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α-Glucosidase inhibitors from the seeds of *Syagrus romanzoffiana*

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Abstract

Bioassay-guided fractionation against α-glucosidase resulted in isolation and characterization of eight active compounds from the EtOH extract of the seeds of *Syagrus romanzoffiana*. Of these, seven are stilbenoids, and two of them, 13-hydroxykompasinol A (**1**) and scirpusin C (**4**), possess potent inhibitory activity against α-glucosidase type IV from *Bacillus stearothermophilus* with the IC_{50} value of 6.5 and 4.9 μM, respectively. The *in vivo* assay on normal Wistar rats using oral sucrose challenge also demonstrated that kompasinol A (**2**) and 3,3′,4,5,5′-pentahydroxy-*trans*-stilbene (**5**) possess significant effect in reducing the postprandial blood glucose level (10.2% and 12.1% at 10 mg/kg, respectively). These results suggest that stilbenoids might be explored for their therapeutic potential as hypoglycemic agents.

Keywords: *Syagrus romanzoffiana*; Arecaceae; α-Glucosidase inhibitor; Stilbenoids; 13-Hydroxykompasinol A; Scirpusin C; Type 2 diabetes

1. Introduction

The worldwide estimation of diabetic patients around 2030 will be more than double from that of 2005, and most of these will be dominated by those suffering from type 2 diabetes (Gershell, 2005). This increasing trend in type 2 diabetes mellitus has become a serious medical concern worldwide that prompts every effort in exploring for new therapeutic agents to stem its progress. Although the drug treatment for type 2 diabetes mellitus has been improved to some extent during the last decade, drug resistance is still a big concern that needs to be dealt with effective approaches. Thus, the pursuit of drugs acting on an unique target, which is also devoid of the tolerance problem is always the ideal aim of researchers. α-Glucosidase inhibitors act against the enzyme in the gut that restrains liberation of glucose from oligosaccharides and thereby reduces the postprandial glucose levels and insulin responses (Casirola and Ferraris, 2006). Such inhibitors, including acarbose and voglibose, are currently used clinically in combination with either diet or other anti-diabetic agents to control blood glucose levels of patients (Van de Laar et al., 2005). To either avoid or decrease the adverse effects of current agents and also to provide more candidates of drug choices, it is still necessary to search for new α-glucosidase inhibitors for further drug development. Natural resources provide a huge and highly diversified chemical bank from which we can explore for potential therapeutic agents by bioactivity-targeted screenings. Thus, we had tried to use bioassay guided approach to find active ingredients from Formosan plants. From preliminary tests, we found that several plant extracts were active against this specific enzyme and among them the defatted EtOH extract of the seeds of *Syagrus romanzoffiana* (Cham.) Glassman (Arecaceae) exhibited potent activity against α-glucosidase type IV from *Bacillus stearothermophilus* ($IC_{50} < 10$ μg/ml). This plant, known as queen palm, is a common and familiar sight in streets, gardens and parks in either subtropical or tropical landscapes. The gum exudate of this plant had

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been found to contain a heteropolysaccharide composed of fucose, arabinose, xylose galactose, glucose and uronic acid (Simas et al., 2006). A few components such as flavonoids and steroids had been isolated from its dried leaves (Idaka et al., 1991; EI-Sakhway, 1998). This plant was also demonstrated to possess hypoglycemic effect and cytotoxic activity (EI-Sakhway, 1998). We followed the fractionation by *in vitro* bioassay against α-glucosidase to isolate active ingredients from this plant. Two of the major active compounds isolated were tested for their hypoglycemic effect on oral sucrose challenged rat model. The followings describe the outcome of these efforts.

2. Results and discussion

Bioassay guided screening indicated that the defatted EtOH extract of the seeds of *S. romanzoffiana* showed 55% inhibitory activity against α-glucosidase at a concentration of 10 μg/ml. Further fractionation indicated the active ingredients to be concentrated in the BuOH soluble fraction, having 73% inhibition at 10 μg/ml level. This fraction was further separated over Sephadex LH-20 and low pressure RP-18 columns that eventually yielded eight active compounds (**1**–**8**). Compounds **5**–**7** were characterized as the known 3,3′,4,5,5′-pentahydroxy-*trans*-stilbene, piceatannol, and resveratrol, respectively, by NMR spectroscopic comparisons (Nakajima et al., 1978). Compound **8** was identified as 4-hydroxybenzoic acid (Scott, 1972).

The ^{1}H NMR spectra of compounds **1** (Table 1) and **2** (Table S1) were very similar, both containing characteristic signals for a 2,6-disubstituted-7,8-benzo-3-oxabicyclo-[3.3.0]octane moiety, and common signals for four aryl protons, appearing as a two-proton singlet and an AX system. The presence of a 2,6-disubstituted-7,8-benzo-3-oxabicyclo[3.3.0]octane moiety was confirmed by the analysis of a COSY spectrum of **1**, displaying the following correlations: δ 4.59 (*d*, H-8) ↔ δ 3.75 (*dd*, H-7) ↔ δ 3.01 (*dq*, H-8′) ↔ δ 4.12 (*d*, H-7′), 4.45 (*t*, H-9′β) and 3.52 (*t*, H-9′α), and δ 4.45 ↔ δ 3.52. Compound **2** was identified as kompasinol A (Kulesh et al., 1995; Kobayashi et al., 1996), based on comparison of the spectroscopic data and the optically inactive property. The ^{1}H NMR spectrum of **1** (Table 1) was slightly different from that of **2** (Table S1) by replacing an ABX system (δ 6.85, *d*, $J = 1.6$ Hz; 6.76, *d*, $J = 8.0$ Hz; and 6.73, *dd*, $J = 8.0$, 1.6 Hz) in that of **2** with a two-proton singlet (δ 6.42). The molecular formula of **1**, $C_{25}H_{24}O_9$, as deduced from HR-FAB-MS, had one additional oxygen atom relative to that of **2**. Pooling these ^{1}H NMR and MS data together would allow characteriza-

Table 1
^{1}H and ^{13}C NMR, and HMBC spectroscopic data for compounds **1** and **4** (CD_3OD, 400 MHz)

Position	**1**			**4**		
	δ_H	δ_C	HMBC (H → C)	δ_H	δ_C	HMBC (H → C)
1		148.6 *s*			141.1 *s*	
2		123.0 *s*		6.19 *d* (2.1)	105.8 *d*	1, 3, 4, 6, 7
3		156.3 *s*			159.4 *s*	
4	6.18 *d* (2.0)	102.9 *d*	2, 3, 5, 6	6.09 *t* (2.1)	102.3 *d*	2, 3, 5, 6
5		160.1 *s*			159.4 *s*	
6	6.25 *d* (2.0)	103.3 *d*	2, 4	6.19 *d* (2.1)	105.8 *d*	1, 2, 4, 5, 7
7	3.75 *dd* (8.8, 4.4)	59.7 *d*	2, 9, 9′	6.57 *d* (16.2)	128.4 *d*	1, 2, 6, 9
8	4.59 *d* (4.4)	89.4 *d*	1, 7, 8′, 9, 9′, 10, 14,	6.62 *d* (16.2)	126.5 *d*	1, 9, 10, 14
9		134.6 *s*			126.6 *s*	
10	6.42 *s*	106.4 *d*	8, 11, 12, 14		121.4 *s*	
11		147.1 *s*			149.6 *s*	
12		133.7 *s*			130.4 *s*	
13		147.1 *s*			147.8 *s*	
14	6.42 *s*	106.4 *d*	8, 10, 12, 13	6.69 *s*	105.6 *d*	8, 10, 12, 13
1′		137.9 *s*			134.4 *s*	
2′	6.31 *s*	105.5 *d*	1′, 3′, 4′, 7′	6.37 *s*	105.7 *d*	1′, 3′, 4′, 7′
3′		149.1 *s*			147.0 *s*	
4′		134.7 *s*			134.1 *s*	
5′		149.1 *s*			147.0 *s*	
6′	6.31 *s*	105.5 *d*	1′, 4′, 5′, 7′	6.37 *s*	105.7 *d*	1′, 4′, 5′, 7′
7′	4.12 *d* (0.8)	52.1 *d*	1, 1′, 2, 2′, 3, 6′, 7, 8′, 9′	5.26 *d* (5.3)	95.4 *d*	10, 11, 1′, 2′, 6′, 8′, 9′
8′	3.01 *dq* (0.8, 8.8)	56.5 *d*	1, 2, 1′	4.39 *d* (5.3)	58.7 *d*	10, 11, 1′, 7′, 9′, 10′, 14′
9′	3.52 *t* (8.5) (α)	75.0 *t*	7′		147.6 *s*	
	4.45 *t* (8.5) (β)		7, 8			
10′				6.16 *d* (1.8)	107.3 *d*	8′, 11′, 12′
11′					159.8 *s*	
12′				6.17 *t*-like	102.7 *d*	10′, 11′, 12′, 13′
13′					159.8 *s*	
14′				6.16 *d* (1.8)	107.3 *d*	8′, 12′, 13′
OCH_3	3.72 *s*	56.7 *q*	3′, 5′			

tion of **1** as 13-hydroxykompasinol A, a new stilbeno-phenylpropanoid. The *cis*-oriented relative stereochemistry of the 3-oxabicyclo[3.3.0]octane moiety in **1**, being the same as that of kompasinol A (**2**) (Kobayashi et al., 1996), was determined by analysis of an NOESY spectrum, which showed the correlations of H-7 to H-8′ and H-10 (H-14); H-8′ to H-7, H-9′β, and H-2′ (H-6′); H-9′α to H-9′β and H-7′. 13-Hydroxykompasinol A (**1**) is optically inactive and has no CD absorption, and thus it is a racemate like kompasinol A. The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) of **1** were assigned unambiguously by analysis of 1D and 2D NMR spectra. For instance, the HMBC spectrum showed the shift correlations of H-8 to C-9′ and C-10 (C-14), H-8′ to C-1, C-1′ and C-2, H-7′ to C-2, C-3 and C-2′, both H-10 and H-14 to C-8 and C-12, and a six-proton singlet (3′- and 5′-OMe) to a two-carbon signal (C-3′, 5′), allowing the assignment of these carbon chemical shifts and confirming the linkage of the 3,4,5-trihydroxyphenyl group to C-8, and the 3,5-dimethoxy-4-hydroxyphenyl group to C-7′.

Compounds **3** and **4** had molecular formulae, $C_{28}H_{22}O_7$ and $C_{28}H_{22}O_{10}$, respectively, as deduced from HR-FAB-MS. Except for the different signals in the aromatic regions, the ^{1}H NMR spectra of **3** (Table S1) and **4** (Table 1) shared common resonances for an AX system in the aliphatic region (in **4**, δ 5.26, 4.39, $J = 5.3$ Hz) and an AB system for the *trans*-coupled olefinic protons (in **4**, δ 6.62, 6.57, $J = 16.2$ Hz). Combination of the MS and the ^{1}H NMR spectroscopic data would suggest a 2,3-stilbeno-*trans*-3,4-diphenylfuran skeleton for **3** and **4**. The ^{1}H NMR spectrum of **3** in the aromatic region showed signals for 12 aryl protons, composed of four coupling patterns, an AA′XX′, an ABX, a *meta*-coupled AX, and an A_2B (Table S1). Compound **3** is optically inactive and was identified as scirpusin A, based on comparison of its physical data to those reported (Nakajima et al., 1978). Compound **4** was obtained as brownish amorphous powder, $[\alpha]_D^{25} + 28.0$. The ^{1}H NMR spectrum of **4** (Table 1) showed signals for nine aryl protons, composed of two A_2B systems [δ 6.19 (2H, *d*) and 6.09 (1H, *t*); and δ 6.16 (2H, *d*) and 6.17 (1H, *t*)], a two-proton singlet (δ 6.37) and a one-proton singlet (δ 6.69). These data suggested the presence of three symmetric moieties in the structure of **4**, i.e. two 3,5-dihydroxyphenyl groups and one 3,4,5-trihydroxyphenyl group. The location of these phenolic substitutions was elucidated by analysis of both HSQC and HMBC spectra. The HSQC spectrum designated the chemical shifts of those protons attached carbons as listed in Table 1. The HMBC spectrum showed key three-bond shift correlations of H-7

Table 2
Inhibitory effect of compounds **1**–**8** against α-glucosidase (IC_{50}, μM)

Compounds	IC_{50}[a]
1	6.5
2	11.2
3	8.3
4	4.9
5	19.2
6	23.2
7	23.9
8	56.4

[a] The IC_{50} values were calculated from the dose–response curve of six concentrations of each test compound in triplicate. IC_{50} of acarbose: 40 nM.

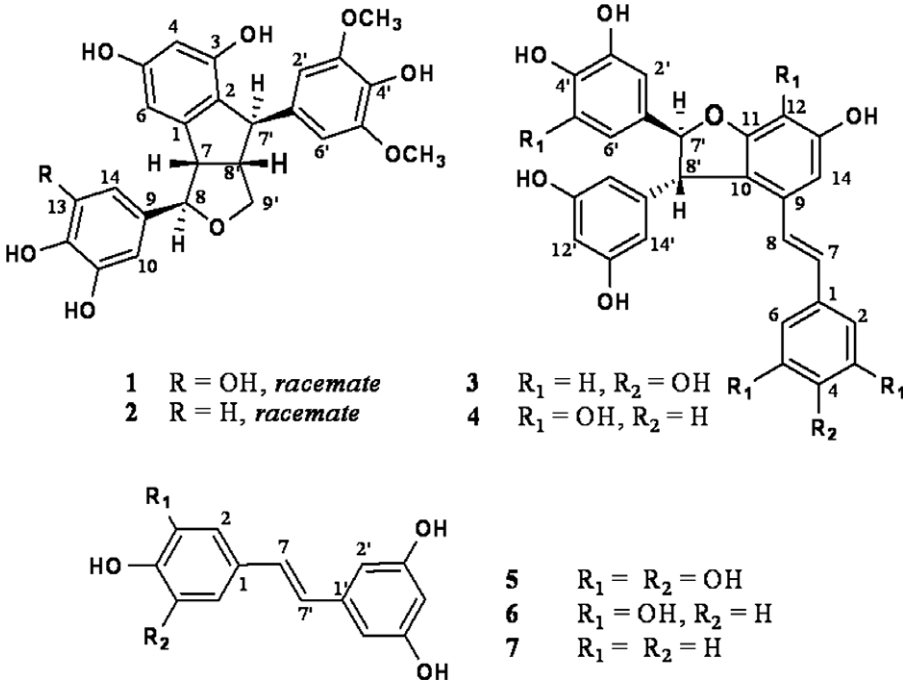

Fig. 1. Structures of stilbenoids **1**–**7** isolated from *Syagrus romanzoffiana*.

(δ 6.57, *d*) to two aryl methine carbons (C-2 and C-6, both at δ 105.8, *d*), H-8 (δ 6.62, *d*) to a nonoxygenated quaternary carbon (δ 121.4, *s*, C-10) and an aryl methine carbon (δ 105.6, *d*, C-14), H-7′ (δ 5.26, *d*) to an oxygenated quaternary aryl carbon (δ 149.6, *s*, C-11) and two aryl methine carbons (C-2′ and C-6′, both at δ 105.7, *d*), and H-8′ (δ 4.39, *d*) to two aryl methine carbons (C-10′ and C-14′. both at δ 107.3, *d*) (Table 1). Pooling these data together, thus, located the phenolic groups at C-3, C-5, C-12, C-13, C-3′, C-4′, C-5′, C-11′ and C-13′. The NOE correlations of H-7′ to H-10′ and H-14′, and H-8′ to H-2′ and H-6′, observed in an NOESY spectrum of **4**, indicated a *trans* orientation between H-7′ and H-8′. The CD data of **4** displayed two positive Cotton effects at λ 269 and 232 nm, arising from the exciton coupling of two phenyl substitutions at C-7′ and C-8′, which were similar to those reported to (+) viniferin (Lins et al., 1986; Li et al., 1996). Hence compound **4** possessed 7′*S*,8′*S*-configuration as depicted in the figure. Compound **4** is a new chemical entity and is named scirpusin C (Fig. 1).

The effect of the isolated compounds against α-glucosidase was evaluated. The results were expressed as IC_{50} values as summarized in Table 2. These data indicated that compounds **1** and **4** possessed high potency with the IC_{50} value of 6.5 and 4.9 μM, respectively. This is the first report that such fused stilbenoids possess inhibitory effect against α-glucosidase. These data also provide an insight into the structure–activity relationship among these stilbenoids. That is the anti-α-glucosidase activity increased along with the increment of phenolic substitutions in the same skeleton, such as **1** vs. **2**, **4** vs. **3**, and **5** vs. **6**. To confirm the *in vitro* activity, the abundant stilbeno-phenylpropanoid **2** and pentahydroxystilbene **5** were further assayed *in vivo* on an oral sucrose challenged normal Wistar rat using acarbose as a positive control. As shown in Fig. 2, the increase of postprandial blood glucose was monitored. After sucrose challenge, blood glucose level reached maximum at 30 min and then decreased gradually. The oral administration of **2** and **5** showed significant reduction in postprandial blood glucose level, 10.2% and 12.1%, respectively, at 30 min with a dose of 10 mg/kg. Furthermore, compound **5** exhibited this suppression effect in a dose-dependent manner, 10.2% and 26.5% at doses of 10 and 30 mg/kg, respectively. The monomeric stilbene, resveratrol (**7**), was reported recently to possess similar *in vitro* activity (Kerem et al., 2006). Piceatannol (**6**) and scirpusin B (3-hydroxyscirpusin A) have been reported to inhibit the related enzyme α-amylase and have shown *in vivo* hypoglycemic activity in a glycogen-loaded mice animal model (Kobayashi et al., 2006). The hypoglycemic effect, either *in vitro* or *in vivo*, of stilbeno-phenylpropanoids (**1**, **2**), however, is observed for the first time from this study.

3. Conclusions

This study discloses that stilbenoids, both monomeric and dimeric, are the active ingredients in *S. romanzoffiana* seeds against α-glucosidase. Two new stilbenoids, including one stilbeno-phenylpropanoid 13-hydroxykompasinol A (**1**) and one dimeric stilbenoid scirpusin C (**4**), were characterized and shown to be potent inhibitors of α-glucosidase. This work demonstrates that stilbenoids, both simple and fused types, might be explored as potential agents in controlling hyperglycemia by reducing the postprandial blood glucose level after food intake.

4. Experimental section

4.1. General

The Optical rotations were measured on a JASCO DIP-370 polarimeter, whereas IR (KBr disc) and UV (MeOH) measurements were performed on a JASCO IR Report-100 Infrared Spectrometer and a Hitachi 150-20 Double Beam Spectrophotometer, respectively, and CD on a J-720 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer (CD_3OD, δ_H 3.30 and δ_C 49.0 ppm). High resolution FAB mass spectra

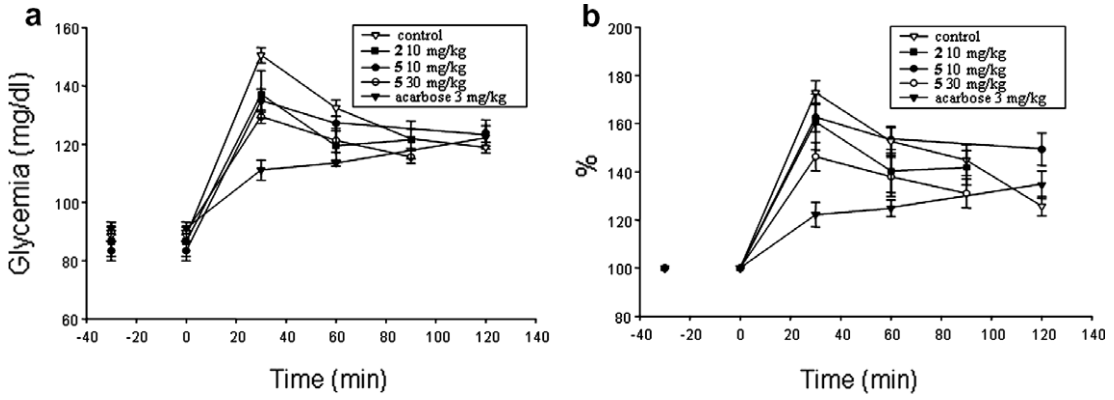

Fig. 2. Effect of **2** (■, 10 mg/kg) and **5** (●, 10 mg/kg; ○, 30 mg/kg) on blood glucose levels after single oral administration of 2 g/kg sucrose (this time point represents as 0 min) in male normal Wistar rats. (a) The *y*-axis represents as glycemia level at mg/dl. (b) *y*-Axis represents as relative % of blood glucose level. Acarbose 3 mg/kg (▼) was used as a positive control. Data are the means ± SEM for six animals.

were measured on a JEOL JMS-700 mass spectrometer. Pre-packed Lobar column (size B, 310 × 25 mm, LiChroprep RP-18, 40–63 μm, Merck) was used for separation. TLC analysis was performed on silica gel plates (KG60-F_{254}, Merck).

4.2. Chemicals and reagents

n-Butanol, *n*-hexane, $CHCl_3$ and MeOH (CAS and HPLC grade) were purchased from Mallinckrodt (KY, USA). Ethanol (95%) was supplied by Taiwan Tobacco and Liquor Corporation. CD_3OD (99.8%) was purchased from Cambridge (MA, USA) and deionized H_2O was prepared from a Barnstead water purification system (Dubuque, IA, USA).

4.3. Plant material

The fully ripe seeds of *S. romanzoffiana* (Cham.) Glassman (Arecaceae) were collected in February 2005 in Ku-Ting Riverside Park, Taipei, Taiwan. The voucher specimen (NTUSP20050201) was authenticated by Prof. Hsieh, Chang-Fu, Department of Life Sciences, National Taiwan University (NTU), and is deposited in School of Pharmacy, College of Medicine, NTU.

4.4. Extraction and isolation

The powdered seeds of *S. romanzoffiana* (2.0 kg) were extracted with 95% EtOH (4 l × 2) to give an EtOH extract (61.4 g) after evaporation under reduced pressure. The EtOH extract (61.4 g) dissolved in MeOH (0.4 l) was partitioned against *n*-hexane (0.3 l × 3) to remove lipids. The MeOH soluble fraction (30.6 g), obtained after evaporation, was partitioned between H_2O (0.3 l) and *n*-BuOH (0.3 l × 3) to give an *n*-BuOH soluble fraction (21.1 g). Part of this *n*-BuOH soluble fraction (14.1 g) was subjected to Sephadex LH-20 CC (180 g, MeOH) to yield 20 fractions. Fr. 6 (521.4 mg) was pure **8**. Fr. 14 (691.0 mg) was pure **5**. Based on the α-glucosidase inhibition assay, the active fractions, Frs. 8, 13, 16, and 17, with inhibitory activity more than 50% at 10 μg/ml, were further separated. Fr. 8 (581.0 mg) was applied to Sephadex LH-20 (100 g)CC, eluted with MeOH–H_2O (3:1), to give six subfractions. Subfr. 3 was pure **2** (83.6 mg). Subfr. 4 (119.1 mg) after a RP-18 Lobar column, eluted with 30–100% MeOH in H_2O stepwise, yielded **1** (17.3 mg) and another crop of **2** (44.2 mg). Fr. 13 (1.8 g) was subjected to further chromatography over a Sephadex LH-20 column (170 g), eluted with MeOH–H_2O (10:3), to yield 10 subfractions. Of these, subfr. 9 was another crop of **5** (230.8 mg) and subfr. 7 was pure **6** (213.9 mg). Subfr. 6 (153.9 mg) yielded compound **7** (3.8 mg) after another Sephadex LH-20 column (20 g, 85% MeOH–H_2O) separation. Lobar CC (RP-18) of fr. 16 (393.0 mg), eluted with 30–100% MeOH in H_2O stepwise, yielded compound **3** (19.0 mg). Fr. 17 (914.0 mg) was subjected to a Sephadex LH-20 column (170 g, MeOH–H_2O 1:1) to give five subfractions. Subfr. 3 after two successive RP-18 Lobar columns (40–100% MeOH–H_2O; 32% MeOH–H_2O) yielded compound **4** (51.2 mg).

4.5. 13-Hydroxykompasinol A (*1*)

4.5.1. {(3*l*3a-trans,3a*l*8a-cis,8*l*8a-trans)-5,7-Dihydroxy-3-(3,4,5-trihydroxyphenyl)-8-(4-hydroxy-3,5-dimethoxyphenyl)-3,3a,8,8a-tetrahydro-1H-2-oxa-cyclopenta[a]indene}

A pale-yellow amorphous solid; $[\alpha]_D^{25}$ 0 (MeOH, *c* 1.00); CD (MeOH, *c* 2.14 × 10^{-5} M) no maximum; IR (KBr) ν_{max} cm^{-1}: 3397, 2928, 1614, 1517, 1462, 1335, 1217, 1114, 1034, 838; UV (MeOH) λ_{max} nm (log ε): 277.5 (3.73); for ^1H and ^{13}C NMR spectra, see Table 1; HR-FAB-MS *m*/*z* 468.1418 $[M]^+$ (Calcd. for $C_{25}H_{24}O_9$: 468.1420).

4.6. Scirpusin C (*4*)

4.6.1. [(2S,3S)-6,7-Dihydroxy-3-(3,5-dihydroxyphenyl)-2-(3,4,5-trihydroxyphenyl)-4-(3,5-dihydroxystyryl)-2,3-dihydrobenzofuran]

Brownish amorphous powder; $[\alpha]_D^{25}$ + 28 (MeOH, *c* 1.00); CD (MeOH, *c* 1.93 × 10^{-5} M): $[\theta]_{269}$ +3 794, $[\theta]_{232}$ +36 335, $[\theta]_{224}$ +37 627; IR (KBr) ν_{max} cm^{-1}: 3332, 1606, 1522, 1452, 1337, 1154, 1022, 957, 840; UV (CH_3CN) λ_{max} nm (log ε): 325.0 (4.05), 284.0 (3.82), 206.0 (4.88); for ^1H and ^{13}C NMR spectra, see Table 1; HR-FAB-MS *m*/*z* 518.1213 $[M]^+$ (Calcd. for $C_{28}H_{22}O_{10}$: 518.1212).

4.7. Biological activities

4.7.1. Assay for α-glucosidase activity

α-Glucosidase activity was assayed according to the reported method with a slight modification (Pistia-Brueggeman and Hollingsworth, 2001). The enzyme solution (3 U/ml) was prepared by dissolving α-Glucosidase type IV (Sigma Co., St. Louis, USA) from *B. stearothermophilus* in 0.5 M phosphate buffer (pH 6.5), containing 0.24 M K_2HPO_4 and 0.26 M KH_2PO_4. The enzyme solution (20 μl) and test compounds/extracts (10 μl) dissolved in MeOH–H_2O (1:9, v/v) were mixed in a 96-well microtiter plate. After 15 min preincubation at 37 °C, the substrate solution [10 μl, 20 mM *p*-nitrophenyl-α-D-glucopyranoside (NPG), Sigma] in the same buffer was added and the solution was incubated for an additional 35 min at 37 °C. The increment of absorbance at 405 nm due to the hydrolysis of NPG by α-glucosidase was measured by a microtiter plate reader (μQuant, Bio-TEK). Acarbose (Bayer AG, Germany) was used as a positive control and averages of three replicates were presented. The inhibition percentage (%) was calculated by the equation: Inhibition (%) = $[1 - (A_{sample}/A_{control})] \times 100$.

4.7.2. In vivo assay for hypoglycemic effect

Wistar rats were taken from the Laboratory Animal Center at College of Medicine, National Taiwan University

(NTU). All protocols were approved by the Institutional Animal Care and Use Committee, College of Medicine, NTU. Six male 8-week-old Wistar rats were used in each group. All rats were housed in a light and temperature controlled room with food and water available *ad libitum*. Test compounds were given to each rat by oral administration at a single dose (10 mg/kg or 30 mg/kg). Acarbose (3 mg/kg) was used as a positive control. After 10 min, sucrose solution at a dose of 2 g/kg was administered to each rat (represented as 0 min at Fig. 2). Rats of the control group were administered the same volume of substrate solution without sample. About 20 μl of blood samples were collected from the tail vein at −30, 0, 30, 60, 90, 120 min relative to the time of oral sucrose challenge. The blood glucose level was measured immediately by a disposable glucose sensor (Biosystems, Barcelona, Spain). Data were expressed as the mean (mg/dl) ± SEM. The significant difference vs. control was examined with an unpaired Student's *t*-test.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2007.12.004.

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