Phytochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Lanostane-type triterpenoids from *Abies faxoniana* and their DNA topoisomerase inhibitory activities

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ARTICLE INFO

Article history:
Received 5 October 2014
Received in revised form 22 April 2015
Available online xxxx

Keywords:
Abies faxoniana
Pinaceae
Triterpenoids
Topoisomerases

ABSTRACT

Nine lanostane-type triterpenoids were isolated from branches and leaves of *Abies faxoniana*, along with 10 known compounds. Two were isolated as inseparable mixtures of epimers at C-23 of the γ-lactone ring that had a lactol structure. The structures of the nine compounds were established by spectroscopic analysis and circular dichroism (CD) data. The absolute configurations at the stereogenic centres of two of the known compounds were confirmed by X-ray crystallography. One compound showed cytotoxic activities against HCT-116, MCF-7, and A549 cells with IC_{50} values of 8.9, 7.6, and 4.2 μM, respectively. The isolated compounds were tested for their effects on human DNA topoisomerases I and II. One was found to be a selective inhibitor of human topo II activity with an IC_{50} value of 53.5 μM, which was comparable to that of the topo II inhibitor etoposide (IC_{50} = 49.6 μM).

1. Introduction

There are approximately 50 species in the genus *Abies* (family Pinaceae), 20 of which occur exclusively in China (Fu et al., 1999). *Abies* plants are a rich source of structurally diverse and biologically important terpenoids (Lavoie et al., 2012; Handa et al., 2013; Huang et al., 1988; Li et al., 2009, 2012; Shan et al., 1988; Yang et al., 2009, 2010a,b). These *Abies* terpenoids have been demonstrated to exert diverse bioactivities, particularly cytotoxic, antitumor and antiinflammatory activities (Matsunaga et al., 1965; Lavoie et al., 2012; Li et al., 2009, 2012; Wada et al., 2002; Yang et al., 2010a,b). *Abies faxoniana* is a woody plant distributed exclusively in China, especially in the northern part of the Sichuan province and the southern part of the Gansu province (Fu et al., 1999). In this study, the branches and leaves of *A. faxoniana* were collected for a systematic chemical investigation, which led to the isolation of 9 new (**1**–**9**) and 10 known triterpenoids (Figs. 1 and 2). Herein, the isolation and structural elucidation of compounds **1**–**9**, their cytotoxic activities against human tumour cell lines, and their inhibitory effects on human DNA topoisomerases I and II are described.

2. Results and discussion

The CH_2Cl_2 soluble part of the EtOH extract from the branches and leaves of *A. faxoniana* was fractionated by silica gel column chromatography, followed by Sephadex LH-20 and preparative HPLC to afford 9 new lanostane-type triterpenoids (**1**–**9**) and 10 known compounds (**10**–**19**). The known compounds were determined to be abieslactone (Matsunaga et al., 1965; Kutney and Westcott, 1971) (**10**), 3β-hydroxy-9β-lanosta-7,24-dien-26,23*R*-olide (Tanaka and Matsunaga, 1991) (**11**), 3-oxo-9βH-lanost-7-en-26,23-olide (Kukina et al., 1998) (**12**), (23*R*,25*R*)-3,4-*seco*-17,14-friedo-9βH-lanosta-4(28),6,8(14)-trien-26,23-olid-3-oic acid (Wada et al., 2002) (**13**), (23*R*,25*R*)-3,4-*seco*-9βH-lanosta-4(28),7-dien-26,23-olid-3-oic acid (Raldugin et al., 1986) (**14**), 7,14,22*Z*,24-mariesatetraen-26,23-olide-3α-ol (Gao et al., 2008) (**15**), abiesatrine D (Yang et al., 2010b) (**16**), firmanoic acid (Hasegawa et al., 1987a) (**17**), neoabieslactone E (Li et al., 2009) (**18**), and spiromarienonol A (Tanaka et al., 2004) (**19**) by comparing their spectroscopic data with those reported in the literature. The absolute configurations at the stereogenic centres of known compounds **10** and **16** were confirmed by X-ray crystallography

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http://dx.doi.org/10.1016/j.phytochem.2015.04.012
0031-9422/

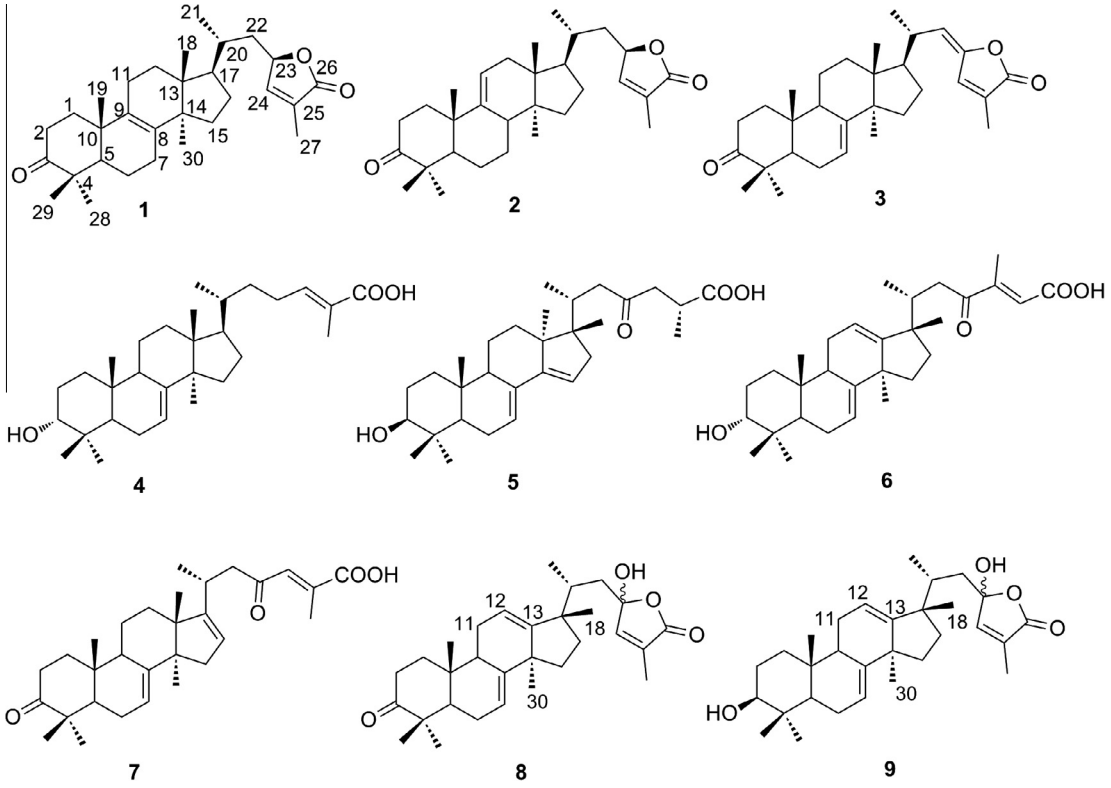

Fig. 1. Chemical structures of new compounds **1**–**9**.

(the single crystal X-ray diffraction data are found in Fig. S70 in the Supplementary data).

Compound **1** was assigned the molecular formula $C_{30}H_{44}O_3$ from its positive HRESIMS with a $[M+H]^+$ ion at a *m*/*z* of 453.3302, indicating a hydrogen deficiency index of nine. Its IR spectrum indicated absorption bands for α,β-unsaturated-γ-lactone (1741 cm^{-1}), carbonyl (1698 cm^{-1}), and olefinic (1646 cm^{-1}) groups. Its ^1H and ^{13}C NMR spectra showed a 4-substituted 2-methyl-2-butenolide ring [δ_H 1.91 (t), 4.98 (ddd), 6.99 (d); δ_C 10.6 (q), 78.9 (d), 129.4 (s), 149.7 (d), 174.4 (s)], a tetrasubstituted double bond [δ_C 135.1 (s), 133.2 (s)], a carbonyl group [δ_C 217.8 (s)], five methyl groups attached to tertiary carbons, a secondary methyl group, and nine methylene groups. The above spectroscopic data of **1** including those of the lactone moiety resembled those of the known compound 3-oxo-9β-lanosta-7,24-dien-26,23*R*-olide (Tanaka et al., 1990), except for the presence of an olefinic group at C-8 and C-9 instead of at C-7 and C-8. Thus, structure **1** was assigned as 3-oxolanosta-8,24-dien-23,26-olide. In the NOESY spectrum, H-17 correlated with H_3-30, suggesting a 17*R** relative configuration of **1**. In the circular dichroism (CD) spectrum of **1** (Fig. S9 in the Supplementary data), a negative Cotton effect at 216 nm (−12.1) was found, which established a 23*R* configuration of the lactone side-chain in **1** (Allen et al., 1971; Tanaka et al., 2004; Li et al., 2009). Accordingly, the structure of compound **1** was proposed to be 3-oxolanosta-8,24-dien-26,23*R*-olide, and it was named neoabieslactone G.

Neoabieslactone H (**2**) shared the same molecular formula as **1**, and they exhibited similar IR and NMR spectroscopic data. However, comparison of the ^1H and ^{13}C NMR spectra of the two compounds showed a difference in the double bond position. HMBC correlations from H_3-19 to C-9 and from H-11 to C-9 and C-12 indicated that the double bond in **2** was located at the C-9 and C-11 positions [δ_H 5.28 (dd); δ_C 147.1 (s), 116.0 (d)], instead of the double bond at the C-8 and C-9 positions of **1**. The 17*R** relative configuration of **2** was established on the basis of the NOESY correlation of H-17 with H_3-30. The 23*R*-configuration of the lactone side-chain in **2** was deduced from CD measurements (Fig. S18 in the Supplementary data), which gave rise to a negative Cotton effect at 216 nm (−9.4) similar to that of **1**. Therefore, compound **2** was deduced to be 3-oxolanosta-9(11),24-dien-26,23*R*-olide.

Compound **3** had a molecular formula of $C_{30}H_{42}O_3$ as determined by positive HRESIMS with a $[M+H]^+$ ion at a *m*/*z* of 451.3127, indicating a hydrogen deficiency index of ten. The IR spectrum indicated absorption bands for α,β-unsaturated-γ-lactone (1745 cm^{-1}), carbonyl (1696 cm^{-1}), and olefinic (1646 and 1648 cm^{-1}) groups. The ^1H and ^{13}C NMR spectroscopic data of **3** showed overall similarities to those of the known compound 3-oxo-9β-lanosta-7,24-dien-26,23*R*-olide (Tanaka et al., 1990), except for the presence of an additional double bond at C-22 and C-23 [δ_H 5.44 (d); δ_C 147.1 (s), 120.1 (d)]. In the NOESY spectrum, H-17 was correlated with H_3-30, indicating that C-17 has a *R** configuration in **3**. Compound **3** was thus assigned as 3-oxo-9β-lanosta-7,22*E*,24-trien-26,23-olide, and given the name neoabieslactone I.

The molecular formula of compound **4** was established to be $C_{30}H_{48}O_3$ on the basis of its $[M-H]^-$ ion at a *m*/*z* of 455.3610 in negative HRESIMS, indicating a hydrogen deficiency index of seven. The IR spectrum suggested the presence of hydroxy (3445 cm^{-1}), carboxylic (1772 cm^{-1}), and olefinic (1646 cm^{-1}) groups. The NMR spectroscopic data were very similar to those of the known compound abiesatrine D (Yang et al., 2010b), except for the presence of a C-3 hydroxy group [δ_C 77.5 (d)] in **4** instead of a carbonyl moiety [δ_C 219.3 (s)]. The hydroxy group in **4** 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 77.5. In the NOESY spectrum (Fig. 3), H-3 was correlated to H_3-19, which established a 3α-OH group in **4**. The 17*R** relative configuration of **4** was established