A new C₁₉-diterpenoid alkaloid from the roots of *Aconitum duclouxii*

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A new C₁₉-diterpenoid alkaloid, ducloudine F (1), was obtained from the roots of *Aconitum duclouxii*, along with eight known alkaloids (2–9) isolated from this species for the first time. Their structures were established on the basis of extensive spectroscopic analyses. The antimicrobial activities of these compounds were investigated.

**Keywords:** *Aconitum duclouxii*; Ranunculaceae; C₁₉-diterpenoid alkaloid; ducloudine F; antimicrobial

1. **Introduction**

*Aconitum duclouxii* Levl. (Ranunculaceae), a perennial herb distributed in Dali Bai Autonomous Prefecture, Yunnan Province of China, has long been used as a folk medicine to treat rheumatism and pains (People’s Government of Dali Bai Autonomous Prefecture 1991). Phytochemical research on it was so limited that only two alkaloids, aconitine and duclouxine, have been isolated from this plant (Wang et al. 1984). In our previous articles, we have reported seven diterpenoid alkaloids isolated from *A. duclouxii* (Yin et al. 2013, 2014). During our further phytochemical investigation on this plant, a new C₁₉-diterpenoid alkaloid, named ducloudine F (1), was obtained, together with eight known alkaloids, N-deethylaconitine (2) (Arlandini et al. 1987), deoxyaconitine (3) (Zhang et al. 2013), takaonine (4) (Sakai et al. 1979), benzoylaconine (5) (Jampani & Alfred 1993), 14-benzoylpseudaconine (6) (Khetwal & Pande 2004), crassicaulidine (7) (Wang & Liang 1985), talatisamine (8) (Pelletier et al. 1985) and chasmanine (9) (Gao et al. 2012) (Figure 1), which were isolated from this species for the first time. Their structures were established on the basis of extensive spectroscopic analyses (1D and 2D NMR, MS, IR, X-ray crystallographic analysis). These nine compounds were tested for their antifungal activities against *Candida albicans* and antibacterial activities against *Escherichia coli* and *Bacillus subtilis*. In this article, the isolation, structural elucidation and biological activity of compound 1 are described. In addition, the complete NMR data of compound 2 and the X-ray crystallographic data of compound 3 are also reported in this article for the first time.

2. **Results and discussion**

Compound 1 was isolated as a white amorphous powder and its molecular formula was deduced to be C₂₄H₃₆NO₆ by HR-ESI-MS at *m/z* 434.2515 [M + H]⁺. The IR spectrum indicated the
presence of an OH group (3428 cm$^{-1}$), a keto-carbonyl (1730 cm$^{-1}$) and a C–C bond (1675 cm$^{-1}$). Inspection of the NMR data indicated an aconitine-type C$_{19}$-diterpenoid alkaloid (Pelletier et al. 1984). The NMR spectra displayed signals of an N-ethyl group ($\delta$H 1.04, t, $J = 7.2$ Hz; 2.33, 2.47, each 1H, m; $\delta$C 12.9, q; 48.6, t), a keto-carbonyl ($\delta$C 216.2, s) and a characteristic disubstituted C–C bond ($\delta$H 5.80, 1H, d, $J = 10.0$ Hz; 5.90, 1H, dd, $J = 10.0$ Hz, 4.8 Hz; $\delta$C 131.8, d; 134.3, d). The $^{13}$C NMR spectrum revealed five oxygenated carbons at $\delta$C 70.5, d; 76.0, t; 80.1, s; 82.3, d; 87.1, d, corresponding to the molecular formula, indicating the presence of two hydroxyl groups and three OMe groups. Three OMe groups (δH 3.27, 3.29, 3.30, each 3H, s; $\delta$C 56.0, 57.2, 59.3, q) were located at C-6, C-16 and C-18, respectively, on the basis of the HMBC correlations from OCH$_3$-6 ($\delta$H 3.30, s) to C-6 ($\delta$C 82.3, d), OCH$_3$-16 ($\delta$H 3.27, s) to C-16 ($\delta$C 87.1, d), OCH$_3$-18 ($\delta$H 3.29, s) to C-18 ($\delta$C 76.0, t) (Figure S1). A hydroxyl group should be located at C-8 according to the HMBC correlation between OH-8 ($\delta$H 4.36, s) and C-8 ($\delta$C 80.1, s), which was further confirmed by the HMBC correlations observed from H-15, H-6 and H-9 to C-8. The other hydroxyl group was located at C-1 on the basis of the COSY correlation between H-1 and H-2, while the $\alpha$-orientation of OH-1 was confirmed in the NOESY spectrum by the correlation between the H-1 (δH 3.89) and H-10 (δH 2.18) (Pelletier & Djarmati 1976; Peng et al. 2005). The HMBC correlations between H-1 to C-2, H-1 to C-3, H-2 to C-4 and H-19 to C-3, demonstrated that the C–C bond is between C-2 and C-3 (Chen et al. 2009). The keto-carbonyl is located at C-14, according to the HMBC correlations from H-9, H-10 and H-13 to C-14. All signals in the $^1$H ($^{13}$C) spectra of compound 1 were assigned based on its 2D NMR spectra. Therefore, the structure of ducloudine F (1) was determined.

On the basis of the detailed NMR investigation ($^1$H, $^{13}$C, COSY, HMBC, HMQC and NOESY NMR experiments), accurate $^{13}$C NMR and detailed $^1$H NMR assignments for compound 2 were accomplished for the first time. Meanwhile, compound 3 yielded a crystal suitable for X-ray analysis, and its crystallographic study was conducted and reported for the first time (Figure 2).

A pathogenic fungus (C. albicans) and two pathogenic bacteria (E. coli and B. subtilis) were selected for antifungal and antibacterial assays, respectively. The results indicated that compound 1 exhibited antibacterial activity against B. subtilis with an MIC (Minimum Inhibitory Concentration) of 147.73 µmol/L and compound 2 exhibited antifungal activity against C. albicans with an MIC of 51.84 µmol/L. However, other compounds did not exhibit significant antimicrobial activities (MIC > 200 µmol/L).
3. Experimental procedures

3.1. General procedure

Melting points were determined on an XRC-1 Melting Point Apparatus (Sichuan University Science Instrument Co., Chengdu, China) and uncorrected. A Shimadzu UV–Vis 2550 spectrometer (Shimadzu, Kyoto, Japan) was used for UV spectra. Optical rotations were measured with a Jasco P-1020 digital polarimeter (JASCO, Tokyo, Japan). A Nicolet Magna-IR 550 spectrometer (Thermo Nicolet Co., Madison, WI, USA) was used for scanning IR spectroscopy with KBr pellets. NMR spectra were recorded on Bruker AM-400 spectrometers (Bruker, Karlsruhe, Germany) using TMS as the internal reference. ESI-MS analyses were recorded with Agilent G3250AA (Agilent, Santa Clara, CA, USA) and Auto Spec Premier P776 spectrometer (Waters, Milford, MA, USA). Silica gel (200–300 mesh, Qingdao Marine Chemical Group Co., Qingdao, China) was used for column chromatography (CC). Fractions were monitored by using TLC and visualised by spraying with modified Dragendorff’s reagent.

3.2. Plant material

The roots of *A. duclouxii* were collected from Dali Bai Autonomous Prefecture, Yunnan Provinces of China, in December 2012. The samples were identified by Professor Shu-Gang Lu, School of Life Sciences, Yunnan University, Kunming, China. The voucher specimen has been deposited at the Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education, Kunming, China (herbarium no. 2012-bcw-1).

3.3. Extraction and isolation

The air-dried and powdered roots (6.5 kg) of *A. duclouxii* were percolated with 0.5% HCl (45 L). The aqueous acidic solution was basified with 10% aqueous NH₃·H₂O to pH 9–10 and then extracted with AcOEt (3 × 15 L). Removal of the solvent under reduced pressure afforded the total crude alkaloids (51.5 g) as yellowish amorphous powder. The total alkaloids were chromatographed over SiO₂-column eluting with a step chloroform–methanol (100:1 to 1:1) gradient system to yield 13 fractions (A–M). Fraction A (0.5 g) was further subjected to CC [petroleum ether (PE)–acetone–diethylamine, 100:1:1 to 100:10:1] to yield ducloudine F (1) (8.5 mg) and takaonine (4) (5.0 mg). Fraction D (2.5 g) was further subjected to CC (PE–acetone–diethylamine, 100:5:1 to 100:20:1) to yield talatisamine (8) (5.5 mg). Further normal-phased CC purification of fraction F (3.6 g) was accomplished by elution with a PE–acetone–diethylamine (100:5:1 to 100:50:1) to afford benzoylaconine (5) (650.5 mg) and chasmanine (9).
(1.0 mg), deoxyaconitine (3) (1235.0 mg). Fraction G (2.8 g) was further subjected to CC (PE–acetone–diethylamine, 100:5:1 to 100:20:1) to yield crassicaulidine (7) (11.0 mg) and 14-benzoylpsuedaconitine (6) (18.0 mg). Fraction H (1.1 g) was chromatographed over SiO2-column with chloroform–methanol (30:1 to 8:1) to provide N-deethyloaconitine (2) (75.5 mg).

3.4. Duclaudine F (1)

White amorphous powder. mp. 125–127°C, [α]D0 + 35.30 (c = 1.5, CHCl3). IR (KBr) νmax: 3428, 2930, 2810, 1730, 1675, 1450. HR-ESI-MS m/z: [M + H]+ calcd for C24H36NO6 434.2543, found: m/z 434.2515. 1H NMR (400 MHz, CDCl3) δ, ppm: 3.89 (1H, d, J = 4.0 Hz, H-1), 5.90 (1H, dd, J = 10.0 Hz, 4.8 Hz, H-2), 5.80 (1H, d, J = 10.0 Hz, H-3), 3.04 (1H, m, H-5), 3.92 (1H, m, H-6), 2.05 (1H, s, H-7), 2.78 (1H, m, H-9), 2.18 (1H, m, H-10), 2.14 (1H, m, H-12), 2.44 (1H, m, H-12), 2.33 (1H, m, H-13), 1.54 (1H, dd, J = 11.2 Hz, 8.8 Hz, H-15), 2.05 (1H, dd, J = 11.2 Hz, 8.8 Hz, H-15), 3.61 (1H, d, J = 7.2 Hz, H-16), 3.12 (1H, br s, H-17), 3.09 (1H, ABq, J = 8.8 Hz, H-18a), 3.34 (1H, ABq, J = 8.8 Hz, H-18b), 1.73 (1H, ABq, J = 11.2 Hz, H-19), 2.30 (1H, ABq, J = 11.2 Hz, H-19), 2.33 (1H, m, H-21), 2.47 (1H, m, H-21), 1.04 (3H, t, J = 7.2 Hz, H-22), 3.30 (3H, s, OCH3-6), 3.27 (3H, s, OCH3-16), 3.29 (3H, s, OCH3-18). 13C NMR (100 MHz, CDCl3) δ, ppm: 70.5 d (C-1), 131.8 d (C-2), 134.3 d (C-3), 39.3 s (C-4), 48.5 d (C-5), 82.3 d (C-6), 48.5 d (C-7), 80.1 s (C-8), 54.2 d (C-9), 41.7 d (C-10), 48.3 s (C-11), 26.1 t (C-12), 47.1 d (C-13), 216.2 s (C-14), 40.8 t (C-15), 87.1 d (C-16), 64.6 d (C-17), 76.0 t (C-18), 52.2 t (C-19), 48.6 t (C-21), 12.9 q (C-22), 57.2 q (OCH3-6), 56.0 q (OCH3-16), 59.3 q (OCH3-18).

3.5. N-deethyloaconitine (2)

White amorphous powder. HR-ESI-MS m/z: [M + H]+ calcd for C32H44NO11 618.2914, found: m/z 618.2904. 1H NMR (400 MHz, CDCl3) δ, ppm: 3.10 (1H, m, H-1), 2.50 (1H, m, H-2), 1.88 (1H, m, H-2), 3.76 (1H, m, H-3), 2.04 (1H, d, J = 4.8 Hz, H-5), 4.02 (1H, d, J = 4.8 Hz, H-6), 2.70 (1H, m, H-7), 2.78 (1H, m, H-9), 1.98 (1H, m, H-10), 2.63 (1H, m, H-12), 2.02 (1H, m, H-12), 4.83 (1H, d, J = 5.0 Hz, H-14), 4.44 (1H, m, H-15), 3.30 (1H, t, J = 6.4 Hz, H-16), 3.25 (1H, m, H-17), 3.58 (1H, ABq, J = 8.0 Hz, H-18), 3.51 (1H, ABq, J = 8.0 Hz, H-18), 2.94 (1H, m, H-19), 2.30 (1H, ABq, J = 11.2 Hz, H-19), 3.30 (3H, s, OCH3-6), 3.27 (3H, s, OCH3-16), 3.29 (3H, s, OCH3-18), 1.31 (3H, s, OAc-8), 7.99 (2H, d, J = 8.0 Hz, 2’, 6’), 7.53 (1H, t, J = 8.0 Hz, 4’), 7.43 (2H, t, J = 8.0 Hz, 3’, 5’). 13C NMR (100 MHz, CDCl3) δ, ppm: 81.3 d (C-1), 34.9 t (C-2), 70.7 d (C-3), 43.8 s (C-4), 46.9 d (C-5), 83.3 d (C-6), 50.8 d (C-7), 91.4 s (C-8), 43.3 d (C-9), 40.6 d (C-10), 49.2 s (C-11), 34.8 t (C-12), 74.1 s (C-13), 78.8 d (C-14), 78.9 d (C-15), 89.7 d (C-16), 53.9 d (C-17), 77.1 t (C-18), 40.6 t (C-19), 55.1 q (OCH3-1), 57.7 q (OCH3-6), 61.1 q (OCH3-16), 59.1 q (OCH3-18), 172.1 s (OAc-8), 21.3 q (OAc-8), 166.0 s (OCO-14), 129.7 s (1’), 129.6 d (2’, 6’), 128.6 d (3’, 5’), 133.3 d (4’).

3.6. Bioassay for antifungal and antibacterial activities

Antifungal and antibacterial assays were performed in 96-well sterilised microplates using a microdilution method described previously (Tian et al. 2013). The 4-day-old spores from C. albicans were transferred to sterile PDB (Potato Dextrose Broth) medium, and the test concentration was 1 × 10^5 spores/mL. The 18-h-old bacterial cultures from E. coli and B. subtilis were added to sterile LB (Luria-Bertani) broth medium to reach 1 × 10^5 colony-forming units/mL. The test compounds were dissolved in DMSO, and their final concentrations ranged from 512 to 0.5 μg/mL by using a twofold serial dilution method. The final concentration of DMSO did not exceed 5%. The wells containing test strains and diluted compounds were incubated at
28°C (4 days) for fungi and 37°C (24 h) for bacteria. The wells containing a culture suspension and DMSO were run as negative controls. Nystatin (Taicheng Pharmaceutical Co., Ltd, Guangdong, China) and kanamycin (Yunke Biotechnology, Kunming, China) were introduced in the experiments as positive controls for antifungal and antibacterial assays, respectively. All experiments were repeated three times. The growth of test strains was observed with a CX21BIM-set5 microscope (Olympus Corp., Tokyo, Japan). MICs were determined as the lowest concentrations that produced complete growth inhibition of the tested microorganisms.

3.7. X-ray analysis and crystal data of deoxyaconitine

A yellow crystal was obtained from a solution of CHCl₃–MeOH. All crystallographic data were collected on a Bruker Smart AXS CCD diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). Empirical absorption corrections were carried out using the SADABS program. The structures were solved by using direct methods and refined on F² by full-matrix least-squares technique using SHELXL-97 software. All the non-hydrogen atoms were located in difference Fourier syntheses and finally refined with anisotropic displacement parameters. Crystallographic data in CIF (Crystallographic Information Files) format of compound 3 (CCDC No. 96569) are available free of charge via www.ccdc.cam.ac.uk/services/structure_deposit/ (or from Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033).

C₃₄H₄₇NO₁₀; F.W. 629.73; monoclinic space group C2/c; unit cell dimensions a = 12.0535 (11) Å, b = 15.8109(15) Å, c = 17.3370(16) Å, V = 3304.0(5) Å³; α = 90°, β = 90°, γ = 90°, Z = 4, dcalc = 1.266 mg/m³, crystal dimensions 0.46 mm × 0.38 mm × 0.36 mm, μ = 0.09 mm⁻¹, F(000) = 1352. The 18842 measurements yielded 5912 independent reflections after equivalent data were averaged, and Lorentz and polarisation corrections were applied. The final refinement gave R₁ = 0.053 and wR₂ = 0.160 [I > 2σ (I)].

4. Conclusion

The phytochemical investigation on A. duclouxii, a species in series Bullatifolia (Ranunculaceae), revealed that A. duclouxii contains almost all aconitine-type C₁₉-diterpenoid alkaloids. This suggested that A. duclouxii might occupy a somewhat advanced position in series Bullatifolia on the basis of a chemotaxonomic view (Xiao et al. 2006).

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S7.

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Note

1. These authors contributed equally to this study.

References

Arlandini E, Ballabio M, Gioia B, Bugatti C, Colombo ML, Tome F. 1987. N-deethylaconitine from Aconitum napellus ssp. vulgare. J Nat Prod. 50:937–939.
Chen FZ, Chen DL, Chen QH, Wang FP. 2009. Diterpenoid alkaloids from Delphinium majus. J Nat Prod. 72:18–23.
Gao F, Li YY, Wang D, Huang X, Liu Q. 2012. Diterpenoid alkaloids from the Chinese traditional herbal ‘Fuzi’ and their cytotoxic activity. Molecules. 17:5187–5194.
Jampani BH, Alfred K. 1993. Isolation and identification of four norditerpenoid alkaloids from processed and unprocessed roots tubers of Aconitum ferox. J Nat Prod. 56:801–809.
Khetwal KS, Pande S. 2004. Constitutens of high altitude Himalayan herbs part XV: a new norditerpenoid alkaloid from the roots of Aconitum balfourii. Nat Prod Res. 18:129–133.
Pelletier SW, Djarmati Z. 1976. Carbon-13 nuclear magnetic resonance: aconitine-type diterpenoid alkaloids from Aconitum and Delphinium species. J Am Chem Soc. 98:2626–2636.
Pelletier SW, Mody NV, Joshi BS, Schram LC. 1984. Alkaloids: chemical and biological perspectives. New York, NY: Wiley; p. 206–462.
Pelletier SW, Srivastava SK, Joshi BS, Olsen JO. 1985. Alkaloids of Aconitum columbianum Nutt. Heterocycles. 23:331–338.
Peng CS, Chen DL, Chen QH, Wang FP. 2005. New diterpenoid alkaloids from roots of Aconitum sinomontanum. Chin J Org Chem. 25:1235–1239.
People’s Government of Dali Bai Autonomous Prefecture. 1991. The natural resources of Chinese medicinal materials of Dali. Kunming: Yunnan Ethnic Publishing House; p. 77.
Sakai S, Takayama H, Okamoto T. 1979. On the alkaloids of Aconitum japonicum Thunb. collected at Mt. Takao. J Pharm Soc Jpn. 99:647–656.
Tian SZ, Pu X, Luo G, Zhao LX, Xu LH, Li WJ, Luo Y. 2013. Isolation and characterization of new p-Terphenyls with antifungal, antibacterial and antioxidant activities from halophilic actinomycte Nocardiopsis gilva YIM 90087. J Agric Food Chem. 61:3006–3012.
Wang CY, Chen JB, Zhu RH. 1984. Studies on the alkaloids of Aconitum duclouxii Levl and their chemical structures. Acta Pharm Sin. 19:445–449.
Wang FP, Liang XT. 1985. Structures of crassicauline B and crassicaulidine. Planta Med. 51:443–444.
Xiao PG, Wang FP, Gao F, Yan LP, Chen DL, Liu Y. 2006. A pharmacophylogenetic study of Aconitum L. (Ranunculaceae) from China. Acta Phytotax Sin. 44:1–46.
Yin TP, Cai L, Lei G, Dong JW, Liu YX, Ding ZT. 2013. Two new diterpenoid alkaloids from the roots of Aconitum duclouxii Levl. Chin J Org Chem. 33:2528–2532.
Yin TP, Cai L, Lei G, He JM, Dong JW, Fang HX, Zhou H, Ding ZT. 2014. Three new diterpenoid alkaloids from the roots of Aconitum duclouxii. J Asian Nat Prod Res. 16:345–350.
Zhang ZT, Wang L, Chen QF, Chen QH, Chen DL, Liu XY, Wang FP. 2013. Revisions of the diterpenoid alkaloids reported in a JNP paper (2012, 75:1145–1159). Tetrahedron. 69:5859–5866.