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Synthesis and Structure−Activity Relationship Study of Deoxybenzoins on Relaxing Effects of Porcine Coronary Artery

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Deoxybenzoins are potent antioxidants and tyrosinase inhibitors with potential to be developed as food preservatives and cosmetic ingredients. To explore the potential in cardiovascular protection, 25 polyphenolic deoxybenzoins were synthesized and evaluated for inhibitory effects on KCl-induced porcine coronary arterial contraction. The results revealed deoxybenzoins are significant inhibitors of KCl-induced arterial contraction. Among those synthesized, two-thirds of the deoxybenzoins exhibited moderate to good efficacy on relaxing contracted artery including 2,4-dihydroxydeoxybenzoin with EC_{50} = 3.30 μM (E_{max} = 100%, n = 7) and 2,4-dihydroxy-4′-methoxydeoxybenzoin EC_{50} = 3.70 μM (E_{max} = 100%, n = 5). Deoxybenzoins displayed an endothelium-dependent relaxing manner on the contracted artery; the contractile responses of neither endothelium denuded nor *L*-NAME deactivated rings were inhibited. The structure−activity relationships of deoxybenzoin on arterial relaxing effects concluded that the 2,4-dihydroxylated deoxybenzoins presented a potential vascular relaxing pharmacophore, with favoring substitution on ring B in the order of H ≥ *p*-OMe > *p*-OH > *o*-OMe > *m*,*p*-diOMe ≥ *m*-OMe.

KEYWORDS: Artery; deoxybenzoin; phytoestrogen; polyphenol; SAR

INTRODUCTION

Natural antioxidants are usually regarded as health beneficial agents in the prevention of oxidative stress related health problems including cancer, nervous system lesion, and many other cardiovascular diseases (*1*, *2*). These cardiovascular ailments, including coronary arterial disease, arrhythmias, hypertension, atherosclerosis, and angiogenesis, are common health problems of modern human society. There is growing evidence that antioxidants are capable of reviving the vascular relaxation of endothelium-defected vascular smooth muscle (*3*). It has been shown that after antioxidant treatment, the endothelium-denuded vascular smooth muscle can release NO to restore relaxation (*4*). Besides, the endothelium-dependent release of NO stimulated by usual plant food polyphenols had also been demonstrated (*5*). Phytoestrogens including resveratrol (stilbene) (*1*, *6*) and phloretin (dihydrochalcone) (*7*, *8*) are well-known natural antioxidants reported to have vascular relaxing and cardiovascular protecting values. Resveratrol was found to enhance arterial relaxation in an endothelium-dependent manner and had no effect on the level of endothelium NO synthase (eNOS) in spontaneously hypertensive rats (*9*). This stilbene can also enhance estrogen-mediated vascular relaxation (*10*), increase the *c*-GMP concentration of vascular smooth muscle (*11*), and promote other manners of arterial relaxation (*12*, *13*). In addition, dihydrochalcone has also been demonstrated to be an endothelium-independent vascular relaxing phytoestrogen in rabbit coronary artery induced by KCl in vitro (*8*). The chemical skeleton of deoxybenzoins (DOBs) was found to be abridged dihydrochalcone (phloretin) and similar to the 1,2-diphenylethylene system of stilbene (resveratrol) in molecular feature, respectively (*14*). It is proper that a C_2-C_3 connected diphenyl polyphenolic system can be the common feature of cardiovascular protecting activities among stilbene and flavonoids. Apparently, DOBs also showed consistency in this structure−activity correlation due to the demonstrated antioxidant capacity (*14*).

DOBs are readily synthesizable small molecules, which have been demonstrated as useful antioxidants and effective inhibitors of mushroom tyrosinase recently (*14*). Besides, some DOBs were sporadically reported to possess activities such as β estrogenic agonist (*15*), antiallergic and anti-inflammatory (*16*), and antimicrobial agents (*17*−*19*). Chemically, DOBs are key precursors for the synthesis of isoflavones (*20*−*23*), but the structure can be more plausibly defined as analogues of dihydrochalcone and stilbene (**Figure 1**) (*14*). Although rare, there are naturally occurring DOB derivatives (*24*−*26*) that could originate from ring-opening followed by decarboxylation of 3-phenyl 4-hydroxycoumarin derivatives (*24*, *25*) and/or by oxidation of stilbenes (**Figure 1**) (*26*). DOBs can also be produced from alkali degradation of related isoflavones (*26*) and are closely related to isoflavone microbial metabolites, angolensinoids (α-methyl DOB) (*27*, *28*).

On the basis of the demonstrated beneficial biological activities (*14*−*16*) and structural analogy to phytoestrogens, DOBs were thought to have potential in the development of cardiovascular protective and chemopreventive agents. Because the potential as antioxidants had been demonstrated (*14*), the arterial

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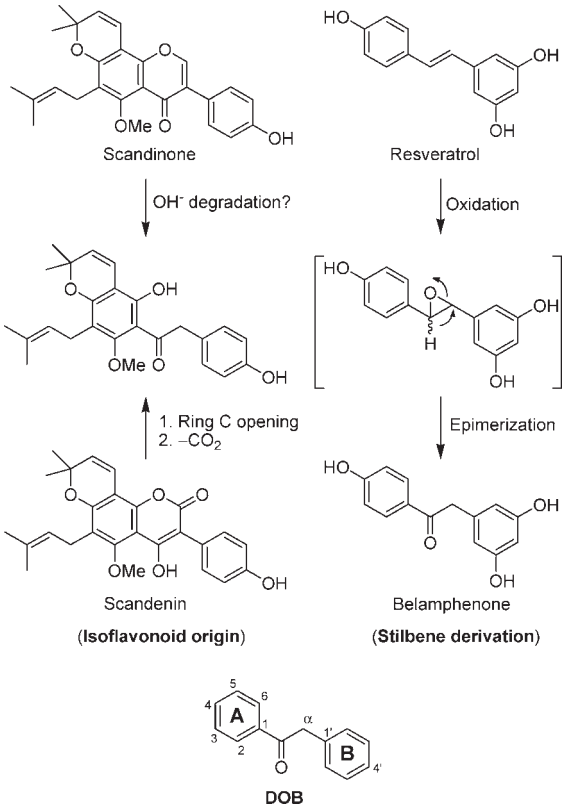


Figure 1. Proposed generation of DOBs found in natural sources.

relaxing evaluation was continued by advanced synthesis of DOBs with diverse substitution. In this study, 25 DOBs were synthesized and evaluated for vascular relaxing potential. Because K^+ ion is an important vascular contracting factor, KCl-induced porcine coronary arterial contracting model was selected for the study. The hit skeleton and structure−activity relationships (SARs) in the inhibition of contracted coronary artery were also investigated. The results displayed that DOBs are powerful inhibitors of arterial contraction and have shown their potential for use in treating cardiovascular diseases.

MATERIALS AND METHODS

Chemistry. The synthetic materials, including resorcinol, 1,2,4-trihydroxybenzene, phenyl acetonitriles, phenylacetic acids, and zinc chloride ($ZnCl_2$), were purchased from Tokyo Chemical Industry (Tokyo, Japan). Boron trifluoride etherate (BF_3-Et_2O) and other phenols (phloroglucinol, pyrogallol, etc.) were purchased from Lancaster Synthesis (Morecambe, U.K.). Melting points (uncorrected) were determined by an Electrothermal 9100 melting point apparatus (Electrothermal Engineering Ltd., Landon, U.K.). Mass spectra were performed on a Shimadzu LCMS-IT-TOF mass spectrometer (Japan) or a Fourier transform mass spectrometer, Bruker APEX II (German). ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained by a Varian Mercury-400 spectrometer (Varian Inc., Palo Alto, CA).

General Procedure for Synthesis of DOBs. The synthesis of DOBs **1**−**18** was performed by condensing respective phenols with equivalent quantities (0.01 mol) of various phenylacetic acids in BF_3-Et_2O at 80−90 °C under nitrogen atmosphere. DOBs **19**−**25** were prepared by condensing 1,2,4-trihydroxybenzene or phloroglucinol (0.02 mol each) and related phenylacetonitriles (0.01 mol) by $ZnCl_2$ (0.01 mol) and saturated HCl (gas, caution!) at 0 °C (**Table 1**). The reaction and quenching details were described in our previous paper (*14*). The DOBs were all purified by column chromatography and recrystallized with proper solvents. Among the DOBs, **1**, **3**−**5**, **11**, **13**, **18**, **23**, and **24** (**Table 1**) were newly synthesized; others were previously reported (*14*). The syntheses was all achieved in high yields at gram scale. On the basis of the LC-MS analysis, the purity of these nine newly synthesized DOBs was >99%. The substitutions of DOBs **1**−**25** are illustrated in **Table 1**. The substitution patterns presented can be classified into 2,4-dioxygenated (**1**−**7**), 3,4-dioxygenated (**8**, **9**), 2,5-dioxygenated (**10**), 2,3,4-trioxygenated (**11**−**16**), 2,4,5-trioxygenated (**17**−**20**), and 2,4,6-trioxygenated (**21**−**25**) series of DOBs with various rings B. DOB **13** was found to be a new compound, and the NMR spectral data of **24** (*29*) were the first presented. DOBs **1**, **3**, and **4** were synthesized by Wähälä et al. (*20*), and the data of **5** and **18** were reported previously in a NMR spectral study by Jha et al. (*30*). The structures of DOBs **11** and **23** were recently reported by Goto et al. (*17*) and Hastings et al. (*31*), respectively.

2,4-Dihydroxydeoxybenzoin (***1***)*:* 2.10 g, 92%; R_f = 0.50 (EtOAc/acetone/*n*-hexane = 2:0.5:7.5); yellowish masses (MeOH + H_2O); mp 116 °C; ^1H NMR (acetone-d_6, 400 MHz, 25 °C) δ 4.29 (2H, s, CH_2), 6.36 (1H, d, J = 2.4 Hz, H-3), 6.46 (1H, dd, J = 2.4, 8.8 Hz, H-5), 7.24 (1H, m, H-4′), 7.33 (4H, m, H-2′,3′,5′,6′), 7.96 (1H, d, J = 8.8 Hz, H-6), 9.54 (1H, s, OH), 12.73 (1H, s, C-2 OH); ^{13}C NMR (acetone-d_6, 100 MHz, 25 °C) δ 44.4 (CH_2), 103.1 (C-3), 108.3 (C-5), 112.9 (C-1), 126.9 (C-4′), 128.7 (C-3′,5′), 129.7 (C-2′,6′), 133.6 (C-6), 135.5 (C-1′), 165.1 (C-2), 166.1 (C-4), 202.7 (C═O); EI-MS m/z 228 $[M]^+$, 137 (*20*).

2,4-Dihydroxy-3′-methoxydeoxybenzoin (***3***)*:* 1.91 g, 74%; R_f = 0.42 (EtOAc/acetone/*n*-hexane = 2:0.5:7.5); colorless plates (acetone + *n*-hexane), mp 128−129 °C; ^1H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 3.77 (3H, s, OMe), 4.26 (2H, s, CH_2), 6.33 (1H, d, J = 2.4 Hz, H-3), 6.45 (1H, dd, J = 2.4, 8.8 Hz, H-5), 6.81 (1H, dd, J = 2.4, 8.8 Hz, H-6′), 6.91 (1H, dd, J = 1.6, 8.4 Hz, H-2′), 6.94 (1H, t, J = 2.0 Hz, H-2′), 7.23 (1H, t, J = 8.0 Hz, H-5′), 7.97 (1H, d, J = 8.8 Hz, H-6), 9.49 (1H, s, OH), 12.70 (1H, s, C-2 OH); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ 44.5 (CH_2), 54.8 (OMe), 103.0 (C-3), 108.2 (C-5), 112.2 (C-4′), 115.2 (C-1), 115.5 (C-2′), 121.8 (C-6′), 129.6 (C-5′), 136.9 (C-1′), 160.2 (C-3′), 165.0 (C-2), 166.1 (C-4), 202.6 (C═O); EI-MS m/z 258 $[M]^+$, 137 (*20*).

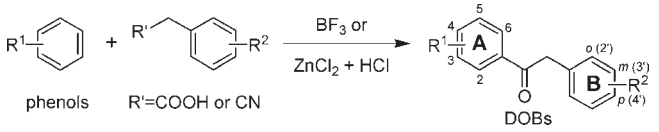
2,4-Dihydroxy-2′-methoxydeoxybenzoin (***4***)*:* 2.24 g, 87%; R_f = 0.45 (EtOAc/acetone/*n*-hexane = 2:0.5:7.5); colorless powders (acetone + *n*-hexane), mp 193 °C; ^1H NMR (acetone-d_6, 400 MHz, 25 °C) δ 3.79 (3H, s, OMe), 4.26 (2H, s, CH_2), 6.34 (1H, d, J = 2.4 Hz, H-3), 6.45 (1H, dd, J = 2.4, 8.8 Hz, H-5), 6.90 (1H, td, J = 7.6, 1.2 Hz, H-5′), 6.98 (1H, dd, J = 0.8, 8.4 Hz, H-3′), 7.21 (1H, dd, J = 2.0, 7.6 Hz, H-6′), 7.25 (1H, td, J = 8.0, 1.6 Hz, H-5′), 7.95 (1H, d, J = 8.8 Hz, H-6), 9.50 (1H, s, OH), 12.70 (1H, s, C-2 OH); ^{13}C NMR (acetone-d_6, 100 MHz, 25 °C) δ 40.3 (CH_2), 56.5 (OMe), 104.3 (C-3), 109.4 (C-5), 112.2 (C-3′), 114.4 (C-1), 121.9 (C-5′), 125.3 (C-1′), 129.8 (C-4′), 132.5 (C-6′), 134.5 (C-6), 158.9 (C-2′), 166.1 (C-2), 167.0 (C-4), 204.1 (C═O); EI-MS m/z 258 $[M]^+$, 137 (*20*).

2-Hydroxy-4,4′-dimethoxydeoxybenzoin (***5***)*:* 1.41 g, 52%; R_f = 0.40 (EtOAc/acetone/*n*-hexane = 2:0.5:7.5); colorless needles (MeOH), mp 110 °C; ^1H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 3.79, 3.83 (each 3H, s, OMe), 4.15 (2H, s, CH_2), 6.42 (1H, d, J = 2.4 Hz, H-3), 6.44 (1H, dd, J = 2.4, 8.8 Hz, H-5), 6.88 (2H, d, J = 8.8 Hz, H-3′,5′), 7.19 (2H, d, J = 8.8 Hz, H-2′,6′), 7.75 (1H, d, J = 8.8 Hz, H-6), 12.74 (1H, s, C-2 OH); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ 43.9 (CH_2), 55.3 (OMe), 55.5 (OMe), 101.0 (C-3), 107.8 (C-5), 113.1 (C-1), 114.2 (C-3′,5′), 126.3 (C-1′), 130.3 (C-2′,6′), 132.0 (C-6), 158.7 (C-4′), 165.8 (C-4), 166.1 (C-2), 202.3 (C═O); EI-MS m/z 272 $[M]^+$, 151 (*30*).

2,3,4-Trihydroxydeoxybenzoin (***11***)*:* 1.93 g, 79%; R_f = 0.43 (EtOAc/acetone/*n*-hexane = 3:0.5:6.5); colorless needles (acetone + H_2O), mp 163−165 °C; ^1H NMR (acetone-d_6, 400 MHz, 25 °C) δ 4.29 (CH_2), 6.50 (1H, d, J = 8.8 Hz, H-5), 7.27 (1H, m, H-4′), 7.33 (4H, m, H-2′,3′,5′,6′), 7.56 (1H, d, J = 8.8 Hz, H-6), 12.72 (1H, s, C-2 OH); ^{13}C NMR (acetone-d_6, 100 MHz, 25 °C) δ 44.4 (CH_2), 107.8 (C-5), 113.2 (C-1), 123.5 (C-6), 126.9 (C-4′), 128.7 (C-2′, 6′), 129.7 (C-3′, 5′), 132.5 (C-3), 135.6 (C-1′), 152.1 (C-2), 152.8 (C-4), 203.4 (C═O); EI-MS m/z 244 $[M]^+$, 153 (*17*).

2,3,4-Trihydroxy-2′-methoxydeoxybenzoin (***13***)*:* 2.14 g, 78%; R_f = 0.35 (EtOAc/acetone/*n*-hexane = 3:0.5:6.5); colorless powders (acetone + *n*-hexane), mp 160 °C; ^1H NMR (acetone-d_6, 400 MHz, 25 °C) δ 3.78 (3H, s, OMe), 4.27 (2H, s, CH_2), 6.50 (1H, d, J = 8.8 Hz, H-5), 6.90 (1H, td, J = 8.4, 1.2 Hz, H-5′), 6.97 (1H, dd, J = 1.2, 8.4 Hz, H-3′), 7.21 (1H, dd, J = 1.6, 8.4 Hz, H-6′), 7.25 (1H, td, J = 8.0, 1.6 Hz, H-5′), 7.55 (1H, d, J = 8.8 Hz, H-6), 8.22 (2H, br, OH), 12.74 (1H, br, OH); ^{13}C NMR (acetone-d_6, 100 MHz, 25 °C) δ 40.3 (CH_2), 56.4 (OMe), 109.0 (C-5), 112.1 (C-3′), 114.7 (C-1′), 121.9 (C-5′), 124.3 (C-6), 125.3 (C-1′), 132.6 (C-6′), 133.7 (C-3), 153.2 (C-4), 153.7 (C-2), 159.0 (C-2′), 204.7 (C═O); EI-MS m/z 274 $[M]^+$,

Table 1. Synthesis of DOBs **1**−**25** and Their Relaxing Effects on Porcine Coronary Arteries[a]



entry	R^1	R^2	EC_{50} (μM)	EC_{max} (%)	pD_2	efficacy[b]
1	2,4-di-OH	H	3.30	100.0	5.48	G
2	2,4-di-OH	*p*-OMe	3.70	100.0	5.43	G
3	2,4-di-OH	*m*-OMe	22.98	100.0	4.64	M
4	2,4-di-OH	*o*-OMe	13.25	99.0	4.88	G
5	2-OH, 4-OMe	*p*-OMe		42.0		ns
6	2,4-di-OH	*m*,*p*-di-OMe	21.89	100.0	4.66	M
7	2,4-di-OH	*p*-OH	6.81	100.0	5.17	G
8	3,4-di-OH	*p*-OMe	28.16	90.1	4.55	M
9	3,4-di-OH	*m*,*p*-di-OMe	33.16	84.3	4.48	M
10	2,5-di-OH-3,4-di-Me	*p*-OMe	4.73	100.0	5.33	G
11	2,3,4-tri-OH	H	54.09	54.4	4.27	M[c]
12	2,3,4-tri-OH	*p*-OMe	15.44	89.8	4.81	M
13[d]	2,3,4-tri-OH	*o*-OMe		25.3		ns
14	2,3-di-OH,4-OMe	*p*-OH		29.0		ns
15	2,3,4-tri-OH	*m*,*p*-di-OMe	21.98	100.0	4.66	M
16	2,3,4-tri-OH	*p*-OH		40.0		ns
17	2,5-di-OH, 4-OMe	H		88.3		W
18	2,5-di-OH, 4-OMe	*p*-OMe		53.1		W
19	2,4,5-tri-OH	*p*-OMe	8.74	99.2	5.06	G
20	2,4,5-tri-OH	*p*-OH	52.21	74.4	4.28	M
21	2,4,6-tri-OH	H	35.83	75.7	4.45	M
22	2,4,6-tri-OH	*p*-OMe	24.82	82.2	4.61	M
23	2,4,6-tri-OH	*m*-OMe		41.1		ns
24	2,4,6-tri-OH	*o*-OMe		13.4		ns
25	2,4,6-tri-OH	*p*-OH		−3.7		ni
resveratrol			36.24	88.2	4.44	
bradykinin			23.42	70.0	4.63	
nifedipine			0.12[e]	97.0	6.92	

[a] Resveratrol, nifedipine, and bradykinin were used as positive controls. The treatments were added in a cumulative manner with concentrations 1, 3, 10, 30, and 100 μM of DOBs and bradykinin. [b] Relaxing efficacy is expressed as "good" (G) when $pD_2 \geq 4.85$, as "moderate" (M) when $4.85 > pD_2 \geq 4$, as "weak" (W) when $pD_2 < 4$, and as "not significant" (ns) when the relative relaxing activity is <50% at a dosage of 100 μM. $n = 4-7$. [c] This compound exhibited the most apparent effect at low concentration (1 μM), $E_{min} = 28.6\%$. [d] New compound. [e] The EC_{50} of this reagent was estimated by the dosages 0.01−3 μM.

153 (base peak); HREI-MS found *m*/*z* 274.0841 (calcd for $C_{15}H_{14}O_5$ = 274.0841).

2,5-Dihydroxy-4,4′-dimethoxydeoxybenzoin (***18***)*:* 1.46 g, 51%; R_f = 0.32 (EtOAc/acetone/*n*-hexane = 2.5:0.5:7); yellow needles (acetone + *n*-hexane), mp 189−190 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 3.78 (3H, s, OMe), 3.90 (3H, s, OMe), 4.11 (2H, s, CH_2), 6.43 (1H, s, H-3), 6.87 (2H, d, J = 8.8 Hz, H-3′,5′), 7.18 (2H, d, J = 8.8 Hz, H-2′,6′), 7.32 (1H, s, H-6), 12.51 (1H, s, C-2 OH); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ 44.3 (CH_2), 55.5 (OMe), 56.4 (OMe), 100.1 (C-3), 111.9 (C-1), 113.8 (C-6), 114.4 (C-3′,5′), 126.5 (C-1′), 130.7 (C-2′,6′), 138.3 (C-5), 154.1 (C-4), 158.9 (C-2), 159.8 (C-4′), 202.6 (C═O); EI-MS *m*/*z* 288[M]$^+$ (*30*).

2,4,6-Trihydroxy-3′-methoxydeoxybenzoin (***23***)*:* 1.86 g, 68%; R_f = 0.23 (EtOAc/acetone/*n*-hexane = 3:0.5:6.5); yellowish plates (MeOH + H_2O), mp 166−168 °C; ^{1}H NMR (CD_3OD, 400 MHz, 25 °C) δ 3.75 (3H, s, OMe), 4.39 (2H, s, CH_2), 5.96 (2H, s, H-3,5), 6.44 (1H, dd, J = 2.4, 8.8 Hz, H-5), 6.75 (1H, ddd, J = 0.8, 2.8, 8.1 Hz, H-6′), 6.79 (1H, t, J = 1.6 Hz, H-2′), 6.80 (1H, m, H-4′), 7.16 (1H, t, J = 8.8 Hz, H-5′); ^{13}C NMR (CD_3OD, 100 MHz, 25 °C) δ 49.3 (CH_2), 54.4 (OMe), 94.6 (C-3,5), 104.2 (C-1), 111.7 (C-4′), 115.3 (C-2′), 121.9 (C-6′), 128.9 (C-5′), 137.7 (C-1′), 159.8 (C-3′), 164.7 (C-2,6), 165.3 (C-4), 203.1 (C═O); EI-MS *m*/*z* 274 [M]$^+$, 153 (*31*).

2,4,6-Trihydroxy-2′-methoxydeoxybenzoin (***24***)*:* 1.94 g, 70%; R_f = 0.18 (EtOAc/acetone/*n*-hexane = 3: 0.5: 6.5); colorless powder (MeOH + H_2O), mp 172 °C; ^{1}H NMR (acetone-d_6, 400 MHz, 25 °C) δ 3.75 (3H, s, OMe), 4.39 (2H, s, CH_2), 5.96 (2H, s, H-3, 5), 6.88 (1H, td, J = 7.6, 1.2 Hz, H-5′), 6.94 (1H, dd, J = 0.8, 8.4 Hz, H-3′), 7.13 (1H, dd, J = 1.2, 7.6 Hz, H-6′), 7.22 (1H, td, J = 8.0, 1.6 Hz, H-5′), 9.31 (1H, br, OH), 11.71 (2H, br, OH); ^{13}C NMR (acetone-d_6, 100 MHz, 25 °C) δ 45.1 (CH_2), 55.0 (OMe), 95.2 (C-3,5), 104.7 (C-1), 110.5 (C-3′), 120.3 (C-5′), 125.2 (C-1′), 128.0 (C-4′), 131.4 (C-6′), 158.2 (C-2′), 164.6 (C-4), 164.7 (C-2,6), 202.9 (C═O); EI-MS *m*/*z* 274 [M]$^+$, 121 (*29*).

Bioassay. *Material and Apparatus*. Isometric tension was measured by Cyber 380 and Digidata 1320A (Axon Instrument, USA) via a force transducer (model FT03, Grass Instrument USA). Krebs−Henseleit (KH) buffer, bradykinin, N^{ω}-nitro-L-arginine methyl ester (L-NAME), resveratrol, and nifedipine were purchased from Sigma Chemical Co. (St. Louis, MO). Porcine hearts were obtained from a local abattoir within 30 min after slaughtering and were immediately transported to the laboratory in KH buffer (pH 7.4). The right coronary and anterior descending branch of the left coronary arteries were dissected and stored overnight at 4 °C in an oxygenated KH buffer solution. The following treatment was performed as previously reported (*32*).

General Contraction Inhibitory Assay of DOBs on Porcine Aortic Rings. Porcine coronary artery is the readily available material for use as an in vitro model of vascular relaxation study (*32*−*34*). Tissue samples were allowed to equilibrate for at least for 1 h before the test began. KCl (30 mM) was added into the organ bath to contract the artery. When the contraction turned stable (usually 15 min), the test solution of DOBs was added into the organ bath to detect inhibitory effect on the contraction. Concentration−response curves were established by cumulative dosages of 1, 3, 10, 30, and 100 μM. The 50% effective relaxing dose is expressed by EC_{50} in micromolar and the treatment leading to a maximum relative relaxing percentage expressed by E_{max} (**Table 1**). However, the EC_{50} of nifedipine (positive control) was estimated from the treatment of 0.01, 0.03, 0.1, 0.3, 1, and 3 μM in cumulative concentration. Contractile responses were calculated by the difference between resting tension and maximum tension developed in response to the KCl stimulation.

The relative relaxing activity of DOBs and positive controls on KCl-induced porcine coronary arterial contraction was calculated by comparison with the control of the respective vehicles according to a previous paper (*32*). When a maximum contraction status is relaxed or less than the resting tension of the aortic ring, the relative relaxation is defined as 100%. Data were expressed as mean ± SEM from at least four experiments. The relative relaxing percentage is calculated by the equation

$$R\% = [(T_i - T_r)/T_i] \times 100\%$$

where R is the relative relaxing percentage, T_i is KCl-induced arterial contractile tension treated with respective DMSO contented solvent, and T_r is contractile tension after treatment of reagent.

A clinical vascular relaxing agent, nifedipine, resverstrol (a famous chemopreventive agent), and bradykinin were used as positive controls. The maximum efficacy (E_{max}) and the pD_2 values ($-\log EC_{50}$) of good to moderate arterial contraction inhibitors of each tested agent are presented that are derived from the regression curves and expressed as 95% confidence intervals. The arterial relaxing efficacy was classified as "good: (G) when $pD_2 \geq 4.85$, as "moderate" (M) when $4.85 > pD_2 \geq 4$, as "weak" (W) when $pD_2 < 4$, and as "not significant (ns) when the relative relaxing effect was $< 50\%$ at the treatment of 100 μM. The EC_{50} and pD_2 values are presented only when the DOBs showed moderate to good efficacy (**Table 1**). However, the relaxing efficacies of positive controls were not evaluated by the above principle.

*Relaxing Assay of DOBs **1** and **7** on Denuded Endothelium Arteries.* Some of the aortic rings were rubbed gently with a wooden probe and cotton to remove the endothelium. To confirm the relaxation, the aortic rings with intact or denuded endothelium were all precontracted by KCl and tested with 10 μM bradykinin (*33*).

*Relaxing Assay of DOBs **1** and **7** on L-NAME Endothelium Deactivated Arteries.* Deactivation of NO signaling in endothelium-intact aortic rings was achieved by adding the NO synthase inhibitor L-NAME (100 μM) solution into the organ baths 5 min before the addition of contraction agent and selected DOBs (10 μM). To confirm the relaxation, the aortic rings with intact or deactivated endothelium were precontracted by KCl and tested with 10 μM bradykinin (*33*).

RESULTS AND DISCUSSION

Inhibitory Effect of DOBs on the KCl-Induced Porcine Coronary Arterial Contraction. It is noteworthy that nearly two-thirds of DOBs exhibited moderate to good efficacy in the inhibition of arterial contraction with pD_2 values of 4.27−5.48 (**Table 1**). Although the activity of DOBs was inferior to that of the clinical agent nifedipine ($pD_2 = 6.92$), they were generally stronger than the contraction inhibitor bradykinin ($pD_2 = 4.63$) and resveratrol ($pD_2 = 4.44$) (**Table 1**). On the basis of the pD_2 values, the order of DOB potency for good inhibitors was **1** ≥ **2** > **10** > **7** > **19** > **4** (**Figure 2**) and for moderate inhibitors was **12** > **6** ≥ **15** ≥ **3** > **22** > **8** > **9** > **21** > **20** > **11** (**Table 1**). DOBs including **1**−**3**, **6**, **7**, and **15** demonstrated an E_{max} of 100% in the contracted coronary artery at the maximum treating concentration (144 μM), and DOBs **2** and **10** even showed a 100% E_{max} by cumulative dose of 44 μM (**Figure 2**). DOBs with the greatest detectable arterial relaxing effect at low concentration (1 μM) were **1** and **11**, which showed E_{min} (the minimum relaxing effect) values of 26.5 and 28.6%.

When scrutinized on the serial relaxing responses based on ring A polyoxygenated system, the potency was found to be 2,4-dioxygenated (**1**−**7**) > 2,4,5-trioxygenated (**17**−**20**) > 2,3,4-trioxygenated (**11**−**16**) > 3,4-dioxygenated (**8**, **9**) > 2,4,6-trioxygenated series (**21**−**25**). The only 2,5-dioxygenated DOB (**10**), although found to be good inhibitor of contracted artery, was not considered in this comparison due to lack of respective derivation. This fact revealed that 2,4-dioxygenated ring A can be the most suitable substitution for arterial relaxing activity of DOBs.

The substitution effect on ring B, however, did not show any enhancement on the arterial relaxing activity of DOBs. It can be differentiated that the *p*-OMe substitution on ring B of DOBs retained the most sensitive activity; *o*-OMe slightly and *m*-OMe largely decreased the activity as shown in **Table 1**. The DOBs with *p*-OH on ring B (**7**, **16**, **20**, and **25**) were generally less active or inactive in inhibiting the arterial contraction when compared with the respective *p*-OMe derivatives (**2**, **12**, **19**, and **22**). The explanation for the good efficacy of **7** and moderate efficacy for **20** could be due to their suitable substitution of ring A. Briefly, the results suggested that 2,4-dihydroxylated DOBs possess favorable potential as candidates for development as relaxing agents of contracted coronary artery.

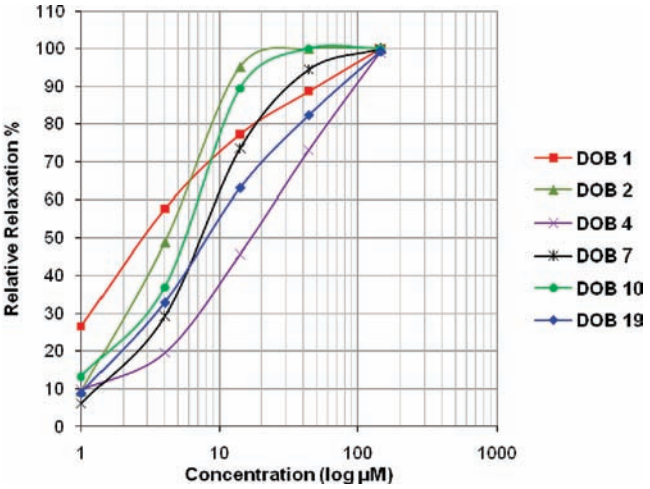


Figure 2. Relative relaxing effects of DOBs with good efficacy ($EC_{50} \leq 14$ μM, $pD_2 \geq 4.85$) on KCl-induced coronary arterial contraction. Values are presented as mean ± SEM ($n = 5$−7). DOB dosages were added in a cumulative manner.

Effects of DOBs 1 and 7 on the Endothelium Denuded and L-NAME Endothelium-Deactivated Aorta Contraction. DOBs **1** (with unsubstituted ring B) and **7** (with *p*-OH ring B) were selected for mechanistic study in the contracted artery relaxation. The former exhibited the most prominent EC_{50} value and an apparent contraction inhibitory effect at low concentration, whereas the latter with a hydroxylated ring B was shown to be the only good arterial relaxant, which has been demonstrated to be a potential β-estrogenic receptor agonist (*15*). It is possible that DOB **7** may have a relaxing manner similar to that of resveratrol (*9*−*13*).

Both of the aortic rings with denuded endothelium and deactivated by L-NAME (NO synthase inhibitor) were used to evaluate the inhibitory pathway. After treatment with 10 μM **1** and **7**, the contraction of normal porcine aortic rings induced by KCl was down-regulated to 19 ± 13 and 37 ± 13%, respectively. When the endothelium was denuded or deactivated by L-NAME, the relaxing effect of the DOBs on coronary artery was completely abolished (**Figure 3**). These results disclosed that the relaxation of DOBs at low concentration (10 μM) on porcine artery could be endothelium-dependent and possibly involved in eNOS activation or NO release.

SARs of DOBs on the Coronary Arterial Contraction Inhibition. The 2,4-dihydroxylated DOB series (**1**−**4**, and **6**−**7**) displayed a distinct arterial contraction inhibitory E_{max} that **1**−**3** and **6**−**7** with 100% inhibition at higher concentration (≥44 μM) and **4** also with an E_{max} of 99% inhibition (**Table 1**). The most suitable skeleton for relaxing effect on contracted coronary artery can be concluded reasonably. Accordingly, the SARs based on the arterial relaxing potency of 2,4-dihydroxylated DOBs can be summarized as follows:

(i) C-4 OH in ring A was an essential substituent with H-bond donating effect, which could be confirmed by the reversed activity

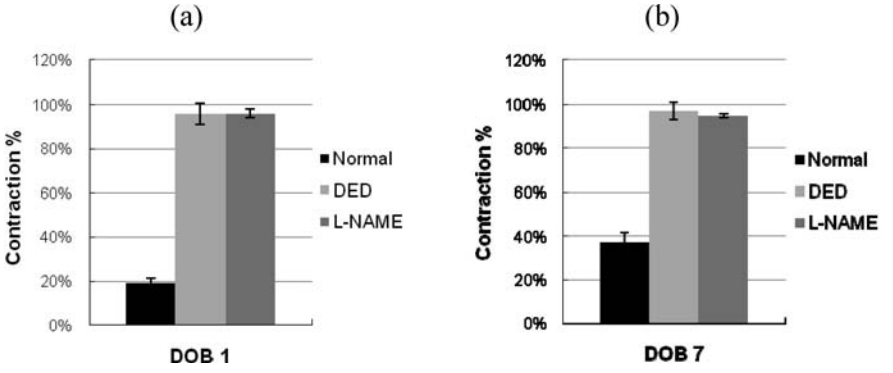


Figure 3. Comparison of contractile responses of porcine aortic rings treated by 10 μM DOBs **1** (**a**) and **7** (**b**) with normal endothelium, denuded endothelium (DED), and L-NAME-treated models. Values are presented as mean ± SEM (n = 6).

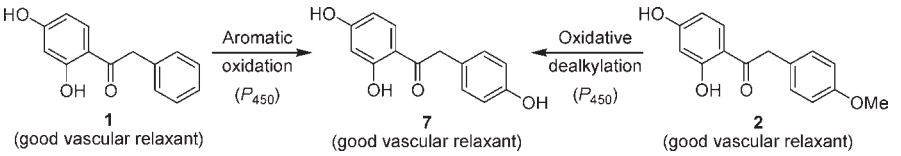


Figure 4. Proposed biotransformation benefits of DOBs **1** and **2** as coronary arterial contraction inhibitors.

of **5** and **18** as compared with that of **2** and **19**. Although DOB **10** lacked a C-4 OH, it was shown to be a good inhibitor. This suggests that C-5 OH could posses an alternative H-bond donating effect similar to those of flavonoids (*34*).

(ii) C-2 OH chelating with carbonyl could have involved both the conformational stabilization and the H-bond donating capability of C-4 OH, which could be similar to that of C-5 OH of flavonoids (*35*); DOBs (**8** and **9**) lacking C-2 OH and DOBs (**11**−**16**) with C-3 OH between C-2 and C-4 appeared to attenuate the vascular relaxing activity. Accordingly, C-2 OH should not be interfered with or deleted for relaxing the contracted artery.

(iii) Additional OH substitution on the 2,4-dihydroxylated ring A seemed to have inverted the arterial relaxing capability of DOBs. For example, additional C-3 OH (such as **11**−**16**) intruding between H-bond donating C-4 OH and chelated C-2 OH led to a large decrease in vascular relaxing capability; C-5 OH (such as **19**) was also found to interfere mildly with C-4 OH (*34*) and may lead to a decrease in vascular relaxing activity of **19** and **20** as compared with **2** and **7**. Furthermore, an additional chelating OH (C-6 OH, **21**−**25**) was also found to have no positive effect on the arterial relaxing activity.

(iv) The most suitable substitution on ring B of 2,4-dihydroxylated DOB was found to be the unsubstituted benzene, although *p*-anisyl is also comparable. Because 2,4-dihydroxylated DOBs were found to be moderate to good inhibitors on the KCl-induced porcine coronary arterial contraction, the order of substitution preference is H ≥ *p*-OMe > *p*-OH > *o*-OMe > *m*,*p*-diOMe ≥ *m*-OMe. It is possible that the electron bulky substituent (OMe) at meta and/or ortho orientations of ring B, for example, **3**, **4**, and **6** versus **2**, are futile substitutions and could be a hindering effect on the receptor interaction more or less.

The arterial contraction inhibitory mechanisms of polyphenolic phytoestrogens are complicated and still controversial (*8*−*13*). Most of the described active molecular features of chemopreventive polyphenols are of two phenolic OH ends (*5*, *7*−*13*, *34*, *35*). The chelated C-2 OH that could be affecting the activity was also found to be an anticipated substitution similar to flavonoids (*34*, *35*). The most active arterial contraction inhibitors among the DOB series can be those with OH at one end (C-4) and unsubstituted or C-4′ oxygenated ring B, alternatively, on the other. The endothelium-dependent arterial relaxing manner of DOBs seemed to be similar to that of resveratrol (*9*, *10*) due to their structural analogy. In addition, the demonstrated release of NO by endothelium stimulation of plant polyphenols could be a support of DOBs used as alternatives of polyphenols (*5*); especially, most of the "good" arterial contraction inhibitors of DOBs exhibited stronger effects than that of resveratrol. However, whether the arterial relaxing power connected with the antioxidant capability requires advanced study because 2,4-dihydroxylated DOBs were not the most potential antioxidants reported previously (*14*).

On the basis of the known metabolic manners of cytochrome P_{450} monooxygenases, either the aromatic oxidation of benzene ring (ring B) of **1** or the oxidative dealkylation of **2** (*36*), the metabolite would be the phytoestrogen **7** (**Figure 4**). DOB **2** may also have a demethylating metabolism similar to that of closely related C-4′ methoxylated isoflavones, that is, biochanin A and formononetin (*37*, *38*). In other words, the "good" vascular relaxing agents **1** and **2** could be transformed into another good arterial contraction inhibitor **7** in vivo, although less active (**Figure 4**). The benefit and efficacy of a clinical agent would be tremendous if both mother chemical and daughter metabolite were active agents. Briefly, 2,4-dihydroxylated DOB could be applied as a pharmacophore and DOBs **1**, **2**, and **7** the hit candidates with potential for advanced development as vascular relaxing agents.

Phytoestrogenic antioxidants have been claimed to be chemopreventive agents and shown to have cardiovascular protecting benefits (*1*,*3*−*6*). In this study, although the synthetic DOBs have demonstrated their benefits in cardiovascular systems (*14*−*16*), enormous efforts are still needed before they can be developed into useful clinical agents or chemopreventives. If detailed studies were to be performed, it is possible that no difference could be noted between synthetic DOBs and natural polyphenols in function of the cardiovascular system (*3*, *5*) and chemoprevention (*1*,*6*). Moreover, the "easily synthesizable small molecule" in essence of DOBs can be their opportunity in competition.

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Supporting Information Available: Synthesis details and additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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