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Lupane Triterpenoids from *Pyrus pyrifolia*

Ji Hye Yoo and Ki Sook Yang*

College of Pharmacy, Sookmyung Women's University

Abstract – Three lupane type triterpenoids were isolated from the methanol extract of *Pyrus pyrifolia* fruit peel through repeated silica gel column chromatography. Based on the spectroscopic methods, their structures were determined to be lupeol (**1**), betulin (**2**), and betulinic acid (**3**).
Keywords – *Pyrus pyrifolia*, triterpene, lupeol, betulin, betulinic acid

Introduction

Pyrus pyrifolia Nakai is a perennial plant belonging to the family of Rosaceae. The fruit of this plant, known as a pear, is common and highly consumed in Korea. The Korean pears, however, have been considered not only fruit but a herbal medicine in East Asia. The fruit and the roots of this plant have been used for the treatment of the fever (Yoo, 1991). Its peels of the fruit have been used to cure abscess, cough, dysentary, and indigestion (Kim, 1988).

Recently, some studies on the fruits of *P. pyrifolia* have led to the isolation of several phenolic compounds such as arbutin, chlorogenic acid (Cui *et al*., 2005) and catechins (Zhang *et al*., 2003). As a part of our ongoing research on bioactive compounds from Korean medicinal plants, we report the isolation and structural elucidation of three lupane triterpenoids from *Pyrus pyrifolia* fruit peel.

Experimental

General experimental procedures – The IR spectra were obtained on a Jasco FT/IR-430 Infrared spectrometer. The EI-MS spectra were obtained using a JEOL JMS-AX505WA, HP5890 Series II mass spectrometer. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded using a Bruker AVANCE 500 NMR spectrometer. Column chromatography was performed using silica gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck).

Plant material – The fruit of *Pyrus pyrifolia* were purchased from Seoul National Agricultural Cooperation Federation in Korea, and its voucher specimen (SMU 0703) has been deposited at the Herbarium of the College of Pharmacy, Sookmyung Women's University, Korea.

Extraction and isolation – Korean pears (40.0 kg) were peeled, and the peels (5.1 kg), pulps (31.3 kg) and cores (4.2 kg) were immediately dropped into methanol, and were extracted 3 times at 80 °C for 4 h, respectively. The MeOH extract was dissolved in distilled water, and filtered by filter paper. This process was to yield to water insoluble part (7.2, 0.9 and 0.2 g) and water soluble part (670.8, 791.2 and 81.7 g) from peel, pulp and core (5.1, 8.3 and 0.9 kg, respectively). The water insoluble part of *Pyrus pyrifolia* fruit peel (7.2 g), which exibited nitrite inhibitory effect, was extracted by EtOAc to give a dark brown extract (4.5 g). The ethyl acetate extract (3.5 g) was chromatographed on silica gel eluting with a Hexane/EtOAc gradient system (4 : 1 → 1 : 50) to obtain Fr. 1 - 7. Among them, Fr. 4 (209.3 mg) was subjected to silica gel chromatography and eluted with Hexane/Acetone/MeOH (7 : 1 : 0.1). Fr. 4-2 ~ 4-4 (38.8, 34.6 and 51.9 mg, respectively) were further purified by silica gel chromatography with Hexane/EtOAc (4 : 1) to yield the compound **1** (77.2 mg). Fr. 5 (180.0 mg) was seperated by silica gel chromatography with $CHCl_3$/MeOH (20 : 1) to give Fr. 5-1 and Fr. 5-2. Fr. 5-2 (90.7 mg) was subjected to repeat chromatography over silica gel to yield the compound **2** (44.5 mg). Fr. 6 (130.0 mg) was further seperated using a silica gel column with $CHCl_3$/MeOH (10 : 1), $CHCl_3$/EtOH (10 : 1) and $CHCl_3$/EtOAc (5 : 1) and compound **3** (21.4 mg) was isolated from $CHCl_3$/EtOAc (5 : 1) elution. Compound **4** (30.3 mg) was obtained from $CHCl_3$/MeOH (10 : 1) elution of Fr. 6-2 (90.6 mg) using the silica gel column. Fr.7 (650.0 mg) was further seperated on a silica gel column with a gradient elution of $CHCl_3$/EtOH (10 : 1 → 5 : 1) to afford compound **5** (16.6 mg).

*Author for correspondence
Tel: +82-2-710-9578; E-mail: ksyang@sookmyung.ac.kr

Lupeol (1) – White needles; EI-MS; *m/z* 426 $[M]^+$; IR (NaCl neat) cm^{-1}: 3430 (-OH), 1640 (C = C) ; ^{1}H-NMR (500 MHz, $CDCl_3$, δ in ppm) : 4.62 (1H, br s, H-30a), 4.50 (1H, br s, H-30b), 3.12 (1H, dd, *J* = 11.4, 4.9Hz, H-3), 2.31 (1H, ddd, *J* = 11.1, 11.08, 5.8Hz, H-19), 1.63 (3H, s, H-29), 0.96 (3H, s, H-26), 0.90 (3H, s, H-23), 0.87 (3H, s, H-27), 0.76 (3H, s, H-25), 0.72 (3H, s, H-24), 0.69 (3H, s, H-28); ^{13}C-NMR (125 MHz, $CDCl_3$, δ in ppm) : 151.2 (C-20), 109.5 (C-30), 79.2 (C-3), 55.5 (C-5), 50.7 (C-9), 48.5 (C-18), 48.2 (C-19), 43.2 (C-17), 43.1 (C-14), 41.1 (C-8), 40.2 (C-22), 39.1 (C-4), 38.9 (C-1), 38.3 (C-13), 37.4 (C-10), 35.8 (C-16), 33.2 (C-7), 29.9 (C-21), 28.2 (C-23), 27.7 (C-2), 27.6 (C-15), 25.4 (C-12), 21.2 (C-11), 19.5 (C-29), 18.5 (C-6), 18.2 (C-28), 16.3 (C-25), 16.2 (C-26), 15.6 (C-24), 14.8 (C-27)

Betulin (2) – White powder; EI-MS; *m/z* 442 $[M]^+$; IR (NaCl neat) cm^{-1}: 2930 (-OH), 1685 (C = C) ; ^{1}H-NMR (500 MHz, $CDCl_3$, δ in ppm) 4.66 (1H, br s, H-30a), 4.62 (1H, br s, H-30b), 3.70 (1H, d, *J* = 10.9Hz, H-28a), 3.23 (1H, d, *J* = 10.9Hz, H-28b), 3.10 (1H, dd, *J* = 10.5, 5.8Hz, H-3), 1.63 (3H, s, H-29), 1.22 (3H, s, H-22), 0.99 (3H, s, H-27), 0.90 (3H, s, H-24), 0.78 (3H, s, H-25), 0.70 (3H, s, H-26); ^{13}C-NMR (125 MHz, $CDCl_3$, δ in ppm) : 149.9 (C-20), 108.5 (C-30), 77.8 (C-3), 58.7 (C-28), 54.7 (C-5), 49.9 (C-9), 49.7 (C-19), 48.5 (C-18), 46.9 (C-17), 46.3 (C-19), 41.6 (C-14), 39.9 (C-8), 38.1 (C-4), 38.0 (C-10), 37.6 (C-1), 36.4 (C-13), 33.6 (C-7), 33.5 (C-22), 29.8 (C-21), 28.9 (C-16), 28.4 (C-23), 26.9 (C-2), 26.1 (C-15), 24.8 (C-12), 20.1 (C-11), 18.2 (C-29), 17.5 (C-6), 15.1 (C-25), 15.0 (C-26), 14.4 (C-24), 13.0 (C-27)

Betulinic acid (3) – White powder; EI-MS; *m/z* 456 $[M]^+$; IR (NaCl neat) cm^{-1}: 3430 (-OH); ^{1}H-NMR (500 MHz, MeOD, δ in ppm) : 4.67 (1H, br s, H-30a), 4.52 (1H, br s, H-30b), 3.10 (1H, t, 8.15Hz), 1.63 (3H, s, H-29), 1.00 (3H, s, H-27), 0.90 (3H, s, H-26), 0.89 (3H, s, H-25), 0.85 (3H, s, H-23), 0.69 (3H, s, H-24); ^{13}C-NMR (125 MHz, MeOD, δ in ppm) : 178.2 (C-28), 149.9 (C-20), 108.5 (C-30), 77.8 (C-3), 56.6 (C-17), 54.6 (C-5), 49.7 (C-9), 48.4 (C-18), 46.2 (C-19), 41.6 (C-14), 39.8 (C-8), 38.0 (C-4), 37.9 (C-1), 37.5 (C-13), 36.3 (C-22), 36.3 (C-10), 33.5 (C-7), 31.4 (C-16), 31.0 (C-15), 28.8 (C-21), 26.9 (C-23), 26.0 (C-2), 24.7 (C-12), 20.0 (C-11), 18.3 (C-29), 17.4 (C-6), 15.1 (C-25), 14.9 (C-26), 13.7 (C-24), 13.0 (C-27)

Results and Discussion

Compound **1** was obtained as white needles and showed a molecular ion peak at $[M]^+$ *m/z* 426 in the EI-MS spectrum, which was consistent with the molecular formula of $C_{30}H_{50}O$. The IR spectrum displayed absorption bands for a hydroxyl group at 3430 cm^{-1} and an olefinic double bond at 1640 cm^{-1}. ^{1}H-NMR spectrum showed signals due to terminal methylene protons at δ 4.50 and 4.62 (each 1H, br s), -carbinol proton at δ 3.12 (1H, dd, *J* = 4.9, 11.4Hz) and seven methyl groups at δ 0.69, 0.72, 0.76, 0.87, 0.90, 0.96 and 1.63. The carbon signals appeared in the ^{13}C-NMR spectrum suggested the presence of two olefinic carbons at δ 109.54 and 151.18, an oxygenated carbon at δ 79.23 and seven methyl carbons at δ 14.33,

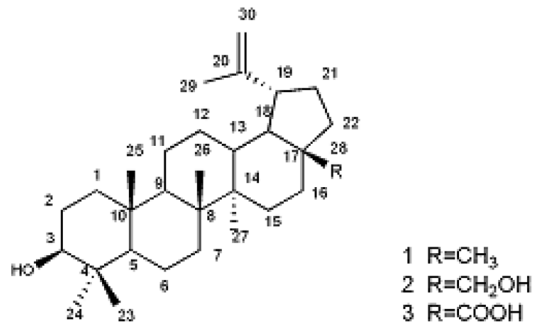

Fig. 1. Isolated compounds from *Pyrus pyrifolia*.

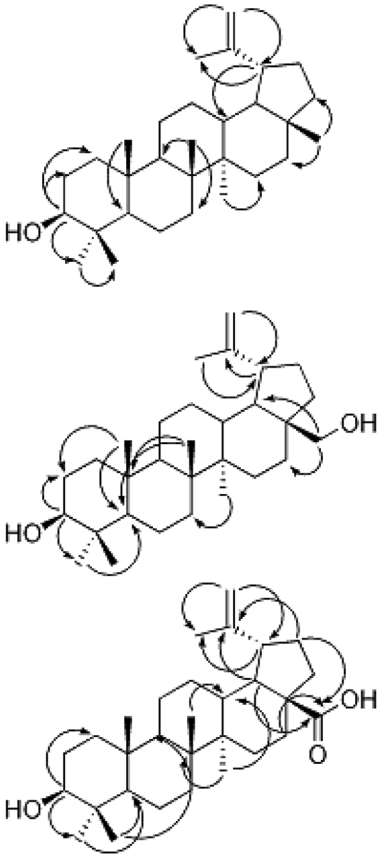

Fig. 2. HMBC connetivities of compounds **1** - **3**.

14.78, 15.58, 15.90, 17.69, 19.53 and 28.34. Based on this observation, together with the data from tracing the connectivities shown in the HMBC spectrum (Fig. 2.) and the data in the literature (Sholichin *et al*., 1980; Im *et al*., 2006), compound **1** was identified to be lupeol, lup-20(29)-en-3β-ol.

Compound **2** showed a molecular ion peak at *m/z* 442 in the EI-MS spectrum suggested a molecular formula to be $C_{30}H_{50}O_2$. The IR spectrum of **2** showed the presence of hydroxy bonds (2930 and 2860 cm^{-1}) and an olefinic bond (1685 cm^{-1}). The ^{1}H-NMR spectrum showed the presense of six methyl protons at δ 0.70, 0.78, 0.90, 0.99, 1.22 and 1.63. Terminal methylene protons at δ 4.66 and 4.62 (each 1H, br s) and an oxygenated methine proton at δ 3.10 (1H, dd, J = 5.8, 10.5Hz) suggested that **2** was a lupane type triterpene derivative. Two oxygenated methylene protons at δ 3.70 and 3.23 (each 1H, d, J = 10.9Hz), rather than a methyl singlet, confirmed the presence of a hydroxy group at C-28. Total 30 carbon signals observed in the ^{13}C-NMR spectrum, including two olefinic carbons at δ 149.90 and 108.59, one oxygenated methine carbon at δ 77.82, one oxygenated methylene carbon at δ 58.74 and six methyl carbons at δ 13.70, 14.43, 14.97, 15.13, 18.23 and 27.74. The HMBC (Fig. 2.) and HMQC spectra were in good agreement to the assigned structure. On the basis of the above evidences, together with the comparison with published literatures (Sholichin *et al*., 1980; Im *et al*., 2006; Siddiqui *et al*., 1988), the structure of **2** was determined to be lup-20(29)-en-3β, 28-diol, commonly known as betulin.

Compound **3** was purified as a white powder. The EI-MS spectrum displayed a $[M]^+$ peak at 456 analysing for $C_{30}H_{48}O_3$. The ^{1}H-NMR spectrum of **3** was almost the same as those of compound **2**, but two oxygenated methylene peaks of C-28 were not observed in the case of compound **3**. The ^{13}C-NMR spectrum of **3** was also similar to those of compound **2**. However, the characteristic carbonyl carbon of carboxylic acid (C-28) was observed at δ 148.24, and the chemical shift of C-17 was shown as a down shift from δ 46.9 to δ 56.6 compared to **2**. Therefore, the structure of compound **3** was elucidated as betulinic acid, lup-20(29)-en-28-oic acid. All assignments in the ^{1}H-NMR and ^{13}C-NMR were based on the measurement of HSQC, HMBC (Fig. 2.) and ^{1}H-^{1}H COSY. The final structure of **3** was confirmed by the comparison of spectroscopic values to those reported in the literature (Sholichin *et al*., 1980; Igoli and Alexander, 2008; Siddiqui *et al*., 1988; Khaliq *et al*., 2007).

The three compounds, lupeol (**1**) (Im *et al*., 2006; Igoli and Alexander, 2008; Lee and Lee, 1999), betulin (**2**) (Im *et al*., 2006; Mehta *et al*., 2003; Siddiqui *et al*., 1988; Rosas *et al*., 2007) and betulinic acid (**3**) (Igoli and Alexander, 2008; Siddiqui *et al*., 1988; Rosas *et al*., 2007; Khaliq *et al*., 2007; Abdel-Kader *et al*., 2007; Kim *et al*., 2008), were purified previously, but this is the first time that they have been isolated from this plant source.

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