Pentacyclic Triterpenes from the Ethyl Acetate Fraction of the Bark of Platanus acerifolia Wild and Antitumor Activities In Vitro

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Abstract

Three pentacyclic triterpenes, named betulinic acid (1), 11α-hydroxy-β-amyrin (2) 3β-acetoxy-29-(29)-lupen-28-aldehyde (3) were isolated from the ethyl acetate fraction of the bark of Platanus acerifolia Wild. The molecular structure of 1 and 2 were established on the basis of various spectroscopic analyses. The molecular structure of 3 was determined by single-crystal X-ray diffraction. Compound (2) and (3) were obtained from the title plant for the first time. Cytotoxicity of the isolated compounds against three human cancer cell lines, HepG-2, MCF-7 and HL-60 were also determined with the cell counting kit-8 (CCK-8) assay. The target compounds showed the high cytotoxicity, with IC₅₀ values in the range 2.2-9.1 µM. These results indicated that pentacyclic triterpenes from the bark of Platanus acerifolia Wild could be explored as potential cancer prevention agents.

Keywords: Pentacyclic triterpenes; Platanus acerifolia Wild bark; Antitumor activity in vitro

Introduction

Platanus acerifolia Wild, one of the famous street and garden trees, is a very large, wide spreading, and long-lived hardwood species native to Eurasia [1]. The bark of Platanus acerifolia Wild has been used as the traditional Chinese medicine in the treatment of dysentery, diarrhea, toothache and tumor [2]. In order to find some bioactive compounds, the chemical constituents of Platanus acerifolia Wild bark were investigated and three compounds, named betulinic acid (1), 11α-hydroxy-β-amyrin (2) and 3β-acetoxy-29-(29)-lupen-28-aldehyde (3) were isolated. The structures of the three compounds were identified by their physicochemical properties and spectral analysis. In addition, the isolated compounds were also evaluated for cytotoxic efficacy against HepG-2, MCF-7 and HL-60 cell lines in vitro.

Materials and Methods

General experimental procedures

Melting points were determined on RD-2 micromelting point apparatus and are uncorrected. The ¹H-NMR (500 MHz) and ¹³C-NMR (500 MHz) spectra were recorded on a Bruker AvanceIII-500 spectrometer and tetramethylsilane (TMS) was used as an internal standard. Silica gel (200-300 mesh for Column Chromatography (CC) and GF₂₅₄ for TLC) was obtained from Qingdao Marine Chemical Company (Qingdao, China). Sephadex LH-20 was obtained from Amershams Biosciences (Uppsala, Sweden). Single-crystal structure of compound 2 was measured on an Enraf-Nonius CAD4 diffractometer.

Plant material

The bark of Platanus acerifolia Wild was collected in Nanjing County, Jiangsu Province, China, in January 2010.

Extraction and isolation

The dried barks of Platanus acerifolia Wild (100 g) were cut into small pieces and extracted with EtOAc (1 liter × 3). The solvent was removed by rotary evaporation and the yellow brown extract (4.0 g) was obtained. The EtOAc extract was subjected to silica gel chromatography using stepwise elution with petroleum ether-CH₂COCH₂ (100:0, 100:1, 100:2, 100:4, 100:8, 100:16, 100:32, 100:100, and 0:100) to afford 90 fractions (F1-F90). F51-F60 (A) was permeated through Sephadex LH-20 using a MeOH-CH₂Cl₂ (1:1) system to give 10 subfractions A1-A10. Fractions A4-A6 were further purified with recrystallization with CH₂Cl₂-CH₂COCH₂ (1:1) system to afford compound (1) (1000 mg); Fraction of F8 was further purified with recrystallization with CH₂Cl₂-CH₂COCH₂-CH₃OH (1:1:1) system to afford compound (2) (10 mg); F11-F20 (B) were permeated through Sephadex LH-20 using a MeOH-CH₂Cl₂ (1:1) system to give 12 subfractions B1-B12. Fractions B4-B7 were further purified with recrystallization with EtOAc-CH₃OH (1:1) system to afford compound (3) (20 mg).

Compounds identification

Compound (1): Compound (1) was readily identified as betulinic acid by the analysis of their NMR spectra and by the comparison with the data reported in literature [3]. ¹H-NMR (500 MHz, DMSO) δ H = 12.08 (1H, brs, H-28), 4.69 (1H, brs, H-29a), 4.56 (1H, brs, H-29b), 4.28 (1H, brs, H-3), 1.64 (3H, s, H-30), 0.93 (3H, s, H-23), 0.88 (3H, s, H-27), 0.87 (3H, s, H-26), 0.76 (3H, s, H-24), 0.68 (3H, s, H-25); ¹³C-NMR (500 MHz, DMSO) δ C = 180.0 (C-28), 150.3 (C-20), 109.5 (C-29), 76.8 (C-3), 55.4 (C-17), 54.9 (C-5), 55.4 (C-5), 49.9 (C-9), 49.5 (C-19), 46.6 (C-18), 42.0 (C-14), 40.2 (C-8), 38.5 (C-4), 38.2 (C-1), 37.5 (C-13), 36.7 (C-10), 36.4 (C-22), 33.9 (C-7), 31.7 (C-16), 30.1 (C-15), 29.2 (C-21), 28.1 (C-23), 27.1 (C-2), 25.1 (C-12), 18.9 (C-30), 17.9 (C-6), 15.9 (C-26), 15.8 (C-24, 25), 14.4 (C-27). Compound (2): The acicular crystal of 11α-hydroxy-β-amyrin was recrystallized in the mixture solution of CH₂Cl₂-CH₂COCH₂-MeOH (1:1:1), and single crystal was obtained in constant temperature (25°C) on the basis of this. m.p: 234–236°C. Elemental Anal. Calcld. (%) for C₂₉H₄₅O₃: C, 81.39; H, 11.38; O, 7.23. Found (%): C, 81.20; H, 11.48; O, 7.32. ¹H-NMR (500 MHz, CDCl₃) δ H = 5.24 (1H, d, H-12), 4.21 (1H, dd, H-12), 3.22 (1H, dd, H-3), 2.06 (1H, m, H-18), 1.94 (2H, m, H-21), 1.23 (3H, s, H-23), 1.08 (3H, s, H-27), 1.02 (3H, s, H-25), 1.02 (3H, s, H-26), 0.87 (3H, s, H-26).

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Results and Discussion

Compound (1) was readily identified as betulinic acid by the analysis of their NMR spectra and by the comparison with the data reported in literature (Figure 1) [3]. 1H-NMR and 13C-NMR spectra showed the typical pattern of pentacyclic triterpene. Especially, the 1H-NMR spectrum of compound was characteristic of the presence of a vinyl proton at 6.24 (1H, d, J = 4.21 (1H, d)), Two C singlets at 6147.1 and 120.7 indicated the presence of C-C double bond. On the basis of the above evidences, compound (2) was suggested to 11α-hydroxy-β-amyrin. The NMR data of compound was in good agreement with the previous data of 11α-hydroxy-β-amyrin (Figure 1) [6]. Compound (3), was obtained as colorless crystals. The NMR data of compound (3) was in good agreement with the previous data of 3β-acetoxy-20(29)-lupen-28-aldehyde [7]. Further single-crystal X-ray diffraction analysis confirmed the molecular structure of compound (3) (Figure 1) [4].

Cell Counting Kit-8 (CCK-8) is a reagent box used to detect cell proliferation, cell survival and cell toxicity based on a water soluble tetrazolium salt, WST-8{2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-benzene disulfonate)-2H-}
tetrazolium monosodium salt). CCK-8 is an alternative to MTT assay. During the process of metabolism of living cells, in the presence of 1-methoxy PMS, the WST-8 in cells produces soluble orange formazan. The formazan generated is proportional to the number of living cells. Compared with MTT, CCK-8 has significant advantages. The formazan generated by CCK-8 solution is water-soluble, and requires specific solvents to dissolve it, such as dimethyl sulfoxide. However, formazan generated by CCK-8 solution is water-soluble, and thus organic solvents need not be used in the experiment. On the other hand, CCK-8 solution is fairly stable, not toxic to cells, and can be used directly [8].

Compound (1), (2) and (3) exhibited cytotoxicity against HepG-2, MCF-7 and HL-60 cancer cell lines by using CCK-8 assays and Taxol as positive control (Table 1). Compound (1), (2) and (3) exhibited cytotoxicity against these cell lines and gave IC50 values in the range 2.2-9.1 µM. Two pentacyclic triterpenes from the ethyl acetate fraction of the bark of Platanus acerifolia Willd showed potent activities against the tested cancer cell lines. Compound (3) was 4.0 times more toxic to HepG-2 and MCF-7 cells than Compound (1),

![Figure 1: Chemical structure of pentacyclic triterpenes.](image-url)
indicating its potential as an anticancer drug. The cytotoxicity of these compounds against some cancer cell lines was previously reported [9-13].

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