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A NEW 18,19-SECOURSANE TRITERPENE FROM THE LEAVES OF *Diospyros kaki*

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A new 18,19-secoursane triterpene together with five known compounds were isolated from the leaves of Diospyros kaki. *The structure of the new compound was elucidated as 18,19-seco-3*β*-hydroxy-urs-12-en-18-one on the basis of spectroscopic methods.*

Keywords: Ebenaceae, *Diospyros kaki*, 18,19-secoursane triterpene.

Diospyros kaki (persimmon), a common and important species of *Diospyros* genus, is widely distributed in east Asia. Its leaves are a traditional plant medicine used for the treatment of hypertension, angina, and internal hemorrhage [1]. Although many quinone compounds have been reported from *Diospyros* plants, recent research shows that the triterpenoids and flavonoids are two kinds of main constituents in persimmon leaves [2–4]. Our previous study has revealed the existence of novel triterpenes with the 18,19-*seco*-ursane structure in the leaves of *Diospyros kaki* [5]. In the continuous chemical investigation of this plant, we described herein the isolation and structural elucidation of a new triterpene compound, 18,19-*seco*-3*β*-hydroxy-urs-12-en-18-one (**1**), together with five known compounds uvaol (**2**) [6], ursolic acid (**3**) [6], oleanolic acid (**4**) [6], (–)-syringaresinol (**5**) [7], and (–)-syringaresinol-4-*β*-D-glucopyranoside (**6**) [7] from the 90% ethanol extract. Although the phenolic compounds are common in *Diospyros* genus and syringaresinol type lignans also have been isolated from many genera, few reports have revealed the existence of lignan constituents till now [8, 9], and compounds **5** and **6** were isolated from this genus for the first time.

Compound **1** was obtained as colorless needles and gave positive reaction with the Lieberman-Burchard test for triterpenoids. Its molecular formula was deduced as $C_{30}H_{50}O_2$ on the basis of the HR-ESI-MS pseudo-ion peak at *m/z* 465.3688 $[M + Na]^+$ (calcd for $C_{30}H_{50}NaO_2^+$, 465.3690), which was also confirmed by distortionless enhancement by polarization transfer (DEPT) analysis. Thus, this compound was revealed to have six degrees of unsaturation. Its IR spectrum gave the absorption band for carbonyl at ν_{max} 1717 cm^{-1}, which was also observed at δ 209.2 s in the ^{13}C NMR spectrum. Since the ^{13}C NMR spectrum also showed two olefinic carbon signals at δ 133.7 d and 138.4 s, which possess one degree of unsaturation, four carbocyclic rings in the structure could be elucidated.

In the intensive analysis of the NMR spectrum of compound **1**, an abnormal double bond with minor chemical shift difference between a tertiary carbon (δ 133.7 d) and a quaternary carbon (δ 138.4 s) was observed in the ^{13}C NMR spectrum, together with a significant downfield olefinic proton signal at δ 6.09 (1H, dd, $J_1 = 10.5$, $J_2 = 7.5$) in the ^1H NMR spectrum, and an unsaturated carbonyl structure could be elucidated. Meanwhile, since the most downfield methyl signal was observed at δ 1.29 (3H, d, J = 6.3), it can be determined that the methyl groups are not attached to carbonyl and double bonds in the structure [10].

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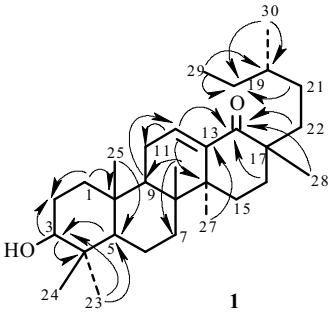
TABLE 1. ^{1}H and ^{13}C NMR Data of **1**[a], J/Hz

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	1.51 (2H, m)	38.8	16	1.31 (1H, m), 1.62 (1H, m)	27.0
2	1.73 (2H, m)	27.3	17		37.2
3	3.19 (1H, dd, J_1 = 10.8, J_2 = 5.3)	79.0	18		209.2
4		38.8	19	1.34 (2H, m)	29.7
5	1.40 (1H, m)	55.3	20	1.66 (1H, m)	28.0
6	1.54 (1H, m), 1.62 (1H, m)	18.2	21	1.29 (2H, m)	40.6
7	1.22 (1H, m), 1.45 (1H, m)	33.9	22	1.59 (2H, m)	44.5
8		40.5	23	0.97 (3H, s)	28.0
9	1.39 (1H, m)	50.4	24	0.94 (3H, s)	15.3
10		47.2	25	0.76 (3H, s)	15.6
11	1.75 (1H, m), 2.10 (1H, m)	25.4	26	0.83 (3H, s)	16.2
12	6.09 (1H, dd, J_1 = 10.5, J_2 = 7.5)	133.7	27	1.25 (3H, s)	26.9
13		138.4	28	0.90 (3H, s)	21.0
14		42.2	29	0.93 (3H, t, J = 6.3)	14.1
15	1.25 (1H, m), 1.56 (1H, m)	26.8	30	1.29 (3H, d, J = 6.3)	20.9

[a]Data were recorded in $CDCl_3$ on a Bruker-ARX-300 spectrometer (^{1}H NMR, ^{13}C NMR, HMQC and HMBC); chemical shifts are shown in δ (ppm) with TMS as internal standard.



1

Fig. 1. Structure and key HMBC correlations (H→C) of compound **1**.

In heteronuclear multiple bond correlation (HMBC) experiments (Fig. 1), the olefinic proton signal at δ 6.09 correlates with the carbonyl group (δ 209.2 s), C-11 (δ 25.4 t), and C-14 (δ 42.2 s), and the two methyl signals of H-27 (δ 1.25 s) and H-28 (δ 0.98 s) show long-range correlations with C-13 (δ 138.4 s) and C-18 (δ 209.2 s), respectively. These results further confirm the unsaturated carbonyl structure. Some important long-range correlations between H-3 (δ 3.19, 1H, dd, J_1 = 10.8, J_2 = 5.3) and C-2 (δ 27.3 t), C-4 (δ 38.8 s); H-29 (δ 0.93, 3H, t, J = 6.3) and C-19 (δ 29.7 t), C-20 (δ 28.0 d); H-30 (δ 1.29, 3H, d, J = 6.3) and C-19, C-20 were also observed in the HMBC spectrum. The detail NMR data assignment was performed by further HMQC and HMBC analysis and is shown in Table 1; due to the free rotation of the σ-bond between C-17 and C-22, the relative configuration of C-20 has not been defined yet. On the basis of above evidence, the structure of compound **1** was identified as 18,19-*seco*-3β-hydroxy-urs-12-en-18-one.

EXPERIMENTAL

Melting points were measured on a 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. IR spectra were obtained on a Bruker IFS-55 spectrometer. NMR spectra were recorded on a Bruker-ARX-300 spectrometer. HR-ESI-MS were taken on a Bruker APEX II FT-ICR MS spectrometer.

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 mm) 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.

Extraction and Isolation. Dried leaves of *Diospyros kaki* (20 kg) were cut into small pieces and extracted with 90% EtOH under reflux. After removal of the solvent by evaporation, the combined extracts (1700 g) were suspended in H_2O and partitioned with petroleum ether and EtOAc. The EtOAc extract was chromatographed on polyamide using MeOH–H_2O (10 and 70%) as eluent to obtain non-flavonoid and phenolic extracts. Then the non-flavonoid extract (110 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 was further chromatographed on silica gel eluting with $CHCl_3$–EtOAc gradient (30:1, 10:1, 2:1) to give compounds **1** (7.9 mg) and **2** (11.2 mg). Fraction 4 was separated by silica gel chromatography and eluted with $CHCl_3$–EtOAc–MeOH (20:3:1) to afford compounds **3** (18.6 mg) and **4** (21.6 mg). Fraction 6 was chromatographed on an ODS column using a mixture of MeOH–H_2O (10, 30, 50, and 70%) as eluent to give fractions I–IV. Fraction II was further purified by Sephadex LH-20 to give compound **5** (7.9 mg). Fraction 8 was separated on an ODS column eluted by MeOH–H_2O gradient to afford compound **6** (9.2 mg).

18,19-*seco*-3*β*-Hydroxy-urs-12-en-18-one. Colorless needles, mp 278–279°C; $[\alpha]_D$ –27.8° (*c* 0.9, MeOH). UV (MeOH, λ_{max}, nm): 269. IR (KBr, ν_{max}, cm^{-1}): 3341, 2925, 1717, 1456. HR-ESI-MS: *m/z* 465.3688 $[M + Na]^+$ (calcd for $C_{30}H_{50}NaO_2^+$, 465.3690). For ^1H NMR and ^{13}C NMR data, see Table 1.

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