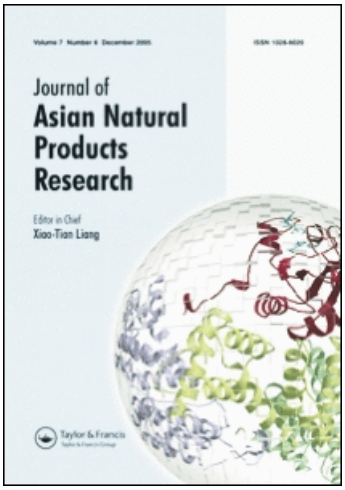

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A novel C-glycosylflavone from the leaves of *Diospyros kaki*

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A novel C-glycosylflavone from the leaves of *Diospyros kaki*

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A novel C-glycosylflavone with unusual α-orientation at the anomeric center of D-glucose was isolated from the leaves of *Diospyros kaki*. Its structure was determined as 8-C-[α-L-rhamnopyranosyl-(1 → 4)]-α-D-glucopyranosylapigenin by chemical methods and spectral experiments.

Keywords: Ebenaceae; *Diospyros kaki*; C-glycosylflavone

1. Introduction

The genus *Diospyros* belongs to family Ebenaceae and comprises about 500 species distributed in the tropical and temperate zones. Although many studies about quinone compounds in the *Diospyros* plants have been reported, there are few reports about constituents in leaves in spite of medicinal uses [1].

Diospyros kaki is widely distributed in East Asia, whose leaves are a traditional plant medicine used for the treatment of hypertension, angina, and internal hemorrhage [2]. It was also reported that in Japan and Korea, the leaves of *D. kaki* are used as health food (persimmon leaf tea) to promote maternal health [3]. Previous phytochemical studies on this plant revealed the presence of triterpenoids, flavonoids, and phenolic compounds [4,5]. As part of our continuous search for potentially active substances from the leaves of *D. kaki*, we described herein the isolation and structural elucidation of a novel C-glycosylflavone, 8-C-[α-L-rhamnopyranosyl-(1 → 4)]-α-D-glucopyranosylapigenin (**1**) together with the six known compounds 2″-*O*-rhamnosyl vitexin (**2**), kaempferol-3-*O*-α-L-rhamnopyranoside (**3**), myricetin-3-*O*-α-L-rhamnopyranoside (**4**), myricetin-3-*O*-β-D-gluco-pyranoside(**5**), blumeol C glucoside (**6**), and byzantionoside B (**7**) from the *n*-BuOH extract of persimmon leaves.

2. Results and discussion

The leaves of *D. kaki* were extracted with 70% EtOH and then concentrated. The concentrated extract was suspended in water and partitioned with $CHCl_3$ and *n*-BuOH, successively. Since the flavonoid and triterpenoid compounds are two kinds of main constituents in persimmon leaves, the *n*-BuOH extract was then chromatographed by polyamide and eluted with 10 and 70% MeOH to give non-flavonoid and phenolic extracts. The phenolic extract was further separated by repeated column chromatography on silica gel, reversed-phase silica gel, Sephadex LH-20, HPLC, and preparative TLC to afford compounds **1**–**7**.

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Compound **1** was obtained as yellow needles and gave positive reactions with $FeCl_3$ and HCl–Mg reagents. Its HR-ESI-MS showed a molecular-ion peak at *m/z* 579.1721 ($[M+H]^+$) indicating the molecular formula $C_{27}H_{30}O_{14}$. Acid hydrolysis of **1** followed by TLC analysis of the hydrolysate and direct comparison with authentic sugars indicated the presence of rhamnose. Its IR spectrum showed absorption bands of hydroxyl group (3396 cm^{-1}) and carbonyl group (1678 cm^{-1}). These evidences, together with UV absorption maxima at 310, 269, and 240 nm, suggested a glycosylflavone structure of compound **1**.

The ^1H NMR spectrum of **1** presented three phenol protons around δ_H 13.0 and 10.5, six aromatic signals at δ_H 7.85 (2H, d, $J = 8.6$ Hz), 6.90 (2H, d, $J = 8.6$ Hz), 6.75 (1H, s), and 6.14 (1H, s) were also observed. ^{13}C NMR spectrum of compound **1** gave five oxygenated carbons at δ_C 164.2, 163.4, 161.6, and 160.4. Meanwhile, the signal of C-6 position at δ_C 99.5 and 12 sugar signals at δ_C 98.5–17.7 including three characteristic C-glycosylflavone sugar signals at δ_C 81.5, 80.9, and 78.9 were also observed. When its ^1H and ^{13}C NMR spectral data were compared with those of 2″-*O*-rhamnosyl vitexin [6], 8-C-(4″-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranosylapigenin [7], and other C-glycosylflavone [8], a C-8-substituted C-glycosylapigenin structure could be deduced. On the basis of acid hydrolysis results and spectral data comparison, the sugar units were assigned as rhamnose and glucose.

Although the signal at δ_H 5.84 (1H, d, $J = 2.3$ Hz) in the ^1H NMR spectrum is significantly downfield than normal anomeric proton of glucose, it could still be observed after D_2O exchange experiment. In the HMQC spectrum of compound **1**, the anomeric proton correlated with the carbon at δ_C 78.9, this anomeric proton also showed cross-peaks with the carbons at δ_C 103.4 and 72.0 in the HMBC experiment. Thus, the glucose should be attached at C-8 position, this is also in accordance with the acid hydrolysis result that no D-glucose was obtained. Since the coupling constant of anomeric proton is 2.3 Hz, α-orientation at the anomeric center of D-glucose was determined. The sugar linkage between rhamnose (C-1‴), whose anomeric proton was observed at δ_H 4.61 (1H, brs) in ^1H NMR spectrum, and glucose (C-4″) was further identified through HMQC, HMBC cross-peaks (Figure 1), and data comparison with the literature data [7]. Finally, the structure of compound **1** was assigned as 8-C-[α-L-rhamnopyranosyl-(1 → 4)]-α-D-glucopyranosylapigenin. Up to now, there is no C-glycosylflavone with α-orientation at the anomeric center obtained from nature, and this is the first report about this novel compound isolated from family Ebenaceae and it could be considered as a chemotaxonomic marker for the genus *Diospyros*.

Six known compounds, 2″-*O*-rhamnosyl vitexin (**2**) [6], kaempferol-3-*O*-α-L-rhamnopyranoside (**3**) [8], myricetin-3-*O*-α-L-rhamnopyranoside (**4**) [8], myricetin-3-*O*-β-D-glucopyranoside (**5**) [8], blumeol C glucoside (**6**) [9], and byzantionoside B (**7**) [9], were also isolated and identified by comparison of their spectral data and TLC behaviors with those of the authentic samples and reported spectroscopic data, of which compounds **6** and **7** were isolated from genus *Diospyros* for the first time.

3. Experimental

3.1 General experimental procedures

Melting points were measured on Yanaco micro-hot-stage apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. UV spectra were obtained on a UV-1201 Shimadzu spectrometer. Bruker IFS 55 performed the IR spectrum. NMR spectra were recorded on a Bruker-ARX-600 spectrometer. HR-ESI-MS were taken on a Bruker APEXII FT-ICR MS spectrometer. HPLC instrument was

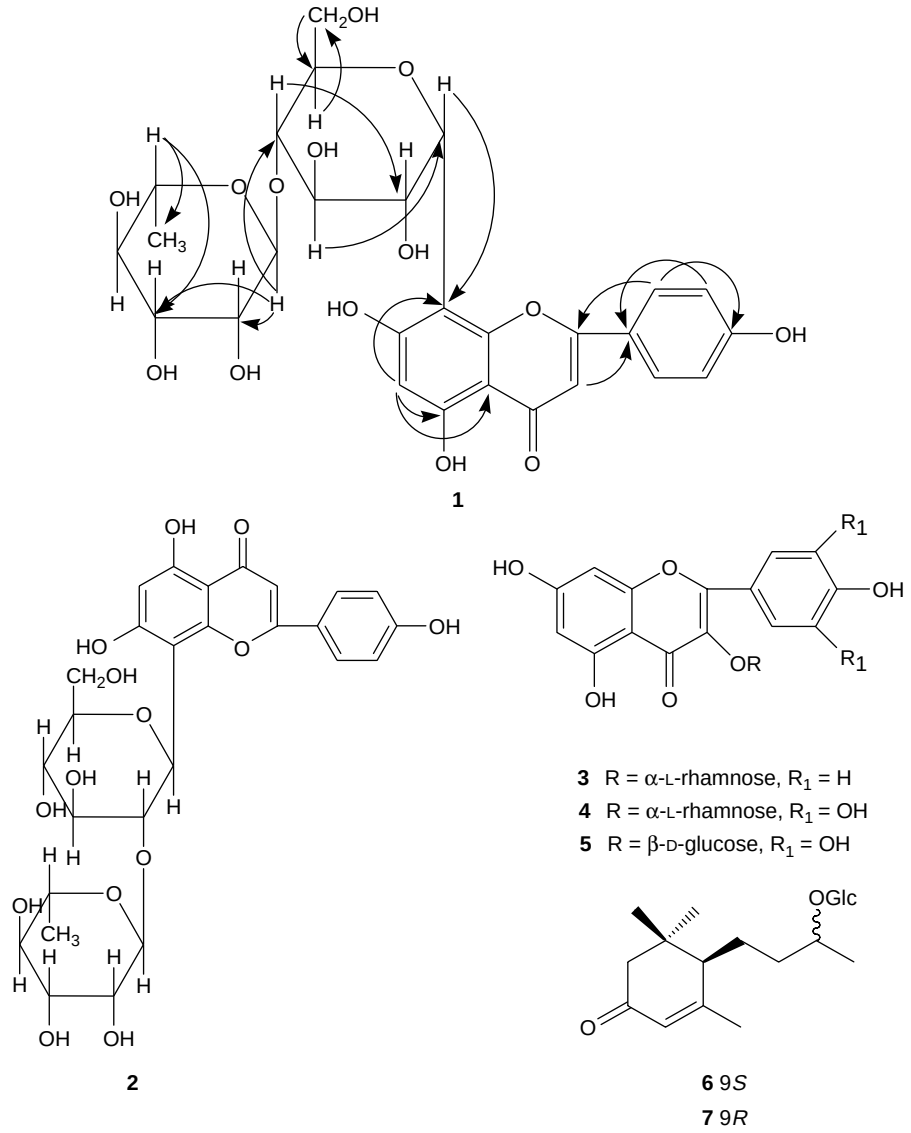

Figure 1. Structures of compounds **1**–**7** and key HMBC correlations of compound **1**.

equipped with an ODS column (250 × 20 mm) from Amersham Pharmacia Biotech (Uppsala, Sweden). The chromatographic silica gel (200–300 mesh) and polyamide (100–140 mesh) were produced by Qingdao Ocean Chemical Factory (Qingdao, China); Sephadex LH-20 was purchased from Amersham Pharmacia Biotech. ODS-A (50 μm) was produced by YMC Co. Ltd (Osaka, Japan). TLC analysis was performed on silica gel 60 F_{254} (Merck, Darmstadt, Germany). All other chemicals and solvents used in this study were of reagent grade.

3.2 Plant material

The leaves of *D. kaki* were collected in Xingtai, Hebei Province of China, in August 2006. The plant was identified by Prof. Xu

Chunquan of Department of Natural Medicines, Shenyang Pharmaceutical University, and a voucher specimen (No. 060801) has been deposited in College of Life Science and Technology, Beijing University of Chemical Technology, Beijing.

3.3 Extraction and isolation

Dried leaves of *D. kaki* (15 kg) were cut into small pieces and extracted with 70% EtOH under reflux. After removal of the solvent by evaporation, the combined extracts (1500 g) were suspended in H_2O and partitioned with $CHCl_3$ and *n*-BuOH, successively. The *n*-BuOH extract was chromatographed on polyamide using MeOH–H_2O (10 and 70%) as eluent, to obtain non-flavonoid and phenolic extracts. Then the phenolic extract (98 g) was concentrated and separated by silica gel chromatography eluting with $CHCl_3$–MeOH gradient (50:1, 30:1, 10:1, 3:1, and 1:1) to yield nine fractions (1–9). Fraction 2 (11.4 g) was subjected to Sephadex LH-20 chromatography eluting with MeOH–H_2O (10, 30, 50, and 70%) to give fractions I–IV. Fraction III (3.8 g) was further separated by HPLC (MeOH–H_2O, 45:55) and purified by preparative TLC ($CHCl_3$–EtOAc–methyl ethyl ketone–MeOH, 5:1:1:1) to afford **6** (10.1 mg) and **7** (11.3 mg). Fraction 4 (10.5 g) was further separated by polyamide chromatography and eluted with $CHCl_3$–MeOH gradient (30:1, 20:1, 10:1, and 5:1) to give **3** (9.2 mg), **4** (7.5 mg), and **5** (9.6 mg). Fraction 6 (15.1 g) was chromatographed on ODS column using a mixture of MeOH–H_2O (10, 30, 50, and 70%) as eluent to give fractions I–IV. Fractions II (2.1 g) and IV (6.8 g) were subjected to Sephadex LH-20 to yield **1** (12.3 mg) and **2** (78.9 mg), respectively.

3.3.1 Compound *1*

Yellow needles (12.3 mg); mp 231–232°C; $[\alpha]_D^{24}$ +14.5 ($c = 0.20$, MeOH); UV (MeOH) λ_{max} (log ε): 310 (3.58), 269 (2.95), 240 (2.78) nm; IR (KBr) ν_{max}: 3396 (OH), 2930 (CH), 1678 (C=O), 1467 (C=C) cm^{-1}; (+) ESI-MS: *m/z* 579 (100) $[M+H]^+$; HR-ESI-MS: *m/z* 579.1721 $[M+H]^+$ (calcd for $C_{27}H_{31}O_{14}$, 579.1714); ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectral data, see Table 1.

3.3.2 Compounds **2**–**7**

Their ^1H and ^{13}C NMR spectral data were identical with those published in the literature.

3.3.3 Acid hydrolysis of compound *1*

Compound **1** (3 mg) was refluxed with 15% HCl–MeOH (4 ml) at 80°C for 4 h. After cooling, the mixture was concentrated and

Table 1. ^1H and ^{13}C NMR spectral data of compound **1** in DMSO-d_6.

Position	δ_H	δ_C
C-2	–	161.6
C-3	6.75 (1H, s)	104.3
C-4	–	182.3
C-5	–	160.4
C-6	6.14 (1H, s)	99.5
C-7	–	160.4
C-8	–	103.4
C-9	–	163.4
C-10	–	103.4
C-1′	–	121.9
C-2′, 6′	7.85 (2H, d, $J = 8.6$ Hz)	128.9
C-4′	–	164.2
C-3′, 5′	6.90 (2H, d, $J = 8.6$ Hz)	116.5
5-OH	13.0 (1H, s)	–
7-OH	10.5 (1H, brs)	–
4′-OH	10.5 (1H, brs)	–
C-1″	5.84 (1H, d, $J = 2.3$ Hz)	78.9
C-2″	2.92 (1H, m)	72.0
C-3″	3.84 (1H, m)	75.2
C-4″	4.32 (1H, m)	80.9
C-5″	4.21 (1H, brd, $J = 7.2$ Hz)	81.5
C-6″	3.51 (1H, brs), 3.63 (1H, brd, $J = 7.2$ Hz)	64.4
C-1‴	4.61 (1H, brs)	98.5
C-2‴	3.50 (1H, m)	71.0
C-3‴	3.41 (1H, m)	70.7
C-4‴	2.81 (1H, m)	71.6
C-5‴	2.13 (1H, m)	68.8
C-6‴	0.58 (3H, brs)	17.7

the residue partitioned with $CHCl_3$–H_2O. The presence of L-rhamnose in this mixture was established by comparison with authentic samples. The HP-TLC in the solvent system MeCOEt–*i*PrOH–Me_2CO–H_2O (20:10:7:6) resulted in the R_f 0.26.

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