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Hemiterpenes of *Ilex macropoda* Miq.

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Abstract – The chromatographic separation of the methanolic extract from the twigs of *Ilex macropoda* (Aquifoliaceae) led to the isolation of three hemiterpenes, and their chemical structures were elucidated by physico-chemical and spectroscopic analysis. Their structures were characterized as 4,5-dihydroxyprenyl caffeate (**1**), 4-(6-*O*-caffeoyl-β-D-glucucopyranosyloxy)-5-hydroxyprenyl caffeate (**2**), and 4-β-D-glucucopyranosyloxy-5-hydroxyprenyl caffeate (**3**), respectively. Compound **1** was isolated for the first time from plant.
Keywords – *Ilex macropoda*, Aquifoliaceae, hemiterpenes

Introduction

Ilex macropoda Miq. is a tree distributed in Korea, Japan and China (Kim, 1995). Earlier investigation on the chemical constituents of *Ilex* species dealt with saponins, terpenoids and phenolic compounds (Kakuno *et al.*, 1991; Taketa *et al.*, 2000; Ouyang *et al.*, 2001; Fuchino *et al.*, 1997; Filip *et al.*, 2001). In a previous study of *I. macropoda*, we reported the isolation and characterization of six pentacyclic triterpenoids (Kim *et al.*, 2002). In continuation of our phytochemical studies of this plant, we have investigated the ethyl acetate- and *n*-BuOH-soluble fractions of methanol extract of the twigs of the plant. A literature survey revealed that no pharmacological studies had been carried out on this plant. Therefore, we were interested in the chemical constituents of this plant for pharmacological study. The chromatographic separation of the ethyl acetate- and *n*-BuOH-soluble fractions of the methanol extract of the twigs of *I. macropoda* led to the isolation of a new hemiterpene and two known hemiterpene glycosides. This paper describes the isolation and structural determination of these compounds.

Experimental

General experimental procedures-^{1}H-and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 and Bruker DMX 600 spectrometers in CD_3OD, and TMS was used as an internal standard. The positive FAB-MS were determined on a JEOL 700 mass spectrometer. IR spectra were obtained on a JASCO FT/IR 410 spectrometer and UV spectra were recorded on Shimadzu UV-1601 UV-Visible spectrophotometer. TLC was carried out on Merck precoated silica gel 60 F_{254} plates (0.25 mm layer thickness), with compounds visualized by spraying plates with 10% (v/v) H_2SO_4 reagent followed by heating at 120 °C for 2-3 min. Silica gel for column chromatography was Kiesel gel 60 (230-400 mesh, Merck). And Sephadex LH-20 was used for column chromatography (Pharmacia, 25-100 μm). All other chemicals and solvents were analyticlal grade and used without further purification.

Plant materials – The aerial parts of *I. macropoda* were collected in October 2000 at Moak mountain, Chonbuk, Korea. A voucher specimen is deposited at the herbarium of college of pharmacy, Woosuk University, Korea (WSU-00-005).

Extraction and isolation – The air-dried plant materials (1.0 Kg) was extracted twice with hot MeOH under reflux. The resultant MeOH extract (210 g) was suspended in water, and then fractionated successively with equal volumes of CH_2Cl_2, ethyl acetate and *n*-BuOH, leaving residual water soluble fraction. Each fraction was evaporated in vaccuo to yield the residues of CH_2Cl_2-soluble fraction (40 g), ethyl acetate-soluble fraction (12 g) and *n*-BuOH-soluble fraction (65 g). The ethyl acetate-soluble fraction (5 g) was chromatographed over silica gel column using solvent system of $CHCl_3$-MeOH (4 : 1) as an eluent to give three subfractions (E1-E3), subfraction E2 was afforded 20 mg of **1** by RP silica gel column

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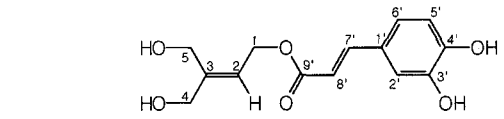
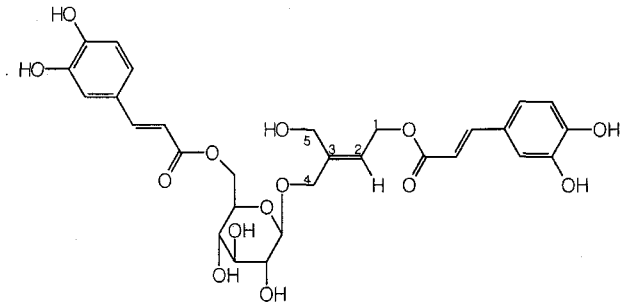
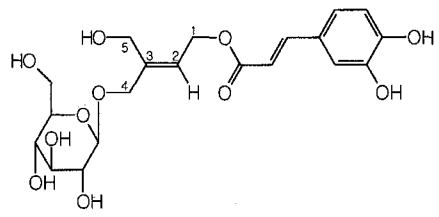


Comp. 1



Comp. 2



Comp. 3

Fig. 1. The structures of compounds **1-3**.

(MeOH : H_2O, 4 : 1). Subfraction E3 was purified by Sephadex LH-20 (MeOH) to yield **2** (35 mg). *n*-BuOH-soluble fraction (10 g) was chromatographed on silica gel column with $CHCl_3$-EtOAc-MeOH (1 : 1 : 1) to give four subfractions (B1-B4). Subfraction B2 was purified by Sephadex LH-20 (MeOH) to yield **3** (55 mg).

4,5-dihydroxyprenyl caffeate (1) – Amorphous powder; $[\alpha]^{24}_D$ −1.2° (c 0.1, MeOH); UV (MeOH) λ_{max} 325, 243, 215; FAB-MS m/z 303.0845 $[M+Na+H]^+$; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.55 (1H, d, J = 15.6 Hz, H-7'), 7.03 (1H, d, J = 1.8 Hz, H-2'), 6.93 (1H, dd, J = 8.4, 1.8 Hz, H-6'), 6.77 (1H, d, J = 8.4 Hz, H-5'), 6.25 (1H, d, J = 15.6 Hz, H-8'), 5.77 (1H, t, J = 6.6 Hz, H-2), 4.84 (2H, d, J = 6.6 Hz, H-1), 4.23 (2H, s, H-4), 4.16 (2H, s, H-5); ^{13}C-NMR (100 MHz, CD_3, OD) see Table 1.

4-(6-O-caffeoyl-β-D-glucucopyranosyl-oxy)-5-hydroxy prenyl caffeate (2) – Amorphous powder; UV (MeOH) λ_{max} 326, 298; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.49, 7.44 (each 1H, d, J = 15.6 Hz, caf-H-7',7"), 6.96, 6.93 (each 1H, d, J = 1.5 Hz, caf-H-2', 2"), 6.84, 6.82 (each 1H, dd, J = 8.4, 1.5 Hz, caf-H-6', 6"), 6.81, 6.68 (each 1H, d, J = 8.4 Hz, caf-H-5', 5"), 6.20, 6.14 (each 1H, d, J = 15.6 Hz, caf-H-8', 8"), 5.73 (1H, t, J = 6.6 Hz, H-2), 4.73 (2H, d, J = 6.6 Hz, H-1), 4.40 (1H, d, J = 12.8 Hz, H-4), 4.29 (1H, d, J = 7.6 Hz, glc-H-1), 4.16 (2H, s, H-5), 4.15 (1H, d, J = 12.8 Hz, H-4); ^{13}C-NMR (100 MHz, CD_3, OD) see Table 1.

Table 1. ^{13}C-NMR spectral data of compounds **1-3** from *Ilex macropoda*

Carbon	1	2	3
1	61.3	61.2	61.2
2	123.0	123.0	125.9
3	144.7	141.0	141.0
4	64.5	71.9	71.4
5	58.5	58.5	58.4
Caf-1	127.7	127.6/126.2	127.5
Caf-2	115.1	116.4/116.4	115.2
Caf-3	149.6	149.5/149.5	149.3
Caf-4	146.8	147.1/147.0	147.0
Caf-5	116.5	116.4/116.4	116.5
Caf-6	122.5	122.9/122.9	123.0
Caf-7	147.0	147.0/146.6	146.5
Caf-8	115.0	114.8/114.7	114.8
Caf-9	169.1	169.0/168.9	169.0
Glc-1		103.0	103.1
Glc-2		75.0	74.8
Glc-3		77.9	77.8
Glc-4		71.7	71.8
Glc-5		75.4	77.6
Glc-6		64.6	62.5

Recorded at 100 MHz in CD_3OD.

4-β-D-glucucopyranosyloxy-5-hydrox-yprenyl caffeate (3) – Amorphous powder; UV (MeOH) λ_{max} 325, 298; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.44 (1H, d, J = 15.6 Hz, H-7'), 6.96 (1H, d, J = 1.5 Hz, H-2'), 6.83 (1H, dd, J = 8.4, 1.5 Hz, H-6'), 6.69 (1H, d, J = 8.4 Hz, H-5'), 6.14 (1H, d, J = 15.6 Hz, H-8'), 5.73 (1H, t, J = 6.6 Hz, H-2), 4.73 (2H, d, J = 6.6 Hz, H-1), 4.34 (1H, d, J = 12.8 Hz, H-4), 4.25 (1H, d, J = 7.6 Hz, glc-H-1), 4.17 (2H, s, H-5), 4.14 (1H, d, J = 12.8 Hz, H-4); ^{13}C-NMR (100 MHz, CD_3, OD) see Table 1.

Results and Discussion

In the course of phytochemical study of the MeOH extract from the twigs of *I. macropoda*, three hemiterpenes were isolated from the ethyl acetate- and *n*-BuOH-soluble fractions. Compounds **1-3** have similar patterns in their NMR spectra. ^{1}H- and ^{13}C-NMR data indicated the presence of a caffeoyl and a β-D-glucopyranosyl moiety in the molecule of **3**, and two caffeoyl and a β-D-glucopyranosyl moiety in the molecule of **2**. These known compounds, 4-(6-*O*-caffeoyl-β-D-glucucopyranosyloxy)-5-

hydroxyprenyl caffeate (**2**) and 4-β-D-glucucopyranosyloxy-5-hydroxyprenyl caffeate (**3**) were identified by physical and spectroscopic data measurement and by comparison with published values (Fuchino *et al.*, 1997).

Compound **1** was obtained as an amorphous powder and formulated as $C_{14}H_{15}O_6$ by high-resolution FAB-MS. ^{1}H-[δ 7.55 (1H, d, J = 15.6 Hz, H-7'), 7.03 (1H, d, J = 1.8 Hz, H-2'), 6.93 (1H, dd, J = 8.4, 1.8 Hz, H-6'), 6.77 (1H, d, J = 8.4 Hz, H-5'), and 6.25 (1H, d, J = 15.6 Hz, H-8')] and ^{13}C-[(δ 169.1 (C-9'), 149.6 (C-3'), 147.0 (C-7'), 146.8 (C-4'), 127.7 (C-1'), 122.5 (C-6'), 116.5 (C-5'), 115.1 (C-2'), and 115.0 (C-8')] NMR data indicated the presence of a caffeoyl moiety in the molecule. Accommodation of the remaining signals, δ_H [5.77 (1H, t, J = 6.6 Hz), 4.84 (2H, d, J = 6.6 Hz), 4.23 (2H, s) and 4.16 (2H, s)], and δ_C (144.7, 123.0, 64.5, 61.3 and 58.5), led to the formulation of a 4,5-dioxygenated prenyl structure, and the signals at 4.84 (2H, d, J = 6.6 Hz) and 5.77 (1H, t, J = 6.6 Hz) were assigned for H-1 and H-2 on the basis of the coupling pattern. Considering the down field shift of the proton signal for C-1, δ_H 4.84, and the correlation with the carboxyl carbon of the caffeoyl group, δ_C 169.1, in long-range ^{1}H-^{13}C shift correlation spectroscopy (HMBC), the position of the caffeoyl group was determined to be at C-1. Other signals were assigned by nuclear overhauser effect correlation spectroscopy (NOESY). On the basis of these observation and careful analysis of the COSY, DEPT, and HMBC NMR data of **1**, compound **1** was identified as 4,5-dihydroxyprenyl caffeate. This is the first report on the isolation of compound **1** from the nature. Recently, as similar compounds **1-3**, two new hemiterpene glucosides named pubescenosides A [2-(*trans*-caffeoyloxy) methyl-3-hydroxy-1-butene-4-*O*-β-D-glucopyranoside] and B (2-hydroxymethyl-3-caffeoyloxy-1-butene-4-*O*-β-D-glucopyranoside) were reported from the root of *I. pubescens* (Jiang *et al.*, 2005). Pharmacological investigation on pubescenosides A and B indicated that both possess potent anti-platelet aggregation activities.

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