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Two new glucosides from the pellicle of the walnut (*Juglans regia*)

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Abstract: A new *α*-tetralonyl glucoside, 6'-*O*-acetyl-juglanoside E (**1**), and a new dihydrophaseic acid glucoside, dihydrophaseic acid 1-*O*-(6-*O*-acetyl)-glucopyranoside (**2**), together with two known ones, juglanoside E (**3**) and dihydrophaseic acid (**4**), were isolated from the pellicle of the walnut (*Juglans regia*). The structures of the new compounds were elucidated by comprehensive spectroscopic analysis, including IR, HRESIMS, 1D and 2D NMR data.

Keywords: walnut, *Juglans regia*, pellicle, *α*-tetralonyl glucoside, dihydrophaseic acid glucoside

Introduction

The *Juglans* genus (family Juglandaceae) comprises several species and is widely distributed all over the world. The walnut tree (*Juglans regia* L.), a well-known member of this genus, is cultivated commercially throughout southern Europe, northern Africa, eastern Asia, United States and western South America. In China, *Juglans regia* is not only an agricultural commodity, but can be also used for different medicinal functions. Walnuts, the seeds of *Juglans regia*, are a highly nutritious food, which have been found to be a rich source of essential unsaturated fatty acids, tocopherols,[1] hormone and strong antioxidant melatonin.[2] Mahoney et al. reported that the activity of inhibiting aflatoxigenesis in *Aspergillus flavus* located entirely in the pellicle (seed coat) of walnuts.[3] However, to our knowledge, few phytochemical investigations on the pellicle of walnuts have been undertaken.[4,5] This paper describes the separation and structural elucidation of a new *α*-tetralonyl glucoside (**1**) and a new dihydrophaseic acid glucoside (**2**), along with two known ones, juglanoside E (**3**) and dihydrophaseic acid (**4**) from the pellicle of this plant (Figure 1).

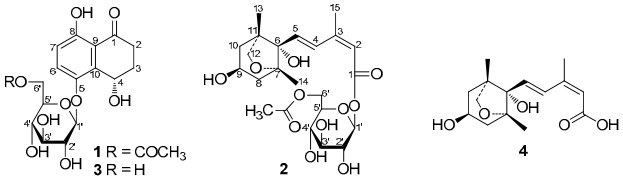

Figure 1. Structures of compounds **1**–**4**

Results and Discussion

Compound **1** was obtained as white amorphous powder with mp 84.5–86.2 °C. The positive HRESIMS spectrum showed the $[M + Na]^+$ ion peak at *m/z* 421.1112 (calcd 421.1110) in accordance with the molecular formula $C_{18}H_{22}O_{10}$, indicating eight degrees of unsaturation. The IR spectrum showed the presence of hydroxy (3390 cm^{-1}) and two carbonyl (1729, 1643 cm^{-1}) groups. The ^{13}C NMR spectrum gave 18 carbon resonances (Table 1). Besides an acetyl signals (δ_C 20.7, 172.6), and a glucopyranose signals (δ_C 104.4, 77.8, 75.6, 75.3, 71.5, 64.6), other 10 resonance signals can be ascribable to a tetrasubstituted phenyl (δ_C 159.3 s, 148.5 s, 135.2 s, 128.7 d, 118.9 d, 116.3 s), two methylenes (δ_C 33.6 t, 30.3 t), a methine (δ_C 61.3 d) and a carbonyl (δ_C 206.5 s), revealing the presence of a trihydroxy-*α*-tetralone.[6] In combination with the ^1H NMR spectrum, proton signals due to an tetrasubstituted aromatic ring at δ_H 6.87 (d, J = 9.0 Hz) and 7.43 (d, J = 9.0 Hz), suggested the 4,5,8-trihydroxy-*α*-tetralone moiety in **1**. The sugar moiety was attached to C-5 of the aglycon on the base of the correlation of H-1′ [δ_H 4.79 (d, J = 7.5 Hz)] with C-5 (δ_C 148.5) in the HMBC spectrum (Figure 2). In the ^1H NMR spectrum, the coupling constant of the anomeric proton at δ_H

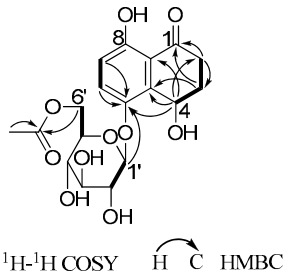

Figure 2. Selected 2D NMR correlations of **1**

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Table 1. NMR spectral data of 1 and 3 in CD_3OD (^1H: 500 MHz; ^{13}C: 125 MHz)

	1		3	
pos.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		206.5, C		206.5, C
2a	3.06, m	33.6, CH_2	3.05, m	33.6, CH_2
2b	2.53, m		2.50, m	
3a	2.23, m	30.3, CH_2	2.23, m	30.2, CH_2
3b	2.20, m		2.20, m	
4	5.35, t (2.8)	61.3, CH	5.35, t (3.0)	61.3, CH
5		148.5, C		148.9, C
6	7.43, d (9.0)	128.7, CH	7.51, d (9.2)	128.5, CH
7	6.87, d (9.0)	118.9, CH	6.86, d (9.2)	119.0, CH
8		159.3, C		159.1, C
9		116.3, C		116.2, C
10		135.2, C		134.7, C
1′	4.79, d (7.5)	104.4, CH	4.78, d (7.6)	104.5, CH
2′	3.50, m	75.3, CH	3.52, m	75.4, CH
3′	3.44, t (9.0)	77.8, CH	3.47, m	77.9, CH
4′	3.35, m	71.5, CH	3.40, m	71.3, CH
5′	3.57, m	75.6, CH	3.39, m	78.3, CH
6′a	4.40, dd (2.0, 11.5)	64.6, CH_2	3.72, m	62.5, CH_2
6′b	4.24, dd (6.0, 11.5)		3.90, m	
OAc	2.03, s	20.7, CH_3		
		172.6, C		

4.79 (d, J = 7.5 Hz) indicated that the sugar moiety was a β-glucopyranose, which was further determined to be β-D-glucopyranose as judged from the specific rotation ($[\alpha]_D^{20}$ + 35.3, c 1.0, H_2O) obtained on acid hydrolysis of **1**.[7] In addition, correlation from H-6′ (δ_H 4.40, 4.24) to carbonyl carbon (δ_C 172.6) of the acetyl group was also observed in the HMBC spectrum, indicating that the acetyl group was linked to C-6′. Therefore, the planar structure of **1** was elucidated and was designated as 6'-*O*-acetyl-juglanoside E.

C-4 of **1** was assumed to be *S* because of co-occurrence of other (4*S*)-4,5,8-trihydroxy-α-tetralone isolated from *Juglans*.[6,8,9] Moreover, (4*R*)-4,5,8-trihydroxy-α-tetralone from microbial metabolites has not been reported in plants yet.[10]

Compound **2** was isolated as white amorphous powder with mp 106.1–108.2 °C. Its molecular formula $C_{23}H_{34}O_{11}$, was derived from HRESIMS ($[M + Na]^+$ 509.1988, calcd. for $C_{23}H_{34}O_{11}Na$, 509.1998). The IR spectrum displayed the absorption bands for OH (3398 cm^{-1}), C=O (1708 cm^{-1}), C=C (1601 cm^{-1}), and C-O-C (1071, 1037 cm^{-1}) functionalities. NMR spectra of **2** (Table 2) revealed the presence of one β-glucosyl moiety [δ_H 5.52 (1H, d, J = 8.2 Hz); δ_C 95.3 d, 77.8 d, 76.1 d, 73.9 d, 71.2 d, 64.5 t] and an acetyl group [δ_H 2.07 (3H, s); δ_C 172.8 s, 20.7 q]. Acid hydrolysis of **1** and comparison of the specific rotation of the acid hydrolysate ($[\alpha]_D^{20}$ + 36.5, c 1.0, H_2O) with an authentic standard of D-glucose established the sugar as D-glucose.[7] The remaining 15 carbons were ascribable for three methyls, three methylenes, four methines, four quaternary carbons, and one carbonyl group, suggesting the presence of a dihydrophaseic acid moiety in **2**.[11] HMBC correlation of H-1′ [δ_H 5.52 (d, J = 8.2 Hz)] with C-1 (δ_C 165.8) established the β-D-glucopyranosyl unit located at C-1. The HMBC correlation of H-6′ [δ_H 4.23 (dd, J = 5.5, 12.0 Hz), 4.39 (d, J = 12.0 Hz)] with the carbonyl signal of acetyl group at δ_C 172.8 revealed that the acetyl group could be located at C-6′ in the glucosyl moiety. Thus, the planar structure of **2** was elucidated as shown.

The cyclohexane ring in **2** appears to be a chair conformation based on the literatures[12,13], the spin-spin splitting patterns, and the coupling constants of H-8b (t, J = 13.0 Hz), H-9 (tt, J = 13.0, 7.0 Hz), and H-10b (t, J = 13.0 Hz).[14] On the beta side of the cyclohexane plane, H-8b (δ_H 1.75) and H-10b (δ_H 1.67) have to be axial because of the NOE correlations of H-5 [δ_H 6.63 (d, J = 16.0 Hz)] with H-8b and H-5 with H-10b (Figure 3),[12] while the absence of NOE correlations of H-9 with H-8b and H-10b indicated H-9 (δ_H 4.12) was also in axial on the alpha side of the ring. The presence of a clear NOE correlation between H-9 and H-12a [δ_H 3.74 (d, J = 7.5 Hz)] which was not observed in *epi*-dihydrophaseic acid derivatives[12] supported above conclusion as well. In addition, oxymethylene carbon C-12 has to be in axial orientation on the alpha side of the ring (Figure 3), as a stereo assumption for a successful ether ring formation in system **2**.[12] OH-6 and the oxymethylene bridge should be on the same side of the ring system due to almost superimposed NMR signals of the two oxymethylene protons (H-12) (Table 2).[13,15] Further NMR data comparison between **2** and both dihydrophaseic acid and *epi*-dihydrophaseic acid could give another evidence.[15] The ^1H NMR data of **2** was in good accordance to that of dihydrophaseic acid (**4**) (Table 2), but exhibited some differences from that of *epi*-dihydrophaseic acid, especially for H-5. In **2**, this signal occur 0.23 ppm further downfield, which was accounted for OH-9 can approach of the side chain.[13] Thus, OH-9 and the C-5 side chain have to be in the same beta side of the cyclohexane ring. At last, the configurations of the double bonds in **2** were determined via 1D and 2D NMR. The (*E*) configuration of C(4)=C(5) bond was assigned based on the coupling constants,[13,16] and a ROESY cross-peak (Figure 3) between H-2 and Me-15 showed the configuration of C(2)=C(3) bond is (*Z*). So far, the relative configuration of **2** was confirmed and named as dihydrophaseic acid 1-*O*-(6-*O*-acetyl)-glucopyranoside.

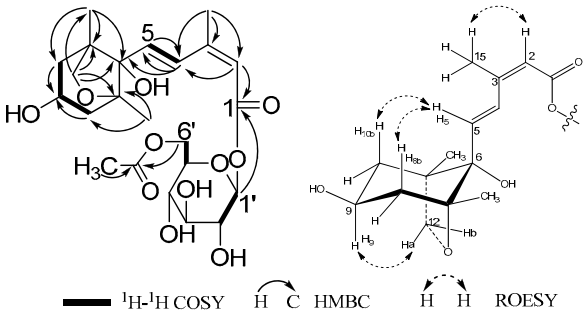

Figure 3. Selected 2D NMR correlations of **2**

Experimental Section

General Experimental Procedures. Melting point was measured on a Dresden HMK micromelting point apparatus and was uncorrected. The high resolution 1D and 2D NMR spectra (HMQC, HMBC, ^1H-^1H COSY and ROESY) were performed using Bruker DRX-500 MHz spectrometers. All chemical shifts (δ) are given in ppm, and TMS was used as an internal standard. MS spectra were measured on a VG Auto Spec-3000 mass spectrometer and Agilent G3250AA LC/MSD TOF spectrometer. IR was measured on a Nicolet FTIR 5DX spectrophotometer with KBr pellets. Specific rotation was obtained on a JASCO P-1020 digital polarimeter. Silica gel (200–300 mesh) was the product of the Qingdao Marine Chemical Ltd. Sephadex LH-20 was purchased from

Table 2. NMR spectral data of 2 and 4 in CD_3OD (^1H: 500 MHz; ^{13}C: 125 MHz)

	2		**4**	
pos.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		165.8, C		169.6, C
2	5.86, s	117.7, CH	5.76, s	119.2, CH
3		154.0, C		151.5, C
4	8.05, d (16.0)	131.7, CH	7.89, d (16.0)	131.8, CH
5	6.63, d (16.0)	136.6, CH	6.52,d (16.0)	135.2, CH
6		83.3, C		83.2, C
7		87.8, C		87.8, C
8a	2.05, overlap	46.0, CH_2	2.03, m	46.0, CH_2
8b	1.75, t (13.0)		1.73, m	
9	4.12, tt (13.0, 7.0)	66.0, CH	4.11, m	66.0, CH
10a	1.85, dd (13.0, 7.0)	44.5, CH_2	1.83, m	44.5, CH_2
10b	1.67, t (13.0)		1.67, m	
11		49.4, C		49.4, C
12a	3.74, d (7.5)	77.3, CH_2	3.70, d (8.8)	77.3, CH_2
12b	3.83, d (7.5)		3.80, d (8.8)	
13	0.96, s	16.3, CH_3	0.92, s	16.4, CH_3
14	1.18, s	19.7, CH_3	1.14, s	19.7, CH_3
15	2.15, s	21.3, CH_3	2.08, s	21.3, CH_3
1′	5.52, d (8.0)	95.3, CH		
2′	3.40, m	73.9, CH		
3′	3.46, t (9.0)	77.8, CH		
4′	3.39, m	71.2, CH		
5′	3.62, m	76.1, CH		
6′a	4.23, d (12.0)	64.5, CH_2		
6′b	4.39, dd (5.5, 12.0)			
OAc	2.07 s	20.7, CH_3		
		172.8, C		

Amersham Biosciences. RP-18 was purchased from Merck (Merck, Darmstadt, Germany). A Waters 600 series instrument equipped with Waters Spherisorb® ODS2 column (5 μm, 4.6 mm × 250 mm) was used for high-performance liquid chromatography (HPLC) analysis, and a semi-preparative Wters Spherisorb® S10 ODS2 column (5 μm, 20 mm × 250 mm) was used for the sample preparation.

Plant Materal. The pellicle of *Juglans regia* was collected in Kunming, Yunnan Province, China, in August 2008, and authenticated by Prof. Shu-Gang Lu from School of Life Science, Yunnan University. A voucher specimen (No. LCS-02) has been deposited in the Key Laboratory of Medicinal Chemistry for Nature Resource of Yunnan University.

Extraction and Isolation. The dried pellicle of *Juglans regia* (1.8 kg) was powdered and extracted three times with 80% aqueous acetone at room temperature. The extracts were combined, and evaporated under reduced pressure. The crude residue was suspended in H_2O, and partitioned with petroleum ether, EtOAc, and *n*-butanol successively. The *n*-butanol fraction (140 g) was subjected to silica gel column eluted gradually with $CHCl_3$/MeOH (200:1–1:1) to yield eight fractions (Fr. C1–C8). Fr. C3 (6.3 g) was chromatographed on a silica gel eluted with EtOAc/MeOH (20:1) and then purified on Sephadex LH-20 (MeOH), yielding **4** (9 mg). Fr. C5 (1.2 g) was subjected to MCI gel, Sephadex LH-20 and semi-preparative HPLC successively to yield **1** (11 mg), **2** (9 mg) and **3** (8 mg).

6'-*O*-acetyl-juglanoside E (1): white powder; mp 84.5–87.2 °C; $[\alpha]_D^{20}$ – 9.02 (*c* 1.28, MeOH); IR (KBr) ν_{max}: 3390, 2921, 1729, 1643, 1465, 1239, 1073 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m*/*z*: 421.1112 $[M + Na]^+$ (calcd for $C_{18}H_{22}O_{10}Na$ $[M + Na]^+$, 421.1110).

Dihydrophaseic acid 1-*O*-(6-*O*-acetyl)-glucopyranoside (2): white amorphous powder; mp 106.1–108.2 °C; $[\alpha]_D^{20}$ – 5.33 (*c* 1.90, MeOH); IR (KBr) ν_{max}**:** 3398, 2931, 1708, 1601, 1236, 1071, 1037 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m*/*z*: 509.1988 $[M + Na]^+$ (calcd for $C_{23}H_{34}O_{11}Na$ $[M + Na]^+$, 509.1998).

Acidic Hydrolysis of 1 and 2. 5 mg of **1** and **2** was added 3 mL of 2 N HCl, and the reaction mixture was heated at 90 °C on a water bath for 2 h. After workup, the reaction mixture was extracted three times with 2 mL of EtOAc. The aqueous phase was concentrated under reduced pressure to give a residue. Comparison of the optical rotations for each hydrolyzed product of **1** ($[\alpha]_D^{20}$ + 35.3, *c* 1.0, H_2O) and **2** ($[\alpha]_D^{20}$ + 36.5, *c* 1.0, H_2O) with an authentic standard confirmed that the glucose was D-glucose.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/ 10.1007/s13659-012-0009-0 and is accessible for authorized users.

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