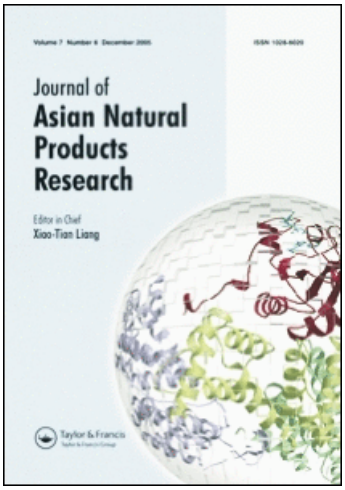

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Guang Chen[a]; Sui-Xu Xu[b]; Hong-Zhong Wang[a]; Rong-Qing Zhang[a]
[a] Department of Biological Science and Biotechnology, Tsinghua University, Beijing, China [b] Department of Natural Medicines, Shenyang Pharmaceutical University, Shenyang, China

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Note

Kakispyrol, a new biphenyl derivative from the leaves of *Diospyros kaki*

GUANG CHEN†, SUI-XU XU‡, HONG-ZHONG WANG† and RONG-QING ZHANG†*

†Department of Biological Science and Biotechnology, Tsinghua University, Beijing 100084, China
‡Department of Natural Medicines, Shenyang Pharmaceutical University, Shenyang 110016, China

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A new biphenyl derivative, 4′,5-dimethoxy-3-β-D-glucopyranosyloxy-4-hydroxy-biphenyl, named kakispyrol (**1**), has been isolated from the leaves of *Diospyros kaki*, together with three known compounds, vitexin (**2**), 2″-*O*-rhamnosyl vitexin (**3**) and isorhamnetin-3-*O*-β-D-glucopyranoside (**4**). The structure of compound **1** has been determined on the basis of spectroscopic evidence.

Keywords: *Diospyros kaki*; Kakispyrol; Biphenyl

1. Introduction

"Shi Ye" is the fresh or dry leaf of *Diospyros kaki* L. (Ebenaceae), which is widely distributed in East Asia. It is used in the treatment of hypertension, angina and internal haemorrhage in China [1], and has been used traditionally in Korea and Japan to promote maternal health [2]. Previous phytochemical studies on this plant have revealed triterpenoids, flavonoids and phenolic compounds [3]. We describe here the isolation and characterization of a new biphenyl derivative, kakispyrol (**1**), together with three known compounds, vitexin (**2**) [4], 2″-*O*-rhamnosylvitexin (**3**) [5], and isorhamnetin-3-*O*-β-D-glucopyranoside (**4**) [6], from this plant. Among them, **2**–**4** were isolated from the *Diospyros* genus for the first time, and this is the first report of the isolation of C-glycosylflavones from the family Ebenaceae.

2. Results and discussion

Compound **1** was isolated from the n-BuOH-soluble fraction of a 70% alcohol extract of the leaves of *Diospyros kaki*.

*Corresponding author. Tel./Fax: +86-10-62772899. E-mail: rqzhang@mail.tsinghua.edu.cn

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Compound **1** was obtained as colorless needles and gave a positive reaction with $FeCl_3$ reagent by TLC. Acid hydrolysis of **1** followed by TLC analysis of the hydrolysate and direct comparison with standard sugars indicated the presence of glucose. HR-ESIMS gave a quasi-molecular ions at *m/z* 409.1488 $[M + H]^+$, 426.1759 $[M + NH_4]^+$ and 834.3139 $[2M + NH_4]^+$, corresponding to the molecular formula $C_{20}H_{24}O_9$. The IR spectrum shows absorption bands for hydroxyl group (3405 cm^{-1}) and benzene skeleton (1608, 1503, 1453 cm^{-1}) that are confirmed by the ^{13}C NMR spectrum.

The ^1H NMR spectrum of **1** exhibits a D_2O-exchangeable phenolic proton at δ 8.25. Four aromatic protons (AA′BB′) at δ 7.57 (2H, d, $J = 8.2$ Hz), 6.94 (1H, d, $J = 8.2$ Hz) occur, indicating a para-substituted benzene. Two aromatic protons at δ 7.06 (1H, br*s*), 6.88 (1H, br*s*) suggest another benzene in the molecule. In addition, two methoxy group signals appear at δ 3.76 (3H, s) and 3.82 (3H, s). In the ^{13}C NMR spectrum, 12 aromatic carbon signals show a biphenyl structure characteristic [7]; six glucose carbon signals and two methoxy group signals (56.1, 55.2) are also observed. An anomeric proton signal was identified at δ 4.72 (1H, d, $J = 6.2$ Hz) by the HMQC spectrum. Its *J* value indicates a β-configuration at the anomeric position of glucose. In the HMBC experiment, the anomeric proton signal at δ 4.72 correlates with the carbon signal at δ 146.1, suggesting that the glucose moiety is attached to C-3. In addition, the phenolic proton signal (δ 8.25) correlates with the carbon signals (δ 146.1, 135.6, 148.4), and correlation also occurs at one of the methoxy group protons (δ 3.82) with a carbon signal (δ 148.4), indicating that the hydroxy and methoxy groups are at C-4 and C-5 respectively. Since the ^1H NMR spectrum indicates a para-substituted benzene moiety and the other methoxy group protons at δ 3.76 are correlated with a carbon signal (δ 158.4), this methoxy group can be attached to C-4′.

Further assignment for the proton and carbon signals was carried out using HMQC and HMBC spectra (table 1). On the basis of the above evidence, compound **1** was assigned as 4′,5-dimethoxy-3-β-D-glucopyranosyloxy-4-hydroxy-biphenyl, named kakispyrol (figure 1).

Table 1. NMR data of compound **1** in DMSO-d_6.

Position	δ_C	δ_H	*HMBC*
1	130.4		H-2′, H-6′
2	107.8	7.06 (1H, br.s)	H-6
3	146.1		H-2, H-1″, 4-OH
4	135.6		H-2, H-6, 4-OH
5	148.4		H-6, 5-OCH_3, 4-OH
6	105.3	6.88 (1H, br.s)	H-2
1′	132.8		H-2, H-6, H-3′, H-5′
2′, 6′	127.4	7.57 (2H, d, $J = 8.2$ Hz)	H-2′, H-6′
4′	158.4		H-2′, H-6′, H-3′, H-5′, 4′-OCH_3
3′, 5′	114.2	6.94 (2H, d, $J = 8.2$ Hz)	H-3′, H-5′
5-OCH_3	56.1	3.82 (3H, s)	
4′-OCH_3	55.2	3.76 (3H, s)	
4-OH		8.25 (1H, s)	
1″	102.7	4.72 (1H, d, $J = 6.0$ Hz)	
2″	73.5	3.28	
3″	77.4	3.30	
4″	70.2	3.15	
5″	76.0	3.30	
6″	61.0	3.41	

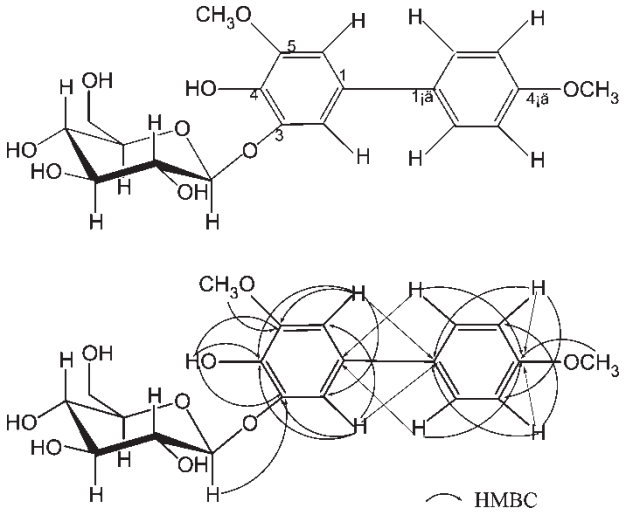

Figure 1. Structure and key HMBC correlations of compound **1**.

Compounds **2**–**4** were identified as vitexin, 2″-*O*-rhamnosylvitexin, and isorhamnetin-3-*O*-β-D-glucopyranoside, respectively, by comparing their spectral data with those of authentic samples. They were isolated from the *Diospyros* genus for the first time, and this is the first report of the isolation of C-glycosylflavones from the family Ebenaceae.

3. Experimental

3.1 General experimental procedures

Melting points were measured on Yanaco micro-hot-stage apparatus and are uncorrected. A Bruker IFS 55 was used to record the IR spectrum. HR-ESI MS were taken on a Bruker APEX II FT-ICRMS spectrometer. NMR spectra were recorded on a Bruker-ARX-300 spectrometer. Chromatographic silica gel (200–300 mesh) and polyamide (100–140 mesh) were produced by Qingdao Ocean Chemical Factory, and ODS and Sephadex LH-20 were purchased from Amersham Pharmacia Biotech. TLC analysis was performed on silica gel 60 F_{254} (Merck), with compounds visualized by spraying with 10% (v/v) H_2SO_4.

3.2 Plant material

Leaves of *Diospyros kaki* were collected in Xingtai, Hebei province of China, in September 2001. The plant was identified by Xu Chunquan, Professor of the Department of Natural Medicines, Shenyang Pharmaceutical University, and a voucher specimen has been deposited in the Herbarium of the Department of Natural Medicines, Shenyang Pharmaceutical University, Shenyang.

3.3 Extraction and isolation

Dried leaves of *Diospyros kaki* (7 kg) were extracted with 70% EtOH under reflux. After removal of solvent by evaporation, the combined extracts (1200 g) were suspended in H_2O,

and then partitioned with light petroleum. The aqueous layer was partitioned again with $CHCl_3$ and n-BuOH. The n-BuOH extract was chromatographed on a silica-gel column, using mixtures of $CHCl_3$ and MeOH as eluent, to give fractions A–G. Fraction C was chromatographed on an ODS column with MeOH–H_2O (20–80%) as solvent to give fractions 1–4; Fraction 2 was then purified by Sephadex LH-20 to give compound **1**. Fraction D was subjected to polyamide chromatography, eluting with $CHCl_3$–MeOH (10:1 to 3:1), to give fractions 1–4; Fraction 2 was then purified by Sephadex LH-20 to give compound **2**, and fraction 3 gave **4**. Fraction F was chromatographed on Sephadex LH-20, eluting with MeOH–H_2O (70%), to furnish **3**.

Compound **1**: colorless needles (15 mg), mp 195–197°C, showed a black spot with 10% H_2SO_4. IR (KBr) ν_{max} (cm^{-1}): 3405, 1608, 1503, 1453; HR-ESIMS: *m/z* 409.1488 $[M + H]^+$ (calcd. for $C_{20}H_{25}O_9$, 409.1493), 426.1759 $[M + NH_4]^+$, 834.3139 $[2M + NH_4]^+$. ^1H NMR (DMSO-d_6, 300 MHz), ^{13}C NMR (DMSO-d_6, 75.0 MHz), HMBC data see table 1.

Compounds **2**–**4**: their ^1H and ^{13}C NMR data are identical to those published in the literature.

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