Triterpenoids from *Abies faxoniana* and their cytotoxic activities

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**ABSTRACT**

Two previously unreported triterpenoids (1 and 2) and four known compounds were isolated from *Abies faxoniana*. Compound 1 has a \(\alpha,\beta\)-unsaturated-\(\gamma\)-lactone ring conjugated with the C-22/23 olefin in the C-17 side chain. The structures of the new compounds were established on the basis of spectroscopic data analysis. These compounds were tested for their cytotoxicities against six human tumour cell lines. Compound 1 showed cytotoxic activities against MCF-7 and A549 cells with IC\(_{50}\) values of 7.5 and 8.7 \(\mu\)M, respectively.

**1. Introduction**

There are approximately 50 species in the genus *Abies* (Pinaceae), 20 of which are indigenous to China (Zheng & Fu 1978). Phytochemical studies have indicated that terpenoids are characteristic constituents of the *Abies* species (Yang et al. 2010, 2014; Lavoie et al. 2012; Li et al. 2012, 2015; Handa et al. 2013; Wang et al. 2015a, 2015b; Belhadj Mostefa et al. 2016; Kim et al. 2016; Wu et al. 2016). These *Abies* terpenoids have been demonstrated to exert diverse biological activities, particularly *in vitro* antitumor activities (Yang et al. 2010; Lavoie et al.)
Abies faxoniana is a woody plant in China distributed exclusively in the mountains of Sichuan and Gansu provinces (Zheng & Fu 1978). Previously, we reported the isolation of eight pairs of epimeric triterpenoids involving a characteristic spiro-E/F ring from A. faxoniana (Wang et al. 2015a). These triterpenoids were obtained as epimeric mixtures arising from tautomerism at C-23 of the spirolactone structure. Herein, the branches and leaves of A. faxoniana were collected for further phytochemical investigation, which led to the isolation of two new triterpenoids (1 and 2) and four known compounds (3–6) (Figure 1). The cytotoxic activities of the isolated compounds against six human tumour cell lines are also described.

2. Results and discussion

Compound 1, obtained as white amorphous powder, had the molecular formula C_{30}H_{42}O_{3} based on 13C NMR data and the m/z 473.3041 [M + Na]^+ ion in the positive HRESIMS, indicating 10° of unsaturation. The IR spectrum indicated absorption bands for conjugated γ-lactone carbonyl (1743 cm−1), hydroxy (3442 cm−1) and olefinic (1644 cm−1) groups (Tanaka et al. 1999). The 1H NMR spectrum of 1 displayed signals for six methyl singlets at δ_H 0.87 (s, 3H), 0.97 (s, 3H), 1.04 (s, 3H), 1.15 (s, 3H), 1.24 (s, 3H) and 1.97 (s, 3H), one methyl doublet at δ_H 0.91 (d, 6.5, 3H), one methine proton signal at δ_H 3.43 (br s, 1H) attributed to a secondary alcoholic function and four olefinic protons at δ_H 5.63 (dd, 8.4, 2.5, 1H), 5.43 (dd, 8.4, 2.5, 1H), 5.11 (d, 10.6, 1H) and 6.97 (s, 1H). The 13C NMR spectrum of 1 showed 30 carbon signals classified by DEPT and HSQC data as eight methine groups (including an oxygenated secondary carbon at δ_C 76.5 and four olefinic tertiary carbons at δ_C 118.6, 119.1, 121.1 and 137.8), nine quaternary carbons (including a carboxyl carbon at δ_C 171.4 and four olefinic quaternary carbons at δ_C 128.8, 146.3, 147.8 and 155.6), six methylene groups at δ_C 23.1, 25.3, 27.5, 29.4, 36.5 and 45.0 and seven methyl groups at δ_C 10.5, 16.7, 22.4, 23.0, 27.0, 27.3 and 28.2. The above 1H and 13C NMR data of 1 exhibited a similar signal to those of the known compound pindrolactone except for the difference in the double bonds.
bond position (Gao et al. 2008). The HMBC correlations from H$_3$-18 to C-13, and from H-12 to C-11 and C-13 indicated that there was a Δ$^{12,13}$ double bond at δ$_C$ 119.1/155.6; δ$_H$ 5.43 (dd) in 1 instead of the Δ$^{9,11}$ double bond in pindrolactone (Figure S17, Supplementary material). In addition, the C-22/23 olefin attached to the butenolide ring in 1 has been shown to have a Z configuration, which was confirmed from the correlations of H-22 with H-24, and H-24 with H$_3$-27 in the NOESY spectrum. The relative configuration of 1 was determined by analysis of a NOESY experiment, which provided correlations of H-3 to H$_3$-19 and H$_3$-29; H-9 to H$_3$-19. This indicated H-3, H-9 and H$_3$-19 were co-facial and were assigned as β-oriented. Moreover, the NOESY correlations of H-5 to H$_3$-28, and H$_3$-30 to H-20 were observed, whereas no NOESY correlations of H-9 to H-5 and H-9 to H$_3$-30 were observed, which demonstrated the α-orientations for H-5 and H$_3$-30, and the β-orientation for H$_3$-18. Therefore, the structure of compound 1, named neoabieslactone L, was proposed to be 3α-hydroxy-9β-lanosta-7,22,24-tetraene-26,23-olide.

The molecular formula of compound 2 was established to be C$_{30}$H$_{48}$O$_2$ on the basis of its [M + H]$^+$ ion at a m/z of 441.3721 in positive HRESIMS, indicating a hydrogen deficiency index of seven. The IR spectrum suggested the presence of hydroxy (3450 cm$^{-1}$), aldehyde (1725, 2820, 2720 cm$^{-1}$) and olefinic (1646 cm$^{-1}$) groups. The $^1$H, $^{13}$C and DEPT NMR spectroscopic data of 2 indicated 30 carbon signals including six methyl singlets, one methyl doublet, nine methylenes, eight methines and six quaternary carbons with the aid of HSQC data, including one aldehyde group at δ$_C$ 195.4; δ$_H$ 9.37 (s, 1H), an oxygenated secondary carbon at δ$_C$ 79.2; and two pairs of double bonds at δ$_C$ 121.5/148.6, 139.0/155.5. By combining the above evidence with comparison of NMR data with those of lanostane triterpenoids from the Abies plants, 2 was found to be similar to the known compound abiesatrine K (Wang et al. 2015b), except for the presence of an aldehyde group at δ$_C$ 195.4; δ$_H$ 9.37 (s, 1H) in 2 instead of a carboxylic moiety. This aldehyde group attached to C-26 was established based on the HMBC correlations from the aldehydic hydrogen at δ$_H$ 9.37 to C-24, C-25 and C-27, and from H-24 and H$_3$-27 to the aldehydic carbonyl at δ$_C$ 195.4 ((Figure S18, Supplementary material)). The hydroxy group in 2 could be located at C-3 because of the HMBC correlations from H$_3$-28 and H$_3$-29 to the oxymethine at δ$_C$ 79.2. The NOESY correlations of H-3 with H$_3$-19, H-3 with H$_3$-29, and H$_3$-19 with H-9 revealed a co-facial relationship of H-3, H$_3$-19 and H-9 and were assigned as β-oriented. No NOESY correlations could be detected between H-9 and H-5, and H-9 and H$_3$-30, indicating that H-5 and H$_3$-30 resided on the opposite side of the plane, and were deduced to be α-orientation. The α-orientation of H-17 was established on the basis of the NOESY correlation of H-17 with H$_3$-30. Consequently, the structure of compound 2, named abiesatrine Q, was determined to be 3α-hydroxy-9β-lanosta-7,24E-diene-26-al.

The known compounds were identified as grandisolide (3) (Allen et al. 1971), 3α-acetoxy-7-oxolanosta-8,24-dien-26,23R-olide (4) (Tanaka et al. 2000), neoabiestrine A (5) (Li et al. 2012) and coccinone A (6) (Wang et al. 2009) by comparison of their $^1$H and $^{13}$C NMR data with the literature data.

All the isolated compounds (1–6) were tested for their cytotoxic activities against the human tumour cells Huh7, HepG2, SMMC7721, HCT-116, MCF-7 and A549 by MTT method (Table 1). Doxorubicin was used as a positive control. Among these compounds, compound 1 showed the strongest cytotoxicity against MCF-7 and A549 cells with IC$_{50}$ values of 7.5 and 8.7 μM, respectively; compounds 3 and 4 demonstrated inhibitory activity against HepG2 cells with IC$_{50}$ values of 17.9 and 15.0 μM, respectively.
3. Experimental

3.1. General experimental procedures

NMR spectra were determined with a Bruker Avance 500 spectrometer with TMS as an internal standard. Optical rotations were measured with a JASCO P-1020 digital polarimeter in MeOH. HR–ESI–MS were performed on an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer. IR spectra were recorded in KBr disk by a Bruker FTIR Vector 22 spectrometer. UV spectra were recorded with a Shimadzu UV-2500 spectrometer. Column chromatography (CC) was carried out on silica gel (Qingdao Haiyang Chemical Co., Ltd, China), YMC 50 μm ODS-A (Milford, USA) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), respectively. TLC was conducted on silica gel plates GF254 (Yan Tai Heng nuo Chemical Technology Co. Ltd, China). HPLC purification (Shimadzu LC-2010A HT, Shimadzu Crop., Kyoto, Japan) was conducted on a semi-preparative RP-C18 silica column (Agilent ZORBAX SB-C18, 5 μm, 9.4 × 250 mm).

3.2. Plant material

The branches and leaves of *A. faxoniana* were collected from Li county, Sichuan province in August 2009. The plant was authenticated by Prof. Hanming Zhang in the Department of Pharmacognosy, Second Military Medical University. A voucher specimen (20090813001) was deposited in the Herbarium of the Department of Phytochemistry, Second Military Medical University, Shanghai, China.

3.3. Extraction and isolation

The petroleum ether (PE), CH₂Cl₂, EtOAc and n-BuOH fractions were obtained from the 90% EtOH extract of the branches and leaves of *A. faxoniana* as reported previously (Wang et al. 2015a). The CH₂Cl₂ fraction was concentrated to yield 100.0 g of residue, which was subjected to silica gel (ϕ 8 × 100 cm, 100–200 mesh, 1000 g) CC eluted with a PE/EtOAc gradient (50:1–1:1) to give 10 fractions (Fr.1–Fr.10). Fr. 8 [PE/EtOAc (3 : 1), 18.0 g] was subjected to RP-C₁₈ silica gel column chromatography (ϕ 4.5 × 60 cm, 50 μm, 300 g) and was eluted with a gradient of MeOH/H₂O (30 : 70 → 100 : 0) to afford 7 subfractions (Fr. 8–1–Fr. 8–7). The Fr. 8–4 (3.0 g) was purified by Sephadex LH-20 column chromatography (ϕ 4.0 × 150 cm, MeOH/ H₂O, 50 : 50) and finally semi-preparative HPLC (MeOH/H₂O, 70 : 30) to furnish 3 (33.8 mg). The Fr. 8–5 (4.0 g) was subjected to RP-C₁₈ silica gel column chromatography (ϕ 2.5 × 60 cm,
50 μm, 150 g) eluted with gradient MeOH/H2O (30: 70 → 100: 0) followed by semi-preparative HPLC (MeOH/H2O, 80: 20) to afford 2 (4.7 mg) and 1 (3.8 mg). Compounds 4 (42.8 mg), 5 (35.8 mg) and 6 (8.4 mg) were isolated after CC over Sephadex LH-20 (ϕ 4.0 × 150 cm, MeOH/H2O, 80: 20) followed by semi-preparative HPLC (MeOH/H2O, 60: 40) from Fr. 8-6 (2.5 g).

Neobieslactone L (1): Amorphous powder; [α]D20 + 22 (c 0.1, CHCl3); UV (MeOH) λmax (log ε) 265.0 (3.00) nm; IR (KBr) νmax 3442, 1743, 1644, 1045, 818 cm−1; 1H NMR (500 MHz, CDCl3) δ 1.59–1.89 (m, 2H, H-1), 1.61–1.91 (m, 2H, H-2), 3.43 (br s, 1H, H-3), 1.46 (dd, 14.0, 3.5, 1H, H-5), 1.84–1.93 (m, 2H, H-6), 5.63 (dd, 8.4, 2.5, 1H, H-7), 1.97–2.15 (m, 1H, H-9), 1.81–2.20 (m, 2H, H-11), 1.24 (s, 3H, H-18), 0.87 (s, 3H, H-19), 1.93–1.97 (m, 1H, H-20), 0.91 (d, 6.5, 3H, H-21), 5.11 (d, 10.6, 1H, H-22), 6.97 (s, 1H, H-24), 1.97 (s, 3H, H-27), 1.04 (s, 3H, H-28), 0.97 (s, 3H, H-29), 1.15 (s, 3H, H-30). 13C nMR (125 MHz, CDCl3) δ 29.4 (C-1), 27.5 (C-2), 76.5 (C-3), 37.0 (C-4), 40.5 (C-5), 23.1 (C-6), 121.1 (C-7), 147.8 (C-8), 51.9 (C-9), 34.8 (C-10), 25.3 (C-11), 155.6 (C-13), 49.6 (C-14), 36.5 (C-15), 45.0 (C-16), 46.7 (C-17), 23.0 (C-18), 16.7 (C-19), 38.4 (C-20), 22.4 (C-21), 118.6 (C-22), 146.3 (C-23), 137.8 (C-24), 128.8 (C-25), 171.4 (C-26), 10.5 (C-27), 27.3 (C-28), 27.0 (C-29), 28.2 (C-30); HReSIMS (positive) m/z 473.3041 [M + Na]+ (Calcd for C30H42O3Na, 473.3134).

Abiesatrine Q (2): Amorphous powder; [α]D20 + 53 (c 0.1, CHCl3); UV (MeOH) λmax (log ε) 205.0 (2.90) nm; IR (KBr) νmax 3450, 2820, 2720, 1725, 1646, 1045, 818 cm−1; 1H NMR (500 MHz, CDCl3) δ 1.54–1.84 (m, 2H, H-1), 1.59–1.95 (m, 2H, H-2), 3.20 (br s, 1H, H-3), 1.45 (dd, 14.0, 3.5, 1H, H-5), 1.85–1.93 (m, 2H, H-6), 5.55 (dd, 6.5, 3.0, 1H, H-7), 1.98–2.18 (m, 1H, H-9), 1.40–1.63 (m, 2H, H-11), 1.32–1.80 (m, 2H, H-12), 1.40–1.78 (m, 2H, H-15), 1.44–1.92 (m, 2H, H-16), 1.47–1.49 (m, 1H, H-17), 0.96 (s, 3H, H-18), 0.83 (s, 3H, H-19), 0.83 (s, 3H, H-28), 0.73 (s, 3H, H-29), 1.73 (s, 3H, H-30). 13C nMR (125 MHz, CDCl3) δ 35.4 (C-1), 27.8 (C-2), 79.2 (C-3), 38.7 (C-4), 48.5 (C-5), 23.0 (C-6), 121.5 (C-7), 148.6 (C-8), 48.3 (C-9), 35.8 (C-10), 22.8 (C-11), 33.3 (C-12), 43.5 (C-13), 52.6 (C-14), 34.5 (C-15), 28.6 (C-16), 53.3 (C-17), 23.5 (C-18), 16.3 (C-19), 36.1 (C-20), 18.1 (C-21), 35.1 (C-22), 26.1 (C-23), 155.5 (C-24), 139.0 (C-25), 195.4 (C-26), 9.1 (C-27), 28.8 (C-28), 24.3 (C-29), 30.4 (C-30); HReSIMS (positive) m/z 441.3721 [M + H]+ (Calcd for C30H49O2, 441.3654).

3.4. Cytotoxic MTT assay

HepG2, Huh7, SMMC7721, HCT-116, MCF-7 and A549 cell lines were obtained from Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Cytotoxicity assay was determined by the MTT method. The test compounds were dissolved in 0.1% DMSO. Cells were seeded in a 96 well plate at 6 × 10^3 cells/well and exposed to the test compounds for 24 h. The cultures were treated with 0.1% DMSO as the vehicle control. After 24-h incubation, 10-μL MTT solution (5 mg/mL) in PBS was added into 100-μL medium, and the plates were incubated for 4 h at 37°C in the incubator chamber. The supernatant was then removed from the formazan crystals, and 100-μL DMSO was added to each well. The absorbance was measured by a microplate reader using a wavelength of 570 nm.

4. Conclusion

In our continuing search for bioactive compounds from Abies species, two new triterpenoids (1 and 2) and four known compounds (3–6) were isolated from the branches and leaves of
**A. faxoniana.** Since triterpenoids from *Abies* plants displayed various interesting biological activities, particularly in cytotoxic and antitumor activities, the cytotoxicities of these isolated compounds (1–6) were evaluated against six human tumor cell lines. Among them, the most potent cytotoxicity against MCF-7 and A549 cells was found for 1 with IC$_{50}$ values of 7.5 and 8.7 μM, respectively. It is therefore interesting to note that an α,β-unsaturated-γ-lactone conjugated with a double bond in C-17 side chain seemed to enhance the cytotoxic activity. However, further investigation on the mechanism of action underlying the *in vitro* antitumor effect of 1 is necessary.

**Supplementary material**

Supplementary material relating to this article is available online, including the HR–ESI–MS and 1D/2D NMR spectra for compounds 1 and 2.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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