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A New Dimeric Lignan from the Stems of *Willughbeia edulis*

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Abstract – As part of our continued study on the chemical constituents of *Willughbeia edulis* stems, a new dimeric lignan named edulignan (**1**) was isolated from its EtOAc-soluble extract. Based on NMR spectroscopic interpretation, the planar structure of **1** has been suggested to have two 2-substituted 4-chromanone subunits with different stereochemical configurations. In addition, the MS/MS analysis of the products obtained by acid-catalyzed hydrolysis of **1** was supportive of its structure. Unfornatually, the new compound **1** did not show α-glucosidase inhibitory activity with an IC_{50} value > 250 μM.
Keywords – *Willughbeia edulis*, Apocynaceae, Dimeric lignan, α-Glucosidase

Introduction

Willughbeia edulis, syn. *Willughbeia cochinchinensis* (Apocynaceae) is a perennial tree sparsely distributed in Vietnam, Cambodia, and Thailand. In Vietnam, *W. edulis* is locationally known as "Gui do". In Cambodia, Laos, and Vietnam, its latex is used as a sore plaster and its stems are used to treat yaws, dysentery, and liver discomfort. In Malaysia, its latex is applied to yaws and its roots are used as a treatment of jaundice, heartburn, and diarrhea.[1]

In a continued study on the screening of medicinal plants for α-glucosidase inhibitory activity,[2-10] the MeOH-soluble extract from the stems of *Willughbeia edulis* showed strong α-glucosidase inhibitory activity, with an IC_{50} value of 1.73 μg/mL. In addition, some known compounds such as epifriedelanol, taraxeryl acetate, ambolic acid, α-amyrin, lupeol acetate, 3-β-*O*-3′-hydroxylignoceryllupeol, 3β-acetoxy-30-norlupan-20-one, 3-β-*O*-behynyllupeol, scopoletin, cleomiscosin A, curcumin, desmethoxycurcumin, pinoresinol, syringaresinol, and alyterinate A were isolated from the stems of *W. edulis* by our research group.[11,12]

The objective of this research was to discover a new compound with potent α-glucosidase inhibitory activity. Thus, a further phytochemical study was carried out, leading to the isolation of a new dimeric lignan, edulignan (**1**) (Fig. 1). In this study, we have reported its isolation and structural elucidation and its α-glucosidase inhibitory activity.

Experimental

General experimental procedures – HRESIMS was performed on a Bruker micrOTOF-QII mass spectrometer (Bruker Singapore Pte., Ltd., Singapore). NMR spectra were taken on a Bruker Avance III 500 spectrometer (Brucker BioSpin AG, Thailand) with acetone-d_6 as an internal standard, and chemical shifts are expressed in δ values. The absorbance (OD) was measured with a Shimadzu UV-1800 UV-Vis spectrophotometer (Shimadzu Pte., Ltd, Singapore). Column chromatography was carried out using Silica gel 60, (40 – 63 μm) (Scharlau, Spain). Analytical and preparative TLCs were carried out on a precoated Kieselgel 60F_{254} plate (Merck KGaA, Germany). α-glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae*, *p*-nitrophenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich (Sigma-Aldrich Pte. Ltd.). Acarbose and DMSO were purchased from Merck (Merck KGaA). Other chemicals were of the highest grade available.

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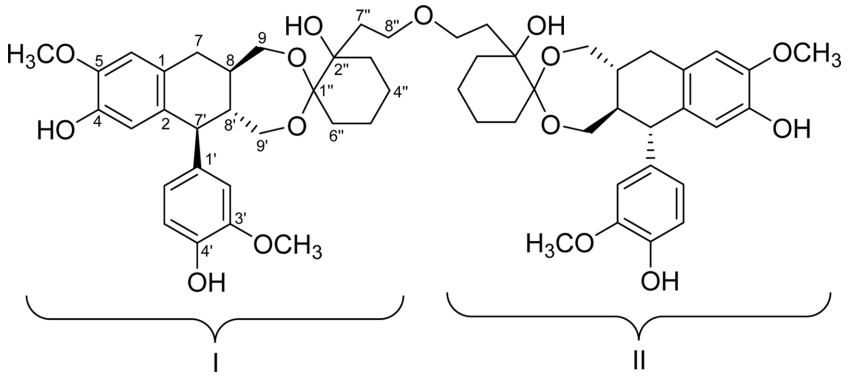

Fig. 1. The structure of compound **1**.

Plant materials – The stems of *Willughbeia edulis* were collected at Phu Quoc City, Kien Giang Province, Vietnam, in July 2013. It was identified by Dr. Ngot Pham Van, Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City, Vietnam. A voucher sample of the stems (MCE003) has been deposited at the Department of Medicinal Chemistry, Faculty of Chemistry, University of Science, Ho Chi Minh City, Vietnam.

Extraction and isolation – The dried powder stems of *Willughbeia edulis* (10 kg) were exhaustively extracted in a Soxhlet extractor with *n*-hexane, EtOAc, and MeOH in turn to yield *n*-hexane- (205 g), EtOAc- (120 g), and MeOH (760 g)-soluble fractions, respectively. The EtOAc-soluble fraction was subjected to a silica gel column and eluted with EtOAc–*n*-hexane mixtures (v/v, 0:100 → 100:0) to obtain 12 fractions: fr.1 (2.8 g), fr.2 (3.4 g), fr.3 (6.5 g), fr.4 (0.5 g), fr.5 (6.3 g), fr.6 (2.2 g), fr.7 (8.5 g), fr.8 (14.7 g), fr.9 (6.3 g), fr.10 (20.4 g), fr.11 (19.1 g), and fr.12 (29.3 g). Fraction fr.6 was chromatographed using Me_2CO–$CHCl_3$ mixtures (v/v, 0:100 → 80:20) as the eluent to afford 6 subfractions (fr.6-1, 70.2 mg; fr.6-2, 450.5 mg; fr.6-3, 524.1 mg; fr.6-4, 161.8 mg; fr.6-5, 274.6 mg; fr.6-6, 207.9 mg). Subfraction fr.6-3 was subjected to a silica gel column by elution with Me_2CO–*n*-hexane mixtures (v/v, 0:100 → 100:20) to furnish 6 sub-subfractions (fr.6-3-1, 19.1 mg; fr.6-3-2, 19.4 mg; fr.6-3-3, 230.2 mg; fr.6-3-4, 57.0 mg; fr.6-3-5, 100.5 mg; fr.6-3-6, 108.0 mg). Sub-subfraction fr.6-3-5 was passed over a silica gel column eluted with EtOAc–*n*-hexane mixtures (v/v, 0:100 → 100:0) to obtain **1** (5.7 mg).

Edulignan (1) – Yellow amorphous powder; ^{1}H (500 MHz, acetone-d_6) and ^{13}C (125 MHz, acetone-d_6) NMR: see Table 1 and Fig. S1–S5; HRESIMS: *m/z* 1023.4739 $[M + H_2O + Na]^+$ (calcd. for 1023.4718, $C_{56}H_{72}O_{16}Na$) and 523.2309 $[½ \times (M + H_2O) + Na]^+$ (calcd. for 523.2308, $C_{28}H_{36}O_8Na$) (Fig. S6).

***α*-Glucosidase inhibitory activity assay** – Thus, 3 mM *p*-nitrophenyl-*α*-D-glucopyranoside (25 μL) and 0.2 U/mL *α*-glucosidase (25 μL) in 0.01 M phosphate buffer (pH = 7.0) were added to the sample solution (625 μL) to start the reaction. Each reaction was carried out at 37 ºC for 30 min and stopped by adding 0.1 M Na_2CO_3 (375 μL). Enzymatic activity was quantified by measuring absorbance at 401 nm. The IC_{50} value was defined as the concentration of an *α*-glucosidase inhibitor that inhibited 50% of *α*-glucosidase activity. Acarbose was used as a positive control.

Acid-catalysed hydrolysis of 1 – 5.7 mg of **1** and 2 mg of iodine in 5 mL of acetone was stirred at refluxing temperature for 5 min. The solvent was then removed under vacuum, and the residue was diluted with 10 mL of dichloromethane. The mixture was washed with 5% aqueous $Na_2S_2O_3$ (10 mL) and H_2O (20 mL). The organic layer was separated and dried over Na_2SO_4. The solvent was removed to give the mixture of **1a** and **1b**, which was purified by preparative TLC to obtain **1a** (2.1 mg) and **1b** (1.0 mg).

Result and Discussion

Compound **1** was obtained as a yellow amorphous powder. The HRESIMS data gave an adduct molecular ion peak at *m/z* 1023.4739 $[M + H_2O + Na]^+$ (calcd. for 1023.4718, $C_{56}H_{72}O_{16}Na$) and a fragment ion as a base peak at *m/z* 523.2309 $[½ \times (M + H_2O) + Na]^+$ (calcd. for 523.2308, $C_{28}H_{36}O_8Na$). The ^{1}H NMR spectrum of **1** displayed signals of two 1,3,4-trisubstituted benzenes [δ_H 6.79 (1H, d, J = 8.0 Hz, H-5′$_I$), 6.74 (1H, d, J = 1.8 Hz, H-2′$_I$), 6.63 (1H, dd, J = 8.0, 1.8 Hz, H-6′$_I$), 6.78 (1H, d, J = 8.0 Hz, H-5′$_{II}$), 6.73 (1H, d, J = 1.8 Hz, H-2′$_{II}$), 6.61 (1H, dd, J = 8.0, 1.8 Hz, H-6′$_{II}$)], two 1,2,4,5-tetrasubstituted

Table 1. NMR spectroscopic data of **1** in CD_3COCD_3

N°	Subunit I		Subunit II		HMBC
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	
1	127.7		127.8		
2	133.6		133.7		
3	117.0	6.15 s	117.0	6.15 s	C-1, C-5, C-7′
4	146.2		146.2		
5	146.7		146.7		
6	112.2	6.65 s	112.2	6.65 s	C-2, C-4, C-7
7a	32.7	2.63 dd (15.6, 3.2)	32.7	2.63 dd (15.6, 3.2)	C-6, C-9, C-8′
7b		2.50 dd (15.6, 11.4)		2.50 dd (15.6, 11.4)	
8	43.3	1.40–1.82 m	43.5	1.40–1.82 m	C-7′, C-9′
9a	66.0	3.76 m	66.2	3.76 m	C-7, C-8′, C-1″
9b		3.54 dd (12.0, 2.6)		3.57 dd (12.0, 2.6)	
1′	137.8		137.9		
2′	113.4	6.73 d (1.8)	113.4	6.74 d (1.8)	C-4′
3′	148.5		148.5		
4′	145.5		145.5		
5′	115.6	6.78 d (8.0)	115.7	6.79 d (8.0)	C-1′, C-3′
6′	122.8	6.61 dd (8.0, 1.8)	122.8	6.63 dd (8.0, 1.8)	C-2′, C-4′
7′	49.2	3.44 d (10.3)	49.2	3.44 d (10.3)	C-3, C-8, C-9′
8′	50.3	1.40–1.82 m	50.4	1.40–1.82 m	C-7, C-1′
9′a	64.4	3.67 m	64.6	3.70 m	C-8, C-7′, C-1″
9′b		3.17 dd (11.7, 2.4)		3.30 dd (11.7, 2.4)	
1″	101.6		101.7		
2″	71.0		71.1		
3″	35.0	1.40–1.82 m	35.1	1.40–1.82 m	C-1″
4″	29.0	1.40–1.82 m	29.2	1.40–1.82 m	
5″	30.8	1.40–1.82 m	30.9	1.40–1.82 m	C-1″
6″	35.2	1.40–1.82 m	35.3	1.40–1.82 m	
7″	44.1	1.40–1.82 m	44.3	1.40–1.82 m	
8″	59.3	3.76 m	59.3	3.76 m	C-2″, C-8″
5-OCH_3	56.3	3.78 s	56.3	3.78 s	C-5
3′-OCH_3	56.4	3.79 s	56.4	3.79 s	C-3′
4-OH		7.12 s		7.12 s	C-3, C-5
4′-OH		7.39 s		7.40 s	C-3′, C-4′
2″-OH		3.60 s		3.61 s	C-1″, C-7″

benzenes [δ_H 6.65 (2H, s, H-$6_{I,II}$), 6.15 (2H, s, H-$3_{I,II}$)], six oxymethylene groups [δ_H 3.76 (6H, m, H-$9a_{I,II}$, H_2-$8''_{I,II}$)], 3.67 (1H, m, H-$9'a_I$), 3.54 (1H, dd, J = 12.0, 2.6 Hz, H-$9b_I$), 3.17 (1H, dd, J = 11.7, 2.4 Hz, H-$9'b_I$)], 3.70 (1H, m, H-$9'a_{II}$), 3.57 (1H, dd, J = 12.0, 2.6 Hz, H-$9b_{II}$), 3.30 (1H, dd, J = 11.7, 2.4 Hz, H-$9'b_{II}$), six methine groups [δ_H 3.44 (2H, d, J = 10.3 Hz; H-$7'_{I,II}$), 1.40–1.82 (4H, m, H-$8_{I,II}$, H-$8'_{I,II}$)], four methoxy group [δ_H 3.79 (6H, s, $3'_{I,II}$-OCH_3), 3.78 (6H, s, $5_{I,II}$-OCH_3], six hydroxy group [δ_H 7.39 (1H, s, $4'_I$-OH), 7.40 (1H, s, $4'_{II}$-OH), 7.12 (2H, s, 4_I-OH, 4_{II}-OH), 3.60 (1H, s, $2''_I$-OH), 3.61 (1H, s, $2''_{II}$-OH)], and the overlapped signals of methylene groups [δ_H 2.63 (2H, dd, J = 15.6, 3.2 Hz, H-$7a_{I,II}$), 2.50 (2H, dd, J = 15.6, 11.4 Hz, H-$7b_{I,II}$), 1.40–1.82 (20H, m, H_2-$3''_{I–II}$, H_2-$4''_{I–II}$, H_2-$5''_{I–II}$, H_2-$6''_{I–II}$, H_2-$7''_{I–II}$] (Table 1). On the other hand, the ^{13}C NMR spectrum of **1** showed resonance signals for 56 carbons including 24 aromatic carbons (δ_C 112.2–148.5), two ketal carbons [δ_C 101.6 (C-$1''_I$), 101.7 (C-$1''_{II}$)], two oxygenated tertiary carbon [δ_C 71.0 (C-$2''_I$), 71.1 (C-$2''_{II}$)], six oxymethylene carbons [δ_C 66.0 (C-9_I), 66.2, (C-9_{II}), 64.4 (C-$9'_I$), 64.6 (C-$9'_{II}$), 2 × 59.3 (C-$8''_{I,II}$)], six methine carbons [δ_C 2 × 49.2 (C-$7'_{I,II}$), 43.3 (C-8_I), 43.5 (C-8_{II}),

50.3 (C-8″$_{I}$), 50.4 (C-8″$_{II}$)], four methoxy carbons [δ_C 2 × 56.3 (5$_{I,II}$-OCH$_3$), 2 × 56.4 (3′$_{I,II}$-OCH$_3$)], and 12 methylene carbons (δ_C 29.0–44.3). These ^{1}H and ^{13}C NMR data with the observed HMBC correlations (Fig. 2) indicated **1** having the ketalization of bis(2-substituted cyclohexanone) with four hydroxy groups at C-9$_{I,II}$ and C-9′$_{I,II}$ of two identical isolariciresinols (Fig. 3). These hydroxy groups may attack carbonyl groups from the *Re* or *Si* face, leading to the different configuration of two subunits of **1**. The HMBC correlations from H$_2$-9$_{I,II}$, H$_2$-9′$_{I,II}$, and 2″$_{I,II}$-OH to the ketal carbons C-1″$_{I,II}$ were supportive of the planar structure of **1**. The (I-8″,*O*,II-8″)-ether linkage was confirmed based on the HMBC correlations from H-8″$_I$ to C-8″$_{II}$ and from H-8″$_{II}$ to C-8″$_I$.

The relative configurations of C-7, C-8, and C-8″ could not be suggested by the NOESY correlations because the signals for H-8 and H-8″ was overlapped. In addition, the configurations of non-hydrogen carbons C-1″ and C-2″ were still undetermined based on the NMR spectroscopic interpretation. The acid-catalyzed hydrolysis of **1** afforded **1a** and **1b**. The ^{1}H NMR data (Fig. S7) and the specific rotation value of **1a** suggested that its structure was (+)-isolariciresinol.[13] Due to a small amount of **1b**, the ^{1}H NMR spectrum of **1b** did not show any clear signal. Thus, the HRESIMS analysis of **1b** was carried out, which revealed the ion peaks at *m/z* 299.3856 $[M + H]^+$ and 159.1032 $[½ × (M + H_2O) + H]^+$ (Fig. S8). The MS/MS analysis of a base peak at *m/z* 159.1032 showed the prominent fragment ions at *m/z* 113.0612, 112.0545, and 101.0628, which were confirmed the presence of a hydroxyethyl group and a 2-hydroxycyclohexane structure.[14] The structure of **1b** was identified as 2,2′-(oxybis(ethane-2,1-diyl))bis(2-hydroxycyclohexan-1-one). Thus, the structure of edulignan (**1**) was suggested as shown. In this study, the relative configuration of C-1″ and C-2″ remained unresolved.

The isolated compound was tested for its α-glucosidase inhibitory activity.[15] Acarbose (IC_{50}, 215 μM) was used as the positive control. Compound **1** showed no inhibitory activity with the IC_{50} value of > 250 μM.

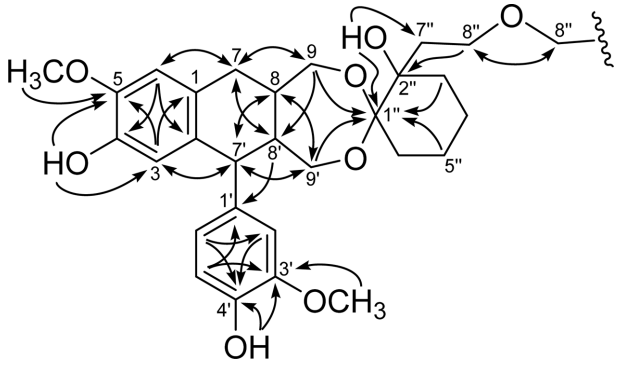

Fig. 2. HMBC correlations observed for **1**.

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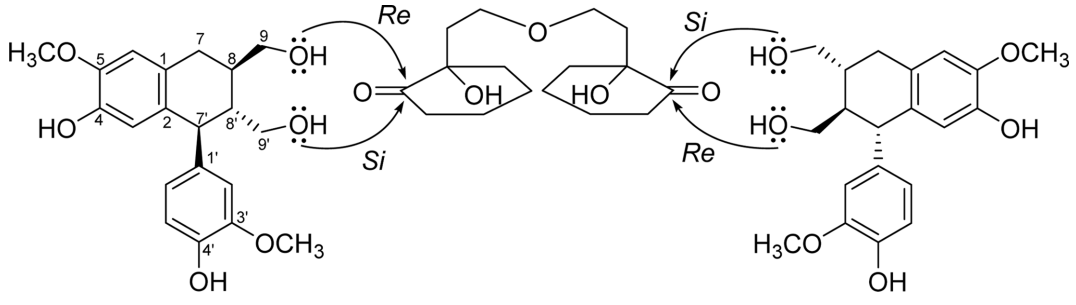

Fig. 3. Proposed formation route of **1**.

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