STUDIES ON THE ALKALOIDS FROM *ACONITUM POLYSCHISTUM* HAND-MAZZ. PART II

Hongcheng Wang and Aina Lao
Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai, China

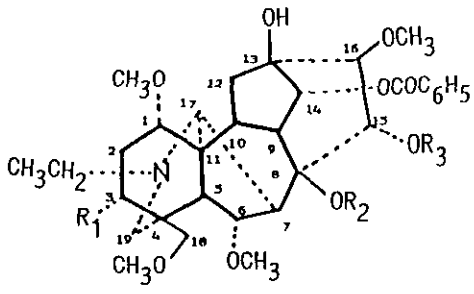
Yasuo Fujimoto*, Kimiko Kobayashi, Toshio Sakurai[φ], and Takashi Tatsuno[§]
The Institute of Physical and Chemical Research, Wako-Shi, Saitama 351-01, Japan

Abstract —— A new alkaloid, polyschistine D (1), a new natural product, benzoyldeoxyaconine (2), and three known alkaloids, benzoylaconine (3), deoxyaconitine (4), and aconitine (5) have been isolated from the roots of *Aconitum polyschistum* Hand-Mazz collected Sichuan province in China. The structures of polyschistine D (1) and benzoyldeoxyaconine (2) were confirmed on the basis of their spectral data and the chemical correlations.

In the previous paper[1], we have reported the isolation and structural elucidation of three new C_{19} diterpenoid alkaloids, polyschistine A, B and C from the roots of *Aconitum polyschistum*. Continued investigation of the constituents of this plants has led to the isolation of a new alkaloid, polyschistine D (1), a new natural product, benzoyldeoxyaconine (2) along with known alkaloids, benzoylaconine (3), deoxyaconitine (4) and aconitine (5).

Polyschistine D showed the molecular ion peak at *m/z* 645.3136 (calc. 645.3136, $C_{34}H_{47}NO_{11}$) in its high resolution mass spectrum. The ^{1}H-nmr spectrum[2] of 1 indicated the presence of an N-ethyl [δ 1.10 (3H, t, J=7.1 Hz)], an acetyl [δ 2.08 (3H,s)], four methoxyls [δ 3.26, 3.29, 3.31, 3.72 (3H each, s)] and five

aromatic protons [δ 7.43-8.08 (5H)]. The signals at δ 4.92 (1H, dd, J=6.1 and 12.9 Hz, H-3) and at δ 5.02 (1H, d, J=5.1 Hz, H-14) suggested that the acetoxyl and benzoyloxy groups should be located at C-3 and C-14 positions, respectively. In the lower field there were two proton signals which could be assigned to H-6 [δ 4.08 (1H, d, J=7.1 Hz)] and H-15[δ 4.55 (1H, dd, J=5.4 and 5.5 Hz)]. When the ^{13}C-nmr spectrum[2] of 1 was compared with those of benzoylaconine (3) and some other aconitine type alkaloids, the chemical shift pattern of 1 is very close to that of 3 except for a few changes (see Table 1).



(1) R_1=$OCOCH_3$, R_2=R_3=H
(2) R_1=R_2=R_3=H
(3) R_1=OH, R_2=R_3=H
(4) R_1=H, R_2=$COCH_3$, R_3=H
(5) R_1=OH, R_2=$COCH_3$, R_3=H
(6) R_1=$OCOCH_3$, R_2=H, R_3=$COCH_3$
(7) R_1=OH, R_2=H, R_3=$COCH_3$
(8) R_1=$OCOCH_3$, R_2=$COCH_3$, R_3=H

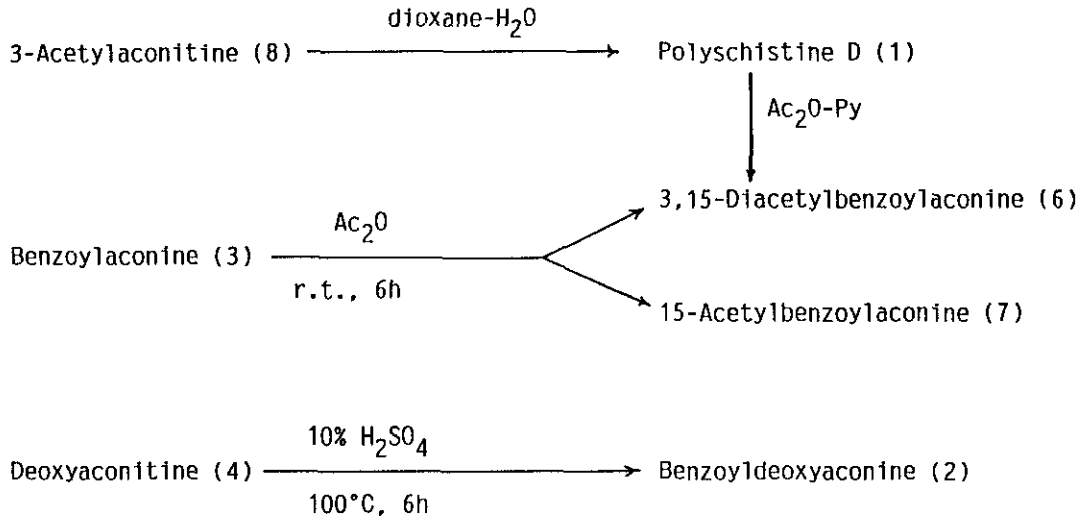


Table 1

The ^{13}C-nmr data (100 MHz, δ, $CDCl_3$) for polyschistine D (1), benzoyldeoxyaconine (2), benzoylaconine (3), deoxyaconitine (4), aconitine (5), 3,15-diacetylbezoylaconine (6), 15-acetylbenzoylaconine (7) and 3-acetylaconitine (8).

	compound							
carbon	1	2	3	4	5	6	7	8
1	83.9	83.9	82.8	85.5	83.7	83.0	82.7	83.8
2	32.0	25.0	32.0	26.7	34.1	31.9	33.7	32.1
3	72.2	36.9	71.0	37.0	71.0	71.9	71.5	71.9
4	42.5	38.9	43.2	39.4	43.4	42.3	43.2	42.5
5	49.6	48.6	48.7	49.5	47.2	49.9	49.5	46.2
6	82.1	82.5	81.8	83.7	82.4	82.0	82.5	82.3
7	46.8	48.7	45.5	45.6	44.5	45.3	45.1	45.6
8	75.9	81.7	78.3	92.5	92.2	76.6	76.7	92.1
9	46.0	45.9	45.8	44.9	45.2	45.9	46.7	44.9
10	41.7	41.6	41.8	41.4	41.4	41.4	41.3	40.8
11	50.1	47.0	50.3	50.3	50.1	49.9	50.3	49.9
12	37.0	36.9	36.1	37.0	36.2	36.5	36.2	36.6
13	75.0	74.9	74.7	74.4	74.2	74.9	74.8	74.3
14	80.1	79.7	79.9	79.2	79.1	79.3	79.4	79.1
15	81.9	81.7	81.5	79.3	78.9	87.3	87.2	79.1
16	91.5	91.0	91.0	90 5	90.3	88.9	88.9	90.4
17	60.5	62.6	62.0	61.0	60.6	60.5	60.8	61.1
18	72.0	79.8	77.4	79.2	77.1	72.1	77.0	71.1
19	49.0	55.6	53.9	53.6	48.8	49.0	48.9	49.1
N-CH_2	47.9	49.6	49.2	49.5	47.2	47.8	47.5	47.5
CH_3	13.3	12.5	12.4	13.7	13.2	13.2	13.1	13.5
1-OCH_3	56.0	56.0	55.3	56 3	55.6	56.0	55.6	56.4
6-OCH_3	58.7	59.1	59.0	59.3	59.0	58.7	59.0	58.4
16-OCH_3	61.3	61.2	61.0	61.4	60.9	61.3	61.3	60.8
18-OCH_3	58.1	58.0	57.8	58.2	57.9	57.7	57.5	58.9
O=C	170.1			172 6	172.2	169.9	173.2	172.5
CH_3	21.0			21.7	21.3	21.0	20.7	21.5
O=C						173.0		170.3
CH_3						20.8		21.3
	166.3	166.3	166.3	166.0	165 9	166.1	166.4	166.2
	132.2	130.0	130.0	130.0	130.2	130.3	130.2	130.1
	129.8*	129.6*	129.9*	129.9*	129.6*	129.8*	129.8*	129.8*
	128.3*	128.4*	128.3*	128.8*	128.5*	128.3*	128.4*	128.4*
	132.9	133.0	132.8	133.4	133.1	132.7	132.9	133.4

* Two carbons

All these spectral data for 1 indicated that polyschistine D should be assigned as 3-acetylbenzoylaconine. Finally, the structure of 1 was further confirmed by the following chemical correlation.

Acetylation of 1 with Ac_2O-Py gave an acetate (6) as a sole product which was also obtained by acetylation of benzoylaconine (3) with Ac_2O. Furthermore, hydrolysis of 3-acetylaconitine (8)[3] with dioxane-H_2O (1 : 1)[4,5] gave a hydrolyzed product which was identical with polyschistine D.

From the results of the chemical reactions mentioned above, the structure of polyschistine D was established as 3-acetylbenzoylaconine (1).

The compound (2) showed the molecular ion peak at m/z 587.3055 (calc. 587.3082, $C_{32}H_{45}NO_9$) in its high resolution mass spectrum. The ^{1}H-nmr spectrum of 2 disclosed the presence of an N-ethyl [δ 1.23 (3H, t, J=7.0 Hz)] and four methoxyls [δ 3.30, 3.33, 3.47 and 3.73 (3H each, s)]. The multiplet signal at δ 7.42-8.10 (5H) and the doublet signal at δ 4.98 (1H, H-14) suggested the presence of a bezoyloxy group to be situated at C-14 position. In comparison of the ^{13}C-nmr spectral data of 2 with those of 3, the chemical shift pattern of 2 is very close to that of 3 except the C-3 signal (36.9 ppm) resonated in the higher field than that (71.0 ppm) of 3 and furthermore, C-2 and C-4 signals were appeared in the slightly higher field than those of 3, thereby suggesting the lack of the C-3 hydroxyl group.

From the above mentioned ms, ^{1}H- and ^{13}C-nmr spectral data, the compound (2) should be benzoyldeoxyaconine which was obtained as a hydrolysis product of lipodeoxyaconine by Kitagawa et al.[5]. The structure of 2 was confirmed by the following chemical reaction[6]. Thus, deoxyaconitine (4) was hydrolyzed with 10% aq. H_2SO_4 to afford a hydrolysis product [mp 230°C, m/z 587 (M^+), $C_{32}H_{45}NO_9$] which was identical with the natural product (2) in comparison of their spectral data. This is the first report on the isolation of benzoyldeoxyaconine (2) from natural sources.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage equipped with a microscope and are uncorrected. Optical rotations were measured on a JASCO DIP-181 digital polarimeter. Mass spectra were recorded on a Hitachi RMU-6M. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL FX-400 and Brucker AC-100 spectrometers,

respectively. High performance liquid chromatography was carried out on an 8 x 300 mm column (Nucleosil 5-NO_2) with a Hitachi pump. Each peak was monitored with a RI detector (Shodex RI SE-11).

Extrtaction and Isolation of Alkaloids

Air dried and powdered roots of *Aconitum polyschistum* Hand-Mazz. (1.4 kg) were extracted with 90% ethanol (7l) by cold percolation. The solvent was evaporated *in vacuo* to give approx. 180 g of concentrates. A half of the residue (90 g) was dissolved in 2% aq. HCl (400 ml). The aquous phase was progressively basified with concentrated ammonia and extracted with $CHCl_3$ to afford the crude alkaloid portions at pH 5 (2.5 g) and at pH 8 (1.5 g), respectively. The pH 5 portion (2.5 g) was subjected to alumina column chromatography and eluted successively with $CHCl_3$, $CHCl_3$-EtOAc (2 : 1), $CHCl_3$-EtOAc (1 : 2), EtOAc and EtOH. Fraction of 300 ml each was collected and checked by tlc. Fractions 11-30 [318 mg, elution with $CHCl_3$-EtOAc (2 : 1)] were further purified by hplc [EtOAc-acetone (1 : 1), flow rate 3 ml/min] to afford polyschistine D (1, 8 mg), benzoylaconine (3, 25 mg) and deoxyaconitine (4, 65 mg), respectively. Fractions 86-171 (173 mg, elution with EtOAc) were further separated by hplc [EtOAc : acetone (1 : 1), flow rate 3 ml/min] to give benzoyldeoxyaconine (2, 13 mg). Fractions 39-85 [50 mg, elution with $CHCl_3$-EtOAc (1 : 2)] were further separated by HPLC [EtOAc-acetone (1 : 1), flow rate 3 ml/min) to afford aconitine (5, 27 mg).

Polyschistine D (1) ——— mp 251-252°C, $[\alpha]_D$ +11.4 ($CHCl_3$), hms : *m/z* 645.3136 (M^+), calc. 645.3146, $C_{34}H_{47}NO_{11}$. ^{1}H-nmr : 1.10 (3H,t, *J*=7.1 Hz, NCH_2CH_3), 2 08 (3H, s, $COCH_3$), 4.92 (1H, dd, *J*=6.1, 12.9 Hz, H-3), 5.02 (1H, d, *J*=5.1 Hz, H-14), 3.26, 3.29, 3.31 and 3.72 (3H each, s, OCH_3), 7.43-8.08 (5H, m, aromatic protons). For the ^{13}C-nmr data see Table 1.

Benzoyldeoxyaconine (2) ——— mp 232-234°C, $[\alpha]_D$ -15.4 ($CHCl_3$), hms : *m/z* 587.3055 (M^+), calc. 587.3092, $C_{32}H_{45}NO_9$. ^{1}H-nmr : 1.23 (3H, t, *J*=7.0 Hz, NCH_2CH_3), 3.30, 3.33, 3.47 and 3.73 (3H each, s, OCH_3), 4.98 (1H, d, *J*=5.1 Hz, H-14), 7.42-8.10 (5H, m, aromatic protons). For the ^{13}C-nmr data see Table 1.

Benzoylaconine (3) ——— hms : *m/z* 603.3048 (M^+), calc. 603.3041, $C_{32}H_{45}NO_{10}$. ^{1}H-nmr : 1.41 (3H, t, *J*=7.1 Hz, NCH_2CH_3), 3.28, 3.36, 3.46 and 3.72 (3H each, s, OCH_3), 4.98 (1H, d, *J*=5.1 Hz, H-14), 7.45-8.07 (5H, m, aromatic protons). Comparison of tlc, ^{1}H-nmr and ^{13}C-nmr of 3 with those of an authentic sample of benzoylaconine showed them to be identical.

Aconitine (5) mp 198-200°C, fdms : m/z 645 (M^+), $C_{34}H_{45}NO_{12}$. ^{1}H-nmr : 1.09 (3H, t, J=7.1 Hz, NCH_2CH_3), 1.39 (3H, s, $COCH_3$), 3.17, 3.27, 3.30 and 3.76 (3H each, s, OCH_3), 7.44-8.04 (5H, m, aromatic protons). Comparison of tlc, mp, ^{1}H-nmr and ^{13}C-nmr data of 5 with those of an authentic sample of aconitine showed them to be identical.

Preparation of Polyschistine D (1) from 3-Acetylaconitine (8) ——A mixture of 3-acetylaconitine (110 mg), dioxane (2 ml) and water (2 ml) was heated under reflux for 1 h. The solvent was evaporated *in vacuo*. The residue was diluted with 1% aq. ammonia (6 ml) and extracted with CH_2Cl_2 (10 ml) to afford a white solid (105 mg) which showed two spot on tlc. It was separated on a preparative tlc plate [silica gel, solvent : hexane-EtOAc-diethylamine (10 : 10: 1)] to give polyschistine D (1, Rf=0.53) as crystalline needles (83 mg from EtOAc, mp 253°C) which was identical with the natural product in comparison of mp, tlc, ms, ^{1}H- and ^{13}C-nmr.

Hydrolysis of Deoxyaconitine (4) ——Deoxyaconitine (125 mg) was heated with 10% sulfuric acid (2 ml) in a sealed tube on a steam bath for 6h. The solution was basified (pH 8) with concentrated ammonia and extracted with CH_2Cl_2 (3 x 4 ml). The solvent was removed to afford a solid which showed two spots on tlc. It was separated on a preparative tlc plate [silica gel, solvent : acetone-petroleum ether (2 : 1)] to give benzoyldeoxyaconine (2, Rf=0.11) as a crystalline solid (35 mg, mp 232°C) which was identical with the natural product in comparison of mp, tlc, ^{1}H- and ^{13}C-nmr.

Acetylation of Benzoylaconine (3) ——Benzoylaconine (145 mg) was acetylated with acetic anhydride (2 ml) at room temperature for 6h. The solvent was evaporated *in vacuo* to give a white solid which showed three spots on tlc plate. The solid was separated on a preparative tlc plate [silica gel, solvent : hexane-EtOAc-diethylamine (6 : 4 : 1)]. The band (Rf=0.67) was cut and extracted with CH_2Cl_2-methanol to afford 3,15-diacetylbenzoylaconine (6)[7] as a crystalline solid (72 mg from ethanol). 6 : mp 203-204°C, $[\alpha]_D$ -10.4 ($CHCl_3$), ms : m/z 687 (M^+), calc. for $C_{36}H_{49}NO_{12}$. ^{1}H-nmr : 1.15 (3H,t, J=7.1 Hz, NCH_2CH_3), 2.06 (3H, s, $COCH_3$), 2.18 (3H, s, $COCH_3$), 4.92 (1H, dd, J=6.1, 12.9 Hz, H-3), 4.94 (1H, d, J=5.1 Hz, H-14), 3.21, 3.24, 3.26 and 3.61 (3H each, s, OCH_3), 5.41 (1H, d, J=6.4 Hz, H-15), 7.42-8.06 (5H, m, aromatic protons). For the ^{13}C-nmr data see Table 1. The band (Rf=0.53) was cut and extracted with CH_2Cl_2-methanol to afford 15-acetylbenzoylaconine (7) as a crystalline solid (35 mg from $CHCl_3$-ether). 7 :

mp 183-185°C, $[\alpha]_D$ -16.2 ($CHCl_3$), ms : m/z 645 (M^+), calc. for $C_{34}H_{45}NO_{11}$. ^{1}H-nmr : 1.35 (3H, t, J=7.1 Hz, NCH_2CH_3), 2.16 (3H, s, $COCH_3$), 3.18, 3.21, 3.26 and 3.64 (3H each, s, OCH_3), 4.86 (1H, d, J=5.2 Hz, H-14), 5.40 (1H, d, J=6.4 Hz, H-15), 7.36-7.96 (5H, m, aromatic protons). For the ^{13}C-nmr data see Table 1.

Acetylation of Polyschistine D (1) —— Polyschistine D (4.5 mg) was acetylated with acetic anhydride (0.5 ml) and pyridine (3 drops) at room temperature for overnight. The solvent was removed *in vacuo* to give an acetate which was identical with 3,15-diacetylbenzoylaconine (6) in comparison of tlc and ^{1}H-nmr.

REFERENCES AND NOTES

* To whom correspondence should be addressed.

ψ Present address: Faculty of Education, Shinshu University, Nishinagano, Nagano 380, Japan.

§ Present address: Department of Chemistry, Faculty of Sciences, Science University of Tokyo, 1-3 Kagurazaka, Shinjuku, Tokyo 162, Japan.

1. Hongcheng Wang, Aina Lao, Y. Fujimoto, and T. Tatsuno, *Heterocycles*, 23, 803 (1985).
2. The ^{1}H- (400 MHz, δ) and ^{13}C-nmr (100 MHz, δ) data in the text were taken in $CDCl_3$ containing TMS as an internal standard.
3. X. Chang, H. Wang, L. Liu, Y. Zhu, and R. Zhu, *Acta Pharmaceutica Sinica*, 16, 474 (1981).
4. H. Sato, C. Yamada, C. Konno, Y. Ohizumi, K. Endo, and H. Hikino, *Tohoku, J. Exp. Med.*, 128, 175 (1979).
5. I. Kitagawa, Z. L. Chen, M. Yoshihara, and M. Yoshikawa, *Yakugaku Zasshi*, 104, (8), 848 (1984).
6. H. Wang, Y. Gao, R. Xu and R. Zhu, *Acta Chimica Sinica*, *39*, *869* (*1981*).
7. W. R. Dunstan first obtained 3,15-diacetylbenzoylaconine (6) by acetylation of benzoylaconine, but the published structure was erroneous. W. R. Dunstan and F. H. Carr, *J. Chem. Soc.*, 67, 459 (1985).

Received, 26th October, 1987