New alkaloids from *Aconitum taipaicum* and their cytotoxic activities

Zeng-Jun Guo*, Ying Xu, Hui Zhang, Meng-Yi Li and Ke Xi

Faculty of Pharmacy, Medical College of Xi’an Jiaotong University, Xi’an 710061, P.R. China

(Received 12 July 2013; final version received 28 October 2013)

Three new aconitine-type C$_{19}$-diterpenoid alkaloids, taipeinines A–C (1–3), were isolated from the roots of *Aconitum taipaicum*. The chemical structures of these three compounds were established as (1α,6α,8α,14α,16α)-20-ethyl-8,14-dihydroxy-1,6,16-trimethoxy-4-(methoxymethyl)-aconitane (1), (1α,6α,8α,14α,16β)-20-ethyl-8,14-dihydroxy-1,6,16-trimethoxy-4-(methoxymethyl)-aconitane (2) and (1α,6α,8α,14α,16α)-20-ethyl-1,8,14-trihydroxy-6,16-dimethoxy-4-(methoxymethyl)-aconitane (3), respectively, on the basis of spectroscopic analyses, mainly MS, 1D and 2D NMR. The cytotoxic activities of these compounds were also assayed, and the results were quite impressive.

**Keywords:** *Aconitum taipaicum*; aconitine-type C$_{19}$-diterpenoid alkaloids; cytotoxic activities

1. Introduction

Herb *Aconitum taipaicum* Hand. –Mazz. (Ranunculaceae) is an endemic plant in the Taibai Mountains of Shaanxi Province in China and its roots have been used in Chinese folk medicine as anti-inflammatory, gout, rheumatic, cardiotonic, diuretic and analgesic drug for a long period of time (Pelletier & Mody 1980; Singhuber et al. 2009; Kawasaki et al. 2011). As an aconitum plant, alkaloids should be the main constituents of this herb, but in previous reports, only 10 alkaloids (Wang & Fang 1982; He et al. 2008), including yunaconitine, neoline, talatisamine, chasmanine, isodelelatine, atisine, delfissinol, liangshanine, hypaconitine and delelatine, have been reported. The potential medicinal importance of this herb and our interest in the chemistry of alkaloids prompted us to investigate more alkaloids from this plant. The result of our research is that three new aconitine-type C$_{19}$-diterpenoid alkaloids (1–3, see Figure 1) and two other kinds of alkaloids which we reported (Xu et al. 2010) were isolated. The diterpenoid alkaloids from *Aconitum* plants are believed to be the main bioactive compounds (Gao et al. 2007). Therefore, in this study, the cytotoxicity of three new compounds against HL-60 and K-562 is assayed in order to find a suitable drug with low toxicity.

2. Results and discussion

Compound 1 was obtained as a white amorphous powder. The MS ([M + H]$^+$ at *m/z* 451.2938) and $^{13}$C NMR spectrum of the compound indicated a molecular formula of C$_{25}$H$_{41}$NO$_6$. The $^1$H NMR spectrum displayed signals for an $N$-ethyl group at $\delta$(H) 1.06 (t, $J = 7.2$ Hz, 3H), 2.47 (m,1H) and 2.52 (m,1H), and four methoxyl groups at $\delta$(H) 3.24, 3.30, 3.34, 3.30 (each 3H, s). These characteristic data suggested that 1 was a C$_{19}$-diterpenoid alkaloid (Chen et al. 2003; Gao et al. 2007; Jiang et al. 2012). Four moieties: C(1)−C(2)−C(3), C(9)−C(14)−C(13)−C(12)−C

*Corresponding author. Email: guozj@mail.xjtu.edu.cn

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(10), C(6)–C(7)–C(17) and C(15)–C(16)–C(13) were obtained from the $^1$H, $^1$H COSY spectrum. The HMBC experiment showed the following correlations: H–C(15)/C(16), C(7), C(8), C(9), and C(13); H–C(1)/C(2), C(10), C(11) and C(17); H–C(10)/C(1), C(5), C(8), C(9), C(11), C(12) and C(17); H–C(5)/C(6), C(8), C(10), C(12), C(13), C(14) and C(15), which connected the four moieties. Thus the connectivity of 1 was determined: it was an aconitine-type C$_{19}$-diterpenoid alkaloid. In the HMBC experiment, correlations between δ(H) 3.24, 3.30, 3.34 and 3.30 and C(1), C(6), C(16) and C(18), respectively, indicated that the four methoxyl groups were located at C(1), C(6), C(16) and C(18). The relative configuration of 1 was established by observing the NOSEY experiment as follows: correlations between H–C(1), H–C(5), MeO–C(6), H–C(7), H–C(9), H–C(10) H–C(13), HO–C(14) and MeO–C(16) were determined to be α, β, α, β, β, β, α, β, respectively. The correlation between HO–C(14) and HO–C(8) in $^1$H–$^1$H NOSEY spectrum suggested that the orientation of HO–C(8) was α-configuration. Therefore, the structure of 1 was determined and named as taipeinines A.

Compound 2 was obtained as a white amorphous powder. The MS ([M + H]$^+$ at m/z 452.2937) and $^{13}$C NMR spectrum of the compound indicated a molecular formula of C$_{25}$H$_{41}$NO$_6$. The $^1$H NMR spectrum displayed signals for an N-ethyl group at δ(H) 1.08 (t, J = 7.2 Hz, 3H), 2.53 (m,1H) and 2.55 (m,1H), and four methoxyl groups at δ(H) 3.25, 3.31, 3.34, 3.31 (each 3H, s). These characteristic data suggested that 2 was a C$_{19}$-diterpenoid alkaloid (Chen et al. 2003; Gao et al. 2007; Jiang et al. 2012). The $^1$H and $^{13}$C NMR data of 2 were quite similar to those of compound 1 except that H–C(16) showed no correlation with any H$_B$ in the NOSEY experiment, which indicated that MeO–C(16) was β-configuration. Thus, the structure of compound 2 was established and it was named as taipeinines B.

Compound 3 was obtained as a white amorphous powder. The MS ([M + H]$^+$ at m/z 437.2774) and $^{13}$C NMR spectrum of the compound indicated a molecular formula of C$_{24}$H$_{39}$NO$_6$. The $^1$H NMR spectrum displayed signals for an N-ethyl group at δ(H) 1.12 (t, J = 7.2 Hz, 3H), 2.48 (m,1H) and 2.57 (m,1H), and three methoxyl groups at δ(H) 3.31, 3.31, 3.33 (each 3H, s). These characteristic data suggested that 3 was a C$_{19}$-diterpenoid alkaloid (Chen et al. 2003; Gao et al. 2007; Jiang et al. 2012). The $^1$H and $^{13}$C NMR data were quite similar to those of compound 1 except that C(1) was substituted by hydroxyl group in compound 3 but by methoxyl group in compound 1. The deduction was further confirmed by another 2D NMR spectrum. Therefore, the structure of 3 was determined and named as taipeinines C.

Cytotoxicity of compounds 1–3 against cells HL-60 and K562 was examined and the IC$_{50}$ values are listed in Table 1 with adriamycin used as a positive control. Compound 1 potently suppresses the proliferation of cells HL-60 and K562. On the basis of these results, compounds from A. taipeicum could be potential anti-leukaemia agents.

| IC$_{50}$ (μg/mL) | Compound 1 | Compound 2 | Compound 3 | Adriamycin |
|------------------|------------|------------|------------|------------|
| K562             | 0.2 ± 0.05 | 17.8 ± 0.32| 19.9 ± 0.17| 2.0 ± 0.05 |
| HL-60            | 0.7 ± 0.15 | 21.3 ± 0.29| 21.6 ± 0.23| 2.0 ± 0.06 |
3. Experimental

3.1. General experimental procedures
Silica gel GF254 (10–40 mm) for thin-layer chromatography was purchased from Qingdao Marine Chemical Co. Ltd, China. Silica gel (200–300 mesh) for column chromatography was purchased from Qingdao Marine Chemical Co. Ltd, China. Polyvinyl sulphonionic ion exchange resin (H-form; cross linking 001 × 7) was bought from Xi’an Sunresin Technology Ltd, China. NMR spectra were measured with TMS as the internal standard on a Bruker AVANC_III-500M NMR Spectrometer. HR-ESI-MS spectra were recorded on Micross Mass Autospec-UltimaE TOF mass spectrophotometer.

3.2. Plant material
The A. taipaicum was collected in the Taibai Mountains of Shaanxi Province, China, and was authenticated by Professor Junxian Wang, Xi’an Jiaotong University. A voucher specimen (20030708) of the plant has been deposited in the herbarium of the Faculty of Pharmacy, Xi’an Jiaotong University.

3.3. Extraction and isolation
The powdered roots (2.25 kg) of A. taipaicum were percolated with 0.05 mol/L HCl (22 L) using the method reported in the literature (Fang & Huo 1966). The percolated filtrate (18 L) was added to polyvinyl sulphonic ion exchange exchange resin column at a speed of 5 mL/min and then washed repeatedly with deionised H2O. The air-dried resin was then alkalised with 10% aqueous NH4OH (0.5 L) and extracted with ethanol (2.0 L), and evaporated to give the total crude alkaloids (14.8834 g). The crude alkaloids (14 g) were chromatographed over silica gel (550 g) column which was eluted with CHCl3–MeOH (50:1 to 3:5) gradient system to give fractions A1–A25. Fractions A2 (0.0847 g) and A3 (0.1492 g) were separated on a silica gel column eluted with petroleum–ethyl acetate (20:1 and 10:1) to afford the known compound β-sitosterol (8.7 mg). Fractions A4 (0.7625 g) and A5 (0.9316 g) were separated on a silica gel column eluted with CHCl3–MeOH (7:1) to afford fraction B4 (0.1076 g). Further silica gel chromatography of fraction B4 eluted with petroleum–acetone produced compound 3 (14.3 mg). Fraction A6 (0.0673 g) was chromatographed over silica gel column eluting with ethyl acetate–MeOH (23:1) to give compound 1 (5.7 mg). Fractions A7–A9 (1.9541 g) were chromatographed over silica gel column eluting with ethyl acetate–MeOH (20:1) to give fraction B7 (0.2187 g). Sephadex LH-20 column chromatography of fraction B7 with CHCl3–MeOH (1:1) as the eluent afforded fraction C7 (0.1620 g). Fraction C7 was chromatographed on a silica gel column (CHCl3–MeOH) to provide compound 2 (27.9 mg).

3.3.1. (1α,6α,8α,14α,16α)-20-Ethyl-8,14-dihydroxy-1,6,16-trimethoxy-4-(methoxymethyl)-aconitane (1)
C25H41NO6, white amorphous powder. m.p. 84.8–86.0°C. MS m/z: 451.2938 ([M + H]⁺, calcd. for C25H41NO6, 451.2933). ¹H NMR (CDCl3, 500 MHz) δ: 3.02 (1H, dd, H-1), 1.92 (1H, m, H-2a), 2.27 (1H, m, H-2b), 1.52 (1H, m, H-3a), 1.71 (1H, m, H-3b), 2.21 (1H, m, H-5), 4.20 (1H, d, H-6), 2.05 (1H, m, H-7), 2.05 (1H, m, H-9), 1.73 (1H, m, H-10), 1.85 (1H, m, H-12a), 1.96 (1H, m, H-12b), 2.31 (1H, m, H-13), 4.12 (1H, t, J = 4.5 Hz, H-14), 2.10 (1H, m, H-15a), 2.43 (1H, m, H-15b), 3.40 (1H, m, H-16), 3.14 (1H, s, H-17), 3.32 (1H, abq, H-18a), 3.71 (1H, abq, H-18b), 2.55 (1H, m, H-19a), 2.66 (1H, m, H-19b), 2.55 (1H, m, H-21a), 2.55 (1H, m, H-21b), 1.08 (1H, t, J = 7.2 Hz, H-22), 3.25 (3H, s, 1-OCH3), 3.31 (3H, s, 6-OCH3), 3.34 (3H, s, 16-OCH3), 3.31 (3H, s, 18-OCH3); ¹³C NMR (CDCl3, 500 MHz) δ: 86.51 (C-1), 25.80 (C-2), 35.50 (C-3), 86.51 (C-1), 25.80 (C-2), 35.50 (C-3),
3.3.2. (1α,6α,8α,14α,16β)-20-Ethyl-8,14-dihydroxy-6,16-trimethoxy-4-(methoxymethyl)-aconitane (2)

C_{25}H_{41}NO_{6}, white amorphous powder. HR-ESI-MS: 452.2937 ([M + H]^+, calc. for C_{25}H_{41}NO_{6}, 451.2933). \(^1\)H NMR (CDCl\(_3\), 500 MHz) δ: 3.00 (1H, dd, H-1), 1.94 (1H, m, H-2a), 2.27 (1H, m, H-2b), 1.52 (1H, m, H-3a), 1.66 (1H, m, H-3b), 2.21 (1H, m, H-5), 4.20 (1H, d, H-6), 2.04 (1H, m, H-7), 2.03 (1H, m, H-9), 1.72 (1H, m, H-10), 1.85 (1H, m, H-12a), 1.98 (1H, m, H-12b), 2.30 (1H, m, H-13), 4.12 (1H, t, J = 4.5 Hz, H-14), 2.08 (1H, m, H-15a), 2.45 (1H, m, H-15b), 3.40 (1H, m, H-16), 3.13 (1H, s, H-17), 3.33 (1H, abq, H-18a), 3.71 (1H, abq, H-18b), 2.50 (1H, m, H-19a), 2.63 (1H, m, H-19b), 2.47 (1H, m, H-21a), 2.52 (1H, m, H-21b), 1.06 (1H, t, J = 7.2 Hz, H-22), 3.24 (3H, s, 1-OCH\(_3\)), 3.34 (3H, s, 6-OCH\(_3\)), 3.30 (3H, s, 16-OCH\(_3\)), 3.30 (3H, s, 18-OCH\(_3\)). \(^{13}\)C NMR (CDCl\(_3\), 500 MHz) δ: 86.21 (C-1), 25.97 (C-2), 35.27 (C-3), 39.44 (C-4), 48.65 (C-5), 82.35 (C-6), 52.59 (C-7), 72.53 (C-8), 50.36 (C-9), 45.59 (C-10), 50.25 (C-11), 28.34 (C-12), 37.88 (C-13), 75.58 (C-14), 38.81 (C-15), 82.05 (C-16), 62.66 (C-17), 80.77 (C-18), 53.80 (C-19), 49.33 (C-21), 13.75 (C-22), 56.20 (1-OCH\(_3\)), 57.33 (6-OCH\(_3\)), 56.42 (16-OCH\(_3\)), 59.24 (18-OCH\(_3\)).

3.3.3. (1α,6α,8α,14α,16α)-20-Ethyl-1,8,14-trihydroxy-6,16-dimethoxy-4-(methoxymethyl)-aconitane (3)

C_{24}H_{39}NO_{6}, white amorphous powder. HR-ESI-MS: 437.2774 ([M + H]^+, calc. for C_{24}H_{39}NO_{6}, 437.2777). \(^1\)H NMR (CDCl\(_3\), 500 MHz) δ: 3.65 (1H, dd, H-1), 1.70 (1H, m, H-2a), 2.05 (1H, m, H-2b), 1.62 (1H, m, H-3a), 1.90 (1H, m, H-3b), 2.17 (1H, m, H-5), 4.17 (1H, d, H-6), 2.00 (1H, m, H-7), 2.18 (1H, m, H-9), 2.28 (1H, m, H-10), 1.51 (1H, m, H-12a), 1.49 (1H, m, H-12b), 1.86 (1H, m, H-13), 4.21 (1H, t, J = 4.5 Hz, H-14), 2.08 (1H, m, H-15a), 2.38 (1H, m, H-15b), 2.38 (1H, m, H-16), 2.67 (1H, s, H-17), 3.26 (1H, abq, H-18a), 3.65 (1H, abq, H-18b), 2.31 (1H, m, H-19a), 2.70 (1H, m, H-19b), 2.48 (1H, m, H-21a), 2.57 (1H, m, H-21b), 1.12 (1H, t, J = 7.2 Hz, H-22), 3.33 (3H, s, 6-OCH\(_3\)), 3.33 (3H, s, 16-OCH\(_3\)), 3.33 (3H, s, 18-OCH\(_3\)). \(^{13}\)C NMR (CDCl\(_3\), 500 MHz) δ: 72.22 (C-1), 29.3 (C-2), 29.37 (C-3), 38.12 (C-4), 44.84 (C-5), 83.12 (C-6), 52.16 (C-7), 72.24 (C-8), 48.29 (C-9), 40.33 (C-10), 49.47 (C-11), 29.87 (C-12), 44.10 (C-13), 76.01 (C-14), 42.82 (C-15), 81.83 (C-16), 63.76 (C-17), 80.25 (C-18), 57.01 (C-19), 48.27 (C-21), 13.05 (C-22), 57.88 (6-OCH\(_3\)), 56.31 (16-OCH\(_3\)), 59.18 (18-OCH\(_3\)).

3.4. Cell culture and cytotoxicity assay

In the cytotoxicity assays, adriamycin at concentration of 4 μg/mL was chosen as the reference drug. Cell line HL-60 and cell line K562 were maintained in RPMI-1640 medium (Gibco BRL) with 3.7 g/L sodium bicarbonate, supplemented with 10% heat-inactivated FBS, seeded in 96-well tissue culture plates, and maintained in a humidified atmosphere of 5% CO\(_2\) and 95% air at 37°C for 3–6 days before experimentation. The cytotoxicity of the compounds was assayed by the methyl thiazolyl tetrazolium (MTT) method. The cells were diluted to 2 × 10^3 cells/mL with fresh medium and mixed. the test compound was dissolved in dimethyl sulfoxide (DMSO). If the compound was active at 50 μg/mL, a series of solutions were prepared by twofold dilution, and exposed to cells as mentioned above, in order to obtain IC\(_{50}\) values. Plates were incubated at 37°C under 5% CO\(_2\) atmosphere for 24 h. After incubation, 10% MTT was added and incubated...
at 37°C for 4 h. The pure formazan product was solubilised with 150 μL DMSO for 10 min at room temperature. The plate was read at 590 nm in a plate reader, and all of the reported experiments were carried out at least three times. IC$_{50}$ value was calculated using non-linear regression analysis. The percent inhibitions were obtained from the equation:

$$\% \text{ Inhibition} = \left[ \frac{A_{\text{test}} - A_{\text{negative}}}{A_{\text{adriamycin}} - A_{\text{negative}}} \right] \times 100.$$ 

4. Conclusion

Three new aconitine-type C$_{19}$-diterpenoid alkaloids, taipeinines A–C (1–3), were isolated and the structures of the three new compounds were elucidated. The cytotoxicity of the three compounds against cells HL-60 and K562 was assayed and compound 1 exhibited stronger cell growth inhibitory than adriamycin. On the basis of these results, compounds from A. taipaicum could be potential anti-leukaemia agents.

Supplementary material

The spectra of the three new compounds are available online, alongside Figures S1 and S2.

Acknowledgements

We thank Professor Zhongjun Ma of Zhejiang University for measuring NMR spectra. This study was supported by a grant from the National Natural Science Foundation of China (Grant No. 81172957).

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