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α-GLUCOSIDASE INHIBITORY CONSTITUENTS FROM Trichosanthis Radix

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One new compound, β-*D-apiofuranosyl-(1→6)-1-ethyl*-O-β-*D-glucopyranoside (**1**), and 11 known compounds were isolated from Trichosanthis Radix. Their structures were mainly elucidated by NMR and MS spectra. All the isolated compounds were tested for their inhibitory activities against* α-*glucosidase, and compound **11** exhibited potential inhibitory activity with an* IC_{50} *value of 36.41 ± 0.54* μ*M.*

Keywords: Trichosanthis Radix, hypoglycemic activities, α-glucosidase.

Trichosanthis Radix is the dry roots of *Trichosanthes kirilowii* Maxim. or *Trichosanthes rosthornii* Harms, which is an important traditional Chinese medicine for treating diabetes and fever. Modern biological studies have demonstrated that this herb has hypoglycemic [1], anti-inflammatory [2], antiherpes virus [3], and antitumor activity [4]. Previous phytochemical studies showed that this herb was rich in trichosanthin [5], lectins [6], polysaccharide [7], triterpenes [8], and amino acid [9]. The reported hypoglycemic components of Trichosanthis Radix are trichosans A, B, C, D, and E [10] and lectin [11]. In this paper, the isolation of one new compound and 11 known compounds from the water fraction of Trichosanthis Radix and their α-glucosidase inhibitory activities are reported.

Compound **1** was isolated as a yellow liquid. It was found to possess the molecular formula $C_{13}H_{24}O_{10}$ by HR-ESI-MS (*m/z* 375.1061 $[M + Cl]^-$), which was confirmed by ^{13}C and DEPT NMR spectra. The ^{13}C NMR spectra of compound **1** showed the presence of one CH_3, four CH_2, and seven CH, as well as one quaternary carbon atom.

Based on the comparison of compound **1** with the literature, the ^1H NMR and ^{13}C NMR spectra of **1** (Table 1) were similar to those of methyl β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside [12]. The major differences between compound **1** and methyl β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside were the new signals of δ_C 67.2 (C-1), 16.4 (C-2), and the disappearance of δ_C 56.7 (OCH_3) in compound **1**. In the ^1H–^1H COSY spectrum, the correlations of δ 1.27 (3H, t, J = 7.2 Hz, H-2) with δ 3.96 (1H, m, H-1α) and δ 3.64 (1H, m, H-1β) suggested the presence of $[CH_2CH_3]$. In the HMBC spectrum, the correlations of δ_H 3.96 (1H, m, H-1α) and δ_H 3.64 (1H, m, H-1β) with δ_C 105.0 (C-1′), and the correlations of δ 3.64 (1H, m, H-1β) with δ_C 77.7 (C-5′) indicated that the ethyl was connected to C-1′. Thus, the structure of **1** was established as β-D-apiofuranosyl-(1→6)-1-ethyl-*O*-β-D-glucopyranoside.

Careful analysis of the 1D NMR and MS spectral data resulted in the identification of the other 11 known compounds as methyl-α-D-fructofuranoside (**2**) [13], 3-(β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**3**) [14], uridin (**4**) [15], uracil (**5**) [16], allantoin (**6**) [17], 2,2′-(leucyl azanediyl)-bis-(3-methylpentanoic acid) (**7**) [18], L-phenylalaninamide (**8**) [19], *N*-methyl-2-pyrrolidinone (**9**) [20], tyrosine (**10**) [21], 3,3′,5,5′-tetra-tert-butyl-2,2′-dihydroxybiphenyl (**11**) [22], and 5α,6β-dihydroxy daucosterol (**12**) [23].

Except for compounds **5** and **8**, all the other 10 compounds were isolated from this plant to our knowledge for the first time. In our investigation, it was found that compound **11** exhibited potential inhibitory activity (IC_{50} = 36.41 ± 0.54 μM) against α-glucosidase (positive control acarbose IC_{50} = 0.17 ± 0.0036 μM), and it might be developed as an α-glucosidase inhibitory candidate to control the blood sugar level of diabetics.

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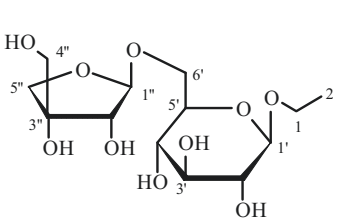
TABLE 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for **1** (CD_3OD, δ, ppm, J/Hz)

C atom	δ_H	δ_C	HMBC
1	3.96 (1H, m); 3.64 (1H, m)	67.2	C-1′, 2
2	1.27 (3H, t, J = 7.2)	16.4	C-1
1′	4.28 (1H, d, J = 7.8)	105.0	C-2′, 5′, 1
2′	3.18 (1H, dd, J = 9.0, 9.0)	75.9	
3′	3.36 (1H, dd, J = 9.0, 9.0)	78.9	
4′	3.29 (1H, dd, J = 9.0, 9.0)	72.6	
5′	3.42 (1H, m)	77.7	C-1′, 6′
6′	4.01 (1H, dd, J = 11.4, 1.8)	69.5	C-1″, 4′
	3.62 (1H, dd, J = 11.4, 1.8)		
1″	5.03 (1H, d, J = 2.4)	111.9	C-2″, 4″, 6′
2″	3.93 (1H, d, J = 2.4)	78.8	
3″	–	81.4	
4″	4.00 (1H, d, J = 9.6)	75.8	
	3.79 (1H, d, J = 9.6)		
5″	3.61 (1H, d, J = 5.4)	66.4	
	3.60 (1H, d, J = 5.4)		



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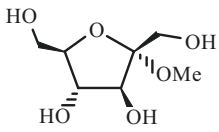

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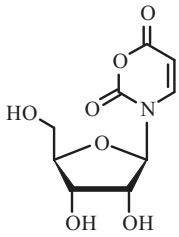

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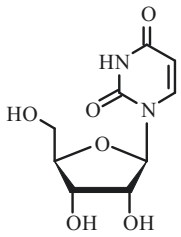

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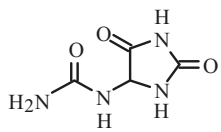

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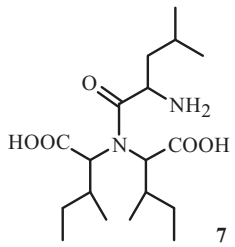

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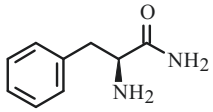

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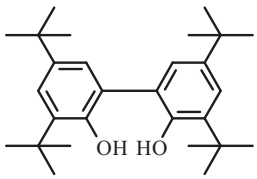

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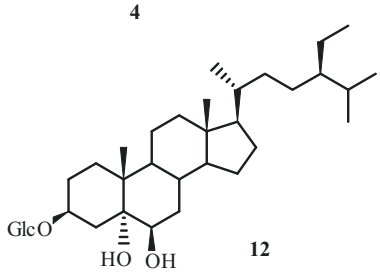

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EXPERIMENTAL

General. NMR spectra were recorded on a Bruker-Avance III-600 MHz spectrometer with TMS as internal standard. MS data were obtained on a MS Waters AutoSpec Premier P776 mass spectrometer. HR-ESI-MS data were acquired using a UPLC-IT-TOF MS spectrometer. UV data were obtained using a UV-2700 series spectrophotometer. IR data were recorded on a NiCOLET iS10 spectrophotometer. Optical rotation data were obtained using a Autopol VI spectrometer.

Plant Material. The dried roots of *Trichosanthes kirilowii* Maxim, Trichosanthis Radix, were purchased from Hele Chinese Medicine Co., Ltd., in August, 2019, and identified by Dr. Yumei Zhang from Xishuangbanna Tropical Botanical Garden, Chinese Academy of Science. A voucher specimen (No. 20190815) for Trichosanthis Radix was deposited in the Innovative Drug Research Group of Xishuangbanna Tropical Botanical Garden.

Extraction and Isolation. The air-dried and powdered roots of *T. kirilowii* (20 kg) were extracted with 90% methanol and filtered at room temperature. The filtrate was concentrated and extracted with petroleum ether, then extracted by ethyl acetate, BuOH, and H_2O. The water fraction (514.9 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (20:1–0:1) to generate six fractions (Frs. 1–6). Fraction 1 (16.1 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (30:1–0:1), Sephadex LH-20 column chromatography and eluted with $CHCl_3$–MeOH (1:1) to yield compounds **5** (28.0 mg), **4** (8.9 mg), and **12** (5.1 mg). Fraction 2 (18.8 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (30:1–0:1) and repeatedly crystallized to yield compound **8** (7.2 mg).

Fraction 3 (7.2 g) was separated by Sephadex LH-20 with MeOH–$CHCl_3$ (1:1) and a BUCHI RP-C18 medium pressure column with MeOH–H_2O (10:90) to yield compound **1** (4.0 mg). Fraction 4 (14.7 g) was subjected to silica gel column chromatography and eluted with petroleum ether–ethyl acetate (10:1–1:1) and ethyl acetate–MeOH (20:1–1:1) to yield compounds **6** (3.6 mg), **9** (2.8 mg), **3** (2.3 mg), and **11** (2.8 mg). Fraction 5 (77.1 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (15:1–1:1) and repeatedly crystallized to yield compounds **7** (31.0 mg) and **10** (61.0 mg). Fraction 6 (44.0 g) was further chromatographed over a BUCHI RP-C18 medium-pressure column with MeOH–H_2O (10:90–95:5) to yield compound **2** (4.3 mg).

***β*-D-Apiofuranosyl-(1→6)-1-ethyl-*O*-*β*-D-glucopyranoside (1)**, $C_{13}H_{24}O_{10}$, yellow liquid, $[\alpha]_D^{24.8}$ –26.1° (*c* 0.105, DMSO). UV/Vis (MeOH, λ_{max}, nm) (log ε): 196 (3.61). IR (KBr, ν, cm^{-1}): 3407, 2928, 2886, 1638, 1406, 1054, 1023, 577. For ^1H and ^{13}C NMR data, see Table 1. HR-ESI-MS *m/z* 375.1061 $[M + Cl]^-$ (calcd 375.1063).

***α*-Glucosidase Inhibitory Activities**. The sample to be tested (final concentration 20 μg/mL), enzyme solution (final concentration 0.025 U/mL), buffer solution and substrate (final concentration 1 mM) were sequentially added to the 96-well cell culture plates, fully mixed, and repeated with two wells. At the same time, the blank control and acarbose (final concentration 1 μg/mL) positive control were set up. Incubated at 37°C for 50 min, the optical density value at 405 nm was measured by an enzyme labeling instrument, and the inhibition rates of *α*-glucosidase activities were calculated.

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