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A New Naphthoquinone from *Pyrola japonica*

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A new naphthoquinone, 5,8-dihydro-2,7-dimethyl-[1,4]naphthoquinone (**1**), which was named 5,8-dihydrochimaphilin, isolated from an ethyl acetate soluble fraction from the root of *Pyrola japonica*, together with chimaphilin (**2**). Compound **1** was transformed rapidly to **2** upon exposure to air by HPLC analysis. This fact supported that chimaphilin (**2**) may be an artifact from **1**.

Key words: *Pyrola japonica*, 5,8-dihydro-2,7-dimethyl-[1,4]naphthoquinone, chimaphilin

INTRODUCTION

The root of *Pyrola japonica* Klenze ex Alefeld (Pyrolaceae) has been used for the treatment of arthritic disease (Kosuge *et al.*, 1985) and hemostasis (Kagawa *et al.*, 1992) in Chinese folk medicine. We have isolated chimaphilin from the root of *P. japonica* (Bae *et al.*, 1996), a 1,4-naphthoquinone derivative, which was shown to have significant cytotoxicities against L1210 and K-562 tumor cell lines with ED_{50} values of 1.17 and 0.88 μg/ml, respectively (Bae *et al.*, 1996).

This paper reports the isolation and characterization of two naphthoquinone compounds (**1, 2**) based on spectroscopic and chemical evidence.

MATERIALS AND METHODS

General

^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on NMR DRX300 spectrometer in $CDCl_3$ with TMS as an internal standard. IR spectra were recorded on IR Report-100 (JASCO). UV spectra were obtained on Schimadzu UV-260 UV/Visible spectrophotometer (Shimadzu). FAB-MS was obtained on Nermag R10-10 spectrometer (direct infusion) using NH_3 as ionizing gas. TLC and column chromatography were carried out on pre-coated Silica-gel F_{254} plates (Merck) and Kieselgel 60 (230 400 mesh, Merck).

Plant material

Pyrola japonica was collected in Taejon, Korea, in April 1999. It was identified by one of authors (Prof. K. Bae). A voucher specimen (CNU1584) is deposited at the Herbarium of College of Pharmacy, Chungnam National University.

Extraction and isolation

The air-dried root (170 g) was extracted with methanol (1 L) by refluxing for 4 h. The extract was evaporated *in vacuo* to afford a syrupy dark-brown residue (25 g), which was suspended in water (700 mL) and then extracted with ethyl acetate (700 mL × 3) to obtain an ethyl acetate-soluble fraction (11 g) after evaporation. A portion (10 g) of the ethyl acetate fraction was chromatographed on a Silica-gel (100 g) column eluted with hexane-EtOAc (4:1) to give four fractions, fr. 1 (3.1 g), fr. 2 (1.6 g), fr. 3 (1.1 g) and fr. 4 (0.6 g). Fr. 2 (1.6 g) was rechromatographed on a silica-gel (60 g), eluted (5 mL/min) with hexane-EtOAc (4:1), to afford **1** (200-400 mL, 61 mg) and **2** (400-700 mL, 150 mg). All fractions eluted were pooled and concentrated at nitrogen-charged evaporator to avoid autoxidation.

5,8-dihydro-2,7-dimethyl-1,4-naphthoquinone (1): yellow

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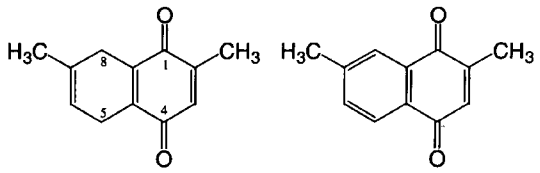
needles (hexane-EtOAc); m.p. 111-113°C; Negative-mode FAB-MS *m/z*: 187 $[M-H]^-$; UV λ_{max} nm (MeOH): 250, 285; IR ν_{max} cm^{-1}: 3020, 2960, 2920, 2870, 1640; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.5 (1H, s, H-3), 5.5 (1H, m, H-6), 3.0 (4H, overlap, H-5,8), 2.0 (3H, s, H-11), 1.8 (3H, s, H-12); ^{13}C-NMR (75 MHz, $CDCl_3$): 183.2 (C-1), 145.3 (C-2), 132.9 (C-3), δ 184.3 (C-4), 28.6 (C-5), 116.7 (C-6), 130.1 (C-7), 24.8 (C-8), 139.1 (C-9), 139.3 (C-10), 22.8 (C-11), 15.7 (C-12).

2,7-dimethyl-1,4-naphthoquione (2, chimaphilin): yellow needles (hexane-EtOAc); m.p. 114-115 °C; Negative-mode FAB-MS (*m/z*): 185 $(M-H)^-$; UV λ_{max} nm (MeOH): 253, 338; IR ν_{max} cm^{-1}: 3450, 2958, 2920, 1660, 1600; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.9 (1H, d, *J*= 8.1 Hz, H-5), 7.9 (1H, s, H-8), 7.5 (1H, d, *J*= 8.1 Hz, H-6), 6.8 (1H, m, H-3), 2.5 (3H, s, H-12), 2.2 (3H, d, *J*= 1.7 Hz, H-11); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 186.5 (C-1), 144.6 (C-2), 134.3 (C-3), 185.9 (C-4), 135.7 (C-5), 126.2 (C-6), 147.8 (C-7), 126.8 (C-8), 134.0 (C-9), 131.4 (C-10), 21.8 (C-11), 16.3 (C-12).

RESULTS AND DISCUSSION

Compound **1** was obtained as yellow needles. The molecular weight of 188 was obtained from the negative FAB-MS, which showed the quasimolecular ion $[M-H]^-$ peak at *m/z* 187 and could be deduced for the molecular $C_{12}H_{12}O_2$ with the combination of ^{13}C-NMR spectrum. The UV absorption at 250 nm and the IR band at 1640 cm^{-1} suggested the presence of a conjugated carbonyl group. The ^{1}H-NMR spectrum of **1** showed signals assignable for two methyls at δ 1.8 (H-12) and 2.0 (H-11) and two methylenes at δ 3.0 (H-5,8), in addition to two olefinic protons at δ 5.5 (H-6) and 6.5 (H-3). The ^{13}C-NMR spectrum demonstrated characteristic signals for two carbonyls at δ 183.2 (C-1) and 184.3 (C-4) and four quaternary carbons at δ 130.1, 139.1, 139.3, and 145.3. These data of **1** was closly related to chimaphilin (**2**), a 1, 4-naphtoquinone analogue. The signals at δ 28.6 (C-5) and δ 24.8 (C-8) of **1** appeared higher field than the corresponding resonances at δ 135.7 (C-5) and 126.8 (C-8) in **2**, indicating the presence of methylenes at C-5 and C-8. This was further supported by the ^{1}H-NMR spectral data, which showed two methylene signals at δ 3.00 (H-5,8). Consequently, the structure of **1** is determined as 5,8-dihydro-2,7-dimethyl-[1,4]naphthoquinone. This is the first report on **1** as a natural product. Compound **2** was identified as chimaphilin (**2**), by comparison with the reported spectral data (Wang *et al.*, 1988).



1 2

Fig. 1. Structures of compounds isolated from *Pyrola japonica*

Compound **1** was transformed rapidly to **2** upon exposure to air. After 2 h exposure to air, a mixture of **1** (21.2 min, 60%) and **2** (18.0 min, 40%) was identified by HPLC analysis (YMC-Pack ODS-AQ, 250×4.6 mm I. D., S-5 μm, 75% acetonitrile, 1.5 mL/min, 250 nm). This fact supported that chimaphilin (**2**) may be an artifact from 5,8-dihydro-2,7-dimethyl-[1,4]naphthoquinone (**1**).

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