Arch Pharm Res Vol 26, No 8, 591-593, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

A New Phenylpropanoid Glucoside from the Fruits of *Illicium verum*

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(Received May 16, 2003)

One new phenylpropanoid glucoside (**3**), along with one known phenylpropanoid (**1**), and one known alkyl glucoside (**2**) were isolated from the fruits of *Illicium verum* and their chemical structures were elucidated on the basis of spectroscopic studies.

Key words: The fruits of *Illicium verum*, Illiciaceae, Phenylpropanoid glucoside, Phenylpropanoid, Alkyl glucoside

INTRODUCTION

The fruits of *Illicium verum* Hook f. (Star anise, Illiciaceae) have been used as a traditional medicine for treatment of stomach disease, pain, etc., in eastern Asian countries (Claus and Tyler, 1965; Okuyama *et al.*, 1993). Several phenylpropanoids, sesquiterpenes, 4-ethoxyphenol, anisy ketone, and veranisatins A-C from this plant have been reported (Okuyama *et al.*, 1993; Nakamura *et al.*, 1996 Sy and Brown, 1998a, 1998b). In this paper, we report the structural elucidation of one new phenylpropanoid glucoside (**3**), along with one known phenylpropanoid (**1**), and one known alkyl glucoside (**2**) from the fruits of *I. verum*.

MATERIALS AND METHODS

General experimental procedures

Melting point was measured by Fisher-Johns melting point apparatus and is uncorrected. Optical rotations were measured using a JASCO DIP-1000 (Tokyo, Japan) automatic digital polarimeter. FT-IR spectra were recorded on a JASCO FT-IR 300E spectrophotometer, and UV spectra on a JASCO V-550 spectrophotometer. The NMR spectra were recorded on Bruker 300 MHz (ARX 300), and Bruker 600 MHz (DMX 600) spectrometers. Samples were dissolved in either acetone-d_6 or CD_3OD and chemical shifts were reported in ppm downfield from TMS. The 2D NMR spectra were recorded by using Bruker's standard pulse program. The FAB-MS spectra were measured with a VG TRIO 2A mass spectrometer. The GC column chromatography was performed on a Hewlett Packard GC 6890 with an HP-5 column (crosslinked 5%-phenyl methyl silicone, 25 m×0.32 mm×0.17 μm) and N_2 as carrier gas (N_2 flow: 3 mL/min; air flow: 3 mL/min), oven temp.: 200°C; inlet temp.: 290°C; FID detector temp.: 250°C. Retention time for trimethylsilyl esters of methyl-2-(polyhydroxyalkyl)-thia-zolidine-4(*R*)-carboxylate of standard D-glucose was 17.1 and 19.6 min, and 10.7 and 11.2 min for L-rhamnose. Stationary phases for column chromatography (Silica gel 60, 70-230, and 270-400 mesh, and Lichroprep RP-18 gel, 40-63 μm, Merck) and TLC plates (Silica gel 60 F_{254} and RP-18 F_{254}) were purchased from EM Scientific. Spots were detected under UV radiation and by spraying with 10% H_2SO_4, followed by heating. D-glucose, L-rhamnose, and *N*-TMS-imidazole (*N*-trimethysilyimidazole) were purchased from Sigma Chemicals Co. Ltd. and Tokyo Kasei Kogyo Co. Ltd., respectively. All other chemicals and solvents were analytical grade and used without further purification.

Plant material

Dried fruits of *I. verum* were purchased in November 1996 from a folk medicine market "Yak-ryong-si" in Daegu and the material was confirmed taxonomically by Professor Gi-Hwan Bae, Chungnam National University in Daejeon, Republic of Korea. A voucher specimen (YNS-96-02) is

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preserved at the College of Pharmacy, Yeungnam University.

Extraction and isolation

The dried fruits of *I. verum* (9 kg) were extracted twice with 90% MeOH (2×12 L) under reflux for 12 h. The MeOH solution was evaporated to dryness (1.13 kg) and the residue partitioned between H_2O and *n*-hexane. The resulting H_2O layer was extracted with EtOAc and *n*-BuOH successively. The *n*-BuOH extract (246.8 g) was chromatographed on a silica gel column (No.9385, 230-400 mesh, 8.0 cm×80 cm) with *n*-BuOH-EtOAc-H_2O (100:1:1, 50:1:1, 10:1:1, 4:1:1) in a stepwise gradient mode. The fractions (100 mL in each flask) were combined on the basis of silica gel TLC and 7 fractions (F1-7) were obtained. The fraction F2 (10.9 g) was rechromatographed over a silica gel column (No.9385, 230-400 mesh, 4.0 cm×60 cm) with CH_2Cl_2-CH_3OH-H_2O (15:2:0.1, 12:2:0.1, 10:2:0.1, 8:2:0.1, 7:3:0.1, 4:6:0.1) in a stepwise gradient mode. 19 fractions (F2-1-2-19) were obtained from this column. The fraction F2-4 (493.7 mg) and F2-6 were rechromatographed over a reverse phase column (40-63 μm, 2.0 cm×75 cm) with CH_3OH-H_2O in a stepwise gradient mode, and gave compounds **1** (F2-4-1) and **2** (F2-6-1), respectively. The fraction F2-11 (657.5 mg) was rechromatographed over a reverse-phase column (40-63 μm, 2.0 cm×75 cm) with CH_3OH-H_2O (3:7, 4:6, 1:1) in a stepwise gradient mode to give compound **3** (F2-11-6).

Compound **1** (6 mg): Colourless oil; $[\alpha]_D^{25}$ 11.95° (c = 0.206, MeOH); ^{1}H-NMR (acetone-d_6, 300 MHz) δ 7.31 (2H, d, *J* = 8.5 Hz, H-2'/ H-6'), 6.87 (2H, d, *J* = 8.5, H-3'/ H-5'), 4.60 (1H, d, *J* = 6.1 Hz, H-1), 3.77 (3H, s, 4'-OCH_3), 3.61 (1H, m, H-2), 3.47 (1H, m, H-3a), 3.36 (1H, m, H-3b); ^{13}C-NMR (acetone-d_6, 75 MHz) δ 159.8 (C-4'), 135.4 (C-1'), 128.8 (C-2'/C-6'), 114.1 (C-3'/C-5'), 77.2 (C-2), 74.3 (C-1), 63.8 (C-3), 55.4 (4'-OCH_3); Positive FAB-MS *m/z* 221 $[MNa]^+$.

Compound **2** (6.3 mg): Brown solid; 74-75°; $[\alpha]_D^{25}$ 42.58° (c = 0.225, MeOH), {lit. $[\alpha]_D^{20}$ 44.5° (c = 1.0, H_2O) (Veibel and Lillelund, 1938)}; ^{1}H- and ^{13}C-NMR data are consistent with literature values (Crout *et al.*, 1990; Sigurskjold *et al.*, 1992); positive FAB-MS *m/z* 237 $[MH]^+$.

Compound **3** (31.1 mg): Yellow amorphous powder; mp: 109-111°; $[\alpha]_D^{25}$ 52.92° (c = 0.225, MeOH); UV (MeOH) λ_{max} (log e) 224.2 (3.99), 280.6 (3.48); IR (KBr) ν_{max} 3396.9, 2932.2, 1613.1, 1513.8, 1457.9, 1249.6 cm^{-1}; ^{1}H-NMR (CD_3OD-d_4, 600 MHz) δ 7.07 (1H, d, *J* = 8.9 Hz, H-6'), 6.97 (1H, d, *J* = 3.0 Hz, H-3'), 6.89 (1H, dd, *J* = 16.1, 1.5 Hz, H-1), 6.73 (1H, dd, *J* = 8.9, 3.0 Hz, H-5'), 6.22 (1H, dt, *J* = 16.1, 6.6 Hz, H-2), 4.89 (1H, s, H-1'"), 4.71 (1H, s, H-1'"), 4.67 (1H, d, *J* = 7.6 Hz, H-1"), 4.00 (1H, dd, *J* = 11.0, 1.5 Hz, H-6"a), 3.83 (1H, m, H-3'"), 3.75 (3H, s, 4'-OCH_3), 3.67 (1H, dd, *J* = 9.5, 3.4 Hz, H-2'"), 3.62 (1H, dd, *J* = 9.6, 6.2 Hz, H-5'"), 3.59 (1H, dd, *J* = 11.0, 6.6 Hz, H-6"b), 3.47 (2H, m, H-2", H-5"), 3.43 (1H, t, *J* = 9.1 Hz, H-3"), 3.37 (1H, m, H-4'"), 3.35 (1H, m, H-4"), 1.87 (3H, dd, *J* = 6.6, 1.5 Hz, H-3), 1.22 (3H, d, *J* = 6.2 Hz, H-6'"); ^{13}C-NMR (CD_3OD-d_4, 150 MHz) δ 156.7 (C-4'), 149.6 (C-2'), 130.6 (C-1'), 127.2 (C-2), 126.7 (C-1), 119.6 (C-6'), 114.0 (C-5'), 111.5 (C-3'), 103.9 (C-1"), 102.2 (C-1'"), 78.2 (C-3"), 76.8 (C-5"), 75.1 (C-2"), 73.9 (C-4'"), 72.3 (C-2'"), 72.1 (C-3'"), 71.6 (C-4"), 69.8 (C-5'"), 68.1 (C-6"), 56.0 (4'-OCH_3), 18.8 (C-3), 17.9 (C-6'"); HR-FAB-MS *m/z* 473.1983 (calcd. for $C_{22}H_{33}O_{11}[M+H]^+$ 473.2013).

Determination of Sugars in 2 and 3

Hydrolysis, derivatization and GLC analysis of sugar residues of **2** and **3** were performed by the reported method (Hara *et al.*, 1987).

RESULTS AND DISCUSSION

The MeOH extract of the fruits of *I. verum* was partitioned with *n*-hexane, EtOAc, *n*-BuOH and H_2O, successively, which were then dried. The *n*-BuOH fraction was chromatographed on Silica-gel column. A major fraction from this column was rechromatographed on a reverse-phase column, which afforded compounds **1-3**.

The molecular formula of **3**, $C_{22}H_{32}O_{11}$ was established from HR-FAB-MS, ^{13}C-NMR, and DEPT spectral data. The ^{1}H-NMR spectrum of **3** indicated the presence of *trans*-olefinic protons, doublet-doublet at δ 6.89 (*J* = 16.1, 1.5 Hz), and doublet-triplet at δ 6.22 (*J* = 16.1, 6.6 Hz). After acid hydrolysis of **3**, sugars were converted to the trimethylsilyl esters of methyl-2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylate and were then determined by GLC

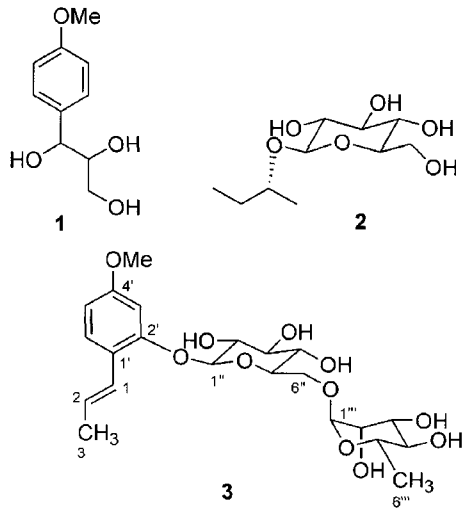

Fig. 1. Compounds isolated from the fruits of *Illicium verum*

analysis to be D-glucoses and L-rhamnose (Enoki *et al.*, 1981). The ^1H,^1H-homonuclear COSY spectrum of **3** showed connectivities between H-5' and H-6', and among H-1, H-2, and H-3. The sugar linkages were determined by heteronuclear multiple-bond correlations (HMBC), which established the locations to be connected among the aglycone of **3** and the two sugar moieties on the basis of two cross peaks, one between C-2' and H-1", and one between C-6" and H-1'". The configurations of anomeric protons of **3** were deducted on the basis of coupling constants of the proton peaks to be b and a form (7.6 Hz and 0 Hz) (Harborne, 1993). Thus the structure of **3** was confirmed.

Compound **1** was elucidated as 1-(4'-methoxyphenyl)-1,2,3-trihydroxypropane from FAB-MS, ^1H- and ^{13}C-NMR data. In addition, compound **1** was suggested to be an *threo*-isomer on the basis of the coupling constant value 6.1 Hz of the benzylic proton H-1 signal at δ 4.60 (Hottori *et al.*, 1987; Ishimaru *et al.*, 1987; Ishida *et al.*, 1996; Mohamed, 2001). Compound **1** was reported previously (Miki *et al.*, 1987; Sakamoto *et al.*, 1999) and isolated for the first time from this plant, and its ^1H- and ^{13}C-NMR data are presented here for the first time as far as we were able to determine.

Compound **2** were identified to be (*R*)-*sec*-butyl-β-D-glucopyranoside by comparison of physical and spectroscopic data (optical rotation value, ^1H- and ^{13}C-NMR) with those in the literature (Veibel and Lillelund, 1938; Crout *et al.*, 1990; Sigurskjold *et al.*, 1992).

ACKNOWLEDGMENT

This research was financially supported, by the Ministry of Health and Welfare of the Republic of Korea (HMP-99-O-11-0008-C).

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