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Structurally Diverse Terpenoids from the Resins of *Populus euphratica*

Yong-Hui Yang [1], Yang Yu [1,2], Shu-Ting Zhang [1], Bai-Xiang Cai [1*] and Ju-Tao Wang [*1,2,3]

[1] *School of Pharmacy, Anhui University of Chinese Medicine, Hefei, 230012, PR China*

[2] *Institute of Medicinal Chemistry, Anhui Academy of Chinese Medicine, Hefei 230012, PR China*

[3] *Anhui Province Key Laboratory of Research & Development of Chinese Medicine, Hefei, 230012, PR China*

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Abstract: Phytochemical analysis of *Populus euphratica* resins has resulted in the isolation and identification of two undescribed terpenoids, including a new triterpene (**1**), a new sesquiterpene (**2**), and nine additional terpenoid (**3-11**) compounds. The structures of these novel compounds were determined using HR-ESI-MS and NMR (1D and 2D) spectroscopic analyses, combined with ECD calculations. The inhibitory activity of these terpenoids against *α*-glucosidase was also evaluated, and the results revealed that none of the compounds exhibited significant *α*-glucosidase inhibitory activity at a concentration of 50 μM. Only compound **1** displayed weak inhibitory activity (1.84 ± 8.96%) compared to the positive control.

Keywords: *Populus euphratica* resins*;* sesquiterpenoids*;* triterpenoids.

1. Introduction

Populus euphratica, a deciduous plant belonging to the Salicaceae family, is a tree species commonly found in wasteland [1]. In China, this species is primarily found in the western region, specifically in non-living environments such as deserts or saline and alkaline lands [2,3]. *Populus euphratica* resin is used in both traditional Chinese and Uyghur medicine. Ancient Chinese texts indicate that it was primarily used in China for the treatment of sore throat, toothache, scrofula, and duodenal ulcer swelling [4,5]. This genus is known for synthesizing diverse compounds, including steroids, diterpenoids, sesquiterpenoids, triterpenoids, volatile oils, and phenolic groups, which exhibit cytotoxic, anti-inflammatory, antiviral, and neuroprotective properties [6-9]. In our ongoing phytochemical investigation of natural compounds, we obtained two undescribed (**1-2**) and nine known terpenoids (**3-11**) (Figure 1) from the resins of *Populus euphratica*. The inhibitory activities of these terpenoids against *α*-glucosidase were also evaluated to gain an in-depth understanding of the biological activities of the secondary constituents. This study presents the isolation, structural elucidation, and *α*-glucosidase-inhibitory activity of these compounds.

*Corresponding authors: E- Mail: caibx103@126.com (Bai-Xiang Cai); wjt591@ahtcm.edu.cn (Ju-Tao Wang).

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2. Expermental

2.1. General Experimental Procedures

The isolated compounds were tested on a 500 MHz NMR spectrometer (Bruker Corporation, Germany) using residual solvent signals as the internal standard. HR-MS data were acquired using an LCMS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). A Waters 1525 HPLC system (Waters Company, USA) equipped with a Waters Sunfire series C_{18} chromatographic column (4.6 mm × 250 mm, 5 μm) was used for the analytical liquid phase separation. Column chromatography silica gel (200-300 mesh) and thin-layer chromatography silica gel plates were manufactured by Shanxi Nuotai Silica Gel Reagent Factory. The reverse filling material used in this study was (20-45 mm, Fuji Silysia Chemical, Japan) RP-18 silicone. Pharmacia Sephadex LH-20 gel material was manufactured by Sweden Amersham Biosciences Company. The colorants used were sulfuric acid-ethanol and sulfuric acid-vanillin.

2.2. Plant Materials

The Chinese Pharmacy of the Second Affiliated Hospital of Anhui University of Chinese Medicine procured the resins of *Populus euphratica*, which were subsequently identified by Yang Qing-Shan, Associate Professor, School of Pharmacy, Anhui University of Chinese Medicine. The samples were stored at the Natural Medicinal Chemistry Laboratory, School of Pharmacy, Anhui University of Traditional Chinese Medicine, China.

2.3. Extraction and Isolation

The resins of *Populus euphratica* (10 kg) were pulverized and extracted with 100% methanol (25 L) at room temperature for three days, and the extraction process was repeated four times. A crude methanol extract (9.5 kg) was obtained by evaporating the methanol using a rotary evaporator at low pressure. The crude extract (1 kg) was fractionated into 13 fractions (Fr.1-Fr.13) by silica gel column chromatography (CC) and eluted with a CH_2Cl_2/ MeOH (1:0-1:1, v/v) gradient. Fraction 4 (4.1 g) was subjected to C_{18} MPLC using MeOH-H_2O (70-100%, v/v) to obtain nine subfractions (C1-9). Compound **4** (3.47 mg) was obtained from subfraction C3 by chromatography on a Sephadex LH-20 (MeOH) column and further purified on an HPLC preparative column using CH_3CN-H_2O (40-60%, v/v). The purification process was repeated for subfraction C4 using CH_3CN-H_2O (55-70%, v/v) to obtain compounds **2** (1.93 mg), **5** (5.51 mg), **8** (4.56 mg), and **9** (2.58 mg). Subfraction C5 was chromatographed and further purified by HPLC with CH_3CN-H_2O (55-70%) to obtain compounds **6** (1.31 mg) and **7** (0.47 mg). Finally, subfraction C6 was purified to obtain compounds **3** (3.96 mg), **10** (1.49 mg), **1** (2.64 mg), and **11** (2.15 mg) using chromatography and an HPLC preparative column with CH_3CN-H_2O (65-80%).

*Compound **1***: Colorless crystal, $[\alpha]_D^{20}$ of 114.6 (c 0.2, CH_3OH). The UV spectrum [(CH_3OH), λ_{max} (log ε)] maximum absorption peaks at 250 (4.07) and 196 (3.69) nm. ^1H and ^{13}C NMR data are presented in Table 1. HR-ESI-MS: *m/z :* 465.3338 $[M+Na]^+$ ($C_{29}H_{46}O_3Na$, calcd. for 465.3339).

*Compound **2***: Yellowish gum, $[\alpha]_D^{20}$ of -14.4 (c 0.2, CH_3OH). The UV spectrum [(CH_3OH), λ_{max} (log ε)] maximum absorption peaks at 204 (3.06) and 196 (2.22) nm. ^1H and ^{13}C NMR data are presented in Table 2. HR-ESI-MS: *m/z :* 277.1773 $[M+Na]^+$ (calcd. for $C_{15}H_{26}O_3Na$, 277.1774).

2.4. α-Glucosidase Assay

α-Glucosidase inhibitory activities were evaluated in accordance with a previously published study [6,10,11]. The *α*-glucosidase inhibition assay was performed using 0.1 M sodium phosphate

buffer (SPB, pH 7.5). Solutions of *α*-glucosidase (2.0 U/mL) and *p*-NPG (10 mM) were prepared using this buffer. In each well, 10 μL of DMSO stock solution of the samples (50 μM), 90 μL of SPB, and 80 μL of *α*-glucosidase solution were added. The samples were shaken for 2 min and incubated at 37 °C for 15 min. Then, a 20 μL aliquot of the *p*-NPG solution was added to initiate the reaction. Enzyme inhibition was determined by measuring the OD values at 405 nm using a microplate reader. Acarbose (MedChemExpress, China) was used as a positive control.

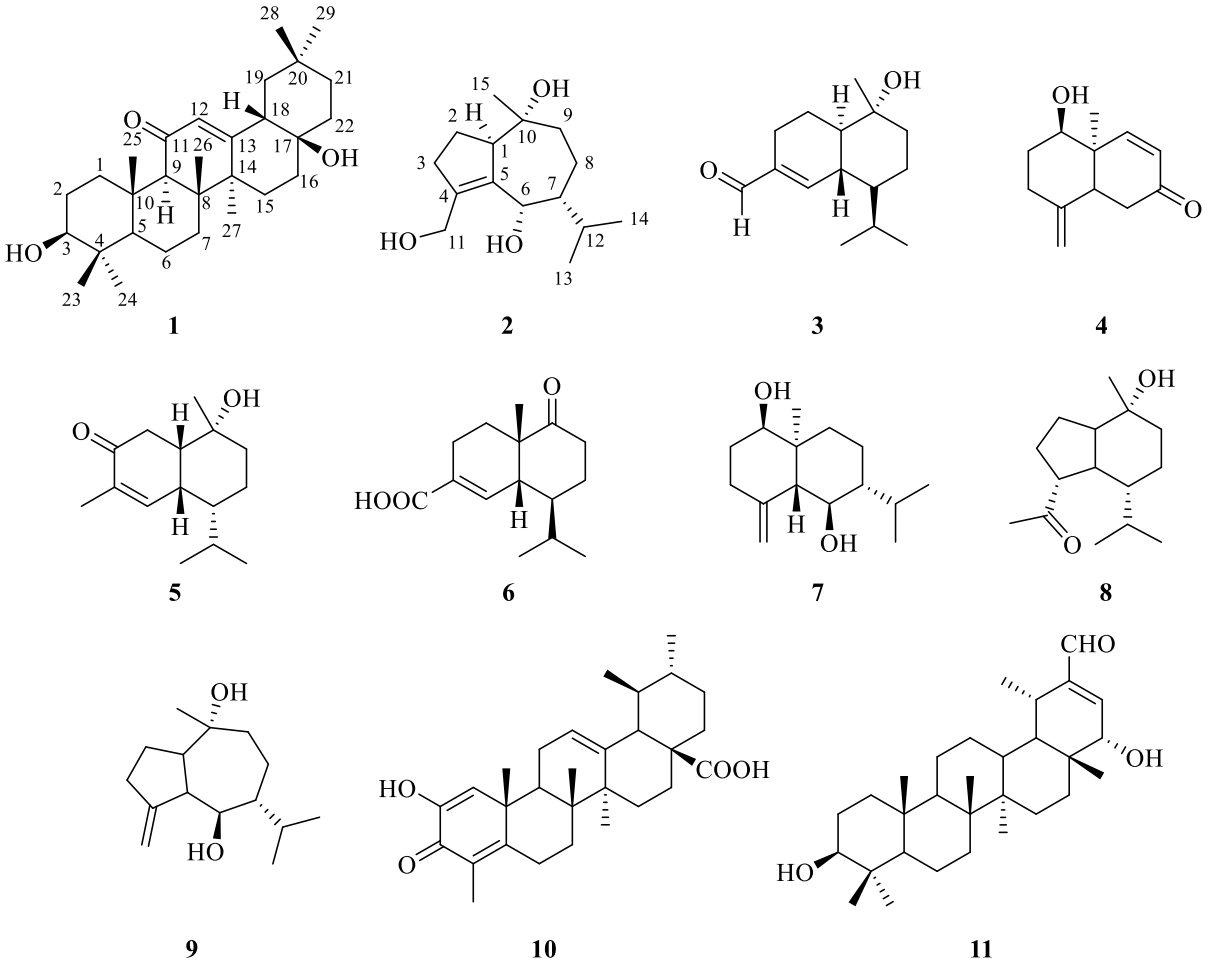

Figure 1. The chemical structures of compounds **1**-**11**.

3. Results and Discussion

Compound **1** was isolated as colorless crystals. It exhibited an $[M+Na]^+$ ion with *m/z* 465.3338 ($C_{29}H_{46}O_3Na$, calculated for 465.3339) in conjunction with ^{13}C NMR data, which corresponded to the molecular formula $C_{29}H_{46}O_3$, indicating seven degrees of unsaturation. The ^1H NMR data (Table 1) revealed the presence of one olefinic protons at δ_H 5.50 (1H, s) and seven methyl groups at δ_H 1.37 (s), 1.13 (s), 1.11 (s), 1.00 (s), 1.79 (s), 0.93 (s) and 0.79 (s). The ^{13}C NMR and DEPT spectra of compound **1** showed 29 carbon resonances classified into seven methyl carbons (δ_C 32.8, 28.7, 24.2, 23.6, 20.3, 16.6, 16.3), nine methylene carbons (δ_C 47.9, 40.2, 38.2, 37.4, 34.3, 27.8, 27.6, 27.3, 18.6), three tertiary carbons (δ_C 63.2, 56.3, 49.5), one olefinic tertiary carbon (δ_C 128.9), one oxygenated tertiary carbon (δ_C 79.4), and eight quaternary carbons including five aliphatic (δ_C 49.1, 46.4, 44.8, 38.6, 31.9), one olefinic (δ_C 174.2), one oxygenated (δ_C 72.4), and one ketone (δ_C 202.9). The data for compound **1** were similar to those for (3*S*, 5*R*, 8*R*, 9*R*, 10*S*, 14*S*, 17*R*, 18*S*)-3,17-dihydroxy-28-norolean-12-en-11,16-dione, an oleanane-type triterpene [12]. However, compound **1** differed from the previously published compound in the absence of a ketone group at C-16. The ^1H-^1H COSY spectrum of compound **1** revealed correlations between the following: H-1/H-2/H-3, H-5/H-6/H-7, H-

15/H-16, and H-21/H-22. HSQC and HMBC spectra were used to analyze the planar structure of compound **1** (Figure 2). The ROESY interactions between H-24, H-5, H-9, and H-27 indicated their co-facial arrangement and established an *α*-orientation (Figure 2). Accordingly, the structure of compound **1** was determined (Figure 1).

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1** (CD_3OD) (δ in ppm, J in Hz)

Position	δ_H	δ_C	Position	δ_H	δ_C
1α	2.75 dt (13.4, 3.6)	40.2	13	-	174.2
1β	1.02m		14	-	31.9
2α	1.70-1.66 m	27.8	15	1.37 m	37.4
2β	1.55-1.50 m		16α	1.23-1.17 m	47.9
3	3.16 dd (11.8, 4.6)	79.4	16β	1.72 m	
4	-	49.1	17	-	72.4
5	0.76 dd (12.0, 2.0)	56.3	18	2.46-2.41 m	49.5
6α	1.47 dt (11.6, 2.8)	18.6	19	2.16 dt (8.4, 2.3)	27.6
6β	1.60 q (3.7, 3.0)		20	-	44.8
7α	1.70-1.66 m	34.3	21α	1.60 q (3.7, 3.0)	27.3
7β	1.47 dt (11.6, 2.8)		21β	1.29-1.27 m	
8	-	46.4	22α	1.75 dd (9.5, 4.3)	38.2
			22β	1.55-1.50 m	
9	2.46-2.41 m	63.2	23	1.79 s	28.7
			24	0.79 s	16.3
10	-	38.6	25	1.11 s	16.6
11	-	202.9	26	1.13 s	20.3
12	5.50 s	128.9	27	1.00 s	24.2
			28	1.37 s	23.6
			29	0.93 s	32.8

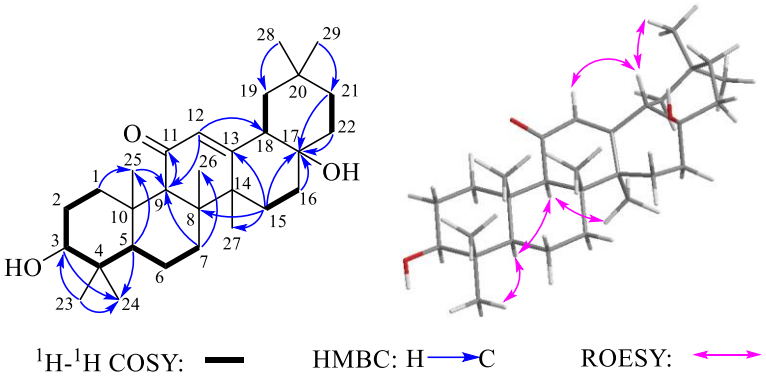

Figure 2. Key HMBC,^{1}H-^{1}H COSY and NOESY correlations for compound **1**

Compound **2** was isolated as a yellowish gum and its molecular formula was determined to be $C_{15}H_{26}O_3$, as deduced from its HR-ESI-MS *m/z* 277.1773 $[M+Na]^+$ (calculated for $C_{15}H_{26}O_3Na$, 277.1774) and ^{13}C-NMR spectra, indicating three degrees of unsaturation. The ^{1}H NMR spectrum displayed signals of three methyl group protons at δ_H 1.06 (s), 0.96 (d, 7.0), 0.80 (d, 7.0), while the ^{13}C NMR and DEPT spectra showed 15 carbon resonances classified into three methyl carbons (δ_C 21.7, 21.7, 16.1), four methylene carbons (δ_C 26.8, 34.6, 21.4, 47.3), one oxygenated methylene carbon (δ_C 59.3), three tertiary carbons (δ_C 56.9, 52.8, 28.9), one oxygenated tertiary carbon (δ_C 69.1), three quaternary carbons including two olefinic (δ_C 145.5, 142.8), and one oxygenated aliphatic (δ_C 77.0). Spectroscopic characteristics indicated that the architecture of compound **2** resembled that of xylaranol B [13], and the ^{1}H-^{1}H COSY spectrum revealed correlations between H-1/H-2/H-3, H-6/H-7/H-8/H-9,

and H-7/H-12/H-13/H-14. The HMBC spectrum revealed correlations between H-2 and C-4 (δ_C 145.5), H-11/H-2 and C-5 (δ_C 142.8), H-13/H-14/H-9 and C-7 (δ_C 52.8), H-1/H-15 and C-9 (δ_C 47.3), and H-3 and C-11 (δ_C 59.3), confirming the presence of a guaiane-type sesquiterpenoid (Figure 1). The relative stereochemistry was determined using the ROESY spectrum, where H-6/H-8/H-12 was associated with H-7, revealing that H-6 was on the same side as H-7 (Figure 3). Furthermore, the planar structure of compound **2** was determined (Figure 1).

Subsequently, the absolute configuration of compound **2** was verified using ECD calculations. As depicted in Figure 4, the experimental ECD curve of compound **2** closely matched the calculated ECD spectrum, enabling explicit assignment of the absolute configuration as 1*R*, 6*S*, 7*R*, and 10*R*.

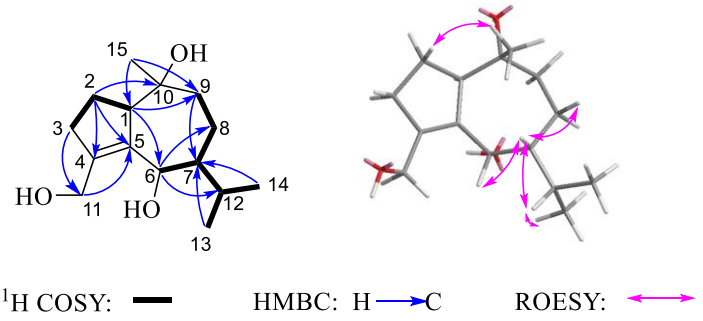

Figure 3. Key HMBC,^{1}H-^{1}H COSY and NOESY correlations for compound **2**

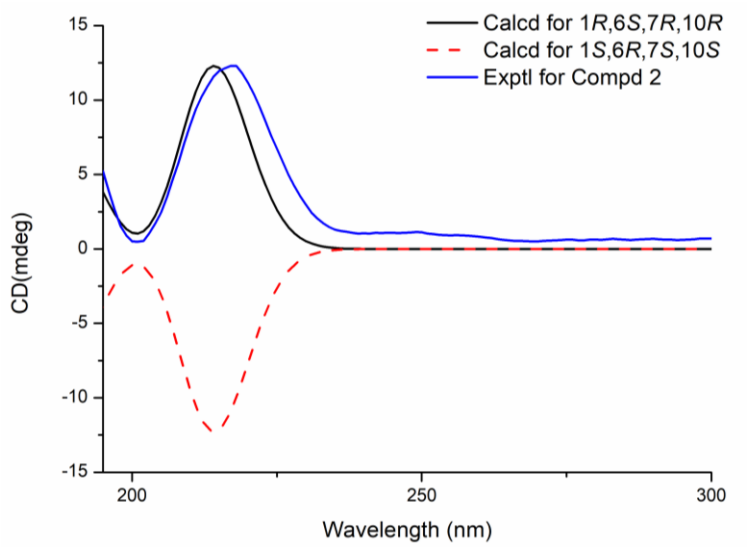

Figure 4. Experimental and calculated ECD spectra of compound **2**

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **2** (CD_3OD) (δ in ppm, J in Hz)

Position	δ_H	δ_C	Position	δ_H	δ_C
1	2.98 d (5.7)	56.9	9α	1.81 dd (8.4, 4.4)	47.3
2α	1.89 m	26.8	9β	1.51 overlapped	
2β	2.16 dd (8.3, 5.0)		10	-	77.0
3α	2.52 m	34.6	11α	4.22 d (8.1)	59.3
3β	2.32 dd (10.3, 6.2)		11β	4.28 d (8.1)	
4	-	145.5	12	2.10 ddt (11.4, 7.1, 4.3)	28.9
5	-	142.8	13	0.80 d (4.3)	16.1
6	4.31 d (6.1)	69.1	14	0.96 d (4.3)	21.7
7	1.47 overlapped	52.8	15	1.06 s	21.7
8α	0.78 m	21.4			
8β	1.48 overlapped				

In addition, known compounds were identified by comparing their spectroscopic data with those reported in the literature. The known compounds were identified as 10-hydroxyl-15-oxo-*α*-cadinol (**3**) [14], 7-trinoreudes-ma-4 (15), 8-dien-1β-ol-7one (**4**) [15], 10*R*-hydroxyamorph-4-en-3-one (**5**) [16], cosmosoic acid (**6**) [17], (1*R*,5*R*,6*R*,7*R*,10*S*)-1,6-Dihroxyeudesm-4(15)-ene (**7**) [18], *ent*-oplopanone (**8**) [19], 6*β*, 10*β*-Dihydroxy-4(15)-guaiene (**9**) [20], jughopanes B (**10**) [21], and (3b,22a)-3,22-dihydroxytaraxast-20-en-30-al (**11**) [22].

Table 3. *α*-glucosidase inhibitory activity of Compounds **1**-**11**

Compounds	Inhibition Rate (%, 50 μM)
1	1.84±8.96
2	-11.06±12.98
3	-12.90±8.88
4	0.46±9.98
5	-18.89±7.59
6	-10.60±10.48
7	-17.05±18.83
8	-16.59±2.14
9	-4.61±4.84
10	-5.53±13.30
11	-29.85±5.74
Acarbose	7.37±6.97

α-Glucosidase inhibitors perform a critical function in the treatment of type 2 diabetes by impeding the degradation of disaccharide and oligosaccharide substrates into monosaccharides [23,24]. Several studies have demonstrated the inhibitory effects of sesquiterpenoids and triterpenoids on *α*-glucosidase [25-27]. Subsequently, compounds **1**-**11** were evaluated for *α*-glucosidase inhibitory activity *in vitro*. The results revealed that these compounds lacked significant *α*-glucosidase inhibitory activity at a concentration of 50 μM (Table 3). Only compound **1** exhibited weak inhibitory activity (inhibition rate: 1.84±8.96%) compared to the positive control. However, further investigations are necessary to elucidate whether these compounds have any effects on other biological profiling mechanisms.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/recordsof-natural-products

ORCID
Yong-Hui Yang: 0009-0000-3681-8080
Yang Yu: 0000-0002-9477-2313
Shu-Ting Zhang:0009-0001-3359-0890
Ju-Tao Wang: 0000-0001-8258-7658
Bai-Xiang Cai: 0000-0002-4550-9666

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