A new $C_{20}$-diterpenoid alkaloid from *Aconitum soongaricum* var. *pubescens*

Lin Chen$^{a,b,c}$, Lianhai Shan$^a$, Wenliang Xu$^a$, Jifa Zhang$^a$, Shuai Huang$^{a,†}$ and Xianli Zhou$^{a,b,†}$

$^a$School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, China; $^b$Key Laboratory of Advanced Technologies of Materials, Ministry of Education, School of Material Science and Engineering, Southwest Jiaotong University, Chengdu, China; $^c$School of Chemistry and Chemical Engineering, China West Normal University, Nanchong, China

**ABSTRACT**

A new denudatine-type $C_{20}$-diterpenoid alkaloid, pubesine (1), along with seven known diterpenoid alkaloids, altaconitine (2), 14-benzoylaconine (3), spicatine A (4), 14-benzoylaconine-8-palmitate (5), 14-O-acetylsenbusine A (6), senbusine A (7) and 14-acetylneoline (8) were isolated from the whole plant of *Aconitum soongaricum* var. *pubescens*. Their structures were elucidated by means of extensive spectroscopic analyses (NMR and HR-ESI-MS) and comparison with data reported in the literature. All compounds were evaluated for their cytotoxicity against H460, MCF-7 and Hep G2 human cancer cell lines.

**1. Introduction**

*Aconitum soongaricum* var. *pubescens* (Ranunculaceae) is a perennial herb distributed mainly in the eastern part of Xinjiang of China, Kashmir and other places. The plant is used as a folk remedy for the treatment of arthritic and rheumatic pain (Wang & Warnock 1979).

In the course of our earlier study, four $C_{20}$-diterpenoid alkaloids and six $C_{19}$-diterpenoid alkaloids had been obtained from roots of this plant (Chen et al. 2015). Pharmacological studies demonstrated that diterpenoid alkaloids were the effective components in the *Aconitum* genus (Liu & Katz 1994; Khetwal & Pande 2004; Wang et al. 2010; Yin et al. 2014; Shyaula et al. 2015). To find more biologically active substances, the whole plant of...
A. soongaricum was phytochemically investigated to afford one new denudatine-type C_{20}-diterpenoid alkaloids, named pubesine (1), together with seven known diterpenoid alkaloids, as altaconitine (2) (Batbayar et al. 1993), 14-benzoylaconine (3) (Wang & Chen 2010), spicatine A (4) (Gao et al. 2006), 14-benzoylaconine-8-palmitate (5) (Bai et al. 1994), 14-O-acetyl senbusine A (6) (Hanuman & Katz 1994), senbusine A (7) (Konno et al. 1982) and 14-acetylneoline (8) (de la Fuente et al. 1989; Figure 1). In this paper, we report the isolation and structure elucidation of these diterpenoid alkaloids, as well as their cytotoxic activities against H460, MCF-7 and Hep G2 human cancer cell lines.

2. Result and discussion

Compound 1 was obtained as a white amorphous solid. Its molecular formula was determined as C_{23}H_{31}NO_{5} by analysis of its HR-ESI-MS spectrum (m/z 402.2279 [M + H]^+ , calcd. for C_{23}H_{32}NO_{5} 402.2280). The IR spectrum showed absorption bands for hydroxyl group (3379 cm\(^{-1}\)) and carbonyl group (1677 cm\(^{-1}\)). The \(^1\)H NMR spectrum showed the presence of a typical N-methyl group (\(\delta_H 2.41\), s, 3H), an acetyl group (\(\delta_H 2.06\), s, 3H) and a tertiary methyl group (\(\delta_H 0.80\), s, 3H). The \(^{13}\)C NMR and DEPT spectra of 1 exhibited the presence of six methylenes (\(\delta_C 23.5, 24.6, 24.7, 29.7, 35.8\) and 45.7), nine methines (\(\delta_C 38.3, 38.6, 45.8, 49.4, 68.3, 70.4, 71.1, 76.8\) and 94.4) and four quaternary carbons (\(\delta_C 37.7, 45.3, 49.7\) and 64.1). The above-mentioned data suggested that compound 1 is a denudatine-type C_{20}-diterpenoid alkaloid (Shen et al. 2009). Selected \(^1\)H and \(^{13}\)C NMR resonances of 1 indicated the characteristic pattern of an epoxy group (\(\delta_H 3.10, 2.42,\) ABq, \(J = 5.4\) Hz; \(\delta_C 64.1\) s, 45.7 t) instead of a typical exocyclic double bond in C_{20}-diterpenoid alkaloids. The NMR signals at \(\delta_H 4.03\) (1H, d, \(J = 5.4\) Hz, H-1β), 3.58 (1H, br s, H-19), 68.3 (d, C-1) and 94.4 (d, C-19) strongly indicated the presence of a C-1-C-19 carbinolamine inner ether (Takayama et al. 1991) in the new alkaloid. Moreover, correlations observed in the HMBC experiment between the signal at \(\delta_H 3.58\) (1H, br s, H-19) with \(\delta_C 68.3\) (d, C-1), 49.4 (d, C-5), 18.7 (q, C-18), 70.4 (d, C-20) and 41.8 (q, C-21) confirmed the C-1-C-19 carbinolamine inner ether linkage (Figure S10). Comparison of the NMR data of 1 with those of gomandonine-13-O-acetate (Kulathaivel & Benn 1988) revealed that they have the same molecular skeleton, except for the presence of a C-1-C-19 carbinolamine inner ether in the compound 1. The difference of 2 mass units in their

![Figure 1. Structures of compounds 1–8.](image-url)
molecular masses also supported the above mentioned difference. The complete planar structure of 1 was further verified by analysis of the HMBC and \(^1\text{H}-^1\text{H}\) COSY spectra (Figure S10).

The relative configuration was deduced from the ROESY spectrum (Figure S10). The hydrogen group at C(1) was assigned an \(\beta\)-orientation based on the signal of H-1 at \(\delta_H 4.03\) (d, \(J = 5.4\) Hz) and the resonance of C-1 at \(\delta_C 68.3\) in the NMR spectra (Pelletier et al. 1984), which was further supported by the cross-peaks between H-C(1)/H-C(20) and H-C(20)/H\(_\alpha\)-C(11) in the ROESY spectrum. Comparison of the NMR data of 1 with those of the known alkaloid Kirinine B (Feng et al. 1998) indicated that ring A possessed a boat conformation, which was confirmed by the cross-peaks between H-C(2)/H-C(5) and H-C(5)/H-C(9) in the ROESY experiment. The 16, 17-epoxy group was assigned \(\alpha\)-orientation, since the epoxy moiety exhibited nearly identical NMR data to those of two known alkaloids bearing a 16, 17-epoxy segment, yesoxine (Bando et al. 1987) and gomandonine (Sakai et al. 1987) whose structures were unambiguously determined by X-ray crystallography. The cross-peaks between H-C(13) with H\(_\alpha\)-C(11) and H-C(15) with H\(_\alpha\)-C(14) in the ROESY experiment revealed that H-C(13) and H-C(15) were \(\alpha\)-oriented. Thus, this compound was assigned to be pubesine.

As far as we know, there were only six denudatine-type C\(_{20}\)-diterpenoid alkaloids that contain 16, 17-epoxy group reported before (Bando et al. 1987; Sakai et al. 1987; Liu et al. 2009; Zhang et al. 2010). Compound 1 was the first C\(_{20}\)-diterpenoid with a denudatine skeleton bearing a 16, 17-epoxy group and a C-1-C-19 carbinolamine inner ether isolated from a natural source, providing a new candidate for further pharmacological investigation. The structures of compounds 2–8 were identified by comparison of their spectral data with those described in the literature.

All compounds were evaluated for their cytotoxicity against MCF-7, Hep G2 and H460 human cancer cell lines. Compound 5 displayed cytotoxicity against the above tumor cells with IC\(_{50}\) values of 11.9, 27.6 and 31.8 \(\mu\)M, respectively, and other compounds were inactive (IC\(_{50}\) > 100 \(\mu\)M, \(n = 3\)).

3. Experimental

3.1. General experimental procedure

Optical rotation was measured on a Perkin-Elmer 341 polarimeter. 1D and 2D NMR spectra were recorded on a Bruker AV 600 NMR spectrometer, and IR spectrum on a ThermoFisher Nicolet 6700 spectrometer (KBr discs, cm\(^{-1}\)). HR-ESI-MS was carried out on a Q-TOF micro mass spectrometer (Waters, USA). Silica gel (Qingdao Haiyang Chemical Co., Ltd., China). TLC plates precoated with silica gel GF254 (Qingdao Haiyang Chemical Co., Ltd, China) were visualised under a UV lamp at 254 nm or by spraying the Dragendorff’s reagent or by iodine.

3.2. Plant material

*Aconitum soongaricum* var. *pubescens* were collected in Houxia, Xinjiang Uygur Autonomous Region of China, in August 2014 and were identified by Prof Qing-Er Yang of the Institute of Botany, Chinese Academy of Sciences. A voucher specimen (C. Ren & L. Wang 705) was deposited in the School of Life Science and Engineering, Southwest Jiaotong University.
3.3. Extraction and isolation

The air-dried powder (6.5 kg) of *A. soongaricum* var. *pubescens* was extracted with 95% EtOH three times at room temperature, with each soaking process lasting three days.

After removal of the solvent by evaporation, the EtOH extract (280 g) was recovered. The extract was treated with 0.5 mol/L hydrochloric acid (2 L) and successively extracted with light petroleum (4 × 1 L) and ethyl acetate (4 × 1 L). Then, 28% aqueous ammonia soln. (2 L) was added to the aq. soln. to bring them to pH 10. The solutions were extracted with CH$_2$Cl$_2$ (4 × 1 L). The CH$_2$Cl$_2$ extract was concentrated to produce the crude alkaloid fraction (20 g).

Column chromatography of the crude alkaloid fraction over silica gel, using a CH$_2$Cl$_2$/MeOH (80:1, v/v) mixture with increasing polarity afforded fractions A–E based on TLC analysis. Fraction A was separated by silica gel CC (light petroleum/Me$_2$CO/Et$_2$N, 8:1:0.1, v/v/v) to obtain 2 (20 mg) and 4 (15 mg). CC (silica gel, CH$_2$Cl$_2$/MeOH, 30:1, v/v) of fraction B afforded 3 (150 mg) and 5 (12 mg). Fraction C was chromatographed on silica gel column and eluted with light petroleum/Me$_2$CO/Et$_2$N (15:1:0.1, v/v/v) to afford 1 (5 mg). Fraction D was subjected to CC on silica gel and eluted with petroleum ether/CH$_2$Cl$_2$ (1:1, v/v) to give 7 (107 mg). Fraction E was subjected to CC on silica gel and eluted with CH$_2$Cl$_2$/Me$_2$CO (10:1–1:1, v/v) to give 6 (73 mg) and 8 (32 mg).

3.4. Cell culture and cytotoxicity assay

The *in vitro* growth inhibitory activities of compounds 1–8 were assayed by the MTS method (Huang et al. 2013; Guo et al. 2014). H460 (human lung adenocarcinoma), MCF-7 (human breast carcinoma) and Hep G2 (hepatocellular carcinoma) cell lines were obtained from ATCC. The cells were cultured in a 96-well plate 24 h before treatment and then exposed to different concentrations of compounds or control for 72 h. Three replicates were prepared for each treatment. DMSO (0.1% v/v) was used as negative control.

3.5. Pubesine (1)

White amorphous solid; [α]$_{20}$ D $\_80$ (c 0.3, MeOH); IR (KBr) $\nu_{\text{max}}$ 3379, 2928, 2856, 1677, 1644, 1568, 1406, 1384, 1353, 1109, 1082, 1063, 1015, 945, 726 cm$^{-1}$. $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$H 4.87 (dd, 1H, J = 3.6, 8.4 Hz, H-13), 4.18 (d, 1H, J = 4.2 Hz, H-15), 4.03 (d, 1H, J = 5.4 Hz, H-1), 3.58 (br s, 1H, H-19), 3.10 (ABq, 1H, J = 5.4 Hz, H-17a), 2.89 (s, 1H, H-20), 2.51 (m, 1H, H-14$\beta$), 2.42 (ABq, 1H, J = 5.4 Hz, H-17b), 2.41 (s, 3H, H-21), 2.38 (1H, overlapped, H-6$\beta$), 2.06 (s, 3H, 13-OAc), 1.96 (m, 1H, H-7), 1.94 (m, 1H, H-9), 1.88 (m, 1H, H-2$\alpha$), 1.77 (1H, overlapped, H-12), 1.75 (m, 1H, H-11$\beta$), 1.62 (1H, overlapped, H-6$\alpha$), 1.55 (m, 1H, H-3$\alpha$), 1.46 (m, 1H, H-2$\beta$), 1.40 (m, 1H, H-14$\alpha$), 1.26 (m, 1H, H-3$\beta$), 1.19 (1H, overlapped, H-5), 1.17 (m, 1H, H-11$\alpha$), 0.80 (s, 3H, H-18). $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$C 170.9 (13-OAc), 94.4 (d, C-19), 76.8 (d, C-15), 71.1 (d, C-13), 70.4 (d, C-20), 68.3 (d, C-1), 64.1 (s, C-16), 49.7 (s, C-10), 49.4 (d, C-5), 45.8 (d, C-7), 45.7 (t, C-17), 45.3 (s, C-8), 41.8 (q, C-21), 38.6 (d, C-12), 38.3 (d, C-9), 37.7 (s, C-4), 35.8 (t, C-14), 29.7 (t, C-3), 24.7 (t, C-6), 24.6 (t, C-2), 23.5 (t, C-11), 21.4 (13-OAc), 18.7 (q, C-18). HR-ESI-MS at m/z 402.2279 [M + H]$^+$ (calcd. for C$_{23}$H$_{32}$NO$_5$ 402.2280).
4. Conclusion

A new diterpenoid alkaloid, pubesine (1), along with seven known diterpenoid alkaloids were isolated from the whole plant of *Aconitum soongaricum* var. *pubescens*. Compound 1 is the first C₂₀-diterpenoid with a denudatine skeleton bearing a 16, 17-epoxy group and a C-1-C-19 carbamidine inner ether isolated from a natural source. All compounds were evaluated for their cytotoxicity against MCF-7, Hep G2 and H460 cell lines. Compound 5 displayed cytotoxicity against the above tumor cells with IC₅₀ values of 11.9, 27.6 and 31.8 μM, respectively, and other compounds were inactive.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81402803]; the Science and Technology Support Programs of Sichuan Province [grant number 2013SZ0083]; the Research Foundation for Educational Commission of Sichuan Province [grant number 15TD0048], [grant number 15ZB0140]; and the Fundamental Research Funds for Central Universities.

References

Bai Y, Desai HK, Pelletier SW. 1994. Long-chain fatty acid esters of some norditerpenoid alkaloids. *J Nat Prod.* 57:963–970.

Bando H, Wada R, Amiya T, Kobayashi K, Fujimoto Y, Sakurai T. 1987. Studies on aconitum species. V: Constituents of *Aconitum yesoense* var. *macroyesoense* (Nakai) Tamura. *Heterocycles.* 26:2623–2637.

Batbayar N, Batsurén D, Tashkhodzhaev B, Yusupova IM, Sultankhodzhaev MN. 1993. Alkaloids of the Mongolian flora. III. *Altaconitine* – a new alkaloid from *Aconitum altaicum*. *Chem Nat Compd.* 29:38–43.

Chen L, Shan LH, Zhang JF, Xu WL, Wu MY, Huang S, Zhou XL. 2015. Diterpenoid alkaloids from *Aconitum soongaricum* var. *pubescens*. *Nat Prod Commun.* 10:2063–2065.

de la Fuente G, Diaz Acosta R, Orribo T. 1989. Diterpenoid alkaloids from *Delphinium pictum* Willd. The structure of pictumine. Heterocycles. 29:205–208.

Feng F, Liu JH, Zhao SX. 1998. Diterpenoid alkaloids from *Aconitum kirinense*. *Phytochemistry.* 49:2557–2559.

Gao LM, Yan HY, He YQ, Wei XM. 2006. Norditerpenoid alkaloids from *Aconitum spicatum* Stapf. *J Integr Plant Biol.* 48:364–369.

Guo ZJ, Xu Y, Zhang H, Li MY, Xi K. 2014. New alkaloids from *Aconitum taipaicum* and their cytotoxic activities. *Nat Prod Res.* 28:164–168.

Hanuman JB, Katz A. 1994. Diterpenoid alkaloids from ayurvedic processed and unprocessed *Aconitum ferox*. *Phytochemistry.* 36:1527–1535.

Huang S, Zhou XL, Wang CJ, Wang HY, Wang YS, Shan LH, Weng J. 2013. New nervogenic acid derivatives from *Liparis nervosa*. *Planta Med.* 79:281–287.

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.

Kulathaivel P, Benn MH. 1988. A 16, 17-epoxy C₂₀-diterpenoid alkaloid from *Aconitum delphinifolium*. *Phytochemistry.* 27:3998–3999.
Liu H, Katz A. 1994. A New Norditerpenoid Alkaloid From Seeds of Aconitum Napellus SSP. vulgare. Nat Prod Lett. 5:147–151.
Liu XY, Chen QH, Wang FP. 2009. Three new C_{20}-diterpenoid alkaloids from Delphinium anthriscifolium var. savatieri. Chinese Chem Lett. 20:698–701.
Pelletier SW, Mody NV, Joshi BS, Schramm LC. 1984. Alkaloids: chemical and biological perspectives. New York: Wiley; Vol. 2, p. 205–462.
Sakai S, Okazaki T, Yamaguchi K, Takayama H, Aimi N. 1987. Structures of torokonine and gomandonine, two new diterpene alkaloids from Aconitum subcuneatum Nakai. Chem Pharm Bull. 35:2615–2617.
Shen XL, Chen QH, Chen DL, Wang FP. 2009. Two novel aconon-type diterpenes derived from C-nor-C 19-diterpenoid alkaloid. Chinese Chem Lett. 20:431–434.
Shyaula SL, Tamang T, Ghouri N, Adhikari A, Marasini S, Bajracharya GB, Manandhar MD, Choudhary ML. 2015. Antileishmanial diterpenoid alkaloids from Aconitum spicatum (Bruhl) Stapf. Nat Prod Res. 1–4. http://dx.doi.org/10.1080/14786419.2015.1114941.
Takayama H, Wu FE, Eda H, Oda K, Aimi N, Sakai S. 1991. Five napelline-type diterpene alkaloids from aconitum liangshannium. Chem Pharm Bull. 39:1644–1646.
Wang FP, Chen QH. 2010. The alkaloids: chemistry and biology. New York: Elsevier Science; Vol. 69, p. 84.
Wang WC, Warnock M. 1979. Flora of China. Beijing: Science press; Vol. 27, p. 310.
Wang FP, Chen QH, Liu XY. 2010. Diterpenoid alkaloids. Nat Prod Rep. 27:529–570.
Yin TP, Cai L, Zhou H, Zhu XF, Chen Y, Ding ZT. 2014. A new C19-diterpenoid alkaloid from the roots of Aconitum duclouxii. Nat Prod Res. 28:1649–1654.
Zhang ZT, Liu XY, Chen DL, Wang FP. 2010. New diterpenoid alkaloids from Aconitum liangshanicum. Helv Chim Acta. 93:811–817.