

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[†], and
Takashi Tatsuno[‡]

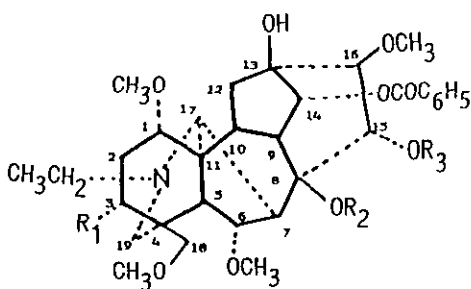
The Institute of Physical and Chemical Research, Wako-Shi,
Saitama 351-01, Japan

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 paper¹, we have reported the isolation and structural elucidation of three new C₁₉ 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, C₃₄ H₄₇ NO₁₁) in its high resolution mass spectrum. The ¹H-nmr spectrum² 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 acetoxy and benzyloxy 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 spectrum² of 1 was compared with those of benzoyleaconine (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) $R_1=OCOCH_3$, $R_2=COCH_3$, $R_3=H$

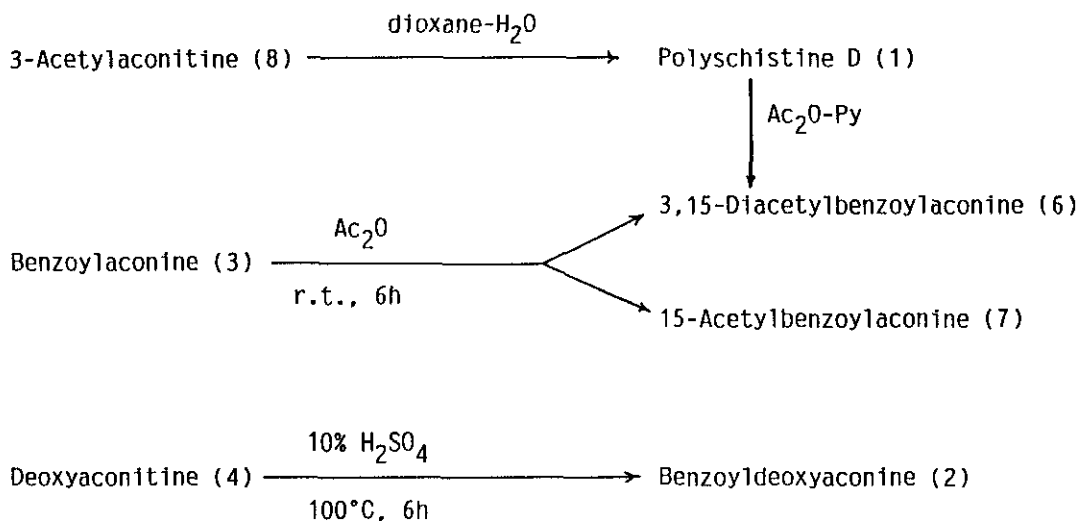


Table 1

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

carbon	compound							
	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 Ac₂O-Py gave an acetate (6) as a sole product which was also obtained by acetylation of benzoylaconine (3) with Ac₂O. Furthermore, hydrolysis of 3-acetylaconitine (8)³ with dioxane-H₂O (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, C₃₂ H₄₅ NO₉) in its high resolution mass spectrum. The ¹H-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 benzoyloxy group to be situated at C-14 position. In comparison of the ¹³C-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, ¹H- and ¹³C-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 reaction⁶. Thus, deoxyaconitine (4) was hydrolyzed with 10% aq. H₂SO₄ to afford a hydrolysis product [mp 230°C, *m/z* 587 (M⁺), C₃₂ H₄₅ NO₉] 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. ¹H- and ¹³C-nmr spectra were recorded on JEOL FX-400 and Bruker AC-100 spectrometers,

respectively. High performance liquid chromatography was carried out on an 8 x 300 mm column (Nucleosil 5-NO₂) with a Hitachi pump. Each peak was monitored with a RI detector (Shodex RI SE-11).

Extraction 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 aqueous phase was progressively basified with concentrated ammonia and extracted with CHCl₃ 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 CHCl₃, CHCl₃-EtOAc (2 : 1), CHCl₃-EtOAc (1 : 2), EtOAc and EtOH. Fraction of 300 ml each was collected and checked by tlc. Fractions 11-30 [318 mg, elution with CHCl₃-EtOAc (2 : 1)] were further purified by hplc [EtOAc-acetone (1 : 1), flow rate 3 ml/min] to afford polyschistine D (1, 8 mg), benzoyleaconine (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 CHCl₃-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, $[\alpha]_D^{25} +11.4$ (CHCl₃), hms : m/z 645.3136 (M⁺), calc. 645.3146, C₃₄H₄₇NO₁₁. ¹H-nmr : 1.10 (3H, t, $J=7.1$ Hz, NCH₂CH₃), 2.08 (3H, s, COCH₃), 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, OCH₃), 7.43-8.08 (5H, m, aromatic protons). For the ¹³C-nmr data see Table 1.

Benzoyldeoxyaconine (2) — mp 232-234°C, $[\alpha]_D^{25} -15.4$ (CHCl₃), hms : m/z 587.3055 (M⁺), calc. 587.3092, C₃₂H₄₅NO₉. ¹H-nmr : 1.23 (3H, t, $J=7.0$ Hz, NCH₂CH₃), 3.30, 3.33, 3.47 and 3.73 (3H each, s, OCH₃), 4.98 (1H, d, $J=5.1$ Hz, H-14), 7.42-8.10 (5H, m, aromatic protons). For the ¹³C-nmr data see Table 1.

Benzoyleaconine (3) — hms : m/z 603.3048 (M⁺), calc. 603.3041, C₃₂H₄₅NO₁₀. ¹H-nmr : 1.41 (3H, t, $J=7.1$ Hz, NCH₂CH₃), 3.28, 3.36, 3.46 and 3.72 (3H each, s, OCH₃), 4.98 (1H, d, $J=5.1$ Hz, H-14), 7.45-8.07 (5H, m, aromatic protons). Comparison of tlc, ¹H-nmr and ¹³C-nmr of 3 with those of an authentic sample of benzoyleaconine showed them to be identical.

Aconitine (5) mp 198-200°C, fdms : m/z 645 (M^+), $C_{34}H_{45}NO_{12}$. 1H -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, 1H -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, $R_f=0.53$) as crystalline needles (83 mg from EtOAc, mp 253°C) which was identical with the natural product in comparison of mp, tlc, ms, 1H - 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, $R_f=0.11$) as a crystalline solid (35 mg, mp 232°C) which was identical with the natural product in comparison of mp, tlc, 1H - 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 ($R_f=0.67$) 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 $C_{36}H_{49}NO_{12}$. 1H -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 ($R_f=0.53$) 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 $\text{C}_{34}\text{H}_{45}\text{NO}_{11}$. ^1H -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 ^1H -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.
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