Three Minor Novel Triterpenoids from the Leaves of *Diospyros kaki*

Guang CHEN,*[,a] Zong-Quan WANG,[b] and Ji-Ming JIA*[,b]

[a] *College of Life Science and Technology, Beijing University of Chemical Technology; Beijing 100029, P. R. China: and* [b] *Hebei Yiling Medicine Institute; Shijiazhuang, Hebei 050035, P. R. China.*

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Two novel 18, 19-secoursane triterpenoids, kakisaponin B (1) and kakisaponin C (2), an ursane type 28-nortriterpene, kakidiol (3) and one known triterpenoid rosamultin (4), were isolated from the leaves of *Diospyros kaki*. The structures of compounds 1 and 2 were determined as 28-*O*-β-D-glucopyranosyl-3α,19,24-trihydoxy-18,19-secours-11,13(18)-dien-28-oic acid (1) and 28-*O*-β-D-glucopyranosyl-2α,3α,19-trihydoxy-18,19-secours-11,13(18)-dien-28-oic acid (2) by chemical methods and spectra experiments. Kakidiol (3) was characterized as a C_{29}-triterpene with an aromatic E-ring in structure. This is the first report of 18,19-secoursane triterpenoids and 28-nortriterpene from family Ebenaceae.

Key words *Diospyros kaki*; 18,19-secoursane triterpenoid; kakisaponin B; kakisaponin C; kakidiol

Diospyros kaki (Ebenaceae) is widely distributed in East Asia, which leaves is a traditional plant medicine for the treatment of hypertension, angina and internal haemorrhage.[1)] It was also reported that in Japan and Korea the leaves of *Diospyros kaki* is used as health food (persimmon leaf tea) to promote maternal health.[2)] Although some reports show that the triterpenoid compounds are the main constituents in genus *Diospyros*,[1)] many research were focused on the triterpene aglycone.[3—5)] Our previous study led to the isolation of a new triterpenoid saponin-kakisaponin A from persimmon leaves,[6)] which indicated the existence of novel triterpenoid saponins in this plant. As part of our continuous search for potentially active substances from the leaves of *Diospyros kaki*, we focused on the minor triterpenoid saponins and reported herein the isolation and structural elucidation of two novel 18,19-secoursane triterpenoids kakisaponin B (**1**) and kakisaponin C (**2**), an ursane type 28-nortriterpene, kakidiol (**3**) and one known triterpenoid rosamultin (**4**). This is the first report of 18, 19-secoursane triterpenoids and 28-nortriterpene from family Ebenaceae.

Results and Discussion

The leaves of *Diospyros kaki* was extracted with 70% EtOH and then concentrated. The concentrated extract residue 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,[1,7)] the *n*-BuOH extract was then chromatographed by polyamide and eluted with 10% MeOH to give the non-flavonoid extract. This 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**—**4**.

Compound **1** was obtained as colourless needles and gave a positive Lieberman–Burchard test for triterpenoids. Its molecular formula was deduced as $C_{36}H_{58}O_{10}$ by high resolution ESI-MS (HR-ESI-MS) (*m*/*z* 673.3917 $[M+Na]^+$) and confirmed by distortionless enhancement by polarization transfer (DEPT) analysis. Thus, eight degrees of unsaturation was determined for this compound. Since acid hydrolysis of **1** followed by TLC analysis of hydrolysate indicated the presence of D-glucose and four olefinic carbon signals (δ_C 143.4, 130.3, 128.2, 127.3), one carbonyl carbon signal (δ_C 175.1) were observed in ^{13}C-NMR spectrum, four carbocyclic rings in structure could be elucidated. In the ^{1}H-NMR spectrum of compound **1** (Table 1), six methyl signals at δ_H 1.60 (3H, s), 1.29 (3H, d, J=6.2 Hz), 1.02 (3H, d, J=6.3 Hz), 0.93 (3H, s), 0.92 (3H, s) and 0.90 (3H, s) were observed. ^{13}C-NMR spectrum of **1** showed six methyl groups at δ_C 23.6, 20.6, 20.0, 18.6, 16.7 and 15.5, two OCH groups at δ_C 70.6, 70.1 and one OCH_2 group at δ_C 65.4 except hexose moiety signals (δ_C 96.2, 79.2, 78.8, 74.2, 71.3, 62.4), which were confirmed in DEPT spectrum. Comparison of the ^{1}H- and ^{13}C-NMR data of compound **1** with those of literature[3,6)] and considering the commonly existing ursane type of pentacyclic triterpenoids in *Diospyros* genus,[1)] a secoursane structure possessing the same A and B ring structure with barbinervic acid was deduced.[8)] The signals at δ_H 6.04 (1H, br d, J=10.2 Hz), 5.79 (1H, br s) and 5.70 (1H, d, J=10.2 Hz) showed correlations with δ_C 130.3, 127.3 and 128.2, respectively in heteronuclear multiple quantum coherence (HMQC) analysis, together with the cross-peaks between δ_H 5.79 and δ_C 143.4, δ_H 6.04 and δ_C 143.4 in heteronuclear multiple bond correlation (HMBC) experiment, suggested a conjugated double bond in structure. Compared the remaining portion NMR data of compound **1** with those of literature,[9)] the similar structure with 2α,3α-dihydroxy-19-oxo-18,19-secours-11,13(18)-dien-28-oic acid except hydroxy group at C-19 position could be elucidated. This can be further verified by long-range correlations from the δ_H 5.79 (H-18) to δ_C 48.0 (C-17), 41.6 (C-14), 38.9 (C-22), δ_H 1.29 (H-29) to δ_C 70.6 (C-19), 41.2 (C-20), δ_H 1.02 (H-30) to δ_C 70.6 (C-19), 41.2 (C-20) in HMBC spectrum. Meanwhile, the glycosidic linkage at C-28 position was determined by the HMBC correlation of anomeric proton at δ_H 6.34 (1H, d, J=7.6 Hz) with δ_C 175.1 (C-28).

The relative configuration of compound **1** was proposed as shown in Fig. 1 by analysis of nuclear Overhauser enhancement and exchange spectroscopy (NOESY) data and comparison of the documented data.[10,11)] Due to the free rotation of σ-bond between C-17 and C-22, the relative configuration of C-19 and C-20 has not been defined yet. On the basis of above evidence, compound **1** was established as 28-*O*-β-D-glucopyranosyl-3α,19,24-trihydoxy-18,19-secours-11,13(18)-dien-28-oic acid and named as kakisaponin B.

Compound **2**, isolated as colourless needles and also gave a positive Lieberman–Burchard test for triterpenoids. Its HR-

* To whom correspondence should be addressed. e-mail: chenguang@mail.buct.edu.cn; jjm_0451@163.com

Table 1. The ^{1}H- and ^{13}C-NMR Data of Compounds **1**—**3** in Pyridine-d_5[a)]

No.	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.45 (1H, m)	33.8	1.83 (1H, m)	42.7	1.43 (1H, m)	34.3
	1.48 (1H, m)		1.88 (1H, m)		1.47 (1H, m)	
2	1.80 (1H, m)	27.6	4.33 (1H, m)	66.0	1.79 (1H, m)	27.4
	1.82 (1H, m)				1.81 (1H, m)	
3	4.44 (1H, m)	70.1	3.75 (1H, J=2.3)	79.4	4.33 (1H, m)	70.0
4	—	44.1	—	38.9	—	44.0
5	1.92 (1H, m)	49.6	1.71 (1H, m)	48.4	1.91 (1H, m)	44.3
6	1.54 (1H, m)	19.0	1.42 (1H, m)	18.3	1.63 (1H, m)	19.0
	1.55 (1H, m)		1.44 (1H, m)		1.65 (1H, m)	
7	1.41 (1H, m)	33.0	1.40 (1H, m)	32.5	2.01 (1H, m)	34.7
	1.43 (1H, m)		1.42 (1H, m)		2.03 (1H, m)	
8	—	37.3	—	38.4	—	40.4
9	2.05 (1H, m)	54.8	2.25 (1H, br s)	54.7	1.72 (1H, m)	48.2
10	—	41.4	—	41.2	—	37.4
11	5.70 (1H, d, J=10.2)	128.2	5.75 (1H, d, J=10.0)	127.8	1.98 (1H, m)	23.8
					2.00 (1H, m)	
12	6.04 (1H, br d, J=10.2)	130.3	6.04 (1H, br d, J=10.0)	130.5	5.54 (1H, br d, J=4.8)	125.7
13	—	143.4	—	143.2	—	139.5
14	—	41.6	—	41.6	—	50.3
15	1.94 (1H, m)	26.4	1.98 (1H, m)	26.5	2.26 (1H, m)	30.0
	1.96 (1H, m)		2.00 (1H, m)		2.28 (1H, m)	
16	2.10 (1H, m)	27.6	2.11 (1H, m)	28.0	2.35 (1H, m)	31.4
	2.13 (1H, m)		2.13 (1H, m)		2.38 (1H, m)	
17	—	48.0	—	48.1	—	138.7
18	5.79 (1H, br s)	127.3	5.79 (1H, br s)	127.5	—	139.0
19	3.84 (1H, m)	70.6	3.86 (1H, m)	70.8	—	133.8
20	1.66 (1H, m)	41.2	1.66 (1H, m)	41.4	—	129.8
21	1.83 (1H, m)	27.9	1.82 (1H, m)	27.6	6.77 (1H, d, J=7.5)	127.7
	1.84 (1H, m)		1.83 (1H, m)			
22	2.02 (1H, m)	38.9	2.02 (1H, m)	38.9	6.90 (1H, d, J=7.5)	122.8
	2.04 (1H, m)		2.04 (1H, m)			
23	1.60 (3H, s)	23.6	1.24 (3H, s)	29.3	1.58 (3H, s)	23.6
24	3.83 (1H, d, J=11.0)	65.4	0.88 (3H, s)	21.7	3.78 (1H, d, J=10.5)	65.9
	4.03 (1H, d, J=11.0)				4.00 (1H, d, J=10.5)	
25	0.92 (3H, s)	20.0	0.90 (3H, s)	16.8	1.06 (3H, s)	20.9
26	0.90 (3H, s)	16.7	0.94 (3H, s)	20.0	1.04 (3H, s)	16.6
27	0.93 (3H, s)	18.6	0.93 (3H, s)	19.2	0.94 (3H, s)	26.6
28	—	175.1	—	175.0	—	—
29	1.29 (3H, d, J=6.2)	20.6	1.29 (3H, d, J=6.2)	20.7	2.31 (3H, s)	17.0
30	1.02 (3H, d, J=6.3)	15.5	1.02 (3H, d, J=6.3)	15.5	2.30 (3H, s)	20.9
Glc						
1	6.34 (1H, d, J=7.6)	96.2	6.32 (1H, d, J=7.8)	96.2		
2		74.2		74.3		
3		78.8		78.8		
4		71.3		71.5		
5		79.2		79.2		
6	4.29 (1H, d, J=9.1)	62.4	4.29 (1H, d, J=9.1)	62.6		
	4.40 (1H, d, J=9.1)		4.40 (1H, d, J=9.1)			

a) δ in ppm and J in Hz. Run at 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR.

ESI-MS spectrum showed a quasimolecular ion peak at *m/z* 673.3914 ($[M+Na]^+$) indicating the molecular formula $C_{36}H_{58}O_{10}$. Inspection of ^{1}H- and ^{13}C-NMR data (Table 1) of **2** revealed that compounds **1** and **2** were closely comparable. The significant difference between these two compounds appeared only in A-ring signals, in which the hydroxy group attached at C-2 position in **2** instead of C-24 position in compound **1**. This assignment can be established by the CH signal at δ_C 66.0 (C-2) in DEPT spectrum and HMBC correlations between δ_H 4.33 (H-2) and δ_C 42.7 (C-1), δ_H 0.90 (H-25) and δ_C 42.7 (C-1), δ_H 1.86 (H-1) and δ_C 66.0 (C-2). Further assignment of this compound and determination of hydroxy groups orientation was carried out by HMQC, HMBC and comparison with literature data.[9)] Therefore, the structure of compound **2** was identified as 28-*O*-β-D-glucopyranosyl-2α,3α,19-trihydoxy-18,19-secours-11,13(18)-dien-28-oic acid and named as kakisaponin C (Fig. 2).

Compound **3** was obtained as white amorphous powder. A molecular formular of $C_{29}H_{42}O_2$ was determined for this compound from the quasimolecular ion peak at *m/z* 423.3247 ($[M+H]^+$) in HR-ESI-MS spectrum. The ^{1}H-NMR spectrum of compound **3** displayed six methyl groups at δ_H 2.31 (3H, s), 2.30 (3H, s), 1.58 (3H, s), 1.06 (3H, s), 1.04 (3H, s) and 0.94 (3H, s), together with an olefinic proton at δ_H 5.54 (1H, br d, J=4.8 Hz). Meanwhile, two unexpected aromatic protons at δ_H 6.77 (1H, d, J=7.5 Hz) and 6.90 (1H, d, J=7.5 Hz)

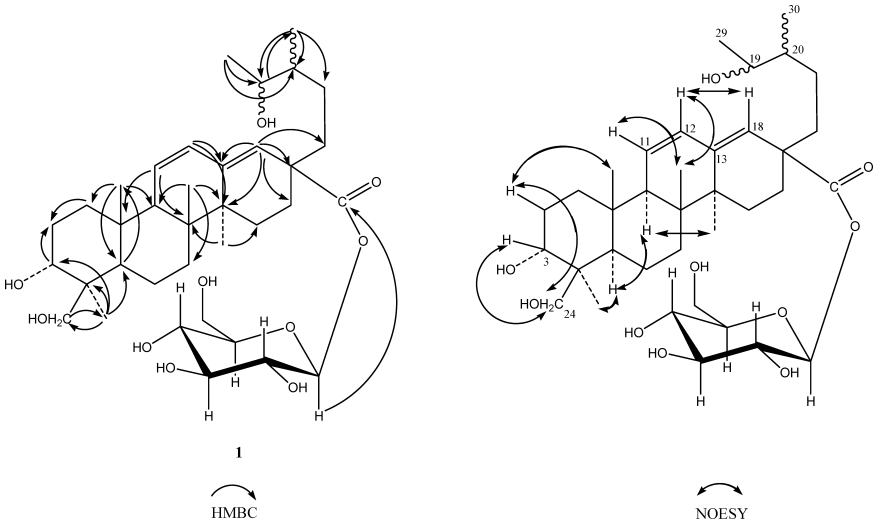

Fig. 1. Structure, Key HMBC and NOESY Correlations of Compound **1**

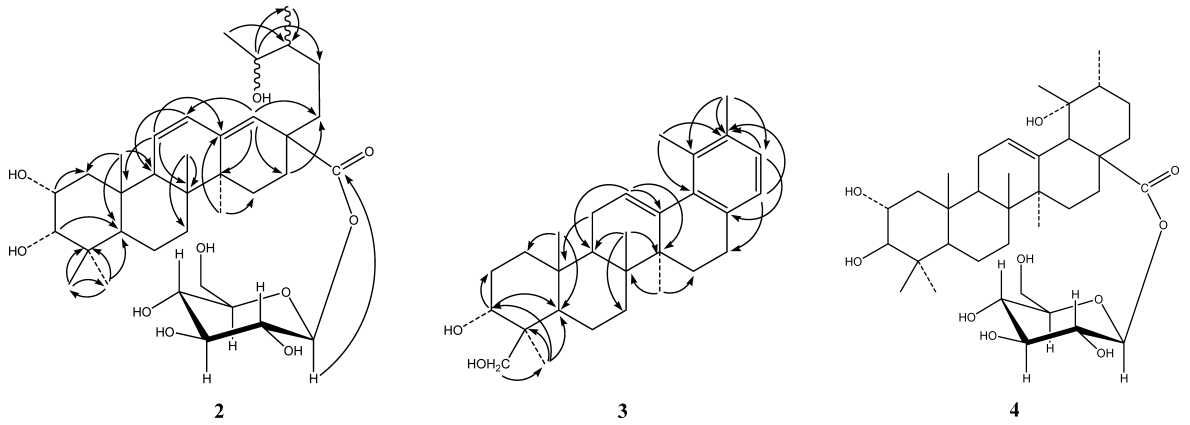

Fig. 2. Structure and Key HMBC Correlations of Compounds **2**—**4**

were also observed, which suggested an aromatic ring in compound **3**. The ^{13}C-NMR spectrum of **3** gave 28 carbon signals. In its DEPT spectrum, two oxygenated methines at δ_C 70.0 (C-3) and 65.9 (C-24) showed CH and CH_2 signals, respectively. Based upon the comparison of ^{13}C-NMR data of **3** with that of compound **1**, the same A-ring structure could be deduced. The HMBC correlations from δ_H 4.33 (H-3) to δ_C 27.4 (C-2), 44.3 (C-5), δ_H 3.78 (H-24) to δ_C 23.6 (C-23) and δ_H 1.58 (H-23) to δ_C 44.0 (C-4), 44.3 (C-5), 70.0 (C-3) also supported this assignment. In the HMBC spectrum of compound **3**, δ_H 6.77 (H-21) correlated with δ_C 138.7 (C-17) and 129.8 (C-20), δ_H 6.90 (H-22) correlated with δ_C 31.4 (C-16) and 129.8 (C-20), the two methyl groups at δ_H 2.31 (H-29) and 2.30 (H-30) also showed correlations with δ_C 139.0 (C-18), 129.8 (C-20) and δ_C 133.8 (C-19), 129.8 (C-20), 127.7 (C-21). These data suggested an aromatic E-ring in structure. After comparing its spectra data with literature[12] and analysis of additional cross-peaks in HMBC spectrum, the formula **3** for the structure of kakidiol could be elucidated (Fig. 2).

The known compound rosamultin (**4**) was also isolated and identified by comparison with the reported data.[13] 18,19-Secoursane type compounds are rarely found in nature. Up to now, only 16 triterpenoids with 18,19-secoursane structure were isolated from three genuses (*Ilex*, *Elsholtzia* and *Rubus*).[9—11,14—17] Meanwhile, present work also obtained an interesting 28-nortriterpene with aromatic E-ring structure and only a few natural products of this kind have been isolated from nature origin.[12,18] This is the first report about these novel compounds isolated from family Ebenaceae and they could be considered as a chemotaxonomic marker for the genus *Diospyros*.

Experimental

General Experiment Procedures TLC was conducted on silica gel plates ($60F_{254}$, Merck). HPLC was performed on Waters-600 prep. HPLC instrument equipped with an Amersham Pharmacia Biotech-ODS (250× 20 mm) column. Column chromatography (CC) were performed on silica gel (200—300 mesh; Qingdao Haiyang, Co., China), Sephadex LH-20 (Pharmacia Biotech AB, S-Uppsala) and ODS-A (50 μm; YMC Co., Ltd., Japan). Melting point was measured on a Yanaco MP-S3 micromelting point apparatus. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. UV spectra were obtained on a UV-1201 Shimadzu spectrometer. IR spectra were recorded on a Perkin-Elmer Spectrum GX spectrometer. NMR spectra were obtained on a Bruker ARX-300 instrument. Chemical shifts are expressed as δ (ppm) with tetramethylsilane (TMS) as the internal standard. ESI- and HR-ESI-MS were recorded on Bruker APEX II FT-ICR mass spectrometers.

Plant Material Dry leaves of *Diospyros kaki* were collected in Xingtai of Hebei Province, P. R. China in Augest 2006 and was identified by Professor Yun.-Zhen. Guo, College of Traditional Chinese Medicines, Shenyang Pharmaceutical University. A voucher specimen for this material (No. 060801) is deposited in College of Life Science and Technology, Beijing University of Chemical Technology.

Extraction and Isolation The dry leaves of *Diospyros kaki* (20 kg) was cut into small pieces and extracted with 70% EtOH under reflux to give an EtOH extract (1200 g). The extract was concentrated *in vacuo* then suspended in 4 l of H_2O and partitioned successively with 4 l of $CHCl_3$ and 4 l of *n*-BuOH. The *n*-BuOH extract (150 g) was subjected to polyamide column chromatography (700 g 7×100 cm column) eluting with 10% MeOH to give non-flavonoid extract. Then this extract (67 g) was concentrated and adsorbed on silica gel (95 g) and separated by CC (silica gel (1000 g) 9×100 cm column; $CHCl_3$/MeOH gradient) to yield 9 fractions (1—9). Fraction 2 was applied to CC (silica gel (1000 g) 3×80 cm column; $CHCl_3$/EtOAc gradient). The collected fractions were purified by preparative TLC ($CHCl_3$/MeOH 22 : 1): **3** (13.3 mg). Fraction 5 was subjected to reversed-phase silica gel column (ODS (220 g) 3×80 cm column; MeOH/H_2O gradient) to give 5 fractions (I—V). Fraction II was purified by HPLC (MeOH/H_2O 75 : 25): **1** (11.5 mg). Fraction IV was further separated by HPLC (MeOH/H_2O 65 : 35): **2** (10.1 mg) and **4** (17.6 mg).

Kakisaponin B (**1**): Colourless needles; melting point (mp) 231—232 °C; $[\alpha]_D^{24}$ −63.5° (*c*=0.15, MeOH); UV λ_{max} (MeOH) nm (log ε): end absorption; IR ν_{max} (KBr) cm^{-1}: 3385, 1711, 1640; ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) data see Table 1; ESI-MS (pos.) *m*/*z*: 673 (100, $[M+Na]^+$); HR-ESI-MS: 673.3917 ($[M+Na]^+$, $C_{36}H_{58}NaO_{10}^+$; Calcd 673.3922).

Kakisaponin C (**2**): Colourless needles; melting point (mp) 258—259 °C; $[\alpha]_D^{24}$ −54.7° (*c*=0.15, MeOH); UV λ_{max} (MeOH) nm (log ε): end absorption; IR ν_{max} (KBr) cm^{-1}: 3390, 1710, 1630; ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) data see Table 1; ESI-MS (pos.) *m*/*z*: 673 (100, $[M+Na]^+$); HR-ESI-MS: 673.3914 ($[M+Na]^+$, $C_{36}H_{58}NaO_{10}^+$; Calcd 673.3922).

Kakidiol (**3**): White amorphous powder; melting point (mp) 147—148 °C; $[\alpha]_D^{24}$ +29.6° (*c*=0.15, MeOH); UV λ_{max} (MeOH) nm (log ε): 243 (3.67); IR ν_{max} (KBr) cm^{-1}: 3385, 1460, 1378, 1220; ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) data see Table 1; ESI-MS (pos.) *m*/*z*: 423 (100, $[M+H]^+$); HR-ESI-MS: 423.3247 ($[M+H]^+$, $C_{29}H_{43}O_2^+$; Calcd 423.3263).

Acid Hydrolysis of 1 and 2 Compounds **1** and **2** (3 mg each) were refluxed with 15% HCl/MeOH (4 ml) at 80 °C for 4 h. After cooling, the mixture was concentrated and the residue partitioned with $CHCl_3$/H_2O. The presence of D-glucose 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 *Rf* 0.26.

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