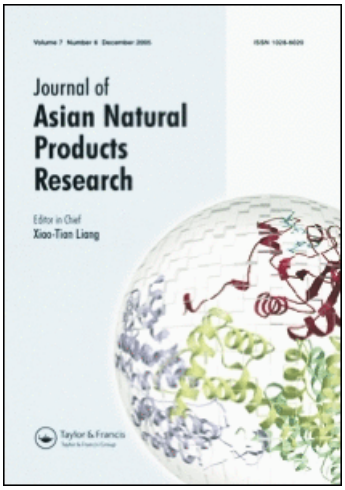

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Constituents from the testas of *Castanea mollissima* Blume with α-glucosidase inhibitory activity

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NOTE

Constituents from the testas of *Castanea mollissima* Blume with α-glucosidase inhibitory activity

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In the screening of biologically active constituents from medicinal plants, the 75% EtOH extract of the testas of *Castanea mollissima* Blume showed potent α-glucosidase inhibitory activity. By means of various chromatographic methods, the extract gave a new dammarane-type triterpene **1** along with 17 known compounds. The structure of **1** was determined to be 3β-acetoxy-20-oxo-21-nordammaran-23-oic acid by HRMS and NMR studies including 2D NMR experiments. The new compound and some known compounds showed potent α-glucosidase inhibitory activity with acarbose as a positive control.

Keywords: *Castanea mollissima* Blume; 3β-acetoxy-20-oxo-21-nordammaran-23-oic acid; α-glucosidase inhibitory activity

1. Introduction

Type 2 diabetes is a multi-factorial disease characterized by high level of blood glucose, insulin, and impaired insulin action. The number of diabetic complaints is continuously growing with a currently estimated worldwide incidence of about 194 million people and is expected to increase to 330 million by 2025 [1]. Inhibitors of glycosidase have the potential for the treatment of various disorders and diseases such as diabetes, cancers, and AIDS [2]. Recently, intestinal α-glucosidase inhibitors were thought to be powerful therapeutic agents in carbohydrate metabolic disorders, especially diabetes mellitus.

Castanea mollissima Blume is a perennial woody plant and widely cultivated in Europe, North America, and Asia (especially in China) as the economic crop for its sweet edible kernels, which has also been used to treat gastroenteritis, bronchitis, and regurgitation in traditional Chinese medicines for centuries [3]. In the screening of biologically active constituents from medicinal plants, the 75% EtOH extract of the testa of *C. mollissima* Blume showed much stronger α-glucosidase inhibitory activity than other plants. By means of various chromatographic methods, the extract gave a new dammarane-type triterpene along with 17 known compounds. This report describes the isolation and the structural elucidation of the constituents from the testas of *C. mollissima*, together with their α-glucosidase inhibitory activity.

2. Results and discussion

The 75% EtOH extract of the testas of *C. mollissima* was portioned between

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petroleum ether (PE), CH_2Cl_2, EtOAc, *n*-BuOH, and H_2O. The PE- and CH_2Cl_2-soluble fractions showed potent α-glucosidase inhibitory activity. These fractions were successively separated by several methods to give a new compound **1** along with 17 known compounds **2**–**18**.

Compound **1** was obtained as colorless needles, with $[\alpha]_D^{20} + 45.3$ ($c = 0.75$, $CHCl_3$). Its molecular formula was established as $C_{28}H_{44}O_5$ by a pseudo-molecular ion peak in HR-FAB-MS at *m/z* 461.3267 $[M+H]^+$. The absorption bands at 3361, 2918, 2850, 1710, 1463, 1216, 1087, 1048, 759 cm^{-1} in the IR spectrum suggested the presence of hydroxyl, alkyl, carboxyl, ester functions, and so on. The ^1H NMR spectrum (600 MHz, $CDCl_3$) showed the presence of six methyl groups at δ_H 0.99, 0.88, 0.86, 0.85, 0.87, 2.06 (each 3H, s), one oxygen-substituted methine group at δ_H 4.46 (1H, dd, $J = 5.7$, 11.1 Hz), and other alkyl proton signals (Table 1). The ^{13}C NMR and DEPT spectra of **1** showed the presence of an acetoxy group (δ_C 21.3, 171.1), five methyl carbons (δ_C 15.5, 16.3, 28.0, 16.5, 15.8), one carbon with oxygenated function (δ_C 80.9), two carbonyl groups (δ_C 176.5, 212.3), along with other alkyl groups including five methine, 10 methylene, and four quaternary carbons. The spectral character and signal patterns of compound **1** (Table 1) indicated that it was a 3β-OH dammarane-type triterpene derivative [4]. The side chain linked with C-17 could be determined by the HMBC experiment completely. In the HMBC spectrum, $H_{a,b}$-22 [δ_H 2.79

Table 1. The ^1H NMR (600 and 150 MHz, $CDCl_3$), ^{13}C NMR, and HMBC spectral data of compound **1**.

No.	δ_H (*J*, Hz)	DEPT	δ_C	HMBC (H → C)
1	1.05 td (5.4, 10.8), 1.67 m	CH_2	38.8	C-19, 2, 10, 9
2	1.62 overlap, 1.63 overlap	CH_2	23.7	C-3, 10, 1
3	4.46 dd (5.7, 11.1)	CH	80.9	CH_3CO, C-26, 4, 25
4		C	37.9	
5	0.83 d (1.8)	CH	55.9	C-4
6	1.46 td (2.4, 6.6), 1.52 m	CH_2	18.1	C-7, 8, 10
7	1.28 m, 1.57 dd (3.6, 7.8)	CH_2	35.5	C-8, 18, 6
8		C	40.5	
9	1.30 m	CH	50.6	C-11, 18, 10, 8
10		C	37.1	
11	1.51 m, 1.26 m	CH_2	21.2	C-12, 10, 8
12	1.61 m, 1.22 m	CH_2	25.5	C-11, 9
13	1.96 m	CH	45.3	C-20, 14, 27
14		C	50.1	
15	1.65 m, 1.15 td (1.6, 9.6)	CH_2	31.6	C-16, 17, 13, 14
16	1.71 m, 1.94 m	CH_2	26.2	C-20, 17, 14, 15
17	2.64 m	CH	53.3	C-13, 12, 22
18	0.99 s 3H	CH_3	15.5	C-14, 8, 9, 7
19	0.88 s 3H	CH_3	16.3	C-1, 10, 9
20		C	212.3	
22	2.79 overlap, 2.80 overlap	CH_2	37.4	C-20, 24
23	2.66 overlap, 2.67 overlap	CH_2	27.5	C-22, 24
24		C	176.5	
25	0.86 s 3H	CH_3	28.0	C-3, 5, 4, 26
26	0.85 s 3H	CH_3	16.5	C-3, 5, 4, 25
27	0.87 s 3H	CH_3	15.8	C-15, 14, 8, 13
$CH_3C{=}O$	2.06 s 3H	CH_3	21.3	$CH_3C{=}O$
$CH_3C{=}O$		C	171.1	

(overlap, 1H), 2.80 (overlap, 1H)] showed correlations with C-20, 24 (δ_C 212.3, 176.5), meanwhile $H_{a,b}$-23 [δ_H 2.66 (overlap, 1H), 2.67 (overlap, 1H)] showed correlations with C-22 (δ_C 37.4) and C-24. In addition, the corresponding cross-peak between H-3 at δ_H 4.46 (1H, dd, $J = 5.7$, 11.1 Hz) and the carbonyl carbon (δ_C 171.1) of the acetoxy group was also found, which suggested that C_3-OH was acylated. The relative configuration of compound **1** was confirmed by its NOESY spectrum (Figure 1). The correlations between H-3/CH_3-25, CH_3-26/CH_3-19/CH_3-18/H-13, and H-17/CH_3-27 indicated the fused form of A–D rings of **1** having the same stereochemistry as that of dammarane-type triterpene derivatives. Based on the above analysis, compound **1** was determined as 3β-acetoxy-20-oxo-21-nordammaran-23-oic acid (Figure 1).

Structures of the known compounds were identified by means of ^{1}H NMR, ^{13}C NMR, and $[\alpha]_D$ data as scoparone (**2**) [5], scopoletin (**3**) [6], *p*-hydroxybenzoic acid (**4**), protocatechuic acid (**5**), gallic acid (**6**), vanillic acid (**7**) [7], eupatilin (**8**) [8], jaceosidin (**9**) [9], 6-methoxyltricin (**10**) [10], stigmast-4-en-6β-ol-3-one (**11**) [11], pulvinatadione (**12**) [12], stigmastane-3β, 6α-diol (**13**) [13], 7-oxo-β-sitosterol (**14**) [14], cycloart-25-ene-3β,24*R*-diol (**15**) [15], 3β-hydroxy-9,19-cycloart-24-oic acid (**16**), clovane-2,9-diol (**17**) [16], and palmitoylglycerol (**18**) [17] (Figure 2).

Table 2 indicated that the plant extract tested by screening showed significant inhibitory activity, and it was found that the activity occurred in all fractions. Each fraction caused a 50% inhibition concentration (IC_{50}) value in a potent effect.

Sixteen compounds **1**–**14**, **17**, and **18** were tested for their α-glucosidase inhibitory activity. The results are shown in Table 3. Compounds **2** and **3** were coumarins and showed potent activity, while compounds **4**–**7** showed no inhibition according to their IC_{50} values. Flavonoid derivatives **8** and **9** exhibited different results, although they have the same skeleton. In addition, the inhibitory activities of compounds **15** and **16** could not be tested for their low yields. Compounds **11**–**14**, with a steroid-type skeleton, showed the most potent inhibitory activity against α-glucosidase. Compound **1**, a nordammarane derivative, showed similar significant activity to that of compounds **11**–**14**, which indicated that the presence of the multiple rings in the structures might be the important factor for their activities.

This result may be helpful to clarify the importance of exploiting its useful resource for this plant.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Perkin-Elmer 341 polarimeter at room temperature. IR spectra were recorded on KBr disks with a Bruker IFS-55 spectrometer (Fallanden, Switzerland). NMR spectra (^{1}H, ^{13}C, and 2D NMR) were

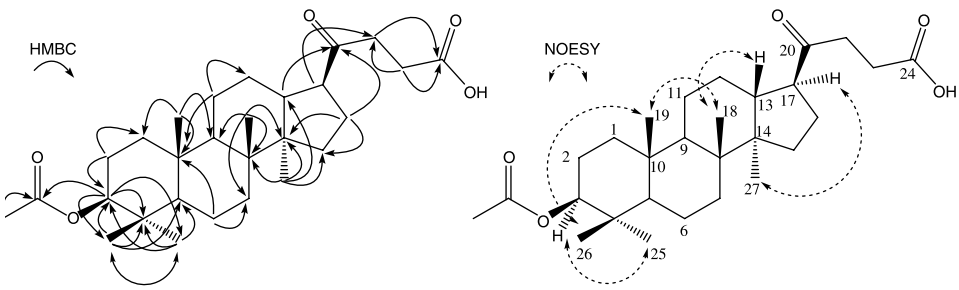

Figure 1. The structure and key HMBC and NOESY correlations of compound **1**.

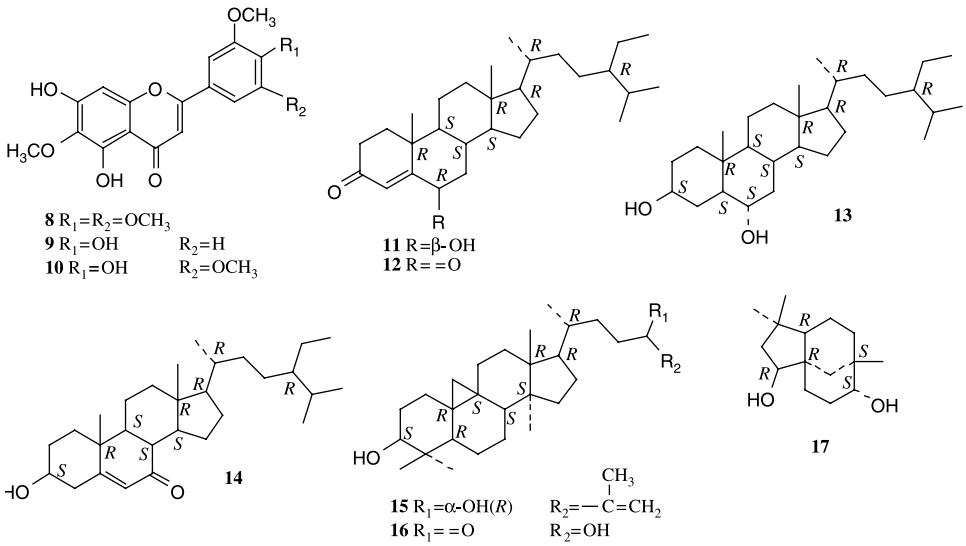

Figure 2. Structures of compounds **8**–**17**.

obtained on a Bruker Avance 600 instrument (Bruker, Rheinstetten, Germany) at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. The high-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on a Bruker micro-TOF-Q mass spectrometer. Silica gel for column chromatography (200–300 mesh) and for TLC plates (GF_{254}) was purchased from Qingdao Marine Chemical Ltd (Qingdao, China). The plates were checked under UV light (254 and 365 nm) and detected with 5% vanillin and 10% H_2SO_4 in EtOH. A molecular device spectrophotometer was used for the measurement of enzyme inhibition.

Table 2. α-Glucosidase inhibitory activities of extracts from the testas of *C. mollissima*.

Extracts	Concentration (mg/ml)	Inhibitory rate (%)	IC_{50} (mg/ml)
75% EtOH	0.9	49.4	0.952
PE	1.5	95.6	0.368
CH_2Cl_2	0.9	87.8	0.346
EtOAc	1.5	78.4	0.963
n-BuOH	0.9	58.1	0.591

3.2 Plant material

Testas of *C. mollissima* Blume were collected in September 2005 at Qianxi County in Hebei Province of China and identified by Prof. Qi Shi Sun (Shen Yang Pharmaceutical University), and the voucher specimen is deposited at the same department (No. ZB2005-027).

3.3 Extraction and isolation

Dried powder of the testas of *C. mollissima* Blume (10 kg) was extracted

Table 3. α-Glucosidase inhibitory activities of some compounds from the testas of *C. mollissima*.

Compounds	IC_{50} (μM)
1	0.01
2	0.45
3	0.91
8	0.49
11	0.13
12	0.19
13	1.05
14	0.12
17	0.74
18	0.46
Acarbose	5.83

with 75% EtOH under reflux successively for three times. The crude extract (800 g) obtained by vacuum concentration was suspended in water (1000 ml) and successively extracted with PE, CH_2Cl_2, EtOAc, and *n*-BuOH. The PE fraction (40 g) was subjected to silica gel column chromatography with a gradient of PE–acetone (100:1–1:1) to afford four fractions. Fraction 1 (1.2 g) was further performed by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to provide compound **11** (49 mg). Fraction 2 (45 mg) was further performed by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to provide compound **15** (5 mg). Fraction 3 (2.1 g) was further performed by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and recrystallized to provide compounds **10** (52 mg), **12** (35 mg), and **16** (4 mg). Fraction 4 (0.7 g) was further subjected to silica gel column chromatography (PE–CH_3COCH_3, 10:1) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to provide compounds **1** (8 mg), **14** (8 mg), and **18** (6 mg).

The CH_2Cl_2 fraction (27 g) was subjected to silica gel column chromatography with a gradient of CH_2Cl_2–MeOH (100:1–1:1) to afford three fractions. Fraction 1 (1.3 g) was further subjected to silica gel column chromatography (PE–CH_3COCH_3, 10:1) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to provide compounds **2** (10 mg) and **3** (8 mg). Fraction 2 (2.2 g) was further performed by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and recrystallized to provide compounds **8** (28 mg) and **9** (35 mg). Fraction 3 (4.1 g) was further subjected to silica gel column chromatography (CH_2Cl_2–MeOH, 12:1) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to provide compounds **7** (12 mg), **13** (15 mg), and **17** (6 mg).

The EtOAc fraction (20 g) was subjected to silica gel column chromatography with a gradient of CH_2Cl_2–MeOH (100:1–1:1) to afford two fractions. Fraction 1 (3.6 g) was further performed by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to provide compound **4** (14 mg). Fraction 2 (12.9 g) was further performed by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and recrystallized to provide compounds **5** (20 mg) and **6** (20 mg).

*3.3.1 3β-Acetoxy-20-oxo-21-nordammaran-23-oic acid (**1**)*

Colorless needles ($CDCl_3$), mp 65–67°C; $[\alpha]_D^{20}$ + 45.3 (c = 0.75, $CHCl_3$); IR (KBr, cm^{-1}): 3361, 2918, 2850, 1710, 1463, 1382, 1216, 1087, 1048, 879, 759, 667; ^1H and ^{13}C NMR spectral data, see Table 1; HR-FAB-MS: m/z 461.3267 $[M+H]^+$ (calcd for $C_{28}H_{45}O_5$, 461.3267).

3.4 Glycosidase inhibition assay

Enzymes of α-glucosidase and *p*-nitrophenylglucopyranoside (PNPG) were purchased from Sigma Chemical Company (Shanghai, China). Acarbose was purchased from Germany Bayer Chemical Ltd (Shanghai, China). The glycosidase inhibition assay was performed according to the reported methods [18] with slight modification. Thirty microliters of 0.02 U/μl α-glucosidase enzyme in 0.2 M phosphate buffer (pH 6.8) and in the presence or absence of various concentrations of test compounds in 0.2 M phosphate buffer (pH 6.8) were incubated in 96-well plates at 37°C for 10 min, and then 140 μl of 0.02 M PNPG in 0.2 M phosphate buffer (pH 6.8) was added, and the plate was incubated at 37°C for another 30 min. The reaction was quenched by the addition of 0.2 M Na_2CO_3 solution (2 ml). Acarbose was tested as a positive control. The increment in absorption at 450 nm due to the hydrolysis of PNPG by glycosidase was monitored on a microplate spectrophotometer (Bio-Rad, Philadelphia, PA, USA). The concentration of samples required to inhibit 50% of their activity under the assay conditions was defined as the IC_{50} value.

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