Systematic Phytochemical Investigation of Abies spectabilis

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Systematical phytochemical investigations on Abies spectabilis afforded 72 chemical constituents. On the basis of physical and spectroscopic data, including 1D and 2D homo- and heteronuclear NMR experiments (heteronuclear single quantum coherence (HSQC), 1H–1H correlation spectroscopy (COSY), heteronuclear multiple bond connectivity (HMBC), and nuclear Overhauser effect spectroscopy (NOESY)), and by comparison with the literature references, they were identified as 3 triterpenoids, 23 diterpenoids, 1 sesquiterpenoid, 13 flavonoids, 12 lignans, and 20 other components. Among these compounds, three were identified as new including abieta-7,13-diene-12α-methoxy-18-oic acid (1), 7α-methoxy-dehydroabietic acid (2), and 5-hydroxy-6-methyl-7,4′-dimethoxyflavone-8-O-β-D-glucopyranoside (3). These three new compounds (1—3) and all the known terpenoids (4—28) were tested for cytotoxic activities against four tumor cell lines: A549, COLO-25, QGY-25, and THP-1. However, none of them showed a positive effect (IC50 >100 μM).

Key words Abies spectabilis; Pinaceae; diterpenoid; flavonoid; cytotoxic activity

Abies is an important genus of the Pinaceae family comprising about 50 species around the world. Plants of this genus show high diversity in their secondary metabolites as well as pharmacological effects.1 Previously, we reported the occurrence of a unique sesquiterpenoid, a novel biflavanol, some diterpenoids, norditerpenoids, triterpenoids, and other compounds from three different Abies plants of A. georgii, A. delavayi, and A. chensiensis.2–9 Abies spectabilis (Don) spach is a tall evergreen tree distributed mainly in East Asia—Himalayas from Afghanistan to Nepal.10 To date, no phytochemical study has been reported on this species. As a continuation of the research analyzing the chemical constituents form Abies species in China, A. spectabilis was selected and subjected to a systematic phytochemical investigation, which led to the isolation of 3 new (Fig. 1) and 69 known chemical components. In this paper, we report the isolation, which led to the isolation of 3 new (Fig. 1) and 69 known compounds. By similar procedures, one new flavone and 34 known compounds were separated from the EtOAc extract.

Compound 1 had a molecular formula C22H32O5, as evidenced by the positive high resolution-electrospray ionization-mass-spectrum (HR-ESI-MS) at m/z 355.2244 [M+Na]+, indicating six degrees of unsaturation. The IR spectrum indicated the presence of carbonyl (a broad band from 2600 to 3451, 1723 cm⁻¹) and conjugated olefinic bonds (1630, 1549 cm⁻¹). The 1H, 13C, and distortionless enhancement by polarization transfer (DEPT) NMR spectra of 1 (Table 1) indicated 21 carbon signals including two singlet methyls [δH 1.23 (3H, s, Me-19), 0.83 (3H, s, Me-20)]; δC 147.7 (q, C-19), 17.5 (q, C-20)], two doublet methyls [δH 1.03 (3H, d, J=6.9 Hz, Me-16), 1.06 (3H, d, J=6.9 Hz, Me-17); δC 22.0 (q, C-16), 22.6 (q, C-17)], one methoxy group [δH 3.36 (3H, s, 12-OMe); δC 56.6 (q, 12-OMe)] and five methylenes; six methines including two vinyls [δH 5.50 (H, br s, H-7), 5.85 (1H, s, H-14); δC 125.1 (d, C-7), 127.6 (d, C-14)] and one oxymethine [δH 3.83 (1H, t, J=2.9 Hz, H-12); δC 77.0 (d, C-12)]; and five quaternary carbons including one carbonyl (δC 182.9, s, C-19) and two olefinic groups [δC 136.1 (s, C-8), 143.2 (s, C-13)]. In the double quantum fluorescein (DQF) correlation spectroscopy (COSY) experiment, the correlations of H-1 through H-2 to H-3; H-4 to H-6; H-9 through H-11 to H-12, and H-15 to H-16,17 established four fragments. The planar structure can be deduced as shown in Fig. 1 according to the heteronuclear multiple bond connectivity (HMBC) correlations traced from four methyls (Me-16,17,19,20), methoxyl, and olefinic protons. Based on the small coupling constant of H-12 (JH1,H12=2.9 Hz), the configuration of C12-OMe was deduced as axial-orientation.11 This could also be confirmed by the nuclear Overhauser effect spectroscopy (NOESY) correlations of H-12 to H-19/H-11 and H-9 to H-5/12-OMe. Therefore, compound 1 was concluded to be abieta-7,13-diene-12α-methoxy-18-oic acid.

Compound 2 was found to possess the molecular formula...
C₃H₂O₃, as shown from the positive HR-ESI-MS at m/z 353.2087 [M+Na]⁺. Its IR spectrum showed the presence of carboxyl (a broad band from 2700 to 3438, 1737 cm⁻¹) and benzene moiety (1630, 1513, 1442, 1383 cm⁻¹). The 1D NMR spectra of 2 (Table 1) indicated 21 carbon signals including four methyls [δ_H 1.21 (6H, d, J=7.0 Hz, Me-16,17), 1.26 (3H, s, Me-5,6,17), 1.25 (3H, s, Me-20); δ_C 24.3 (q, C-16), 24.4 (q, C-17), 17.4 (q, C-19), 24.8 (q, C-20)], one methoxy moiety [δ_H 3.40 (3H, s, 7-OMe); δ_C 56.3 (q, 7-OMe)]; four methylenes; six methines including one oxymethine [δ_H 4.30 (1H, dd, J=3.8, 1.5 Hz, H-7); δ_C 78.6 (d, C-7)] and three from an ABX benzoid moiety [δ_H 7.20 (1H, d, J=8.2 Hz, H-11), 7.15 (1H, dd, J=8.2, 1.9 Hz, H-12), 7.08 (1H, d, J=1.9 Hz, H-14); δ_C 125.1 (d, C-11), 127.6 (d, C-12), 129.8 (d, C-14)]; and six quaternary carbons including one carbonyl (δ_C 183.2, s, C-18) and three from the rest of the ABX benzoid moieties [δ_C 135.2 (s, C-8), 148.7 (s, C-9), 147.2 (s, C-13)]. These signals were very similar to those of 7α-hydroxydehydroacetic acid except for the presence of an additional methyl at 7-OMe. This was confirmed by the long-range correlation of 7-OMe to C-7 in the HMBC spectrum (Fig. 2). Accordingly, compound 2 was identified as 7α-methoxy-dehydroacetic acid.

Compound 3 was isolated as a yellow amorphous powder. The positive HR-ESI-MS at m/z 353.2087 [M+Na]⁺ gave its molecular formula as C₃H₂O₃. The IR spectrum showed the presence of hydroxyls (3424 cm⁻¹) and aromatic rings (1629, 1509 cm⁻¹). The ¹H- and ¹³C-NMR spectroscopic data of 1 (Table 1) were very similar to those of 5-hydroxy-7,4'-dimethoxy-6-methylflavone except for an additional glucopyranose moiety. On acid hydrolysis, 3 afforded α-glucose which was detected by TLC with an authentic sample, and the configuration was determined by measurement of the optical rotation value. The β-anomeric configuration was judged by the large coupling constant (7.8 Hz) of the anomeric proton. In the HMBC spectrum, a long-range correlation was found for the anomeric proton (δ 4.87, d, J=7.8 Hz) of the anomeric proton. Therefore, the β-O-glucopyranoside is apparently attached at the C-8 position of the flavone unit. Thus the structure of compound 3 was determined as 5-hydroxy-6-methyl-7,4'-dimethoxyflavone-8-O-β-D-glucopyranoside.

These three new compounds (1—3) and all the terpenoids (4—28) were tested for antitumor activities against the four tumor cell lines: A549, COLO-25, QGY-25, and THP-1. However, none of them showed positive effect (IC₅₀ > 100 μM).

**Experimental**

**General Experimental Procedures**

Optical rotations were recorded using a Perkin-Elmer 341 polarimeter, whereas UV spectra were obtained by a Shimadzu UV-2550 spectrometer. IR spectra were recorded on a Bruker Vector 22 spectrometer with KBr pellets. NMR spectra were recorded on Bruker Avance 300, 400, 500 or 600 NMR spectrometers in CDCl₃ or DMSO-d₆ using a Perkin-Elmer 341 polarimeter, whereas UV spectra were obtained by a Shimadzu UV-2550 spectrometer. IR spectra were recorded on a Bruker Vector 22 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker Avance 300, 400, 500 or 600 NMR spectrometers in CDCl₃ or DMSO-d₆. ESI-MS were acquired on an Agilent LC/MSD Trap XCT mass spectrometer. HPLC analysis was carried out on a Shimadzu LC-2010A pump and a 490014 mass spectrometer. RP-MPLC was carried out on a Shimadzu LC-2010A pump and a 490014 mass spectrometer. RP-MPLC was carried out on a Shimadzu LC-2010A pump and a 490014 mass spectrometer.
MeOH, and subjected to repeated prep. TLC (petrolum ether–EtOAc, 4 : 1) in 526, 500, 294, 261, 199, 169, 183, 161, 160, 131, 89, 70, 54, 30, 19, 9, 8, 7, 6, 5, 2 nm. Compounds were visualized by exposure to UV light at 254 nm.

**Plant Material**

The aerial parts of A. spectabilis (D. Don) spach were collected from Sejila Hills in Tibet in May 2007 and authenticated by Prof. Han-Ming Zhang in the Department of Pharmacognosy, Second Military Medical University. A voucher specimen (20070513002) was deposited at the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai, China.

**Extraction and Isolation**

The air-dried and powdered aerial parts (17 g) of A. spectabilis (D. Don) spach were extracted with 80% ethanol three times each for 3 h. After filtering, the extract was partitioned sequentially with CHCl₃ (20 l), EtOAc (40 l) and n-ButOH (30 l), respectively. The CHCl₃ extract was subjected to column chromatography over silica gel eluting with a gradient petroleum ether–CHCl₃ (95 : 5–90 : 10–75 : 25–20 : 5) to give three fractions (Fr. 1–Fr. 3). Fr. 3 (10.9 g) was subjected to RP-MPLC eluting with (H₂O–MeOH, 50 : 100), then purified by Sephadex LH-20 eluting with MeOH, and subjected to repeated prep. TLC (petrol ether–EtOAc, 4 : 1) to afford 12-cmc-methoxy-7,13-diene-18-oic acid (1, 8.8 mg), 7α-methoxy-dehydroabietic acid (2, 15.2 mg), dehydroabietic acid (5, 214.3 mg), 13, 17, 17-dimethyl-8,11,13-trien-4-ol (24, 18.5 mg), 13, 17, 17-dimethyl-8,11,13-triene-6-methyl ester (6, 110 mg), 15, 18-dihydroxyabietane-8,11,13-trien-7-one (7, 53 mg), 15-hydroxy-7-oxo-8,11,13-abietatrin-18-oic acid (8, 26 mg), 13, 17-dihydroabietane (9, 102.4 mg), 15-hydroxydehydroabietic acid (10, 68.9 mg), abietane dione (11, 115.7 mg), 13, 17-dihydroxy-8(14)-abiet-18-oic acid (12, 62.5 mg), abietane X (14, 286 mg), and 13, 17-dihydroabietane (15, 34.6 mg).

**Acid Hydrolysis of Compound 3**

The experiment was carried out according to a previous method. Briefly, compound 3 (10 mg) was dissolved in 5 ml HCl at 105 °C, and kept refluxing at 57 °C. After 2 h, the reaction was stopped and the mixture was extracted by EtOAc. The remaining aqueous phase was concentrated to afford 4.4 mg of n-glucose, which was detected by TLC with an authentic sample. The configuration was determined by measurement of the optical rotation value, [α]D30 +38.0 (c=0.22, H₂O).

**Antitumor Assays**

A549, COLO-25, QGY-25, and THP-1 were obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The antitumor experiments were conducted according to previously reported procedures.

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**References**


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22) TLC (0.4—0.5 mm) was conducted with glass plates precoated with silica gel GF₂₅₄ (Yantai). Compounds were visualized by exposure to UV light at 254 nm.