New Antioxidant Phenolic Glucosides from *Viburnum dilatatum*

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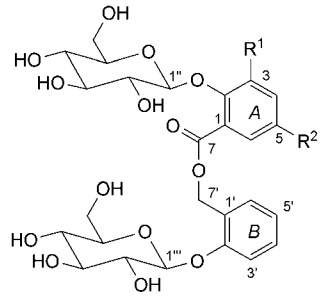
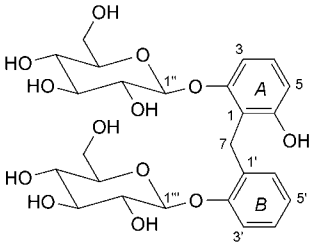
Chemical investigation of the fruits of *Viburnum dilatatum* THUNB. resulted in the isolation and characterization of four new phenolic glycosides, jiamiziosides A–D (**1**–**4**), together with five known compounds. Their structures were established by spectroscopic means and by comparison with the literature values. The antioxidant activities of the new isolates were determined against 2,2-diphenyl-1-picrylhydrazyl (=2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazinyl; DPPH) and superoxide radicals. Among the compounds tested, jiamizioside C (**3**) possesses the most potent inhibitory scavenging effect on DPPH and superoxide radicals with IC_{50} values of 16.8 and 17.8 μM, respectively.

Introduction. – *Viburnum dilatatum* THUNB. (jiamizi in Chinese, gamazumi in Japanese, and snowball tree in English), a wild deciduous low tree belonging to Caprifoliaceae, is found widely in the hills of northern China and Japan. Its small fruits which ripen in autumn have a crimson color and are edible [1][2]. Previous studies showed that the squeezed extract of *V. dilatatum* fruits had strong antioxidant and radical-scavenging activities as measured by the XYZ-dish method [3] and electron-spin resonance [4]. The pharmacological activities of the crude fruit extract have been examined *in vivo*, and a preventive effect on oxidative damages was found in rats subjected to stress [4][5] and in streptozotocin-induced diabetic rats [6]. Previous phytochemical investigations on this plant showed the presence of anthocyanins, phenolic compounds, triterpenoids, and norisoprenoids [2][5][7–10]. The previous studies revealed that phenolic compounds were the main constituents accounting for antioxidant and antiradical activities of the *V. dilatatum* fruits [3–6]. Phenolic compounds are known to possess anti-inflammatory and antioxidant activities [11–13]. This article deals with the chemical investigation of *V. dilatatum* fruits, which resulted in the isolation and characterization of four new minor phenolic glycosides **1**–**4**, along with five known compounds, lanceoloside A, quercetin, quercetin 3-(*β*-glucopyranoside), 5-*O*-caffeoyl-4-*O*-methylquinic acid, and 5-*O*-caffeoylquinic acid. In the present study, we also investigated the antioxidant activities of the new compounds **1**–**4**. Among them, jiamizioside C (**3**) showed most potent antioxidant activities *in vitro*.

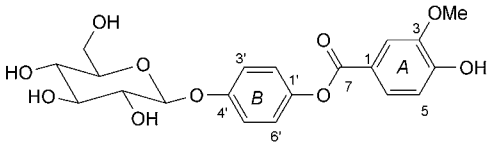
Results and Discussion. – The BuOH-soluble fractions of the MeOH extract of the fruits of *V. dilatatum* THUNB. were subject to repeated column chromatography to afford the new compounds **1**–**4**.



	R^1	R^2
1[1])	OH	H
2[1])	H	OH



3[1])



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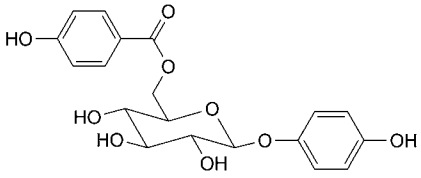

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Compound **1** was obtained as a yellowish oil. Its HR-FT-ICR-MS exhibited the molecular-ion peak at *m/z* 585.1817 ($[M+H]^+$), indicating that the molecular formula is $C_{26}H_{32}O_{15}$. The IR absorption at 3445 cm^{-1} for OH groups, and 1625, 1596, and 1463 cm^{-1} for aromatic rings suggested that compound **1** was a phenolic compound, and the UV absorption at 210, 254, 280, and 305 nm also indicated the presence of one aromatic moiety or more. The sugars obtained by the acidic hydrolysis of **1** were identified as glucose by co-TLC on SiO_2. The structure of **1** was elucidated by analysis of its ^{1}H- and ^{13}C-NMR (*Table 1*), ^{1}H,^{1}H-COSY, HMQC, and HMBC data and comparison with known compounds as a new phenolic glycoside, [2-(β-D-glucopyranosyloxy)phenyl]methyl 2-(β-D-glucopyranosyloxy)-3-hydroxybenzoate, and was assigned the trivial name jiamizioside A.

The ^{13}C-NMR spectrum of **1** showed the presence of 12 signals for two terminal β-glucopyranose moieties, with the remaining 14 signals representing two aromatic rings, one ester C=O group, and one oxygenated CH_2 group. The ^{1}H-NMR spectrum showed the presence of seven aromatic H-atoms, and its ^{1}H,^{1}H-COSY plot suggested the presence of two series of aromatic H-atoms, one with three and the other one with four aromatic H-atoms. From these ^{1}H- and ^{13}C-NMR data, together with those of an HMQC experiment, compound **1** was presumed to be an ester adduct of a salicin (=2-(hydroxymethyl)phenyl β-D-glucopyranoside) and an *O*-glucosylsalicylic acid (=2-(glucosyloxy)benzoic acid) moiety, similar to henryoside isolated from *Viburnum henryi* [14]. Ring *A* and *B* of **1** were inferred to be 1,2,3-trisubstituted (δ(H) 6.57 (*dd*, *J* = 8.2, 2.1 Hz, H–C(4)), 7.19 (*t*, *J* = 8.2 Hz, H–C(5)), and 6.66 (*dd*, *J* = 8.2, 2.1 Hz, H–C(6))) and 1,2-disubstituted (δ(H) 7.14 (*dd*, *J* = 8.0, 2.3 Hz, H–C(3′)), 7.27 (*td*, *J* = 8.0, 2.3 Hz, H–C(4′)), 7.03 (*td*, *J* = 8.0, 2.3 Hz, H–C(5′)), and 7.52 (*dd*, *J* = 8.0, 2.3 Hz, H–C(6′))) from the analysis of the ^{1}H,^{1}H-coupling pattern and the ^{1}H,^{1}H-COSY plot. The arrangement of the substituents of ring *A*

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

Table 1. *^{1}H- and ^{13}C-NMR Data* ((D_6)DMSO) *of Compounds* **1** *and* **2**[1]). δ in ppm, J in Hz.

	1		**2**	
	δ(C)[a])[b])	δ(H)[c])	δ(C)[a])[b])	δ(H)[c])
C(1)	110.0 (*s*)		115.0 (*s*)	
C(2)	156.1 (*s*)		152.5 (*s*)	
C(3) or H–C(3)	155.0 (*s*)		119.2 (*d*)	7.13 (*d*, *J* = 8.3)
H–C(4)	112.7 (*d*)	6.57 (*dd*, *J* = 8.2, 2.1)	120.8 (*d*)	6.93 (*dd*, *J* = 8.3, 2.3)
H–C(5) or C(5)	128.2 (*d*)	7.19 (*t*, *J* = 8.2)	149.7 (*s*)	
H–C(6)	106.0 (*d*)	6.66 (*dd*, *J* = 8.2, 2.1)	116.6 (*d*)	7.09 (*d*, *J* = 2.3)
C(7)	166.5 (*s*)		166.3 (*s*)	
C(1′)	126.0 (*s*)		125.5 (*s*)	
C(2′)	155.9 (*s*)		155.3 (*s*)	
H–C(3′)	115.2 (*d*)	7.14 (*dd*, *J* = 8.0, 2.3)	115.3 (*d*)	7.17 (*dd*, *J* = 8.0, 2.2)
H–C(4′)	129.2 (*d*)	7.27 (*td*, *J* = 8.0, 2.3)	129.6 (*d*)	7.29 (*td*, *J* = 8.0, 2.2)
H–C(5′)	122.4 (*d*)	7.03 (*td*, *J* = 8.0, 2.3)	122.3 (*d*)	7.03 (*td*, *J* = 8.0, 2.2)
H–C(6′)	131.7 (*d*)	7.52 (*dd*, *J* = 8.0, 2.3)	128.9 (*d*)	7.49 (*dd*, *J* = 8.0, 2.2)
CH_2(7′)	62.0 (*t*)	5.40 (*s*)	62.2 (*t*)	5.44 (*s*)
2-GlcO:				
H–C(1″)	100.8 (*d*)	4.87 (*d*, *J* = 7.5)	101.4 (*d*)	4.86 (*d*, *J* = 7.5)
H–C(2″)	73.9 (*d*)	3.23–3.28 (*m*)	73.8 (*d*)	3.25–3.29 (*m*)
H–C(3″)	77.7 (*d*)	3.30–3.34 (*m*)	77.6 (*d*)	3.33–3.37 (*m*)
H–C(4″)	70.2 (*d*)	3.17–3.22 (*m*)	70.2 (*d*)	3.15–3.19 (*m*)
H–C(5″)	77.1 (*d*)	3.28–3.32 (*m*)	76.9 (*d*)	3.30–3.35 (*m*)
CH_2(6″)	61.3 (*t*)	3.68–3.71, 3.45–3.49 (2*m*)	61.2 (*t*)	3.68–3.72, 3.42–3.47 (2*m*)
2′-GlcO:				
H–C(1‴)	101.6 (*d*)	4.92 (*d*, *J* = 7.5)	102.7 (*d*)	4.94 (*d*, *J* = 7.5)
H–C(2‴)	73.9 (*d*)	3.25–3.29 (*m*)	73.9 (*d*)	3.27–3.33 (*m*)
H–C(3‴)	77.7 (*d*)	3.35–3.40 (*m*)	77.6 (*d*)	3.32–3.36 (*m*)
H–C(4‴)	70.3 (*d*)	3.16–3.20 (*m*)	70.2 (*d*)	3.18–3.23 (*m*)
H–C(5‴)	77.4 (*d*)	3.30–3.34 (*m*)	77.0 (*d*)	3.28–3.32 (*m*)
CH_2(6‴)	61.3 (*t*)	3.68–3.72, 3.47–3.52 (2*m*)	61.2 (*t*)	3.70–3.76, 3.46–3.50 (2*m*)

[a]) Recorded at 125 MHz. [b]) Multiplicities inferred from DEPT and HMQC experiments. [c]) Recorded at 500 MHz.

was determined by a HMBC experiment. Thus, the location of the OH group at C(3) was deduced from the correlations H–C(5) (*t*)/C(1) and H–C(6)/C(7) in the HMBC plot. H–C(6) also showed a cross-peak with the oxygenated C(2) which was correlated with one of the two anomeric H-atoms, confirming that the glucosyloxy moiety at ring *A* was located at C(2), as in henryoside [14]. HMBC Cross-peaks from another anomeric H-atom signal at δ(H) 4.92 (*d*, *J* = 7.5 Hz) and the CH_2 signal at δ(H) 5.40 (*s*, CH_2(7′)) to the C-atom at δ(C) 155.9 (*s*, C(2′)) indicated that the glucosyloxy moiety at ring *B* was attached to C(2′). The configurations of the glycosidic linkages of the glucopyranoside moieties in **1** were determined to be β on the basis of the *J* values (δ(H) 4.92 (*d*, *J* = 7.5 Hz) and 4.87 (*d*, *J* = 7.5 Hz)) of the two anomeric H-atoms.

Compound **2**, a yellowish oil, had a molecular formula $C_{26}H_{32}O_{15}$ (HR-FT-ICR-MS: *m*/*z* 585.1814 ($[M+H]^+$)), and should, therefore, be an isomer of **1**. Analysis of the ^{1}H- and ^{13}C-NMR (*Table 1*), HMQC and HMBC data indicated that the structure of ring *B* was the same as that of **1**, and further HMBC and NOESY data allowed to elucidate the

structure of **2** as [2-(β-D-glucopyranosyloxy)phenyl]methyl 2-(β-D-glucopyranosyloxy)-5-hydroxybenzoate, which was named jiamizioside B.

The ^{1}H-NMR spectrum of **2** displayed a *ABX*-type coupling pattern, indicating that ring *A* was 1,3,4-trisubstituted (δ(H) 7.13 (*d*, *J* = 8.3 Hz, H−C(3)), 6.93 (*dd*, *J* = 8.3, 2.3 Hz, H−C(4)), and 7.09 (*d*, *J* = 2.3 Hz, H−C(6))). No long-range correlation was observed from H−C(4) (*dd*) to C(1), indicating that C(2) and C(5) in ring *A* were oxygenated. This inference was confirmed by the cross-peak H−C(6) (*d*)/C(7)=O. The glucosyloxy moiety of ring *A* was deduced to be located at C(2), which was derived from that of **1** isolated from the same plant, on the assumption that the two compouds are biogenetically related. This assignment was confirmed by a HMBC cross-peak H−C(6)/C(2), C(2) being correlated with the anomeric H-atom signal at δ(H) 4.86 (*d*, *J* = 7.5 Hz), and by a NOESY correlation from H−C(3) to the anomeric H-atom.

Compound **3** was obtained as a yellowish oil. The HR-FT-ICR-MS exhibited the molecular-ion peak at *m*/*z* 563.1738 ($[M+Na]^+$), corresponding to the molecular formula $C_{25}H_{32}O_{13}$. Its ^{13}C-NMR spectrum (*Table 2*) showed the presence of 12 signals for two terminal β-glucopyranose moieties, and 12 signals representing two aromatic rings, as in compounds **1** and **2**. The sugars obtained by acidic hydrolysis of **3** were identified by co-TLC, and their structures confirmed as glucose. The ^{1}H- and ^{13}C-NMR data of **3** (*Table 2*) and detailed analysis of 2D-NMR spectra allowed to elucidated the structure of **3** as 2-{[2-(β-D-glucopyranosyloxy)-6-hydroxyphenyl]methyl}phenyl β-D-glucopyranoside, and was given the common name jiamizioside C.

The presence of a benzylic CH_2 signal which was not oxygenated and the absence of ester C=O and oxygenated CH_2 signals in the ^{13}C-NMR spectrum indicated that **3** was a diphenylmethan derivative. The linkage between the two benzene rings *via* the benzylic CH_2(7) was confirmed by the HMBC cross-peaks δ(H) 4.03 (*d*, *J* = 16.0 Hz, H_a−C(7)) and 3.85 (*d*, *J* = 16.0 Hz, H_b−C(7))/δ(C) 115.4 (*s*, C(1)) of ring *A* and 130.2 (*s*, C(1′)) of ring *B*. Since only one aromatic H-atom signal at δ(H) 6.66 (*dd*, *J* = 8.2, 2.2 Hz, H−C(6′)) showed a cross-peak to the benzylic C-atom at δ(C) 22.8 (*t*, C(7)) in the HMBC plot of **3**, C(2) and C(6) in ring *A* and C(2′) in ring *B* were inferred to be oxygenated. This inference was confirmed by the HMBC cross-peaks CH_2(7)/C(2), C(6), and C(2′). The configurations of the glycosidic linkages of the glucopyranoside moieties in **3** were determined to be β on the basis of the *J* values (δ(H) 4.74 (*d*, *J* = 7.6 Hz) and 4.84 (*d*, *J* = 7.6 Hz)) of the two anomeric H-atoms.

Compound **4** was obtained as a yellowish oil. The HR-FT-ICR-MS of **4** exhibited a molecular-ion peak at *m*/*z* 423.1289 ($[M+H]^+$), indicating that the molecular formula is $C_{20}H_{22}O_{10}$. Its ^{13}C-NMR spectrum (*Table 2*) showed the presence of six signals for a terminal β-glucopyranose moiety, with the remaining 14 signals representing two aromatic rings, one ester C=O group, and one MeO group. The sugar obtained by the acidic hydrolysis of **4** was identified by co-TLC, and its structure confirmed as glucose. The structure of **4** was elucidated as 4-(β-D-glucopyranosyloxy)phenyl 4-hydroxy-3-methoxybenzoate by further spectroscopic data, and was given the common name jiamizioside D.

The presence of C=O and absence of the CH_2 C-atom in the ^{13}C-NMR spectrum of **4** indicated that **4** was a phenyl benzoate derivative. The locations of the glucosyloxy moiety and the OH and MeO groups were deduced from the detailed analysis of the coupling patterns and the HMBC cross-peaks. The ^{1}H-NMR and ^{1}H,^{1}H-COSY plots of **4** revealed that ring *A* was 1,3,4-trisubstituted (δ(H) 7.42 (*d*, *J* = 2.1 Hz, H−C(2)), 6.88 (*d*, *J* = 8.3 Hz, H−C(5)), and 7.48 (*dd*, *J* = 8.3, 2.1 Hz, H−C(6))), and ring *B* was 1,4-disubstituted (δ(H) 6.55 (*dd*, *J* = 8.2, 2.2 Hz, H−C(2′,6′)) and 6.85 (*dd*, *J* = 8.2, 2.2 Hz,

Table 2. *^{1}H- and ^{13}C-NMR Data* ((D_6)DMSO) *of Compounds* **3** *and* **4**[1]). δ in ppm, J in Hz.

	3		**4**	
	δ(C)[a])[b])	δ(H)[c])	δ(C)[a])[b])	δ(H)[c])
C(1)	115.4 (*s*)		112.5 (*s*)	
C(2) or H–C(2)	157.5 (*s*)		112.5 (*d*)	7.42 (*d*, J = 2.1)
H–C(3) or C(3)	106.1 (*d*)	6.62 (*dd*, J = 8.1, 2.3)	147.5 (*s*)	
H–C(4) or C(4)	127.5 (*d*)	7.03 (*t*, J = 8.1)	152.1 (*s*)	
H–C(5)	109.3 (*d*)	6.54 (*dd*, J = 8.1, 2.3)	115.3 (*d*)	6.88 (*d*, J = 8.3)
C(6) or H–C(6)	156.5 (*s*)		123.5 (*d*)	7.48 (*dd*, J = 8.3, 2.1)
CH_2(7) or C(7)	22.8 (*t*)	4.03 (*d*, J = 16.0, H_a), 3.85 (*d*, J = 16.0, H_b)	165.4 (*s*)	
C(1′)	130.2 (*s*)		152.8 (*s*)	
C(2′) or H–C(2′)	155.5 (*s*)		115.2 (*d*)	6.55 (*dd*, J = 8.2, 2.2)
H–C(3′)	114.6 (*d*)	7.03–7.07 (overlapped)	117.3 (*d*)	6.85 (*dd*, J = 8.2, 2.2)
H–C(4′) or C(4′)	126.5 (*d*)	7.03–7.07 (overlapped)	150.0 (*s*)	
H–C(5′)	121.6 (*d*)	6.76 (*td*, J = 8.0, 2.2)	117.3 (*d*)	6.85 (*dd*, J = 8.2, 2.2)
H–C(6′)	128.5 (*d*)	6.66 (*dd*, J = 8.0, 2.2)	115.2 (*d*)	6.55 (*dd*, J = 8.2, 2.2)
MeO			55.5 (*q*)	3.79 (*s*)
GlcO:				
H–C(1″)	101.5 (*d*)	4.74 (*d*, J = 7.6)	101.2 (*d*)	4.72 (*d*, J = 7.6)
H–C(2″)	73.6 (*d*)	3.27–3.31 (*m*)	73.1 (*d*)	3.28–3.33 (*m*)
H–C(3″)	77.4 (*d*)	3.35–3.39 (*m*)	76.3 (*d*)	3.32–3.37 (*m*)
H–C(4″)	70.1 (*d*)	3.15–3.20 (*m*)	70.1 (*d*)	3.16–3.21 (*m*)
H–C(5″)	76.9 (*d*)	3.26–3.31 (*m*)	76.3 (*d*)	3.31–3.35 (*m*)
CH_2(6″)	61.0 (*t*)	3.68–3.72, 3.44–3.48 (2*m*)	63.8 (*t*)	3.70–3.74, 3.45–3.49 (2*m*)
GlcO:				
H–C(1‴)	101.1 (*d*)	4.84, J = 7.6)		
H–C(2‴)	73.6 (*d*)	3.28–3.33 (*m*)		
H–C(3‴)	77.4 (*d*)	3.34–3.38 (*m*)		
H–C(4‴)	70.1 (*d*)	3.15–3.19 (*m*)		
H–C(5‴)	76.9 (*d*)	3.27–3.31 (*m*)		
CH_2(6‴)	61.0 (*t*)	3.67–3.72, 3.42–3.47 (2*m*)		

[a]) Recorded at 125 MHz. [b]) Multiplicities inferred from DEPT and HMQC experiments. [c]) Recorded at 500 MHz.

H–C(3′,5′))). The *d* of H–C(2) and the *dd* of H–C(6) showed HMBC cross-peaks with the C=O signal, revealing that the C=O was attached at ring *A*. No HMBC cross-peak was observed at δ(H) 7.48 (*dd*, J = 8.3, 2.1 Hz, H–C(6))/δ(C) 147.5 (*s*, C(3)), the latter being correlated with the MeO signal at δ(H) 3.79 (*s*, MeO), indicating that the MeO group was located at C(3). The observation of HMBC cross-peaks from the anomeric H-atom at δ(H) 4.72 (*d*, J = 7.6 Hz) and four aromatic H-atoms at δ(H) 6.55 (*dd*, J = 8.2, 2.2 Hz, H–C(2′,6′)) and 6.85 (*dd*, J = 8.2, 2.2 Hz, H–C(3′,5′)) to the C-atom at δ(C) 150.0 (*s*, C(4′)) indicated that the glucosyloxy moiety was located at C(4′) of ring *B*. The configuration of the glycosidic linkage of the glucopyranoside moiety of **4** was determined to be β on the basis of the J value (δ(H) 4.72 (*d*, J = 7.6 Hz)) of the anomeric H-atom.

Furthermore, five known compounds, lanceoloside A (**5**), quercetin, quercetin 3-(β-glucopyranoside), 5-*O*-caffeoyl-4-*O*-methylquinic acid, and 5-*O*-caffeoylquinic acid were identified by comparison of their spectroscopic data with those reported in

[2][15–17], among which lanceoloside A (**5**) was isolated for the first time from this plant.

All the new compounds **1**–**4**, which are all >95% pure, were tested for their antioxidant scavenging effects on DPPH (=2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazinyl) and superoxide radicals. All new compounds tested showed antioxidant activities (*Table 3*), among which jiamizioside C (**3**) exhibited potent antioxidant scavenging activities against DPPH and superoxide radicals, with IC_{50} values of 16.8 and 17.8 μM; α-tocopherol and butylated hydroxytoluene (BHT) were used as positive controls.

Table 3. *DPPH and Superoxide Radical Scavenging Activities* (IC_{50}) *of Compounds* **1**–**4**

	DPPH scavenging activity IC_{50} [μM][a])	Superoxide scavenging activity IC_{50} [μM][a])
1	46.5 ± 5.6	79.3 ± 7.1
2	83.8 ± 6.4	74.5 ± 3.9
3	16.8 ± 1.2	17.8 ± 2.0
4	58.6 ± 6.2	69.7 ± 2.4
α-Tocopherol[b])	9.9 ± 1.5	8.4 ± 0.7
BHT[b])	15.2 ± 1.3	14.2 ± 1.5

[a]) IC_{50} Values were calculated from regression lines by using six different concentrations in triplicate. [b]) Positive control (BHT = 2,6-di(*tert*-butyl)-4-methylphenol).

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Experimental Part

General. TLC: precoated plates (silica gel (SiO_2) *60 F254*; *Merck*) of 0.25 mm thickness. Column chromatography (CC): SiO_2 (200–300 mesh), *Sephadex LH-20* (*Amersham*). Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Jasco UV-2200* UV/VIS recording spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Avatar-360* FT-IR spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AVANCE-DMX-500* spectrometer at 25°; at 500 (^{1}H) and 125(^{13}C) MHz; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *Bruker Apex-III* spectrometer for HR-FT-ICR-MS; *Bruker Esquire-3000^{plus}* spectrometer for ESI-MS; in *m*/*z*.

Plant Material. The fruits of *V. dilatatum* THUNB. were collected in Linan County, Zhejiang Province, P. R. China, in September 2006, and identified by Prof. *Changxi Zhang*, Jinhua Medical College, Jinhua, P. R. China. A voucher specimen (zju7146) was deposited with the College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, P. R. China.

Extraction and Isolation. The shade-dried fruits (130 kg) of *V. dilatatum* THUNB. were extracted three times at r.t. with MeOH (3 × 60 l). The extracts were concentrated to afford a gummy residue (6 kg). This residue was suspended in H_2O (10 l), and extracted successively with petroleum ether (3 × 10 l), AcOEt (3 × 10 l), and BuOH (3 × 10 l). The BuOH extract (989 g) was adsorbed onto SiO_2 (600 g) and subjected to CC (SiO_2 (20 × 120 cm, 6 kg; 200–300 mesh), $CHCl_3$/MeOH gradient): *Fractions 1–36. Fr. 6* (24 g) was subjected to CC (SiO_2 (4 × 30 cm, 300 g; 200–300 mesh), $CHCl_3$/MeOH gradient). Quercetin (23.5 mg). *Fr. 14* (31 g) was subjected to CC (SiO_2 (4 × 60 cm, 600 g; 200–300 mesh), $CHCl_3$/MeOH gradient): *Fr. 14.A–14.G. Fr. 14.B* (4 g) was subjected to CC (*Sephadex LH-20* (4 × 150 cm, 300 g; *Amersham*), MeOH): **5** (11.0 mg) and quercetin 3-(β-glucopyranoside) (15.6 mg). *Fr. 14.F* (6 g) was applied to CC (*Sephadex LH-20*, MeOH): **1** (8.9 mg), **2** (11.2 mg), **3** (9.7 mg), and **4** (11.9 mg). *Fr. 33*

(17 g) was applied to CC (*Sephadex LH-20*, MeOH): 5-*O*-caffeoyl-4-*O*-methylquinic acid (13.6 mg) and 5-*O*-caffeoylquinic acid (14.1 mg).

Acidic Hydrolysis of Compounds **1**–**4**. Compounds **1**–**4** (5 mg) were each refluxed at 100° with 10% aq. HCl soln. for 3 h. The aq. hydrolyzate was neutralized with $AgCO_3$ and concentrated. Sugars were identified as glucose by co-TLC (SiO_2, AcOEt/i-PrOH/Me_2CO/H_2O 20:10:7:6). The spots on the plate were visualized by spraying with an anisaldehyde/H_2SO_4 soln.

Jiamizioside A (= *[2-(β-*D*-Glucopyranosyloxy)phenyl]methyl 2-(β-*D*-Glucopyranosyloxy)-3-hydroxybenzoate* **1**): Yellowish oil. $[\alpha]_D^{20} = +37$ ($c = 0.001$, MeOH). UV (MeOH): 210 (4.41), 254 (3.58), 280 (3.20), 305 (3.03). IR: 3445, 1720, 1625, 1596, 1463, 1382, 1224, 1044, 756. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 585 ($[M+H]^+$), 583 ($[M-H]^-$). HR-FT-ICR-MS: 585.1817 ($[M+H]^+$, $C_{26}H_{33}O_{15}^+$; calc. 585.1819).

Jiamizioside B (= *[2-(β-*D*-Glucopyranosyloxy)phenyl]methyl 2-(β-*D*-Glucopyranosyloxy)-5-hydroxybenzoate* **2**): Yellowish oil. $[\alpha]_D^{20} = +41$ ($c = 0.001$, MeOH). UV (MeOH): 210 (4.25), 254 (3.51), 283 (3.36), 305 (3.12). IR: 3450, 1720, 1627, 1595, 1466, 1380, 1227, 1043, 753. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 585 ($[M+H]^+$), 583 ($[M-H]^-$). HR-FT-ICR-MS: 585.1814 ($[M+H]^+$, $C_{26}H_{33}O_{15}^+$; calc. 585.1819).

Jiamizioside C (= *{[2-(β-*D*-Glucopyranosyloxy)-6-hydroxyphenyl]methyl}phenyl β-*D*-Glucopyranoside* **3**): Yellowish oil. $[\alpha]_D^{20} = +30$ ($c = 0.001$, MeOH). UV (MeOH): 213 (4.63), 250 (3.35), 280 (3.77). IR: 3515, 2915, 1620, 1593, 1506, 1451, 1379, 1311, 1136, 1055, 958, 752. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 563 ($[M+Na]^+$), 539 ($[M-H]^-$). HR-FT-ICR-MS: 563.1738 ($[M+Na]^+$, $C_{25}H_{32}NaO_{13}^+$; calc. 563.1741).

Jiamizioside D (= *4-(β-*D*-Glucopyranosyloxy)phenyl 4-Hydroxy-3-methoxybenzoate* **4**): Yellowish oil. $[\alpha]_D^{20} = +34$ ($c = 0.001$, MeOH). UV (MeOH): 210 (4.78), 254 (3.49), 280 (3.25). IR: 3450, 1740, 1623, 1596, 1460, 1380, 1231, 1042, 841, 755. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 423 ($[M+H]^+$), 421 ($[M-H]^-$). HR-FT-ICR-MS: 423.1289 ($[M+H]^+$, $C_{20}H_{23}O_{10}^+$; calc. 423.1291).

DPPH Radical Scavenging Assay. The DPPH radical scavenging activity was measured according to the procedure reported previously [18]. The purities of the compounds used for the assay were >95% as checked by HPLC and ^{1}H-NMR. In brief, 100 µl test samples at different concentrations in MeOH and $8.0 \cdot 10^{-5}$ M DPPH in MeOH (300 µl) were added to a 96-well microtiter plate. The plate was shaken for 1 min on a plate shaker, and incubated for 30 min at r.t. in the dark. After incubation, the absorbance was recorded at 517 nm. The tested samples at different concentrations without DPPH soln. were used as a blank control to eliminate the influence of sample color. α-Tocopherol and BHT were used as a positive control, and DPPH soln. in MeOH served as a negative control. All tests were independently performed in triplicate, and the definition of IC_{50} values of the tested compounds is concentration required to scavenge 50% of DPPH radicals. The DPPH radical scavenging activity was calculated according to the following equation: DPPH radical scavenging activity $[\%] = [A_C - (A_S - A_B)/A_C] \times 100$, in which A_B is the absorbance of the blank control, A_C the absorbance of the negative control, and A_S the absorbance of the sample in DPPH soln.

Superoxide (O_2^-) *Radical Scavenging Assay*. Superoxide was generated by xanthine/xanthine oxidase, and measured by the nitroblue tetrazolium (NBT) reduction method [19][20]. A soln. of the sample in 10% DMSO was added to 0.1 ml of a mixture of 0.2 mM hypoxanthine and 0.1 mM NBT in 50 mM potassium phosphate buffer (pH 7.5) containing 0.05 mM EDTA in a 96-well flat-bottom microplate. Xanthine oxidase (0.1 ml), diluted with 50 mM phosphate buffer (pH 7.5) to 0.08 U/ml, was added to the mixture, and the microplate was incubated at 37° for 20 min. The addition of 0.1 ml of 1N HCl to the mixture terminated the reaction, and the absorbance at 540 nm was measured. The IC_{50} value was defined as the concentration of the sample required to reduce the NBT by 50%.

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