsine (3) were isolated from the roots of *Aconitum contortum*. Structures of these alkaloids were determined on the basis of spectral data and X-ray analyses.

Aconitum contortum, chinese name "Hei-xin-jie", is one of the chinese *Aconitum* plants. In the previous study[1] we have reported the isolation and structures of eight diterpene alkaloids from the roots of *A. contortum*. In the continuous investigation on the constituents of *A. contortum*, we have isolated three new alkaloids, contorine (1), contortine (2) and contorsine (3).

Alkaloids (1, 2 and 3) were assumed as the derivatives of episcopalidine (4)[2] because the ^{1}H and ^{13}C nmr spectral data of 1, 2 and 3 showed similarities with those of 4 except for their ester group located at C-3.

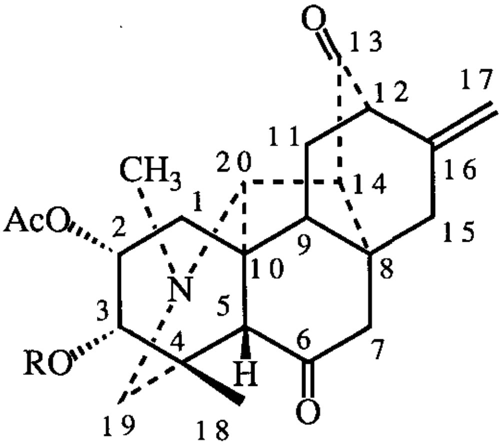
Contorine (1), mp 238 °C (decomp.), showed the molecular ion peak at m/z 533.2392

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

carbon	1	2	3	4[1]
1	34.5	34.3	34.4	34.5
2	67.6	67.3	67.2	67.4
3	75.8	75.2	75.3	76.1
4	41.8	41.5	41.5	41.7
5	58.2	58.2	58.1	58.1
6	n.d.	n.d.	n.d.	200.8
7	50.3	49.3	49.7	50.2
8	44.3	44.4	44.3	44.1
9	49.7	49.6	49.6	49.6
10	41.9	41.9	41.9	41.8
11	22.9	22.8	22.8	21.8
12	52.7	52.6	52.7	52.7
13	211.5	211.0	211.2	210.4
14	63.1	62.5	62.8	63.0
15	34.7	34.5	34.6	34.6
16	141.9	141.6	141.8	141.8
17	110.9	111.1	111.0	110.9
18	25.7	25.4	25.4	25.6
19	56.3	56.9	56.5	56.3
20	70.6	70.8	70.6	70.6
N-CH_3	43.3	42.9	43.1	43.2
C=O	169.3	169.4	169.4	169.1
CH_3	21.2	21.3	21.3	21.2
R	165.2	175.4	175.9	165.1
	113.6	11.5	18.8	128.5
	122.1	16.4	18.9	129.5
	131.6	26.5	34.1	129.7
	163.7	41.2		133.3
	55.5			

n.d. not detected

All assignments were confirmed by ^{13}C-^{1}H COSY and ^{1}H-^{1}H COSY spectra.

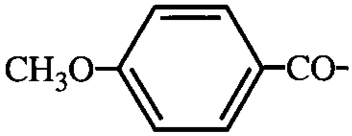


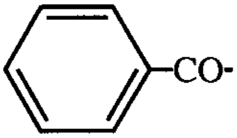
1 : R = As

2 : R = $CH_3CH_2(CH_3)CHCO$

3 : R = $(CH_3)_2CHCO$

4 : R = Bz

As =


Bz =


($C_{31}H_{35}NO_7$) in its high resolution mass spectrum. The uv absorption (λ_{max}^{EtOH} 258 nm) and ir absorption ($\nu_{max}^{CHCl_3}$ 1740 cm^{-1}) showed the presence of aromatic ester in **1**. The appearance of methoxyl signal (δ 3.86, 3H, s) and four aromatic proton signals (δ 6.92 and 7.93 each 2H, d, J=9.0 Hz) in the ^{1}H-nmr of **1** indicated that the ester group at C-3 was anisoyl group. The base peak appeared at m/z 382 ($[M-CH_3OC_6H_4COO]^+$) in the mass spectrum supported the presence of anisoyl group in **1**. These spectral data indicated that 1 should be assigned as 2-acetyl-3-anisoylhetidine.

Contorsine (**2**) and contortine (**3**) obtained as a mixture by the chromatography on the alumina and silica gel. The isolation of them was carried out by hplc on ODS.

Contortine (**2**), mp 230 - 233 °C, showed the molecular ion peak at m/z 483.2632 ($C_{28}H_{37}NO_6$) in its high resolution mass spectrum. The appearence of primary methyl signal (δ 0.89, 3H, t, J=7.4 Hz) and secondary methyl signal (δ 1.13, 3H, d, J=7.0 Hz) in the ^{1}H-nmr of **2** showed that the ester group at C-3 was 2-methylbutyryl group. The base peak appeared at m/z 382 ($[M-C_4H_9COO]^+$) in the mass spectrum supported the presence of 2-methylbutyryl group in **2**. The configuration at C-2 of 2-methylbutyryl group was determined as S configuration by the X-ray analysis (Figure 1). These spectral data indicated that **2** should be assigned as 2-acetyl-3-(S)-2-methylbutyrylhetidine. The C_{20}-diterpenoid alkaloid bearing (S)-2-methylbutyryl group was obtained from *Aconitum yesoense*.[3]

Contorsine (**3**), mp 203 - 206 °C, showed the molecular ion peak at m/z 469.2441 ($C_{27}H_{35}NO_6$) in its high resolution mass spectrum. The appearance of two secondary methyl signals (δ 1.15 and 1.16, each 3H, d, J=7.0 Hz) in the ^{1}H-nmr of **3** showed that the ester group at C-3 was isobutyryl group. The base peak appeared at m/z 382 ($[M-C_3H_7COO]^+$) in the mass spectrum supported the presence of isobutyryl group in **3**. These spectral data indicated that **3** should be assigned as 2-acetyl-3-isobutyrylhetidine.

Finally the structures of **1**, **2** and **3** were confirmed by X-ray crystal analysis as

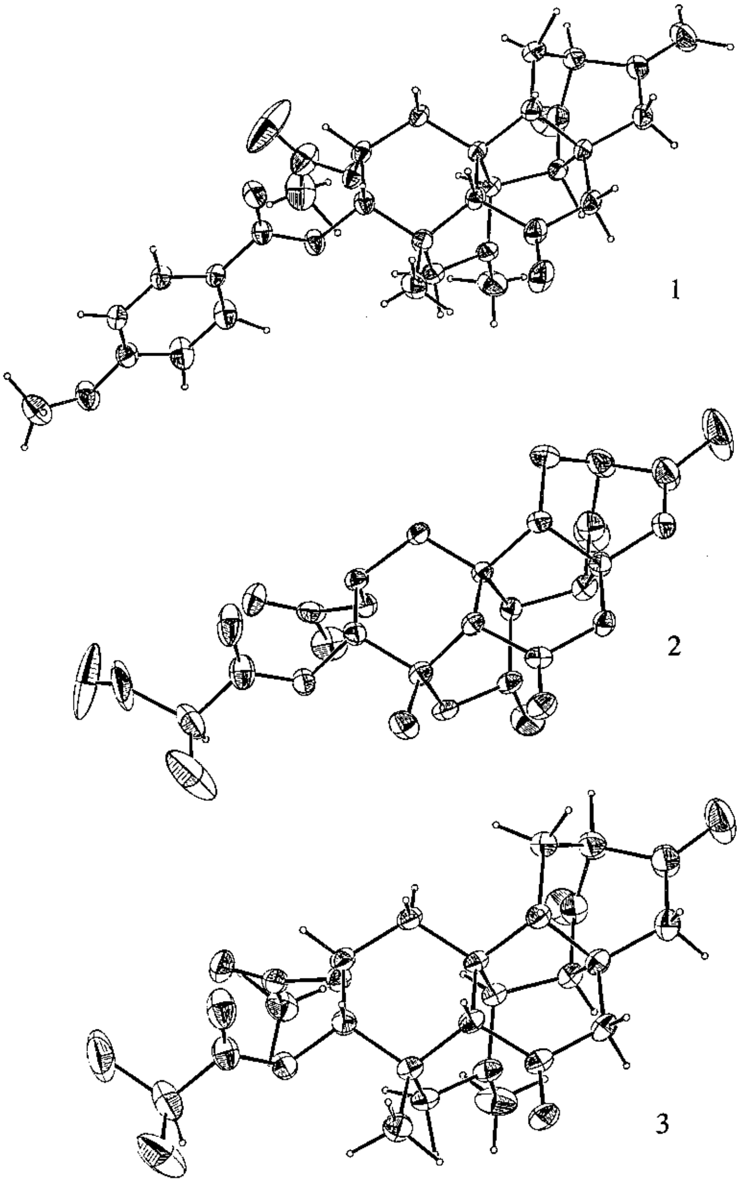


Figure 1. ORTEP drawing of alkaloids

shown in Figure 1.

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 spectrometer. Mass spectra were determined with JEOL JMS-DX300 mass spectrometer. X-Ray crystal analysis was performed with Enruf-Nonius CAD-4 diffractometer. Prep. hplc was performed on Waters deltaprep 3000.

Plant material and Extraction procedure were dealt in our previous paper.[1]

Isolation of alkaloids --- The crude alkaloid(68 g) 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). Purification of fraction 3 by C.I.G. column system[4] (silica gel, cyclohexane - EtOAc - diethylamine, 50:10:1) gave contorine (**1**, 80 mg) and a mixture of contortine (**2**) and contorsine (**3**). The mixture of **2** and **3** was purified with prep. hplc (0.005M PIC B-7 reagent in H_2O - MeOH - acetonitrile, 2:2:1) to give contortine (**2**, 67 mg) and contorsine (**3**, 19 mg).

Contorine (1) --- Colorless prisms from acetone. mp.238 C (decomp.). $[\alpha]_D$ -44.9° (c=0.35, EtOH). Hr-ms : calcd for $C_{31}H_{35}NO_7$ 533.2412, found 533.2392. Ms m/z : 533(M^+), 382(100%), 322, 135. Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 1740, 1714, 1608, 1258. Uv λ_{max}^{EtOH} nm (logε) : 258 (4.21). ^{1}H-Nmr ($CDCl_3$) δ : 1.57, 2.04, 2.44 (each 3H, s), 3.30 (1H, d, J=12.1 Hz), 3.86 (3H, s), 4.83 (1H, ddd, J=2.2, 2.2, 0.7 Hz), 4.85 (1H, d, J=4.5 Hz), 4.99 (1H, t, J=2.3 Hz), 5.51 (1H, ddd, J=4.5, 4.5, 2.3 Hz), 6.92 (2H, d, J=9.0 Hz), 7.93 (2H, d, J=9.0 Hz). ^{13}C-Nmr : shown in Table 1.

X-Ray crystallographic analysis of 1 --- The size of crystal was 0.5 x 0.4 x 0.4mm, monoclinic, space group $P2_1$, a=8.963(3)Å, b=15.701(3)Å, c=10.139(3)Å, V=1418(1)$Å^3$, Z=2, $D_{calc.}$=1.25gcm^{-3} and μ(Cu Kα)=6.8cm^{-1}. Reflections were measured with $\omega/2\theta$ scan mode and using graphite monochromate Cu Kα radiation(λ=1.54184Å). Cell constants were determined by least squares refinement using 24 centered reflections in the range 20° $<\theta<$ 27°. Intensities were measured for 2939 independent reflections in the range $2\theta \leq$ 140°, of which 2834 reflections were considered as observed [$I > 3\sigma(I)$]. The data were corrected for Lorentz and polarization effects. No absorption correction was made. The structure was solved by the direct-methods program Multan[5] and was refined by full-matrix least-squares, using the Enraf-Nonius SDP program.[6] Hydrogen atoms were located on a difference Fourier synthesis map. The last difference Fourier map was essentially featureless with no peaks greater than 0.31eÅ^{-3}. The final discrepancy index was R=0.052.

Contortine (2) --- Colorless prisms from acetone. mp 230 - 233 °C. $[\alpha]_D$ -82.1° (c=0.45, EtOH). Hr-ms : calcd for $C_{28}H_{37}NO_6$ 483.2626, found 483.2632. Ms m/z : 483(M$^+$), 382(100%), 322. Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 1738, 1244, 1146, 1104, 1076, 1026. ^{1}H-Nmr ($CDCl_3$) δ : 0.89 (3H, t, J=7.4 Hz), 1.13 (3H, d, J=7.0 Hz), 1.52, 2.07, 2.45 (each 3H, s), 2.50 (1H, d, J=12.0 Hz), 3.21 (1H, d, J=12.0 Hz), 4.64 (1H, d, J=4.4 Hz), 4.83 (1H, t, J=2.0 Hz), 4.98 (1H, t, J=2.4 Hz), 5.41 (1H, ddd, J=4.4, 4.4, 2.3 Hz), ^{13}C-Nmr : shown in Table 1.

X-Ray crystallographic analysis of 2 --- The size of crystal was 0.5 x 0.3 x 0.25mm, monoclinic, space group $P2_1$, a=13.9038(3)Å, b=7,7758(9)Å, c=13.8157(6)A, V=1328(4)Å^3, Z=2, $D_{calc.}$=1.21gcm^{-3} and μ(Cu Kα)=6.5cm^{-1}. Reflections were measured with $\omega/2\theta$ scan mode and using graphite monochromate Cu Kα radiation(λ=1.54184Å). Cell constants were determined by least squares refinement using 25 centered reflections in the range 17° $<\theta<$ 22°. Intensities were measured for 2790 independent reflections in the range $2\theta \leq$ 140°, of which 2621 reflections were considered as observed [$I > 3\sigma(I)$]. The data were corrected for Lorentz and polarization effects. No absorption correction was made. The structure was solved by the direct-methods program Multan and was refined by full-matrix least-squares, using the Enraf-Nonius SDP program. The positions of 15 hydrogen atoms were located on a difference Fourier synthesis map. The positions of the other hydrogen atoms were not appeared. The last difference Fourier map was essentially featureless with no peaks greater than 0.34eÅ^{-3}. The final discrepancy index was R=0.102.

Contorsine (3) --- Colorless prisms from acetone. mp 203 - 206 °C. $[\alpha]_D$ -88.1° (c=0.62, EtOH). Hr-Ms : calcd for $C_{27}H_{35}NO_6$ 469.2452, found 469.2441. Ms m/z : 469(M$^+$), 382(100%), 322. Ir $\nu_{max}^{CHCl_3}$ cm^{-1} : 1740, 1244, 1152, 1104, 1074, 1026. ^{1}H-Nmr ($CDCl_3$) δ : 1.15, 1.16 (each 3H, d, J=7.0 Hz), 1.50, 2.07, 2.42 (each 3H, s), 2.46 (1H, d, J=11.7 Hz), 3.16 (1H, d, J=11.7 Hz), 4.62 (1H, d, J=4.5 Hz), 4.82 (1H, t, J=2.0 Hz), 4.98 (1H, t, J=2.5 Hz), 5.41 (1H, ddd, J=4.5, 4.5, 2.3 Hz). ^{13}C-Nmr : shown in Table 1.

X-Ray crystallographic analysis of 3 --- The size of crystal was 0.5 x 0.3 x 0.3mm, monoclinic, space group $P2_1$, a=13.766(2)Å, b=7.743(1)Å, c=13.858(2)Å, V=1301.8(7)Å^3, Z=2, $D_{calc.}$=1.20gcm^{-3} and μ(Cu Kα)=6.5cm^{-1}. Reflections were measured with ω/2θ scan mode and using graphite monochromate Cu Kα radiation(λ=1.54184Å). Cell constants were determined by least squares refinement using 23 centered reflections in the range 17° <θ < 24°. Intensities were measured for 2663 independent reflections in the range 2θ ≤ 140°, of which 2545 reflections were considered as observed [$I > 3\sigma(I)$]. The data were corrected for Lorentz and polarization effects. No absorption correction was made. The structure was solved by the direct-methods program Multan and was refined by full-matrix least-squares, using the Enraf-Nonius SDP program. The positions of hydrogen atoms were located on a difference Fourier synthesis map. The positions of the hydrogen atoms bonded to isobutyl and olefinic methyl carbons were not appeared. The last difference Fourier map was essentially featureless with no peaks greater than 0.24eÅ^{-3}. The final discrepancy index was R=0.052.

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