Dimerization of piceatannol by *Momordica charantia* peroxidase and α-glucosidase inhibitory activity of the biotransformation products

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ABSTRACT

Stilbenes, especially those oligomers, have great potential to be antihyperglycemic agents. In this study, eight stilbene dimers, including five new ones, were obtained by biotransformation of piceatannol using *Momordica charantia* peroxidase (MCP) for the first time. Their structures were established on the basis of spectroscopic evidences. These piceatannol dimers displayed potential α-glucosidase inhibitory activities, and *trans* double bond, tetrahydrofuran ring, and free adjacent phenolic dihydroxyls were found to be important for their activities. Enzymatic biotransformation of stilbenes by *M. charantia* peroxidase (MCP) was showed to be a prominent way to produce oligomeric stilbenes for antihyperglycemic development.

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1. Introduction

Stilbenes continue to receive substantial interest because of their varying structures and an array of biological activities. These compounds and their derivatives are important in drug research and development due to their apparent activities such as antioxidant, anticancer, and preventive role for Alzheimer's disease.[1–4] Moreover, increasing body of reports have demonstrated that stilbenes also possess conspicuous hypoglycemic activities, for example, resveratrol analogues from traditional medicinal plants *Pterocarpus marsupium* and *Rheum undulatum*,[5,6] and stilbene glycosides from rhubarb and *Rumex bucephalophorus*.[7,8]

Recently, oligomeric stilbenes have been reported to inhibit amylase and α-glucosidase,[9,10] with even stronger activity than their corresponding monomers, making them the fascinating lead compounds for new antihyperglycemic development.[11] Oligomeric stilbenes are not amply distributed in plant kingdom.[1] This fact and the powerful catalytic ability of *Momordica charantia* peroxidase (MCP) to transform wider range of monomer into their oligomers,[12–14] prompted us to investigate the enzymatic biotransformation of stilbenes by MCP and their in vitro α-glucosidase inhibitory activities of the biotransformation products. In this study, the parental compound used is piceatannol, also known as 3-hydroxyresveratrol, that possessed various biological activities,[15–17] including α-glucosidase inhibitory activity.[11] As a result, eight stilbene dimers including five new ones were obtained.

Herein, we reported the enzymatic biotransformation process, the isolation and structural elucidation of these biotransformation products (Fig. 1), as well as their in vitro α-glucosidase inhibitory effects.

2. Results and discussion

2.1. Synthesis of piceatannol

Piceatannol (1) was synthesized via a key Wittig–Horner reaction using the procedure as shown in Scheme 1.[18] The starting material, 5-(hydroxymethyl)benzene-1,3-diol (10), was benzyl protected to obtain 11 in 85% yield, and then converted to its bromide 12 in 35% yield. The bromide 12 was converted into a phosphorus ylide 13, which reacted with 3,4-bis(benzyloxy)benzaldehyde (15), prepared from 3,4-dihydroxybenzaldehyde (14) in 92% yield, to give benzyl protected piceatannol (16) in 40% yield. Piceatannol (1) was finally obtained by successive deprotection of the benzyl groups of 16 in a yield of 60%.

2.2. Structure elucidation of biotransformation products

MCP used in this study is a kind of peroxidase that isolated from the fruits of *M. charantia*. With favorable enzyme pH stability (pH 3.8–8.0) and thermostability (20–45 °C), it has found wider applications than its counterpart peroxidases.[12–14] Catalyzed by MCP, piceatannol in this investigation was successfully transformed to eight its dimers, including five new ones, the structures of which were elucidated by analysis of spectroscopic data.

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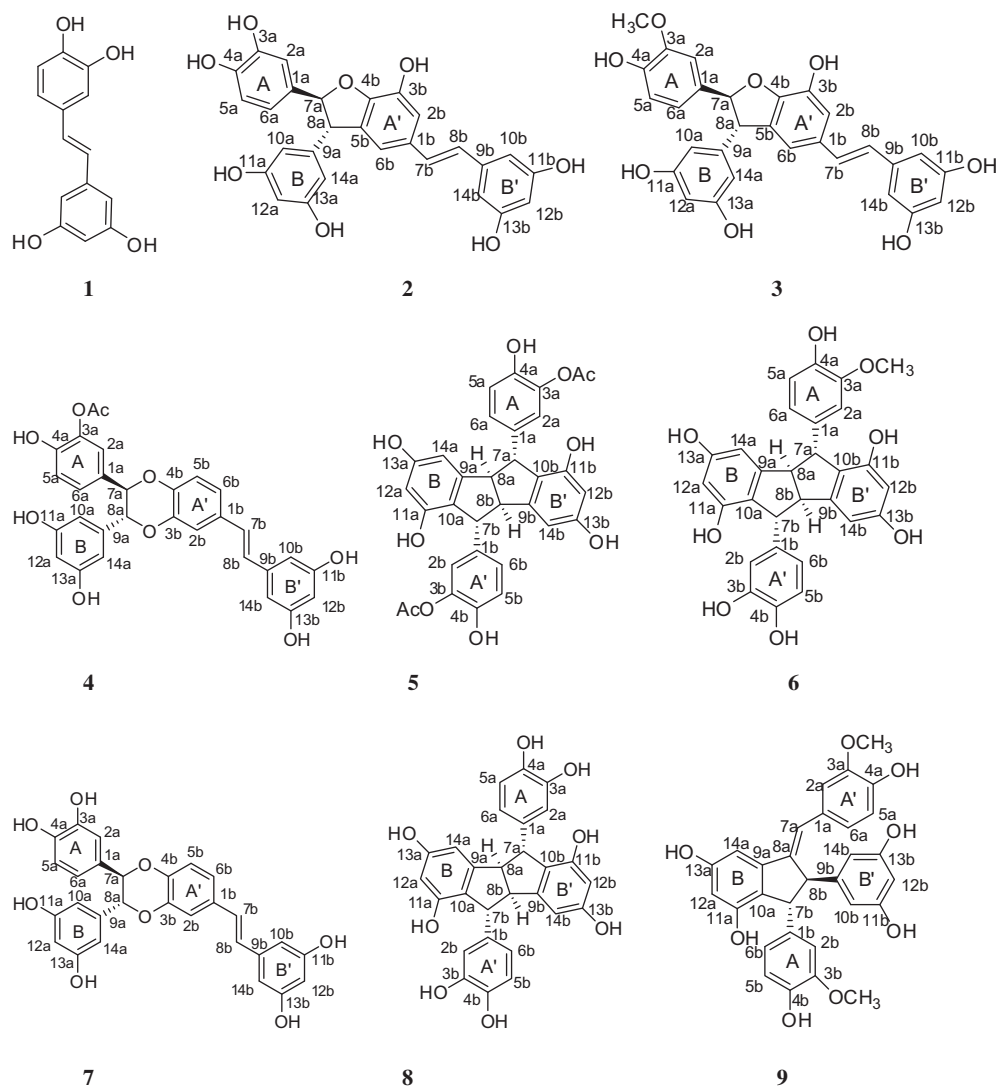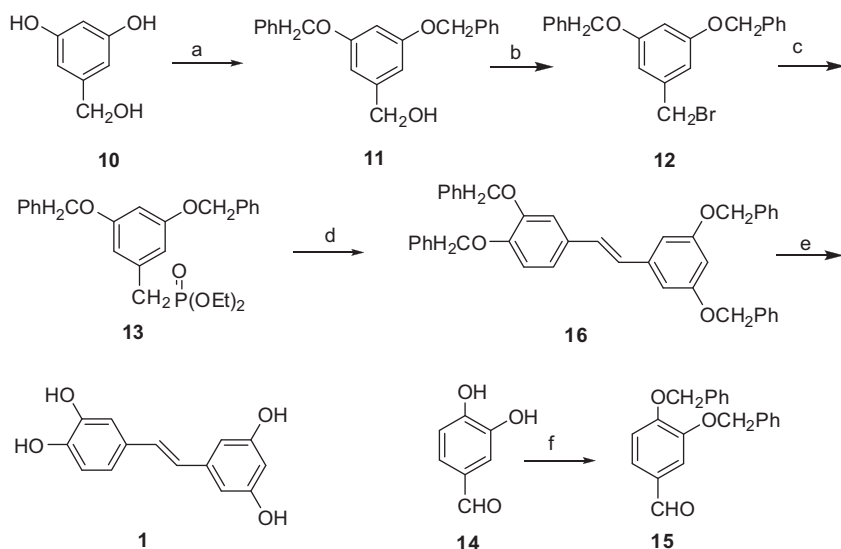E-mail address: cpu_lykong@126.com (L.-Y. Kong).

Figure 1. Chemical structures of compounds **1–9**.

Scheme 1. Synthesis of piceatannol (**1**). Reagents and conditions: (a) PhCH₂Cl, K₂CO₃, KI, CH₃COCH₃, rt; (b) PBr₃, CH₂Cl₂, 0 °C; (c) PO(OEt)₃, rt; (d) **15**, EtONa, 0 °C; (e) BBr₃, CH₂Cl₂, 0 °C; (f) PhCH₂Cl, K₂CO₃, KI, CH₃COCH₃, rt.

Compound **2** was obtained as a white powder. Its molecular formula was determined as $C_{28}H_{22}O_8$ by positive HR-ESI-MS, indicating the formation of a piceatannol dimer. A total of 28 carbon signals (Table 1) were observed as eight oxygen-bearing and five carbon-substituted aromatic quaternary carbons, eleven aromatic and two *trans* olefinic methines, and two aliphatic methines according to its ^{13}C NMR and HSQC spectra. Further inspection of its ^1H NMR data (Table 1) with the help of HMBC spectrum revealed the presence of two 1,3,5-tri-substituted aromatic fragments (B, B′) (Fig. 1), one 1,3,4-tri-substituted aromatic fragment (A), 1,3,4,5-tetra-substituted aromatic fragment (A′), one *trans* double bond, and two mutually coupled aliphatic protons.

The HMBC spectrum also enabled the connection of these partial fragments. The HMBC correlations (Fig. 2) from H-7a (δ_H 5.45, d, 7.8) to C-4b (δ_C 145.3), C-2a (δ_C 117.1) and C-6a (δ_C 121.8), from H-8a (δ_H 4.45, d, 7.8) to C-5b (δ_C 130.6) and C-10a (δ_C 106.4), suggested the existence of a benzofuran ring among rings A, A′ and B with a C-1a–C-7a–C-8a–C-9a linkage. The HMBC cross-peaks of H-7b/C-2b (6b) and H-8b/C-10b (14b) demonstrated that rings A′ and B′ were connected through the *trans* double bond at C-1b and C-9b. The *trans* orientation of H-7a and H-8a in the furan ring was inferred by the observed large coupling constants ($J = 7.8$ Hz) for them and confirmed by correlations (Fig. 2) between H-7a with H-10a and H-14a, and between H-8a with H-2a and H-6a in the NOESY spectrum. Compound **2** was thus elucidated as 4-{*trans*-3-(3,5-hydroxyphenyl)-5-[(E)-2-(3,5-dihydroxyphenyl)vivyl]-7-dihydro-2,3-dihydro-1-benzofuran-2-yl]}benzene-1,2-diol.

Compound **3** was obtained as a white powder. Its molecular formula was determined to be $C_{29}H_{24}O_8$ by the observed pseudo molecular ion at 523.1355 [M+Na]$^+$, 14 mass units more than **2**, in the positive HR-ESI-MS. Its ^1H and ^{13}C NMR data resembled

those of **2** except for the presence of an additional methoxyl group (δ_H 3.84, 3H, s; δ_C 56.2) and chemical shift discrepancy in fragment A (Table 1): the down-field shifted of H-2a signal ($\Delta\delta$ 0.23), and up-field shifted of H-5a ($\Delta\delta$ 0.51) and H-6a ($\Delta\delta$ 0.39) signals. The methoxyl group placing at C-3a was determined by its observed correlation with C-3a in the HMBC spectrum. Therefore, **3** was determined to be 2-methoxy-4-{*trans*-3-(3,5-hydroxyphenyl)-5-[(E)-2-(3,5-dihydroxyphenyl)vivyl]-7-dihydro-2,3-dihydro-1-benzofuran-2-yl]}phenol.

Compound **4** was obtained as a white powder. The molecular formula was determined to be $C_{30}H_{24}O_9$ by positive HR-ESI-MS. Its ^1H and ^{13}C NMR spectra (Table 1) revealed the existence of two 1,3,5-tri-substituted aromatic rings (B, B′), two 1,3,4-tri-substituted aromatic rings (A, A′), one *trans* double bond, one acetyl group, along with two mutually coupled aliphatic protons, which combined with HMBC cross-peaks of H-7a/C-4b and H-8a/C-3b (Fig. 3), suggesting the presence of a dioxane ring.

Analysis of its 1D and 2D data allowed the assignments of all proton and carbon signals of **4** (Table 1). The HMBC correlations from H-2a (6a) to C-7a and from H-8a to C-10a (14a) suggested that aromatic rings A and B were joined at C-1a and C-9a, respectively, through C-7a and C-8a of the dioxane ring (Fig. 3). The HMBC correlations from H-7b to C-2b (6b) and from H-8b to C-10b (14b) demonstrated that fragments A′ and B′ were connected by a *trans* double bond between C-1b and C-9b. Compared with **2**, the chemical shifts of H-2a, H-5a and H-6a were down-field shifted by $\Delta\delta$ 0.37, 0.20 and 0.28, respectively, which combined with its molecular formula, suggested that 3-OH was acetylated. The *trans* orientation of H-7a and H-8a was determined by the observed NOESY cross peaks (Fig. 3) from H-7a to H-10a, H-14a and from H-8a to H-2a, H-6a. The structure of **4** was thus elucidated

Table 1
^1H and ^{13}C NMR data for compounds **2–4**

Position	2		3		4	
	$\delta_H{}^a$	$\delta_C{}^b$	$\delta_H{}^a$	$\delta_C{}^b$	$\delta_H{}^a$	$\delta_C{}^b$
1a		129.7		130.2		130.1
2a	6.82 (d, 2.1)	117.1	7.05 (d, 1.8)	117.1	7.17 (d, 1.5)	126.3
3a		146.3		144.5		145.1
4a		145.9		144.4		144.9
5a	6.79 (d, 8.7)	117.5	6.28 (d, 8.1)	117.9	6.90 (d, 7.8)	115.7
6a	6.67 (dd, 8.7, 2.1)	121.8	6.28 (d, 8.1)	121.1	6.84 (dd, 7.8, 1.5)	123.4
7a	5.45 (d, 7.8)	94.3	5.45 (d, 7.8)	94.2	4.85 (d, 8.1)	81.3
8a	4.45 (d, 7.8)	57.6	4.54 (d, 7.8)	57.4	4.83 (d, 8.1)	81.7
9a		144.2		144.2		140.2
10a	6.06 (d, 1.8)	106.4	6.20 (d, 1.5)	106.4	6.23 (d, 1.8)	107.7
11a		158.9		158.8		159.5
12a	6.13 (d, 1.8)	101.7	6.26 (d, 1.5)	101.6	6.24 (t, 1.8)	103.8
13a		158.8		158.8		159.5
14a	6.06 (d, 1.8)	106.4	6,20 (d, 1.5)	106.3	6.23 (d, 1.8)	107.7
1b		131.1		131.0		132.4
2b	6.80 (d, 2.1)	129.1	6.80 (d, 1.8)	129.0	7.16 (d, 1.5)	115.7
3b		145.8		145.7		145.5
4b		145.3		145.2		145.1
5b		130.6		130.5	6.93 (d, 8.4)	118.2
6b	6.89 (d, 2.1)	127.9	6.90 (d, 1.8)	127.8	7.10 (d, 8.4, 1.5)	121.1
7b	6.84 (d, 16.8)	128.3	6.84 (d, 16.2)	128.2	7.02 (d, 15.6)	129.0
8b	6.95 (d, 16.8)	126.7	6.92 (d, 16.2)	126.6	6.95 (d, 15.6)	128.6
9b		139.6		139.5		140.9
10b	6.39 (d, 1.8)	104.8	6.39 (d, 1.8)	104.7	6.57 (d, 1.8)	106.1
11b		159		158.9		159.9
12b	6.12 (d, 1.8)	102.2	6.12 (t, 1.8)	102.1	6.27 (d, 1.8)	103.2
13b		159		158.9		159.9
14b	6.39 (d, 1.8)	104.8	6.39 (d, 1.8)	104.7	6.57 (d, 1.8)	106.1
OMe			3.84 s	56.2		
OAc					2.28 s	20.6
C=O						168.9

^{a} Data were measured at 500 MHz.
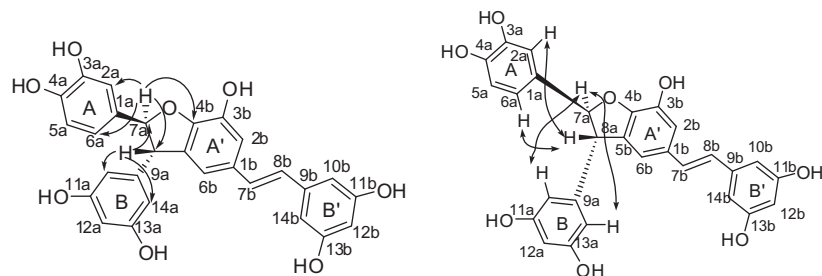^{b} Data were measured at 125 MHz.

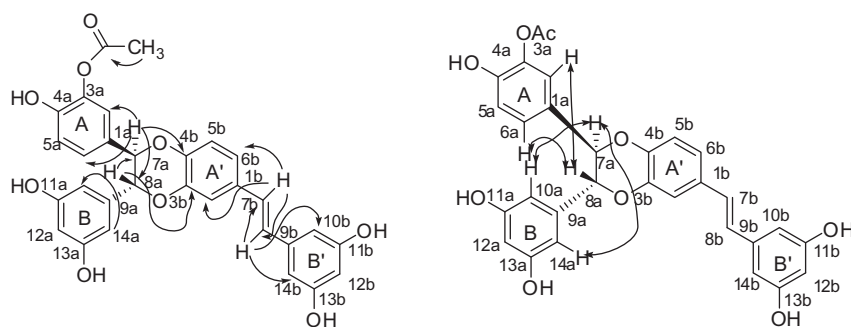**Figure 2.** Key HMBC (H→C) and key NOESY (H↔H) for compounds **2**.

Figure 3. Key HMBC (H→C) and key NOESY (H↔H) for compounds **4**.

as 5-{trans-3-(3,5-dihydroxyphenyl)-6-[(E)-2-(3,5-dihydroxyphenyl) vivyl]-2,3-dihydro-1,4-benzodioxin-2-yl}2-hydroxyphenyl aceate.

Compound **5**, a white powder, has a molecular formula of $C_{32}H_{26}O_{10}$ as determined by the positive HR-ESI-MS ion at m/z $[M+Na]^+$ (calcd 593.1424). The ^1H and ^{13}C NMR spectra (Table 2) showed the presence of only 10 protons and 16 carbons suggested the symmetric nature of **5**. In the ^1H NMR spectrum, 1,2,3,5-tetra-substituted aromatic fragment (B), 1,3,4-tri-substituted aromatic fragment (A′), two aliphatic protons, and one acetoxyl group were observed. In addition to the acetoxyl group, the ^{13}C NMR spectrum resolved 14 carbon signals including four oxygen-substituted aromatic carbons, three carbon-substituted aromatic carbons, five aromatic methine carbons, and two aliphatic carbons.

In the HMBC spectrum (Fig. 4), the correlations from H-8a to C-9a, C-14a, and C-8b (8a), and from H-7b to C-10a and C-8b (8a), suggested the existence of a benzocyclopentane fragment. The cross peaks from H-7b to C-2b and C-6b also confirmed the connection of the 1,3,4-tri-substituted aromatic fragment at C-7b. Moreover, the HMBC correlation from H-3b to the acetyl carbonyl at δ_C 168.8, combined with the downshift of H-2b, H-5b and H-6b by $\Delta\delta$ 0.37, 0.20, and 0.28, respectively, suggested the acetylation of hydroxyl at C-3b. In light of the symmetry of **5** and the methine nature of C-8a and C-8b, the fusion of the two halves of the whole structure at C-8a and C-8b was established. The adoption of trans orientations of H-7a/H-8a and H-7b/H-8b were determined by the ROESY correlations (Fig. 4) from H-8a to H-2a and H-6a, and from H-8b to H-2b and H-6b. The ROESY correlation between H-8a and H-8b demonstrated cis orientations of H-8a and H-8b. Therefore, the structure of **5** was elucidated as 5,5′-(1,3,6,8-tetra-hydroxy-4b,5,9b,10-tetrahydroindeno[2,1-a]indene-5,10-diyl)-bis(2-hydroxy-5,1-phenylene) diacetate.

Compound **6**, had the molecular formula $C_{29}H_{24}O_8$ as established by the positive HR-ESI-MS at 523.1360 $[M+Na]^+$ (calcd for $C_{29}H_{24}O_8Na$, 523.1369). The NMR signals (Table 2) indicated that

Table 2
^1H and ^{13}C NMR data for compounds **5** and **6**

Position	5		6	
	δ_H [a]	δ_C [b]	δ_H [a]	δ_C [b]
1a		128.1		138.1
2a	6.94 (d, 2.1)	124.4	6.83 (d, 2.1)	111.6
3a		136.6		147.9
4a		145.2		145.2
5a	6.70 (d, 8.1)	115.3	6.67 (d, 8.1)	115.3
6a	6.58 (d, 8.1, 2.1)	128.8	6.55 (d, 8.1, 2.1)	120.1
7a	4.47 s	54.4	4.68 s	54.4
8a	3.86 s	60.6	3.86 s	60.6
9a		150.2		150.2
10a		123.1		122.9
11a		155.2		155.1
12a	6.20 (d, 2.1)	102.4	6.15 (d, 2.1)	102.3
13a		159.2		159.1
14a	6.64 (d, 2.1)	103.3	6.57 (d, 2.1)	103.3
1b		128.1		128.8
2b	6.94 (d, 2.1)	124.4	6.57 (d, 2.1)	115.1
3b		136.6		145.5
4b		145.2		143.8
5b	6.70 (d, 8.1)	115.3	6.67 (d, 8.1)	115.8
6b	6.58 (d, 8.1, 2.1)	128.8	6.50 (d, 8.1, 2.1)	119.2
7b	4.47 s	54.4	4.57 s	54.1
8b	3.86 s	60.6	3.86 s	60.6
9b		150.2		150.2
10b		123.1		122.9
11b		155.2		155.1
12b	6.20 (d, 2.1)	102.4	6.15 (d, 2.1)	102.3
13b		159.2		159.1
14b	6.64 (d, 2.1)	103.3	6.57 (d, 2.1)	103.3
OAc	2.28 s	20.8		
OAc	2.28 s	20.8		
C=O		168.8		
OMe			3.78 br s	56.1

[a] Data were measured at 500 MHz.
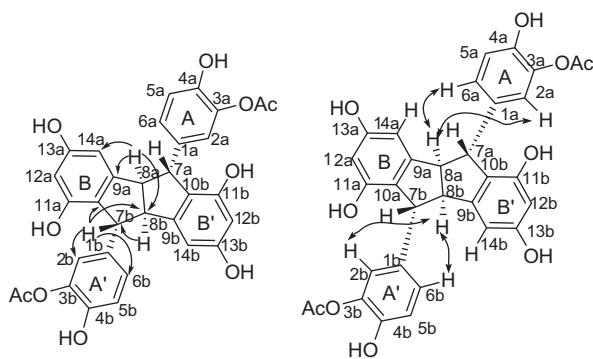[b] Data were measured at 125 MHz.

Figure 4. Key HMBC (H→C) and key NOESY (H↔H) for compounds **5**.

it was an analogue of **5**, however, with different substituents: the absence of two acetoxyl protons and, instead, the appearance of one methoxyl protons. Since HMBC correlations from methoxyl protons (δ_H 3.78, br s) to C-3a (δ_C 147.9) were observed, the methoxyl group was assigned at C-3a. The structure of **6** was further confirmed by 2D NMR experiments, and thus determined to be 5-(3,4-dihydroxyphenyl)-10-(4-hydroxy-3-methoxyphenyl)-4b,5,9b,10-tetrahydroindeno[2,1-*a*]inden-1,3,6,8-tetrol.

The known compounds maackin (**7**), cararosinol D (**8**) and gnetulin (**9**) were identified by comparison of their physical and spectroscopic data with those reported in the literatures.[19–21] These dimers were obtained for the first time by direct biotransformation from piceatannol, which would be rather difficult by common organic reactions.

Biotransformation catalyzed by MCP was presumed to be based on radical reaction (Scheme 2): induced by hydrogen peroxide, piceatannol was dehydrogenated and rearranged to form different piceatannol radicals, which, catalyzed by MCP, were combined to different dimers. Radical reactions were generally not stereo-selective. As a result, these dimers obtained should be racemes, which was consistent with the zero values of optical rotations and was further confirmed by the zero cotton effects in circular dichroism (CD) spectra of compounds **2**, **4**, and **6** (Figs. S4, S11, and S18, Supplementary data).

2.3. α-Glucosidase inhibitory effect in vitro

As shown in Table 3, all the eight dimers inhibited α-glucosidase to various degrees. The three dimers, **2**, **3** and **7**, that have a *trans* double bond showed stronger activity than the parental compound, piceatannol (**1**), which suggested the essential role of this unit for the α-glucosidase inhibitory ability. Moreover, the impressive inhibitory effects of compounds **2** and **3** with IC$_{50}$ values of 1.13 and 3.04 μM, respectively, showed the importance of tetrahydrofuran ring for this activity. Activity disparity for dimers with similar structure, **2/3**, **4/7**, and **5/6/8**, indicated the significance of free hydroxyls at C-3 and C-4.

3. Conclusion

Eight piceatannol dimers including five new ones were obtained by *M. charantia* peroxidase (MCP) catalyzed biotransformation of piceatannol. Raceme nature of these dimers was inferred by the zero values of their optical rotations and cotton effects in the CD spectra, suggesting a radical mechanism involved in the MCP catalyzed biotransformation. These piceatannol dimers displayed potential α-glucosidase inhibitory activities, and *trans* double bond, tetrahydrofuran ring, and free hydroxyls at C-3 and C-4 were found to be important for their activities. Our study favored the

enzymatic biotransformation of stilbenes by MCP as a prominent way to produce oligomeric stilbenes for antihyperglycemic development.

4. Experimental section

4.1. General

Optical rotations were carried out on a JASCO P-1020 polarimeter. CD spectra were obtained on a JASCO 810 spectropolarimeter. UV spectra were measured on a Shimadzu UV-2501 PC spectrophotometer. ^{1}H- and ^{13}C-NMR, and NOESY spectra were recorded with Bruker ACF-300 and Bruker ACF-500 (300 MHz and 125 MHz for ^{1}H- and ^{13}C-NMR, respectively) spectrometer in acetone-d_6 or DMSO-d_6, chemical shifts were reported in ppm as δ value relative to tetramethylsilane (internal standard). Mass spectra were carried out on an Agilent Micro Q-TOF mass spectrometer. Column chromatography was carried out with Sephadex LH-20 (20–100 μm, Pharmacia) and ODS-C18 (100–200 μm, Waters). Preparative HPLC was carried out using Agilent 1100 Series with Shim-park RP-C18 column (200 × 20 mm i.d.) and 1100 Series Multiple Wavelength Detector. DMSO and α-glucosidase (E.C.3.2.1.20) were purchased from Sigma. Other reagents and solvents used were of analytical grade and were purchased from reliable commercial sources.

4.2. Synthesis of piceatannol (1)

4.2.1. [3,5-Bis(benzyloxy)phenyl]methanol (11)

To a solution of 5-(hydroxymethyl)benzene-1,3-diol (**10**, 0.01 mol) in acetone (5 mL), K$_2$CO$_3$, KI, and (chloromethyl)benzene were added and stirred at rotation (rt) for 4 h. The reaction solution was filtrated and evaporated to dryness under reduced pressure, and washed with 0.5 mol/L NaOH and water, respectively. The crude product was purified by recrystallisation to afford [3,5-bis(benzyloxy)phenyl]methanol (**11**, 0.0085 mol). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.10–7.43 (10 H), 6.56 (2H, d, J = 2.1 Hz), 6.46 (1H, t), 5.05 (4H, br s), 4.53 (2H, s)]. HPLC analysis 97.7% (MeOH: H$_2$O = 80: 20 (*V/V*), t_R = 10.58 min).

4.2.2. 1,3-Bis(benzyloxy-5-(bromomethyl)benzene (12)

Phosphorus tribromide was added to the CH$_2$Cl$_2$ solution (10 mL) of [3,5-bis(benzyloxy)phenyl]methanol (**11**, 0.008 mol) at 0 °C for 2 h. Ice water was added to reaction solution, and extracted with diethyl ether. The crude product was evaporated and purified by silica-gel chromatography using petroleum/ethyl acetate 15:1 as an eluent to give bromide **12** (0.0028 mol, 35%). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 7.11–7.43 (10H), 6.65 (2H, d, J = 2.1 Hz), 6.55 (1H, s), 5.03 (4H, br s), 4.35 (2H, s). HPLC analysis 96.9% (MeOH: H$_2$O = 85: 15 (*V/V*), t_R = 8.67 min).

4.2.3. 3,4-Bis(benzyloxy)benzaldehyde (15)

To a solution of 3,4-dihydroxybenzaldehyde (**14**, 0.01 mol) in ethanol (5 mL), K$_2$CO$_3$, KI, and (chloromethyl)benzene were added and stirred at rt for 4 h. The reaction solution was filtrated and evaporated to dryness under reduced pressure, and washed with 0.5 mol/L NaOH and water. The crude product was purified by recrystallisation to afford 3,4-bis(benzyloxy)benzaldehyde (**15**).

4.2.4. 1,2-Bis(benzyloxy)-4-{(E)-2-[3,5-bis(benzyloxy)phenyl] vinyl}benzene (16)

To a mixture of bromide (**12**, 0.0026 mol), triethyl phosphite (5 mL) was added, and stirred at 160 °C for 2 h. The reaction solution was evaporated at 100 °C under reduced pressure to obtain phosphonate (**13**). A solution of phosphonate (**13**) in DMF (10 mL) was added dropwise to a suspension of NaOCH$_3$ (3 mL)
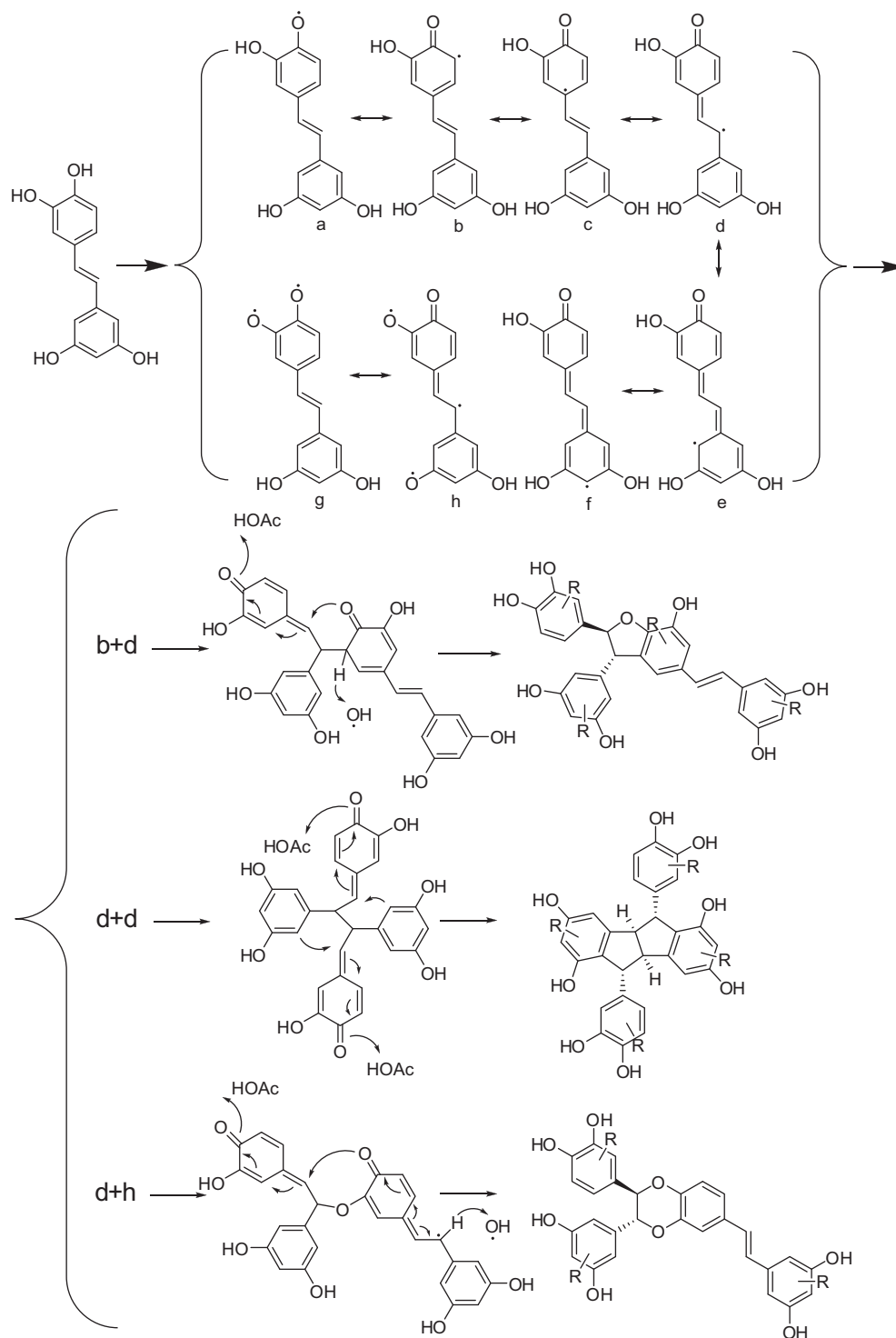
Scheme 2. Proposed mechanism of the biotransformation of piceatannol.

at 0 °C, and 3,4-bis(benzyloxy)benzaldehyde (**15**) was added into the reaction mixture, then stirred at 0 °C for 3 h. Thirty percent of methanol–water was added to the mixture and stirred. Then reaction mixture was vacuum filtrated, and the crude product was recrystallizated to afford 1,2-bis(benzyloxy)-4-{(E)-2-[3,5-bis(benzyloxy)phenyl]vinyl} benzene (**16**). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 7.11–7.57 (10H), 7.04 (2H, d, J = 15.6 Hz), 6.98 (2H, br s), 6.85 (1H, d, J = 15.6 Hz), 6.74 (2H, br s), 6.58 (2H, s), 5.07–5.19 (8H, br s). The compound purity was analyzed by HPLC to be 97.1% (MeOH: H$_2$O = 88: 12 (V/V), t_R = 13.15 min).

4.2.5. Piceatannol (1)

To a solution of compound **16** (0.0012 mol) in CH$_2$Cl$_2$ (20 mL), a mixture of BBr$_3$ and cyclohexane (5 mL, 1:1) was added, and stirred at 0 °C for 2 h. Ice water was added to the reaction solution and solution was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate, and evaporated. The crude product was purified by silica-gel chromatography using CHCl$_3$/MeOH 10:1 as an eluent to give piceatannol (**1**) (0.0098 mol, 79.0%). ESI-MS: [M–H]$^-$ 242.9; ^{1}H NMR (acetone-d_6, 300 MHz) δ: 7.42 (1H, d, J = 8.1 Hz), 7.04 (1H,

Table 3
Inhibitory effect of compounds **1–9** against yeast α-glucosidase (IC$_{50}$, μM)

Compounds	IC$_{50}$ (μM)
2	1.13
3	3.04
4	69.32
5	131.07
6	108.58
7	23.22
8	79.11
9	70.16
1 (Piceatannol)	34.32
1-Deoxynorjirimycin	20.6

br s), 6.99–6.82 (3H, m), 6.55 (2H, br s), 6.36 (1H, br s) HPLC analysis 97.6% (MeOH: H$_2$O = 55: 45 (V/V), t_R = 11.33 min).

4.3. Plant material

Fruits of *M. charantia* were collected at suburb of Nanjing, China, and identified by Professor Minjian Qin, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (No. 000804) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

4.4. Purification of *M. charantia* peroxidase (MCP)

MCP was purified from fruits of *M. charantia* to electrophoretic homogeneity by consecutive treatment of ammonium sulfate fractionation, ion exchange chromatography on DEAE-Sepharose FF, affinity chromatography on Con A Sepharose and gel filtration on Sephadex G-150 as described in our previous report.[12] The purified MCP exhibited a specific activity of 7757 E.U. of peroxidase per mg of protein, which was 46-fold higher than that of the crude extract.

4.5. Biotransformation of piceatannol in aqueous acetone

A solution of piceatannol (300 mg) in acetone (20 mL) and a solution of MCP (4 × 10^3 U) in buffer (100 mM NaOAC–HOAC, pH 7.3, 50 mL) were mixed and treated with hydrogen peroxide (0.3%, 10 mL) at 37 °C and stirred for 5 h.

4.6. Isolation and identification

The reaction mixture was evaporated to dryness at 40 °C under reduced pressure and then dissolved in water, partitioned with ethyl acetate. The ethyl acetate extract was washed with water, dried with anhydrous Na$_2$SO$_4$ and evaporated to dryness at 40 °C under reduced pressure to afford a brown powder (310 mg), which was subjected to RP-C18 column chromatography (Φ 3.0 × 50, 80 g) eluted with MeOH/H$_2$O (40:60 to 60:40, v/v) to afford fraction 1 (52 mg), fraction 2 (55 mg), and fraction 3 (90 mg). Fraction 1 was subjected to Sephadex LH-20 column chromatography (Φ 1.5 × 100, 70 g) eluted with MeOH. Subfractions of 7 to 9 (35 mg) was submitted to prep-HPLC (MeOH-0.05% TFA in H$_2$O, 35:65, v/v) to give **2** (5 mg) and **7** (13 mg). Fraction 2 was subjected to Sephadex LH-20 column chromatography (Φ 1.5 × 100, 70 g, 8 mL each) eluted with MeOH. The elution of 12–14 fractions (37 mg) was submitted to prep-HPLC (MeOH-0.05% TFA in H$_2$O, 47:53, v/v) to give **8** (10 mg) and **9** (6 mg). Fraction 3 was subjected to Sephadex LH-20 column chromatography (Φ 1.5 × 100, 70 g eluted with MeOH. The elution of 10 to 11 fractions (65 mg) was submitted to prep-HPLC (MeOH-0.05% TFA in H$_2$O, 57:43, v/v) to give **3** (6 mg), **4** (9 mg), **5** (5 mg), and **6** (5 mg).

4.6.1. 4-{*Trans*-3-(3,5-hydroxyphenyl)-5-[(*E*)-2-(3,5-dihydroxyphenyl)vivyl]-7-dihydro-2,3-dihydro-1-benzofuran-2-yl]}benzene-1,2-diol (2)

White powder, [α]$_D^{20}$ 0° (c 0.24, MeOH). HR-ESI-MS m/z 509.1220 [M+Na]$^+$ (calcd for C$_{28}$H$_{22}$O$_8$Na, 509.1212). UV λ$_{max}$ (MeOH) nm: 322, 280, 218. ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) see Table 1.

4.6.2. 2-Methoxy-4-{*trans*-3-(3,5-hydroxyphenyl)-5-[(*E*)-2-(3,5-dihydroxyphenyl)vivyl]-7-dihydro-2,3-dihydro-1-benzofuran-2-yl]}phenol (3)

White powder, [α]$_D^{20}$ 0° (c 0.24, MeOH). HR-ESI-MS m/z 523.1355 [M+Na]$^+$ (calcd for C$_{29}$H$_{24}$O$_8$Na, 523.1359). UV λ$_{max}$ (MeOH) nm: 322, 280, 218. See the ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) in Table 1.

4.6.3. 5-{*Trans*-3-(3,5-dihydroxyphenyl)-6-[(*E*)-2-(3,5-dihydroxyphenyl)vivyl]-2,3-dihydro-1,4-benzodioxin-2-yl}2-hydroxyphenyl aceate (4)

White powder, [α]$_D^{20}$ 0° (c 0.24, MeOH). HR-ESI-MS m/z 551.1315 [M+Na]$^+$ (calcd for C$_{30}$H$_{24}$O$_9$Na, 551.1318). UV λ$_{max}$ (MeOH) nm: 322, 280, 218. ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) see Table 1.

4.6.4. 5,5′-(1,3,6,8-Tetrahydroxy-4b,5,9b,10-tetrahydroindeno[2,1-a]indene-5,10-diyl)bis(2-hydroxy-5,1-phenylene) diacetate (5)

White powder, [α]$_D^{20}$ 0° (c 0.20, MeOH). HR-ESI-MS m/z 593.1414 [M+Na]$^+$ (calcd for C$_{32}$H$_{26}$O$_{10}$Na, 593.1424). UV λ$_{max}$ (MeOH) nm: 272, 254, 216. ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) see Table 2.

4.6.5. 5-(3,4-Dihydroxyphenyl)-10-(4-hydroxy-3-methoxyphenyl)-4b,5,9b,10-tetrahydroindeno[2,1-a]inden-1,3,6,8-tetrol (6)

White powder, [α]$_D^{20}$ 0° (c 0.14, MeOH). HR-ESI-MS m/z 523.1360 [M+Na]$^+$ (calcd for C$_{29}$H$_{24}$O$_8$Na, 523.1369). UV λ$_{max}$ (MeOH) nm: 254, 216. ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) see Table 2.

4.6.6. Maackin (7)

White powder, ^{1}H NMR (acetone-d_6, 300 MHz) δ: 7.16 (1H, d, J = 1.5 Hz), 7.10 (1H, dd, J = 8.4, 1.5 Hz), 7.02 (1H, d, J = 15.6 Hz), 6.95 (1H, d, J = 15.6 Hz), 6.93 (1H, d, J = 8.4 Hz), 6.80 (1H, d, J = 1.5 Hz), 6.70 (1H, d, J = 7.8 Hz), 6.57 (2H, d, J = 1.8 Hz), 6.56 (1H, dd, J = 7.8, 1.5 Hz), 6.27 (1H, d, J = 1.8 Hz), 6.24 (1H, t, J = 1.8 Hz), 6.23 (2H, d, J = 1.8 Hz), 4.85 (1H, d, J = 8.1 Hz), 4.83 (1H, d, J = 8.1 Hz). ^{13}C NMR (acetone-d_6, 125 MHz) δ: 160.0, 160.0, 159.5, 159.5, 146.6, 146.0, 145.5, 145.1, 140.9, 140.6, 132.4, 129.5, 129.0, 128.6, 121.0, 121.0, 118.2, 116.1, 116.0, 115.7, 107.7, 107.7, 106.0, 106.0, 81.7, 81.3.

4.6.7. Cararosinol D (8)

White powder, ^{1}H NMR (acetone-d_6, 300 MHz) δ: 6.67 (2H, d, J = 8.1 Hz), 6.57 (2H, d, J = 2.1 Hz), 6.57 (2H, d, J = 2.1 Hz), 6.50 (2H, dd, J = 8.1, 2.1 Hz), 6.15 (1H, d, J = 2.1 Hz), 4.46 (2H, s), 3.76 (2H, s). ^{13}C NMR (acetone-d_6, 125 MHz) δ: 159.2, 159.2, 155.3, 155.3, 150.3, 150.3, 145.6, 145.6, 143.8, 143.8, 138.8, 138.8, 123.1, 123.1, 60.6, 60.6, 54.1, 54.1.

4.6.8. Gnetulin (9)

Brown powder, ^{1}H NMR (acetone-d_6, 300 MHz) δ: 7.05 (1H, s), 6.89 (1H, d, J = 1.8 Hz), 6.83 (1H, d, J = 8.1 Hz), 6.78 (1H, d, J = 2.1 Hz), 6.74 (1H, d, J = 1.8 Hz), 6.69 (1H, d, J = 8.1 Hz), 6.64 (1H, d, J = 8.1 Hz), 6.51 (1H, dd, J = 8.1, 1.8 Hz), 6.34 (2H, d, J = 1.8 Hz), 6.31 (1H, d, J = 2.1 Hz), 6.20 (1H, t, J = 1.8 Hz), 4.27

(1H, s), 4.19 (1H, s), 3.71 (3H, s), 3.57 (3H, s). ^{13}C NMR (acetone-d_6, 125 MHz) δ: 159.9, 159.8, 159.8, 155.4, 149.1, 148.1, 148.0, 147.2, 146.6, 145.8, 142.8, 138.4, 130.2, 124.5, 123.8, 123.2, 120.0, 119.5, 117.7, 115.6, 112.2, 106.3, 106.3, 103.8, 101.5, 98.4, 60.7, 57.9, 56.1.

4.7. α-Glucosidase inhibition assay

Yeast α-glucosidase inhibition assay was performed according to the slightly modified method of Chapdelaine et al.[22] The inhibition was measured spectro-photometrically at pH 6.8 and at 37 °C for 10 min, using 0.01 M p-nitrophenyl α-D-glucopyranoside (PNPG) as a substrate and 1 U/mL of enzyme, in 0.067 M KH_2PO_4–Na_2HPO_4 buffer. Acarbose (98.5%, HPLC) was used as positive control. The increment in absorption at 410 nm due to the hydrolysis of PNPG by α-glucosidase was monitored continuously with an auto multi-functional micro plate reader (BIORAD680).

Acknowledgment

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.07.032. These data include MOL files and InChiKeys of the most important compounds described in this article.

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