JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Oligomers of Resveratrol and Ferulic Acid Prepared by Peroxidase-Catalyzed Oxidation and Their Protective Effects on Cardiac Injury

BEI-BEI YU, XIU-ZHEN HAN, AND HONG-XIANG LOU*

School of Pharmaceutical Sciences, Shandong University, Jinan 250012, People's Republic of China

Peroxidase extracted from *Momordica charantia* was used for the oligomerization of *trans*-resveratrol and ferulic acid on a preparative scale. One new heterocoupling oligomer, *trans*-3*E*-3-[(4-hydroxy-3-methoxyphenyl)methylene]-4-(3,5-dihydroxyphenyl)-5-(4-hydroxyphenyl)tetrahydro-2-franone (**6**), and six resveratrol dimers, leachianol G (**1**), restrytisol B (**2**), parthenostilbenins A (**3**) and B (**5**), 7-*O*-acetylated leachianol G (**4**), and resveratrol *trans*-dehydrodimer (**8**), and one known ferulic acid dehydrodimer, (3α,3aα,6α,6aα)tetrahydro-3,6-bis(4-hydroxy-3-methoxyphenyl)-1*H*,4*H*-furo[3,4-c]furan-1,4-dione (**7**) were obtained. Bioactive experiments showed that compounds **6**–**8** have strong free radical scavenging effects and also have protective effects on doxorubicin-induced cardiac cell injury when tested in vitro.

KEYWORDS: Biotransformation; peroxidase; resveratrol; ferulic acid; cardiac cell injury

INTRODUCTION

Plant peroxidases, which are oxidoreductases utilizing hydrogen peroxide or organic oxide to catalyze oxidative reactions, were found to play an important role in plant physiology such as defense mechanisms against pathogens (*1*), lignification, cell wall biosynthesis, auxin catabolism, and wound healing (*2*). They have also attracted great attention because of their catalytic activity in biotransformation and, more specifically, carbon–carbon bond formation of small complexes (*3*). *Momordica charantia* peroxidase (MCP), purified from the fruits of *M. charantia*, was an easily obtainable peroxidase. The diverse structures formed by the biotransformation of ferulic acid and sinapic acid with MCP had ever been investigated (*4*, *5*) Ferulic acid and sinapic acid are both widespread phenolic derivatives with antiinflammatory and antioxidative capabilities. Their oligomers, afforded by oxidative coupling biotransformation, showed even stronger activities than the parent compounds.

Resveratrol is a stilbenic phytoalexin produced by plants via a metabolic sequence induced in response to biotic or abiotic stress factors. It is one of the most popular phytochemical compounds present in grapes and many other medicinal plants (*6*). There is growing evidence that resveratrol prevents or slows the progression of a wide variety of illnesses, including mammary and skin cancers, cardiovascular disease, and ischemic injuries, as well as enhances stress resistance and extends the lifespans of organisms from yeast to vertebrates (*7*). In addition to resveratrol, its derivatives, such as stilbenoligomers and stilbenolignans, found in the families Vitaceae, Dipterocarpaceae (*8*), and Gnetaceae (*9*), are formed as the result of infection or stress and exhibit a wide range of biological functions including antimicrobial, antiinflammatory, anti-HIV, and anticancer effects (*10, 11*).

Resveratrol is also a good substrate for peroxidases. Langcake and Pryce reported the treatment of resveratrol with horseradish peroxidase to afford resveratrol *trans*-dehydrodimer (*12*). Takaya et al. also investigated the oxidative coupling of resveratrol with peroxidases from soybean, fungus, and horseradish. According to their results, resveratrol *trans*-dehydrodimer and (±)-pallidol were obtained as major products and leachianols F and G and quadrangularins B and C were obtained as minor products (*13*).

In this paper, one easy way to prepare the heterocoupling oligomer of resveratrol and ferulic acid by the easily obtained MCP was investigated. The mixture of resveratrol and ferulic acid, when subjected to peroxidase-mediated oligomerization in the presence of MCP, which was freshly obtained from the fruits of *M. charantia* (*14*), afforded one new heterocoupling product as well as six known resveratrol oligomers and one known ferulic acid dimer. The free radical scavenging abilities and protective effects on doxorubicin (Dox)-induced cardiac cell injury brought about by some of the oligomers were also investigated.

MATERIALS AND METHODS

General Experimental Procedures. The fresh fruits of *M. charantia*, identified by Dr. Xuesen Wen of Shandong University, were purchasd before use from local supermarket. *trans*-Resveratrol and ferulic acid were purchased from Yinhe Pharmaceutic Industry. NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer (^{1}H, 600 MHz, and ^{13}C, 150 MHz), and chemical shifts are reported in ppm as δ relative to Me_4Si (internal standard). Electrospray ionization mass spectroscopy (ESI-MS) was taken by a HP1100 HPLC/MS system equipped with ESI mode. α,α-Diphenyl-β-picrylhydrazyl (DPPH) and

* To whom correspondence should be addressed. Tel: +86-531-88382019. Fax: +86-531-88382019. E-mail: louhongxiang@sdu.edu.cn.

10.1021/jf0711486 CCC: $37.00
Published on Web 08/16/2007

Table 1. ^{1}H and ^{13}C NMR Data of **6** (at 600 and 150 MHz, Respectively) in Acetone-d_6[a]

no.	δ_H	δ_C
1a		125.5
2a	7.01 (d,2.0)	112.6
3a		147.1
4a		148.7
5a	6.82 (d,8.3)	114.7
6a	7.10 (dd,8.3,2.0)	126.3
7a	7.70 (d,2.0)	138.8
8a		123.0
9a		171.6
$-OCH_3$	3.63 (s)	55.0
1b		131.4
2b (6b)	7.22 (d,8.5)	126.9
3b (5b)	6.88 (d,8.5)	115.3
4b		157.4
7b	5.29 (d,2.4)	85.9
8b	4.47 (d,2.4)	52.6
9b		143.3
10b (14b)	6.34 (d,2.1)	105.1
11b (13b)		159.1
12b	6.29 (t,2.1)	101.4

[a] Chemical shifts δ in ppm and coupling constants J in Hz.

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazo-liumbromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO). Rat cardiac H9C2 (2-1) cells were obtained from American Type Culture Collection (ATCC, Rockville, MD).

Extraction of MCP from *M. charantia.* Fruits of *M. charantia* were homogenized with buffer (0.02 mol/L tris-HCl, pH 8.5) in a blender and extracted with stirring overnight. The resulting suspension was filtered and centrifuged (10000*g*, 4 °C) to remove insoluble residues. Solid ammonium sulfate was next added to the supernatant, and the 40–80% saturated ammonium sulfate precipitate was collected, dialyzed against distilled water, and equilibrated with buffer (0.02 mol/L tris-HCl, pH 8.5). After centrifugation (4 °C), the supernatant obtained containd MCP as the main constituent. The protein content was detected using Bradford method (*15*), and its peroxidative activity was determined using a guaiacol colorimetric method (*16*).

High-Performance Liquid Chromatography (HPLC)-MS Analysis of the Reaction Products of Resveratrol and Ferulic Acid. The LC/MS system was composed of an Agilent HPLC system (1100 series, United States) equipped with an online degasser and API 4000 triple-stage quadrupole mass spectrometer (Applied Biosystems, United States). Analysis was carried out employing ESI at the negative ion mode with the following settings: acquisition, 12 and 80 eV; capillary voltage, 3.5 kV; source voltage, −4.9 kV; detector voltage, 650 V; and source temperature, 600 °C. A column C_{18} (Phenomenex, 5 μm, 250 mm × 4.60 mm) was used as the stationary phase. The temperature of the column oven was maintained at 35 °C. The mobile phase consisted of phase A (acetonitrile) and phase B [water contain 0.1% (v/v) formic acid]. Samples were eluted, respectively, by the following gradient time program: 0–20 min 25% A, 25 min 40% A, 40 min 45% A, and 60 min 95% A at a flow rate of 1 mL/min. The injection volume was 10 μL, and the elute was monitored at 210 nm. The HPLC-UV chromatograms, total ion current, and the mass chromatograms were recorded.

Oxidative Coupling of Resveratrol. Crude MCP (20 mL) was dialyzed against NaAc–HAc buffer (100 mmol/L, pH 5.0). Resveratrol (1 g) dissolved in acetone (10 mL) was dripped in the dialysate. To this reaction mixture, H_2O_2 was added (in five consecutive additions) up to a final concentration of 0.3% (w/v). After electromagnetic stirring for 8 h at 30 °C, the reaction mixture was extracted by ethyl acetate and evaporated to dryness. The residue was applied to a column of Sephadex LH-20 and eluted with $CHCl_3$–CH_3OH 1/1 to provide two fractions, A and B, which were further subjected, respectively, to silica gel column chromatography eluted with petroleum ether–acetone 2/1 to afford A_1 and B_{1-5}. Fraction A_1 was subjected to semipreparative HPLC (YMC-Pack ODS-A, 250 mm × 20 mm, S-10 μm, 12 nm) eluted with CH_3OH–H_2O (6.5/3.5) to give compound **8** (40.6 mg) in 23.9 min at a flow rate of 4.0 mL/min. Fraction B_{1-5} was then applied to HPLC (Phenomenex, 5 μm, 250 mm × 4.60 mm) with 25% acetonitrile as the eluant, and five compounds, **1** (8.0 mg), **2** (7.9 mg), **3** (5.7 mg), **4** (6.7 mg), and **5** (4.6 mg), were obtained in 7.36, 13.51, 15.76, 17.54, and 19.01 min, respectively, at a flow rate of 1.0 mL/min.

Oxidative Coupling of Resveratrol and Ferulic Acid. Crude MCP (20 mL) was dialyzed against NaAc–HAc buffer (100 mmol/L, pH 5.0). Resveratrol (457.50 mg) and ferulic acid (388.36 mg) were codissolved in acetone (10 mL), and the mixture was dripped in the dialysate. To this reaction mixture, H_2O_2 was added (in five consecutive additions) up to a final concentration of 0.3% (w/v). After electromagnetic stirring for 10 h at 30 °C, the reaction mixture was extracted by ethyl acetate and evaporated to dryness. The residue was applied to silica gel column chromatography with $CHCl_3$–CH_3OH 15/1 as eluant to afford fractions C and D. Fraction C was recrystallized in methanol to give compound **7** (29.3 mg), while fraction D was applied to HPLC using a preparative column (YMC-Pack ODS-A, 250 mm × 20 mm, S-10 μm, 12 nm) eluted with 55% methanol at a flow rate of 4.0 mL/min to give compound **6** (78.3 mg) in 24.9 min.

Compound 6. Yellowish amorphous powder. ESI-MS: m/z = 421 $[M + H]^+$, 443 $[M + Na]^+$. IR υ_{max}^{KBr} (cm^{-1}) = 3436, 2929, 1749, 1608, 1579, 1514, 1435, 1351, 1245, 1184, 1054, 1027, 964, 823. For ^{1}H and ^{13}C NMR data, see **Table 1**.

Free Radical Scavenging Activity. The DPPH radical scavenging activity of compounds **6**–**8** was evaluated according to Tagashira and Ohtake (*17*) with modification. A methanol solution (0.1 mL) of the samples at various concentrations was added to 3.9 mL (0.025 g/L) of the DPPH solution. The sample tube was shaken gently and placed in the dark for 30 min at room temperature. The absorbance was then measured at 517 nm. The percentage of inhibition was calculated using the following formula: Percent inhibition = 100 × [OD (DPPH) − OD (DPPH + sample)]/OD (DPPH). All assays were carried out in triplicate, and the readings were averaged.

Rat Cardiac H9C2 Cell Culture. Rat cardiac H9C2 (2-1) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin in plastic culture flasks at 37 °C in a humidified atmosphere of 5% CO_2. The cells were fed every 2–3 days and subcultured once they reached 70–80% confluence. Cells were dislodged for both passaging and harvesting by a brief incubation in 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid.

Detection of Cell Viability with MTT Reduction Assay. Cell viability was determined by a slightly modified MTT cytotoxicity assay as described by Cao and Li (*18*). In brief, cells were plated into 96 well tissue culture plates in a range of 3000 cells/well in a final volume of 100 μL of medium and were allowed to attach overnight. First, the cells were incubated with compounds **6**–**8** of different concentrations in DMEM supplemented with 10% fetal bovine serum at 37 °C for 24 h, followed by incubation with 20 μmol/L Dox for 4 h. After that, 50 μL of MTT [2 mg/mL phosphate-buffered saline (PBS)] was added to each well. The plates were incubated at 37 °C for another 2 h. Media were removed, and wells were rinsed with PBS. To each well, 100 μL of dimethyl sulfoxide was added at room temperature to solubilize the formazan crystals, and the absorbance was measured at 570 nm.

Statistical Analysis. All data are expressed as means ± standard errors of the mean (SEM) from at least three independent experiments. Differences between two groups were analyzed by Student's t test. Statistical significance was considered at $p < 0.05$.

RESULTS

Extraction of Peroxidase from *M. charantia.* Crude MCP was obtained by combining consecutive treatment of tris-HCl buffer extraction and ammonium sulfate fractionation. The protein content was 0.86 mg/mL, and the specific activity was 3786.05 U/mg.

HPLC-MS Analysis of the Reaction Products of Resveratrol and Ferulic Acid. Three samples were eluted as follows: sample I, the oxidation products of 1/1 (mol/mol) mixture of

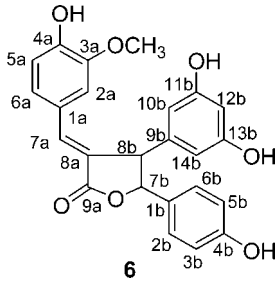

Figure 1. Chemical structures of **6**.

resveratrol and ferulic acid; sample II, the oxidation products of resveratrol; and sample III, the oxidation products of ferulic acid.

The HPLC chromatogram of sample I revealed the formation of eight major products (**Figures 1** and **2**). The retention times of peaks **1**–**5** and peak **8** were similar with those of sample II. The Q_1 scan spectra of LC elute in the negative ion mode showed molecular ion peaks at *m*/*z* 471, 471, 485, 513, 485, and 453, respectively, which suggested that peaks **1**–**5** and **8** were coupling products of resveratrol itself. In the same way, peak **7** was the product resulting from ferulic acid coupling. Compounds **1**–**5**, **7**, and **8** were isolated and identified to be leachianol G (**1**), restrytisol B (**2**), parthenostilbenins A (**3**) and B (**5**), 7-*O*-acetylated leachianol G (**4**), (3α,3aα,6α,6aα)tetrahydro-3,6-bis(4-hydroxy-3-methoxyphenyl)-1*H*,4*H*-furo[3,4-c]furan-1,4-dione (**7**), and resveratrol *trans*-dehydrodimer (**8**), respectively, by comparing their NMR data with those reported before (*14*, *19*–*21*)

On the contrary, peak **6**, which was not found in the HPLC chromatograms of both sample II and sample III, represents a heterocoupling dimer of resveratrol and ferulic acid with a molecular ion peak at *m*/*z* 419 $[M - H]^-$.

Identification of Compound 6. Compound **6** was obtained as a yellowish amorphous powder, which exhibited strong blue fluorescence under UV light at 254 nm. The positive ESI-MS showed a molecular ion peak at *m*/*z* 421 $[M + H]^+$, corresponding to the structure of a dehydrodimer of resveratrol and ferulic acid. The IR spectrum showed characteristic absorption bands for lactone moiety (1749 cm^{-1}), hydroxyl (3436 cm^{-1}, broad), and aromatic (1608 and 1514 cm^{-1}) groups.

The occurrence of the resveratrol moiety in compound **6** was determined by the presence of one set of aromatic protons coupled in an AX_2 system [δ 6.34 (d, 2, $J = 2.1$ Hz) and δ 6.29 (t, 1, $J = 2.1$ Hz)], one set of aromatic protons coupled in an AA′XX′ system [δ 7.22 (d, 2, $J = 8.5$ Hz) and δ 6.88 (d, 2, $J = 8.5$ Hz)], and two saturated carbon signals (δ 85.8 and δ 52.6) in the ^1H and ^{13}C NMR spectra, while the existence of one set of aromatic protons on a 1,3,4-trisubstituted aromatic fragment [δ 7.01 (d, 1, $J = 2.0$ Hz), δ 7.10 (dd, 1, $J = 8.3$ and 2.0 Hz), and δ 6.82 (d, 1, $J = 8.3$ Hz)], two olefin carbon signals (δ 138.8 and δ 123.0), and one lactone carbonyl signal (δ 171.6) determined the presence of ferulic acid moiety. The saturated carbon signal (δ 52.6) and the oxygenated carbon signal (δ 85.8) at relatively higher field indicated that the resveratrol moiety was connected to the ferulic acid moiety through C-7b and C-8b. By combination with the correlations of H-7a to C-8b, H-8b to C-8a, and H-7b to C-9a in the HMBC spectrum, the two moieties were determined to be linked through a γ-lactone moiety [C-8a-CO(C-9a)-O-C-7b-C-8b].

The stereochemistry of olefin carbon (C-7a) was determined to be *E*-configuration for the nuclear Overhauser effect (NOE) correlation peak between H-8b and H-2a in the NOESY spectrum. The relative configuration of H-7b/H-8b was deduced to be a trans relationship, because the NOE correlation peaks between H-8b and H-2(6)b and H-7b and H-10 (*14*)b were detected and the coupling constant of H-7b/H-8b was 2.4 Hz (*23*). No Cotton effect in the circular dichroism indicated that **6** might be a racemic mixture. According to the above analysis, the structure of **6** was elucidated as (±)-*trans*-(3)*E*-3-[(4-hydroxy-3-methoxyphenyl)methylene]-4-(3,5-dihydroxyphenyl)-5-(4-hydroxyphenyl)tetrahydro-2-furanone.

Table 2. Radical Scavenging Activities of Compounds **6**–**8**

product and standard	IC_{50} value (μmol/L)
resveratrol	27.44
ferulic acid	27.90
8	94.42
7	26.07
6	23.05

Free Radical Scavenging Activity. The free radical scavenging ability of compounds **6**–**8**, the major oxidative coupling products of resveratrol and ferulic acid, was assessed. For a more descriptive evaluation, the tests were carried out on a comparative basis with the parent molecules (resveratrol and ferulic acid), which were both well-studied radical scavengers.

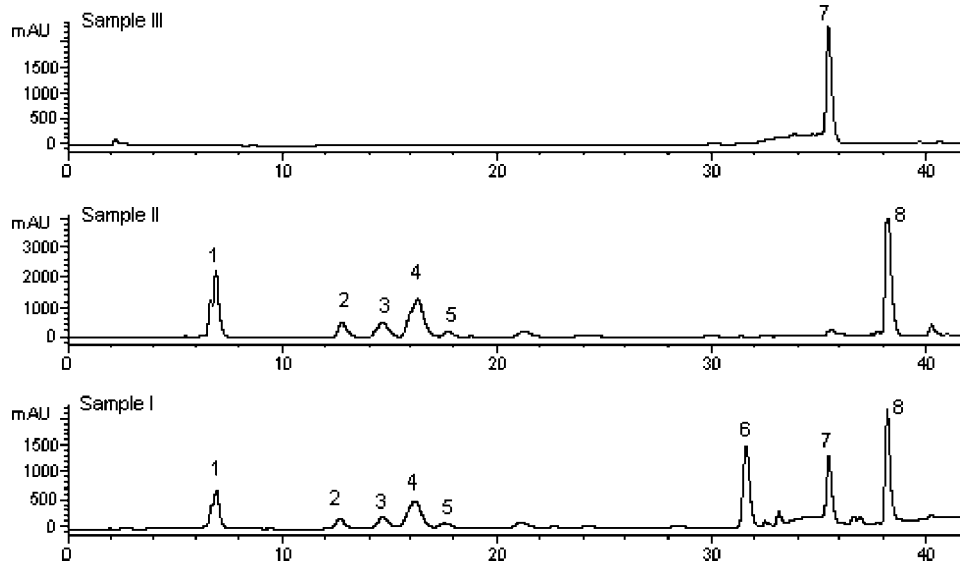

Figure 2. HPLC-UV chromatograms of coupling products of resveratrol and ferulic acid.

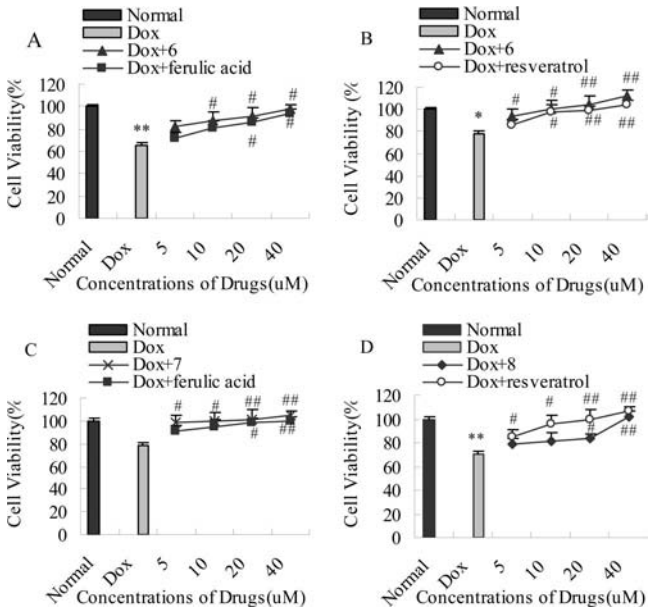

Figure 3. Effects of compounds **6**–**8** on Dox-induced cytotoxicity. (**A**) Comparison between **6** and ferulic acid, (**B**) comparison between **6** and resveratrol, (**C**) comparison between **7** and ferulic acid, and (**D**) comparison between **8** and resveratrol. Cells were incubated with different concentrations of samples for 24 h, followed by incubation with Dox for 4 h. After this incubation, cell viability was determined using the MTT reduction assay. Values represent means ± SEM from six independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$, as compared with a normal group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$, as compared with the Dox group.

Positive DPPH tests suggested that the samples were all free radical scavengers. Among the three products, **6** and **7** exhibited stronger activity, whereas **8** showed moderate activity. **Table 2** showed the 50% inhibitory concentration (IC_{50} value) of these compounds. On the basis of this test, the order of activity was **6** > **7** > resveratrol > ferulic acid > **8**.

Protective Effects of 6–8 on Dox-Induced Cytotoxicity in Cardiac H9C2 Cells. Doxorubicin is an effective anticancer agent, which can also elicit severe cardiotoxicity. We investigated if **6**, **7**, or **8** showed cytoprotection effects on Dox-induced cardiac cell injury. The tests were carried out on a comparative basis with the parent molecules (resveratrol and ferulic acid). As shown in **Figure 3**, compounds **6**–**8** significantly provided protective effects on Dox-induced cytotoxicity in a concentration-dependent manner. Compounds **6** and **7** showed higher protective effects than the parent molecules, while the protective effect of **8** was inferior to that of resveratrol.

DISCUSSION

Peroxidases from soybean, fungus, and horseradish have been used for the oxidation of stilbenes to produce oligostilbenes. The present research showed the potential of peroxidase from *M. charantia* as a tool enzyme for the synthesis of oligomers of resveratrol and ferulic acid under very mild reaction conditions. Incubation of resveratrol itself and the mixture of resveratrol and ferulic acid with crude MCP yielded two sets of products, which were isolated and identified as resveratrol or ferulic acid dimers, respectively. For the possible co-occurrence of methyltransferase or acetylase in the unpurified peroxidase mixture, the formation of some methylated or acetylated derivatives, such as parthenostilbenins A and B, and 7-*O*-acetylated leachianol G in the oxidative coupling was explainable.

To obtain heterodimer, the 1/1 (mol/mol) mixture of resveratrol and ferulic acid was incubated with crude MCP. A dehydrodimer of ferulic acid and a heterodimer of resveratrol and ferulic acid were obtained besides those resveratrol dimers. The structure of the heterodimer is based on a novel stilbenolignan skeleton having a stilbene–phenylpropionic acid unit with a γ-lactone moiety. The possible formation mechanism may be rationalized as follows: In the course of an oxidative coupling reaction, resveratrol was presumably converted into free radicals $^{\bullet}$R1 and $^{\bullet}$R2, while ferulic acid provided the radicals $^{\bullet}$F1 and $^{\bullet}$F2 (as shown in **Figure 4**). The coupling of $^{\bullet}$R2 and $^{\bullet}$F2 afforded an unstable intermediate, which generated **6** via spontaneous intramolecular cyclization. The reaction was one-electron oxidative coupling, and the mechanism was coincident with the assumption on the biogenetic pathway of oligostilbenes (*24*).

In the bioactivity tests performed in this study, the radical scavenging ability test suggested that compounds **6**–**8** were all free radical scavengers. The possible explanation for the lower ability of **8** was that the component moieties of **8** had abolished the *p*-hydroxyl group, which played an important role in scavenging radicals. The structure–activity relationship of natural phenolic compounds showed that the number and position of hydroxyl groups and the related glycosylation and other substitutions largely determined the radical scavenging activity. The differences in radical scavenging activity were attributed to structural differences in hydroxylation, glycosylation, and methoxylation (*25*). The more powerful radical scavenging ability of **6** and **7** may be concerned with more hydroxyl and methoxyl groups.

The result of the cell viability assay demonstrated that pretreatment of rat cardiac H9C2 cells with compounds **6**–**8**

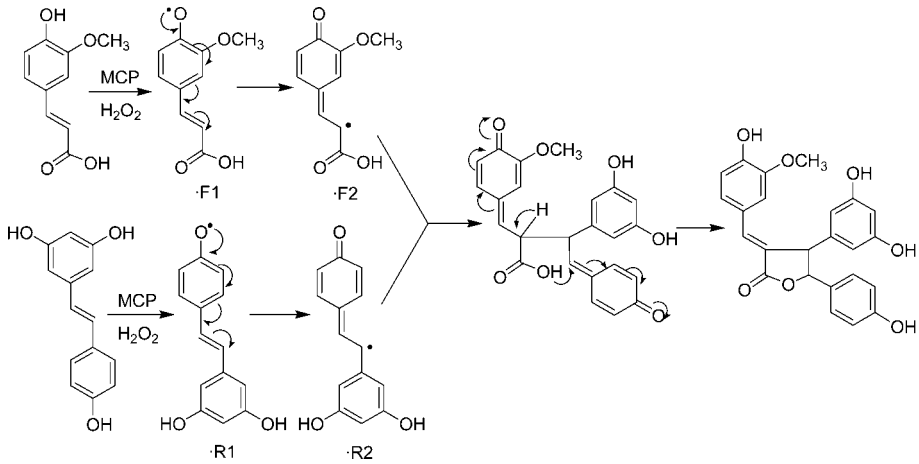

Figure 4. Proposed mechanism for the formation of compound **6**.

led to significant protective effects against Dox-induced decrease in cell viability. Also, the effects of **6** and **7** were more prominent than the monomers, while **8** showed inferior effect, which was coincident with the result of the DPPH radical scavenging activity assay. Dox is a commonly used anticancer drug. It is effective in the treatment of many forms of malignancies. However, the clinical use of Dox is associated with the development of life-threatening cardiomyopathy (*26*). Cardiac mitochondria are implicated as primary targets for Dox toxicity, which is believed to be mediated by the generation of highly reactive free radical species of oxygen from complex I of the mitochondrial electron transport chain (*27*). Because Dox has been shown to be a potential source of reactive oxygen species and free radicals (*28*), free radical scavengers may be effective on Dox-induced cardiac cell injury, which was confirmed by our assays.

In conclusion, this study proposed a tool to diversify compounds with possible pharmacological activity. The extract from the fruit of *M. charantia* with peroxidase activity can be used to catalyze resveratrol and ferulic acid to form their oxidative coupling dimers, and the dimers **6** and **7** showed stronger free radical scavenging activity than their parent compounds, while the effect of **8** was inferior to resveratrol.

ABBREVIATIONS USED

MCP, *Momordica charantia* peroxidase; ESI-MS, electrospray ionization mass spectroscopy; DPPH, α,α-diphenyl-β-picrylhydrazyl; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazoliumbromide.

ACKNOWLEDGMENT

We thank the Central Laboratory of Pharmaceutical Analysis of Shandong University for measurement of NMR, circular dichroism, and mass spectra.

LITERATURE CITED

(1) Takahama, U.; Yoshitama, K. Hydroxycinnamic acid esters enhance peroxidase-dependent oxidation of 3,4-dihydroxyphenylalanine. Differences in the enhancement among the esters. *J. Plant Res.* **1998**, *111*, 97–100.

(2) Hiraga, S.; Sasaki, K.; Ito, H.; Ohashi, Y.; Matsui, H. A large family of class III plant peroxidases. *Plant Cell Physiol.* **2001**, *42*, 462–468.

(3) Zorn, H.; Langhoff, S.; Scheibner, M.; Nimtz, M.; Berger, R. G. A peroxidase from Lepista irina cleaves β,β-carotene to flavor compounds. *Biol. Chem.* **2003**, *384*, 1049–1056.

(4) Liu, H. L.; Kong, L. Y.; Takaya, Y.; Niwa, M. Biotransformation of ferulic acid into two new dihydrotrimers by *Momordica charantia* peroxidase. *Chem. Pharm. Bull.* **2005**, *53*, 816–819.

(5) Liu, H. L.; Wan, X.; Huang, X. F.; Kong, L. Y. Biotransformation of sinapic acid catalyzed by *Momordica charantia* peroxidase. *J. Agric. Food Chem.* **2007**, *55*, 1003–1008.

(6) Mishima, S.; Matsumoto, K.; Futamura, Y.; Araki, Y.; Ito, T.; Tanaka, T.; Iinuma, M.; Nozawa, Y.; Akao, Y. Antitumor effect of stilbenoids from *Vateria indica* against allografted sarcoma S-180 in animal model. *J. Exp. Ther. Oncol.* **2003**, *3*, 283–288.

(7) Baur, J. A.; Sinclair, D. A. Therapeutic potential of resveratrol: the in *vivo* evidence. *Nat. Rev. Drug Discovery* **2006**, *5*, 493–506.

(8) Sotheeswaran, S.; Pasupathy, V. Distribution of resveratrol oligomers in plants. *Phytochemistry* **1993**, *32*, 1083–1092.

(9) Lin, M.; Li, J. B.; Wu, B.; Zheng, Q. T. A stilbene derivative from *Gnetum parvifolium*. *Phytochemistry* **1991**, *30*, 4201–4203.

(10) Cichewicz, R. H.; Kouzi, S. A. Resveratrol oligomers: structure, chemistry, and biological activity. *J. Nat. Prod. Chem.* **2002**, *26*, 507–579.

(11) Ohyama, M.; Tanaka, T.; Ito, T.; Iinuma, M.; Bastow, K. F.; Lee, K. H. Antitumor agents 200. Cytotoxicity of naturally occurring resveratrol oligomers and their acetate derivatives. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3057–3060.

(12) Langcake, B. P.; Pryce, R. J. A new class of phytoalexins from grapevines. *Experientia* **1977**, *33*, 151–152.

(13) Takaya, Y.; Terashima, K.; Ito, J.; He, Y. H.; Tateoka, M.; Yamaguchi, N.; Niwa, M. Biomimic transformation of resveratrol. *Tetrahedron* **2005**, *61*, 10285–10290.

(14) Ou, L.; Kong, L. Y.; Zhang, X. M.; Niwa, M. Oxidation of ferulic acid by Momordica charantia peroxidase and related anti-inflammation activity changes. *Biol. Pharm. Bull.* **2003**, *26*, 1511–1516.

(15) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram guantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(16) Kochba, J.; Spiegel, R. P. Difference in peroxidase activity and isoenzymes in embryogenic orange ovular callus lines. *Plant Cell Physiol.* **1977**, *18*, 463–467.

(17) Tagashira, M.; Ohtake, Y. A new antioxidative 1,3-benzodioxole from *Melissa officinalis*. *Planta Med.* **1998**, *64*, 555–558.

(18) Cao, Z.; Li, Y. Potent induction of cellular antioxidants and phase 2 enzymes by resveratrol in cardiomyocytes: Protection against oxidative and electrophilic injury. *Biochem. Biophys. Res. Commun.* **2002**, *292*, 50–57.

(19) Cichewicz, R. H.; Kouzi, S. A.; Hamann, M. T. Dimerization of resveratrol by the grapevine pathogen *Botrytis cinerea*. *J. Nat. Prod.* **2000**, *63*, 29–33.

(20) Nicotra, S.; Cramarossa, M. R.; Mucci, A.; Pagnoni, U. M.; Riva, S.; Forti, L. Biotransformation of resveratrol: Synthesis of trans-dehydrodimers catalyzed by laccases from *Myceliophtora thermophyla and from Trametes pubescens*. *Tetrahedron* **2004**, *60*, 595–600.

(21) Kim, H. J.; Saleem, M.; Seo, S. H.; Jin, C.; Lee, Y. S. Two new antioxidant stilbene dimers, parthenostilbenins A and B from *Parthenocissus tricuspidata*. *Planta Med.* **2005**, *7*, 973–976.

(22) Ohyama, M.; Tanaka, T.; Iinuma, M. Five resveratrol oligomers from roots of *Sophora leachiana*. *Phytochemistry* **1995**, *38*, 733–740.

(23) Niwa, T.; Doi, U.; Kato, Y.; Osawa, T. Inhibitory mechanism of sinapinic acid against peroxynitrite-mediated tyrosine nitration of protein in *vitro*. *FEBS Lett.* **1999**, *459*, 43–46.

(24) Kobayashi, M.; Mahmud, T.; Yoshioka, N.; Hori, K.; Shibuya, H.; Kitagawa, I. Indonesian medicinal plants. XVIII. Kompasinol A, a new stilbeno-phenylpropanoid from the bark of *Koompassia malaccensis* (Fabaceae). *Chem. Pharm. Bull.* **1996**, *44*, 2249–2253.

(25) Cai, Y. Z.; Sun, M.; Xing, J.; Luo, Q.; Corke, H. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sci.* **2006**, *25*, 2872–2888.

(26) Gewirtz, D. A. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem. Pharmacol.* **1999**, *57*, 727–741.

(27) Oliveira, P. J.; Bjork, J. A.; Santos, M. S.; Leino, R. L.; Froberg, M. K.; Moreno, A. J.; Wallace, K. B. Carvedilol-mediated antioxidant protection against doxorubicin-induced cardiac mitochondrial toxicity. *Toxicol. Appl. Pharmacol.* **2004**, *200*, 159–168.

(28) Ahmed, H. H.; Mannaa, F.; Elmegeed, G. A.; Doss, S. H. Cardioprotective activity of melatonin and its novel synthesized derivatives on doxorubicin-induced cardiotoxicity. *Bioorg. Med. Chem.* **2005**, *13*, 1847–1857.

Received for review April 20, 2007. Revised manuscript received July 2, 2007. Accepted July 13, 2007. This work was supported by National Natural Science Foundation of China (no. 30472072).

JF0711486