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Solanoflavone, A New Biflavonol Glycoside from *Solanum melongena*: Seeking for Anti-Inflammatory Components

Guanghai Shen, Phan Van Kiem[1], Xing-Fu Cai, Gao Li[2], Nguyen Tien Dat, Yeon A Choi[3], Young Mi Lee[3], Yong Ki Park[4], and Young Ho Kim

College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea, [1]Institute of Natural Product Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghiado, Caugiay, Haoi, Vietnam, [2]College of Pharmacy, Yanbian University, Yanji 133000, China, [3]Department of Oriental Pharmacy, College of Pharmacy, Wonkwang University, Iksan, Cheonbuk 540-749, Korea, and [4]Department of Herbology, College of Oriental Medicine, Dongguk University, Kyongju, Kyeongbuk 780-714, Korea

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A new biflavonol glycoside named as solanoflavone (**1**) was isolated from aerial part of *Solanum melongena*. The chemical structure was elucidated as isorhamnetin-3-*O*-β-D-glucopyranoside-(4'→*O*→4''')-galangin-3''-*O*-β-D-glucopyranoside on the basis of physicochemical and spectroscopic methods, including 2D NMR spectral techniques.

Key words: *Solanum melongena*, Biflavonol glycoside, Solanoflavone, Solanaceae

INTRODUCTION

The fruits of *Solanum melongena* (eggplant) are well known as a vegetable all over the world. There are several phenotypes of eggplant as their uses. Of these, the yellow fruit of *S. melongena* (white eggplant, Solanaceae) is commonly used as an ornamental garden plant. Normal eggplant is long and deep purple color, but white eggplant has round form and becomes yellow on ripening. It has the 'cool' property and various pharmacological activities. Crude alkaloidal fraction isolated from the leaves of *S. melongena* exhibited significant analgesic effect and some CNS depression (Vohora *et al.*, 1984). Anthocyanin from eggplant peels of *S. melongena* has antioxidant activity (Noda *et al.*, 1998) and flavonoids from fruits of *S. melongena* have hypolipidermic effects (Sudheesh *et al.*, 1997). We previously have reported that water extract of fruits of *S. melongena* (SMWE) inhibited immunologic and nonimmunologic stimulator-mediated anaphylactic reactions and TNF-α secretion from mast cells (Lee *et al.*, 2001). The SMWE significantly also inhibited PAR2 agonist-induced myeloperoxidase activity and tumor necrosis factor-α expression in paw edema (Han *et al.*, 2003). In spite of pharmacological effects of *S. melongena*, phytochemical research was limited to a few reports. A high content of antioxidant phenolic compounds, hydroxycinnamic conjugates, were identified in the fruit of seven commercial cultivars (Whitaker and Stommel, 2003). An anthocyanin, delphinidine-3-(*p*-cumaroylrutinoside)-5-glucoside (nasunin), was isolated as purple colored crystals from eggplant peels (Noda *et al.*, 1998). In order to identify the anti-inflammatory components from white eggplant, we followed the activity guided fractionation and purification of active compound. This paper reports the isolation and structural elucidation of a new biflavonol glycoside named as solanoflavone (**1**) from aerial part of white eggplant.

Correspondence to: Young Ho Kim, College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
Tel: 82-42-821-5933, Fax: 82-42-823-6566
E-mail: yhk@cnu.ac.kr

MATERIALS AND METHODS

General experimental procedures

Melting points were measured using a Yanaco micro melting point apparatus, optical rotations using a JASCO DIP-370 (Tokyo, Japan) automatic digital polarimeter, UV spectra with a Beckman Du-650 UV-VIS recording spectrophotometer, and FT-IR spectra with a JASCO Report-100 infrared spectrometer. HPLC was carried out using an ODS column (Waters). NMR spectra were recorded using a Bruker DRX 300 and 600 NMR spectrometer. The 2D NMR spectra were recorded by using Bruker's standard pulse program. FAB-MS spectrum was measured with a

JEOL JMS-HX/HX110A tandem mass spectrometer. Column chromatography was performed using silica-gel (*Kieselgel 60, 70-230 mesh and 230-400 mesh*, Merck), Lichroprep RP-C_{18} gel (40-63 μm, Merck) and Sephadex LH-20 (Pharmacia Biotech, Sweden), and thin layer chromatography (TLC) using a pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-C_{18} F_{254} plates (0.25 mm, Merck). Spots were detected under UV radiation and by spraying with 10% H_2SO_4, followed by heating. All other chemicals and solvents were analytical grade and used without further purification.

Plant materials

Dried aerial part of white eggplant was gifted from Mr. Seong Kyeong Kim (Cheongdo, Kyeongbuk, Korea) and identified by Professor Young Mi Lee, College of Pharmacy, Wonkwang University in Cheonbuk, Korea. A voucher specimen (NO 2000-03) is deposited at the Herbarium in College of Pharmacy, Wonkwang University.

Extraction and isolation

The dried aerial part of *S. melongena* L. (0.88 kg) was extracted with MeOH three times under reflux for 12 h to yield 92 g of an extract, 90 g of which was then suspended in H_2O and successively partitioned with dichloromethane, ethyl acetate, and *n*-buthanol. The ethyl acetate soluble fraction (5.66 g) was subjected to chromatography on a silica gel column, using $CHCl_3$-MeOH (10:3) as an eluent, yielding 3 fractions [fr. A (1.76 g), fr. B (1.20 g) and fr. C (2.17 g)]. The fr. B (1.20 g) was subjected to chromatography on a RP-C_{18} column using MeOH-H_2O (9:11) as an eluent, giving 2 subfractions [subfr. 2A (600.0 mg) and subfr. 2B (520.0 mg)]. The subfr. 2B (270.0 mg) was subjected to chromatography on a Sephadex LH-20 column eluted with MeOH to give compound **1** (17.2 mg). Compound **1**: Yellow amorphous powder; m.p.; 165-167 °C; $[\alpha]_D^{28}$-12.0° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 267.0 (4.61); IR (KBr) ν_{max}: 3365, 1652, 1607, 1558, 1507, 1456, 1361, 1207, 1062 cm^{-1}; HR-FAB-MS m/z: 909.2083 $[M+H]^+$ (calcd. for $C_{43}H_{41}O_{22}$: 909.2090); ^{1}H- (600 MHz, CD_3OD) and ^{13}C-NMR (150 MHz, CD_3OD): see Table I.

Determination of sugars in 1

A solution of compound **1** (4 mg) in 5 mL of 10% HCl was heated for 3 h. The reaction mixture was concentrated under reduced pressure and diluted with 30 mL H_2O. The solution was neutralized with Ag_2CO_3 and extracted with EtOAc. The aqueous layer was concentrated, filtered and passed through a NOVA-Pak C_{18} cartridge (Waters) and then repeatedly separated by HPLC [mobile phase: MeCN-H_2O (3:1), flow rate: 0.6 mL/min, detection: refractive index (RI)] to afford D-glucose (1.2 mg). The optical rotation value {$[\alpha]_D^{24}$ +37.2° (*c* 0.12, H_2O)} was a good agreement with that of D-glucose (Crublet *et al.*, 2003, Kiem *et al.*, 2003).

Table I. ^{1}H- and ^{13}C-NMR Data (600 and 150 MHz) for compound **1** in CD_3OD

position	^{1}H δ (*J* in Hz)	^{13}C δ	position	^{1}H δ (*J* in Hz)	^{13}C δ
2		158.42	2"		158.87
3		135.20	3"		135.32
4		179.13	4"		179.23
5		162.80	5"		162.80
6	6.18 (1H, s)	99.85	6"	6.18 (1H, s)	99.85
7		165.89	7"		165.89
8	6.39 (1H, s)	94.71	8"	6.39 (1H, s)	94.71
9		158.23	9"		158.28
10		105.58	10"		105.62
1'		122.95	1"'		122.67
2'	7.92 (1H, d, 2.4)	114.29	2"'	8.05 (1H, d, 9.0)	132.11
3'		148.20	3"'	6.88 (1H, d, 9.0)	115.94
4'		150.64	4"'		161.33
5'	6.87 (1H, d, 8.4)	115.87	5"'	6.88 (1H, d, 9.0)	115.94
6'	7.58 (1H, dd, 8.4, 2.4)	123.71	6"'	8.05 (1H, d, 9.0)	132.11
3'-OCH_3	3.93 (3H, s)	56.78			
1""	5.40 (1H, d, 7.8)	103.66	1"""	5.23 (1H, d, 7.2)	104.11
2""	3.46 (1H, m)	75.88	2"""	3.46 (1H, m)	75.69
3""	3.42 (1H, m)	78.02	3"""	3.42 (1H, m)	78.04
4""	3.31 (1H, m)	71.34	4"""	3.31 (1H, m)	71.47
5""	3.42 (1H, m)	78.33	5"""	3.42 (1H, m)	78.45
6""	3.70 (1H, dd, 9.6, 2.4) 3.74 (1H, dd, 9.6, 2.4)	62.57	6"""	3.55 (2H, m)	62.64

RESULTS AND DISCUSSION

In the course of activity-guided fractionation procedure of the MeOH extract of *S. melongena*, a new biflavonol glycoside (**1**) was isolated in the EtOAc soluble fraction.

Compound **1** was obtained as yellow amorphous power. HR-FAB-MS gave a molecular ion at *m/z* 909.2083 $[M+H]^+$ providing the formula $C_{43}H_{40}O_{22}$ (calcd. for $C_{43}H_{41}O_{22}$: 909.2090). The ^{1}H-NMR and ^{13}C-NMR (two carbonyl resonances at δ_C 179.13 and 179.23), together with IR absorption at 3365 (-OH) and 1652 (conjugated CO) cm^{-1} suggested that compound **1** possessed two flavonoid units (Kuo *et al.*, 2002) and two sugar moieties. In the aromatic region of the ^{1}H-NMR spectra of compound **1**, ^2J coupling between δ_H 6.87 (H-5', d, J = 8.4 Hz) and δ_H 7.58 (H-6', dd, J = 8.4, 2.4 Hz), and ^3J coupling between δ_H 7.92 (H-2', d, J = 2.4 Hz) and δ_H 7.58 (H-6', dd, J = 8.4, 2.4 Hz) indicated an 1,3,4-trisubstituted benzene ring B, and ^2J coupling between two sets of chemically equivalent protons δ_H 8.05 (H-2"'/H-6"', d, J = 9.0 Hz) and δ_H 6.88 (H-3"'/H-5"', d, J = 9.0 Hz) suggested an 1,4-disubstituted

aromatic ring E. The ^{13}C-NMR spectrum of compound **1** exhibited two chemically equivalent aromatic carbons δ_C 132.11 (C-2'''/C-6''') and δ_C 115.94 (C-3'''/C-5'''), and a methoxyl group at δ_C 56.78 (3'-OCH_3). The C-H long range correlations between an anomeric proton H-1'''' (δ_H 5.40) and carbon C-3 (δ_C 135.20), between another anomeric proton H-1''''' (δ_H 5.23) and carbon C-3" (δ_C 135.32), between methoxy protons (δ_H 3.93) and carbon C-3' (δ_C 148.20) were observed in the HMBC spectrum (Fig. 2). The position of the methoxy group was confirmed by the positive NOE effect between H-2' (δ_H 7.92) and 3'-OCH_3 (δ_H 3.93). The above spectral studies suggested that compound **1** could be a biflavonol glycoside consisting of two flavonol groups and two sugar moieties with an ether linkage. Comparison of ^{13}C-NMR spectral data of compound **1** with those of 2,3-dihydroochnaflavone 7,4',7"-tri-*O*-methyl ether showed that C-4' of ring B should be involved in the interflavonoid ether linkage with C-4''' of ring E (Jayakrishna *et al.*, 2003). After acid hydrolysis of **1**, the aqueous layer was separated by HPLC to give only D-glucose, and then optical rotation of the purified glucose was determined (Kiem *et al.*, 2003). The position of sugar linkage was confirmed at C-3 and C-3" by HMBC correlations, respectively. The configurations of anomeric protons of compound **1** were both deduced to be β form on the basis of coupling constants [H-1'''' (J = 7.8 Hz) and H-1''''' (J = 7.2 Hz)]. Based on the above data, the structure of compound **1** was determined as a isorhamnetin-3-*O*-β-D-glucopyranoside-(4'→*O*→4''')-galangin-3"-*O*-β-D-glucopyranoside named as solanoflavone. Its anti-inflammatory activities will be published elsewhere.

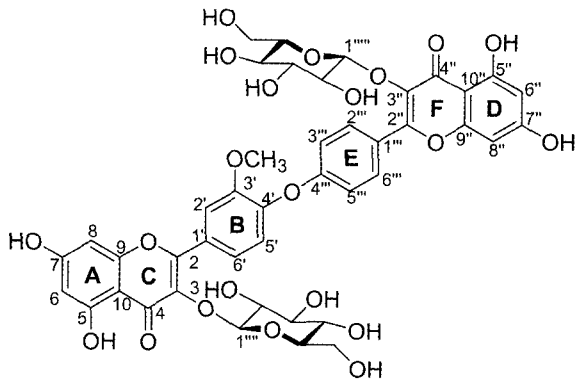

Fig. 1. Structure of compound 1

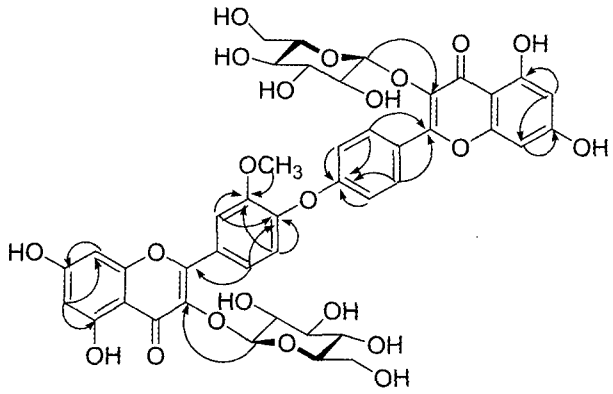

Fig. 2. HMBC correlations of compound 1

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