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α-GLUCOSIDASE INHIBITORY CONSTITUENTS FROM *Toona sinensis*

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Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood glucose levels. One of the therapeutic approaches is to retard absorption of carbohydrates after food uptake by the inhibition of carbohydrate-hydrolyzing enzymes [1]. It has been reported that α-glucosidase inhibitors decrease postprandial hyperglycemia in patients with type II diabetes and delay the development of type II diabetes in patients with impaired glucose tolerance. To date, screening of α-glucosidase inhibitors from plants and synthetic sources is increasing.

Toona sinensis (Meliaceae) is a deciduous tree distributed widely in China, whose bark, seed, flower, and leaf have been used in traditional Chinese medicine for the treatment of heliosis, vomitting, dysentery, lack of appetite, and enteritis in the folk therapy of China, due to their detoxification, antiinflammation, and antioxidant effects [2]. Previous phytochemical investigations showed that the leaves of *T. sinensis* were rich in flavonoids, alkaloids, terpenes, and anthraquinones [3, 4]. However, no study has reported on the chemical constituents of the stem of *T. sinensis*. The present paper describes the structural determination of the compounds from the stem of *T. sinensis* and their α-glucosidase inhibiting activities evaluated *in vitro*.

Plant Materials. The stems of *T. sinensis* were collected from the suburb of Shanghai in winter, and identified by associate Prof. Jun Zhao, Life and Environment Science College, Shanghai Normal University. The voucher specimen (No. 20051207) has been deposited in the Department of Botany, Shanghai Normal University.

Extraction and Isolation. Chopped, air dried stems of *T. sinensis* (3 kg) were extracted in 18 L boiling aq. 95% EtOH (24 h × 3). After evaporation of EtOH in vacuo, the aqueous residue was successively extracted with EtOAc and *n*-BuOH. The EtOAc fraction (65 g) was subjected to silica gel column chromatography using a solvent mixture of $CHCl_3$–MeOH (40:1–1:1) to yield three fractions E1-E3. Repeated column chromatography on silica gel, eluted with petroleum ether–Me_2CO (50:1–4:1), led to the separation of **1** (60 mg), **2** (120 mg), and **3** (8 mg) from fraction E1 (6.3 g). Fraction E2 (19.0 g) was subjected to LH-20 eluted with CH_3OH to give **4** (35 mg), **5** (18 mg), and subfraction E2-1. Further purification was performed by semi-preparative reversed phase HPLC using an Agilent C_{18} (5 μm, i.d. 9.4 × 250 mm) column eluted with a MeOH–H_2O solvent (35:65, v/v, for subfraction E2-1 and 20:80, v/v, for fraction E3) at a flow rate of 2 mL/min, a collective wavelength of 254 nm, and a column temperature of 25°C. Compounds **6** (37 mg) and **7** (9 mg) were isolated from 60 mg of subfraction E2-1 (14.6 g), and **8** (35 mg) and **9** (37 mg) were isolated from 100 mg of fraction E3 (17.5 g).

Acid-catalyzed degradation takes advantage of the readily cleaved interflavanoid C-C linkage in mild acid solution, which yields a flavanol (terminal unit) and a quinone methide (upper unit). The quinonoid intermediate can be captured with a nucleophile such as toluene-r-thiol. Catechin, epicatechin, and their adducts (thiobenzyl ethers) were separated and identified by LC-MS comparing the retention times with the authentic compounds and literature data [5].

Compound **8** released catechin ($[M+H]^+$ at *m/z* 291, R_t 15.8 min) and two catechin-4-thiobenzyl ethers (both $[M+H]^+$ at *m/z* 413, minor and major derivatives eluted at R_t 28.5 and 30.1 min, respectively), thus confirming the sequence as catechin→catechin in **8**. It was previously shown that procyanidin dimers with catechin as the upper unit gave two catechin thiobenzyl ethers (3,4-*trans* and 3,4-*cis*), whereas dimers with epicatechin as the upper unit only produced one epicatechin thiobenzyl derivative (3,4-*trans*) upon thiolytic cleavage of the interflavanoid linkage [6]. Compound **9** yielded epicatechin ($[M+H]^+$ at *m/z* 291, R_t 19.4 min) and two catechin-4-thiobenzyl ethers. It was thus determined to be a catechin→epicatechin dimer.

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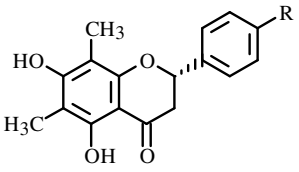
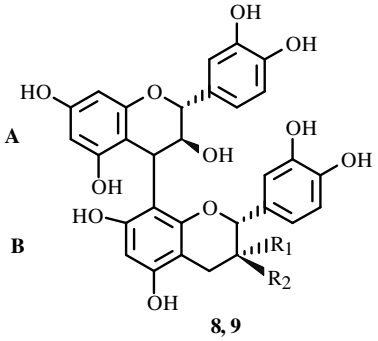
TABLE 1. NMR Data of Compounds **1**, **2** ($CDCl_3$, δ, ppm, J/Hz, TMS) and Compounds **6**, **7** (CD_3OD, δ, ppm, J/Hz, TMS)

C atom	**1** [7]		**2** [7]		**6** [10]		**7** [10]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	78.4	5.34 (dd, J = 12.6, 3.1)	78.7	5.43 (dd, J = 12.8, 3.3)	82.0	4.43 (d, J = 7.1)	78.4	4.81 br.s
3	43.2	2.80 (dd, J = 17.0, 3.1)	43.4	2.85 (dd, J = 17.1, 3.3)	69.0	3.84 m	67.5	4.07 br.s
		3.05 (dd, J = 17.0, 12.6)		3.04 (dd, J = 12.8, 17.1)				
4	196.6		196.3		28.3	2.74 (dd, J = 16.6, 8.5)	29.0	2.76 (dd, J = 16.4, 4.4)
						2.38 (dd, J = 16.6, 5.5)		2.64 (dd, J = 16.4, 3.0)
5	159.3		159.3		157.9		158.0	
6	102.9		103.0		96.6	5.86 (d, J = 1.4)	96.7	5.92 (d, J = 1.3)
7	160.8		160.8		158.3		158.2	
8	102.8		102.8		95.8	5.91 (d, J = 1.4)	96.6	5.95 (d, J = 1.3)
9	102.0		102.0		157.1		157.5	
10	157.8		157.6		101.0		100.8	
1′	131.0		138.9		132.5		132.3	
2′	127.4	7.38 (d, J = 10.4)	125.9	7.38–7.49 m	115.9	6.82 s	115.4	6.95 s
3′	114.1	6.96 (d, J = 10.4)	128.8	7.38–7.49 m	145.5		145.7	
4′	159.8		128.6	7.38–7.49 m	145.7		145.7	
5′	114.1	6.96 (d, J = 10.4)	128.8	7.38–7.49 m	116.4	6.74 (d, J = 7.8)	115.8	6.78 (d, J = 8.0)
6′	127.4	7.38 (d, J = 10.4)	125.9	7.38–7.49 m	119.5	6.70 (d, J = 7.8)	120.1	6.75 (d, J = 8.0)
6-CH_3	7.6	2.05 s	7.6	2.08 s				
8-CH_3	6.8	2.07 s	6.8	2.08 s				
4′-OCH_3	55.3	3.84 s						
5-OH		12.28 s		12.27 s				



1, 2

1: R = H
2: R = OCH_3



8, 9

8: R_1 = H, R_2 = OH
9: R_1 = OH, R_2 = H

***α*-Glucosidase Inhibitory Activity Assay.** The test compounds **1–9** were dissolved in DMSO and diluted serially with 67 mM phosphate buffer (pH 6.8). The assay was done as follows: various concentrations of compound **1–9**, 20 μL were incubated for 5 min with 0.25 mg/mL *α*-glucosidase (0.2 unit/mL) and prepared in 160 μL of 67 mM phosphate buffer (pH 6.8). After 5 minutes of incubation, 10 μL substrate (29 mM PNPG prepared in the same buffer) was added. The sample mixture was incubated at 37 for 10 min. The reaction was terminated by adding 100 μL of 1 M Na_2CO_3 solution. Enzymatic activity was quantified by measuring the *p*-nitrophenol released from PNP-glycoside at 405 nm wavelength in a 96 microplate reader (Biotech Instruments, USA). Enzyme inhibition data were expressed as IC_{50} values (concentration of inhibitor required for 50% inhibition against *α*-glucosidase). All reactions were carried out with three replications. Acarbose was used as reference compound.

Demethoxymatteucinol (**1**): $C_{17}H_{16}O_4$, yellow needle crystal, EIMS *m/z* 284 $[M]^+$, NMR data see Table 1.

Matteucinol (**2**): $C_{18}H_{18}O_5$, yellow needle crystal, EIMS *m/z* 314 $[M]^+$, NMR data see Table 1.

Scopoletin (**3**): $C_{10}H_8O_4$, EIMS *m/z* 314 $[M]^+$, the PMR and ^{13}C NMR data agreed with those published [8].

Gallic Acid (**4**): $C_7H_6O_5$, white needle crystal, EIMS *m/z* 170 $[M]^+$, PMR spectrum (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.06 (2H, s, H-2, 6); ^{13}C NMR spectrum (100 MHz, CD_3OD, δ, ppm): 167.8 (C=O), 146.4 (C-3, 5), 139.5 (C-4), 122.1 (C-1), 110.2 (C-2, 6). These data agreed with those published [2].

TABLE 2. α-Glucosidase Inhibitory Activities of Compounds from *Toona sinensis*

Compound	IC_{50}, μM[a]	Compound	IC_{50}, μM[a]
1	N.i.	**6**	190.7±2.08
2	N.i.	**7**	189.0±2.65
3	N.i.	**8**	111.0±2.65
4	24.3±2.37	**9**	89.0±2.65
5	N.i.	Acarbose (reference)	59.5±4.65

[a]IC_{50}: the concentration of the inhibitor required for the 50% inhibition against α-glucosidase, values represent means ± SD (n = 3); N.i.: no inhibition at 1 mg/mL.

Ethyl Gallate (**5**): $C_9H_{10}O_5$, white powder, EIMS *m/z* 198 $[M]^+$, PMR spectrum (400 MHz, CD_3OD, δ, ppm, J/Hz): 1.36 (3H, t, J = 7.2, CH_3), 4.29 (2H, q, J = 7.2, CH_2), 7.06 (2H, s, H-2, 6); ^{13}C NMR spectrum (100 MHz, CD_3OD, δ, ppm): 167.7 (C=O), 146.6 (C-3, 5), 139.6 (C-4), 121.6 (C-1), 108.9 (C-2, 6), 59.5 (CH_2), 14.4 (CH_3). These data agreed with those published [9].

(+)-Catechin (**6**): $C_{15}H_{14}O_6$, white powder, EIMS *m/z* 290 $[M]^+$, NMR data see Table 1.

(–)-Epicatechin (**7**): $C_{15}H_{14}O_6$, white powder, EIMS *m/z* 290 $[M]^+$, NMR data see Table 1.

Procyanidin B3 (**8**): $C_{30}H_{26}O_{12}$, white powder, ESI-MS (negative) *m/z:* 577 $[M-H]^-$, the PMR and ^{13}C NMR data corresponded with those published [11].

Procyanidin B4 (**9**): $C_{30}H_{26}O_{12}$, white powder, ESI-MS (negative) *m/z:* 577 $[M-H]^-$, the PMR and ^{13}C NMR data agreed with those published [11].

All of the isolated compounds were examined for their α-glucosidase inhibitory activities, and the results are shown in Table 2. It indicated that compounds **4** and **6–9** showed a remarkable inhibitory effect with IC_{50} of 24.3 μM, 190.7 μM, 189.0 μM, 111.0 μM, and 89.0 μM, respectively. Among them, gallic acid (**4**) was better than acarbose. On the other hand, catechin and its analogue or derivatives **6–9** showed strong α-glucosidase inhibitory activities, and they were also present in large amounts in the plant. From the health point of view, *Toona sinensis* is worthy of further development as a food supplement for the treatment of diabetes.

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