Stilbenoids and Phenols in *Acanthopanax brachypus*

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Three new stilbenoids, including α-(3′-*O*-β-D-glucopyranosyl-5′-methoxyphenyl)-2-methoxy-3-methylbenzofuran (**1**), 4-methyl-(*E*)-resveratrol 3-(2″-*p*-hydroxybenzoyl)-*O*-β-D-glucopyranoside (**2**), and 5-*O*-methyl-(*E*)-resveratrol 3-(6″-acetyl)-*O*-β-D-glucopyranoside (**3**), together with six known stilbenoids and phenols, acetovanillone 1-(6′-vanilloyl)-*O*-β-D-glucopyranoside, eugenyl-*O*-β-D-glucopyranoside, α-(3′-hydroxy-5′-methoxy-2′-methylphenyl)-2-hydroxybenzofuran, α-(3′-hydroxy-5′-methoxyphenyl)-2-hydroxybenzofuran, pinosilvin 3-*O*-β-D-glucopyranoside, and (*E*)-resveratrol 3-(6″-galloyl)-*O*-β-D-glucopyranoside were isolated from the EtOH extract of the stem bark of *Acanthopanax brachypus*. Their structures were determined by spectral analysis including extensive 2D-NMR spectral analyses. Compounds **2** and **3** exhibited weak cytotoxicity against human tumor A549 cell line (IC_{50} values of 4.87 and 5.63 μM, resp.).

Introduction. – *Acanthopanax brachypus* (Araliaceae) is distributed in a narrow geographical area, mostly in the loess plateau of the northwest of P. R. China [1]. As a peculiar folk medicinal plant, the root and stem bark are efficient in invigorating the liver and kidney, replenishing the vital essence, soothing the nerves, dispelling rheumatism, and strengthening tendons and bones [2]. Meanwhile, the rhizome extract has been used for the treatment of neurasthenia, male sexual dysfunction, secondary hypertension, hypotension, and leucopenia disease, as well as for cancer prevention and as anticancer agent [3]. Previous investigations have resulted in the isolation of polysaccharides, organic acids, flavonoids, and steroidal and triterpenoid saponins from *A. brachypus* [4–6]. Encouraged by the notable pharmacological properties of *A. brachypus*, we have reinvestigated the constituents of the stem barks and isolated three new stilbenoids, **1**, **2** and **3**, together with six known stilbenoids and phenols (*Fig. 1*). This article deals with the isolation and structure elucidation of these compounds, as well as cytotoxicities of three new compounds against A549 cell line.

Results and Discussion. – Compound **1** was obtained as white amorphous powder, with a molecular formula of $C_{23}H_{26}O_9$ deduced from the $[M + Na]^+$ ion peak at *m/z* 469.1481 (calc. 469.1475) in the HR-ESI-MS and supported by the ^{13}C-NMR data (*Table*). The IR spectrum of **1** exhibited absorptions at 3425 (OH), 1601 and 1516 (aromatic ring), and 964 cm^{-1} (C=C). The UV spectrum of **1** indicated the absorption bands at 218 (4.40), 296 (3.76), 306 (3.68), and 324 (3.37) nm, attributed to a conjugated aromatic system.

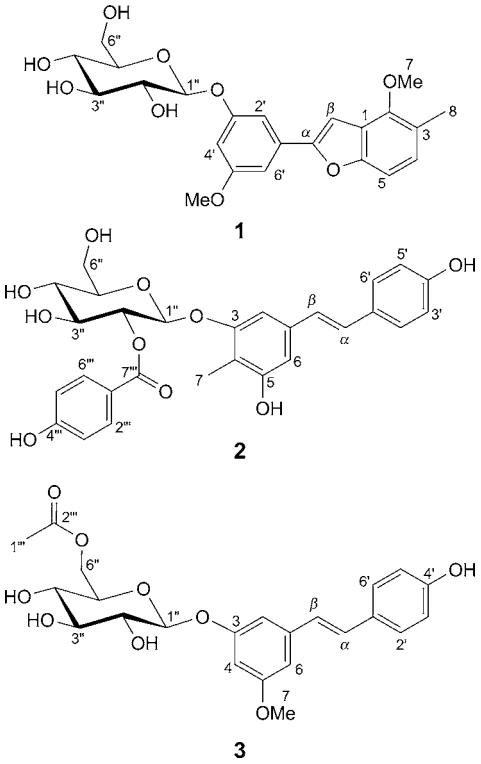

Fig. 1. *Structures of compounds* **1**–**3**

The ^{1}H-NMR spectrum showed the presence of three H-atoms of a 1,3,5-trisubstituted aromatic ring (δ(H) 6.91 (*dd*, $J = 2.0, 1.5$), 6.42 (*dd*, $J = 2.0, 2.0$) and 6.95 (*dd*, $J = 2.0, 1.5$)), two protons of a 1,2,3,4-tetrasubstituted aromatic ring (δ(H) 7.06 (*d*, $J = 7.8$) and 6.98 (*dd*, $J = 7.8, 0.8$)), one 2,4,5-trisubstituted furan-ring H-atom (δ(H) 7.10 (*d*, $J = 0.8$)), two MeO groups (δ(H) 4.01 and 3.86 (*s*, each 3 H)), one Me (δ(H) 2.31 (*s*, 3 H)), and a glucopyranosyl moiety (δ(H) 4.97 (*d*, $J = 7.4$, 1 H), 3.38–3.69 (*m*, 4 H), 3.92 (*dd*, $J = 1.8, 12.1$, 1 H), 3.74 (*dd*, $J = 6.1, 12.1$, 1 H)). The ^{13}C-NMR (DEPT) spectra of **1** showed 23 C-atom signals, including 14 aromatic C-atoms (δ(C) 121.1 (C(1)), 159.9 (C(2)), 117.6 (C(3)), 127.4 (C(4)), 105.3 (C(5)), 154.2 (C(6)), 127.2 (C(1′)), 104.4 (C(2′)), 158.8 (C(3′), 102.1 (C(4′)), 161.5 (C(5′)), 103.4 (C(6′)), 157.2 (C(α)), 100.1 (C(β))), two MeO C-atoms (δ(C) 56.2 (C(7′)) and 59.8 (C(7))), one Me C-atom (δ(C) 15.7 (C(8))), and a glucopyranosyl moiety (δ(C) 102.6 (C(1″)), 74.6 (C(2″)), 75.9 (C(3″)), 71.3 (C(4″)), 76.9 (C(5″)), 62.5 (C(6″))) (*Table*). The long range homoallylic ^{4}J coupling of *ca.* 0.8 Hz was observed between H–C(5) (δ(H) 6.98 (*dd*, $J = 7.8, 0.8$)) and the isolated H–C(β) (δ(H) 7.10 (*d*, $J = 0.8$)) of the furan ring, indicating the presence of benzofuran ring [7][8]. In the HMBC spectrum, H–C(β) (δ(H) 7.10 (*d*, $J = 0.8$)) shows ^{2}J(C,H) to C(α) (δ(C) 157.2) and C(1) (δ(C) 121.1) as well as ^{3}J(C,H) to C(2) (δ(C) 159.9), C(6) (δ(C) 154.2), and C(1′) (δ(C) 127.2), indicating the central annulated heterocyclic moiety of α-phenylbenzofuran skeleton.

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; CD_3OD) *of* **1**, **2**, *and* **3**. δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		121.1 (C)		139.5 (C)		141.3 (C)
2		159.9 (C)	6.63 (br. *s*)	108.2 (CH)	6.65 (br. *s*)	108.1 (CH)
3		117.6 (C)		158.7 (C)		159.4 (C)
4	7.06 (*d*, *J* = 7.8)	127.4 (CH)		109.5 (C)	6.41 (br. *s*)	104.0 (CH)
5	6.98 (*dd*, *J* = 7.8, 0.8)	105.3 (CH)		159.2 (C)		162.3 (C)
6		154.2 (C)	6.70 (br. *s*)	106.7 (CH)	6.71 (br. *s*)	106.9 (CH)
7	4.01 (*s*)	59.8 (Me)	1.99 (*s*)	8.7 (Me)	3.81 (*s*)	55.9 (Me)
8	2.31 (*s*)	15.7 (Me)				
1′		127.2 (C)		130.9 (C)		131.2 (C)
2′	6.91 (*dd*, *J* = 2.0, 1.5)	104.4 (CH)	7.34 (*d*, *J* = 8.8)	128.8 (CH)	7.35 (*d*, *J* = 8.5)	128.9 (CH)
3′		158.8 (C)	6.79 (*d*, *J* = 8.8)	115.9 (CH)	6.80 (*d*, *J* = 8.5)	116.5 (CH)
4′	6.42 (*dd*, *J* = 2.0, 2.0)	102.1 (CH)		157.9 (C)		158.4 (C)
5′		161.5 (C)	6.79 (*d*, *J* = 8.8)	115.9 (CH)	6.80 (*d*, *J* = 8.5)	116.5 (CH)
6′	6.95 (*dd*, *J* = 2.0, 1.5)	103.4 (CH)	7.34 (*d*, *J* = 8.8)	128.8 (CH)	7.35 (*d*, *J* = 8.5)	128.9 (CH)
7′	3.86 (*s*)	56.2 (Me)				
α		157.2 (C)	7.03 (*d*, *J* = 16.5)	129.7 (CH)	7.01 (*d*, *J* = 16.2)	130.2 (CH)
β	7.10 (*d*, *J* = 0.8)	100.1 (CH)	6.77 (*d*, *J* = 16.5)	127.1 (CH)	6.78 (*d*, *J* = 16.2)	126.5 (CH)
1″	4.97 (*d*, *J* = 7.4)	102.6 (CH)	4.94 (*d*, *J* = 7.2)	101.3 (CH)	4.95 (*d*, *J* = 7.5)	102.5 (CH)
2″	3.47–3.50 (*m*)	74.6 (CH)	3.54–3.60 (*m*)	77.8 (CH)	3.42–3.48 (*m*)	74.5 (CH)
3″	3.38–3.43 (*m*)	75.9 (CH)	3.37–3.43 (*m*)	75.1 (CH)	3.38–3.46 (*m*)	75.8 (CH)
4″	3.63–3.69 (*m*)	71.3 (CH)	3.62–3.70 (*m*)	71.5 (CH)	3.62–3.67 (*m*)	71.6 (CH)
5″	3.52–3.57 (*m*)	76.9 (CH)	3.51–3.57 (*m*)	77.1 (CH)	3.52–3.58 (*m*)	75.8 (CH)
6″	3.92 (*dd*, *J* = 1.8, 12.1, H_a), 3.74 (*dd*, *J* = 6.1, 12.1, H_b)	62.5 (CH_2)	3.98 (*dd*, *J* = 1.8, 12.1, H_a), 3.73 (*dd*, *J* = 6.1, 12.1, H_b)	62.9 (CH_2)	4.65 (*dd*, *J* = 1.8, 12.1, H_a), 4.45 (*dd*, *J* = 5.6, 12.1, H_b)	64.9 (CH_2)
1‴				122.6 (C)	2.12 (*s*)	20.8 (Me)
2‴			7.93 (*d*, *J* = 8.8)	132.7 (CH)		172.1 (C)
3‴			6.90 (*d*, *J* = 8.8)	115.6 (CH)		
4‴				162.5 (C)		
5‴			6.90 (*d*, *J* = 8.8)	115.6 (CH)		
6‴			7.93 (*d*, *J* = 8.8)	132.7 (CH)		
7‴				165.7 (C)		

The observed signals are consistent with those of α-(3′-hydroxy-5′-methoxyphenyl)-2-methoxy-3-methylbenzofuran (stemofuran W) [8]. Aromatic H-atoms H–C(5) and H–C(6′) were used to identify C(1), C(3), C(4) and C(6) (^2J(C,H)) as well as C(1′), C(2′), C(4′), C(5′), and C(α) (^3J(C,H)), respectively. Moreover, the HMBCs of Me(7)/C(2); Me(3)/C(3), C(2), and C(4); H–C(6′)/C(2′); and Me(7′)/C(5′) were also helpful to identify the connected aromatic moieties. The coupling constant ($J = 7.4$) of H–C(1″) suggested that the anomeric C-atom of the glucose was β-configured. The D-configuration of the glucose was determined by the HPLC analysis of the acidic hydrolysate of **1**, and the location of the glucose was deduced from the HMBC of H–C(1″)/C(3) and the NOESY correlations of H–C(1″)/H–C(2′) and H–C(4′). Accordingly, compound **1** was a phenylbenzofuran-type stilbenoid, its structure was unambiguously established as α-(3′-*O*-β-D-glucopyranosyl-5′-methoxyphenyl)-2-methoxy-3-methylbenzofuran.

Compound **2** was obtained as white amorphous powder. The HR-ESI-MS exhibited the $[M + Na]^+$ ion peak at m/z 547.1573 (calc. 547.1580), corresponding to the formula $C_{28}H_{28}O_{10}$, which indicated 15 degrees of unsaturation. The UV absorption bands at 218 (4.41), 305 (4.38), and 326 (4.47) nm suggested a stilbene-conjugated system [9]. The IR spectrum showed absorbances for a OH group (3368 cm^{-1}), an ester (1691 cm^{-1}), an olefinic bond (964 cm^{-1}), and aromatic groups (1602, 1510, and 1450 cm^{-1}). The ^{1}H-NMR spectrum of **2** showed the presence of ten aromatic H-atoms (δ(H) 6.63 and 6.70 (br. *s*, each 1 H); 7.34 and 6.79 (*d*, $J = 8.8$, each 2 H); 7.93 and 6.90 (*d*, $J = 8.8$, each 2 H)), two *trans*-olefinic H-atoms (δ(H) 7.03 and 6.77 (*d*, $J = 16.5$, each 1 H)), one Me group (δ(H) 1.99 (*s*, 3 H)), and a β-glucopyranosyl moiety (δ(H) 4.94 (*d*, $J = 7.2$, 1 H), 3.37–3.70 (*m*, 4 H), 3.98 (*dd*, $J = 1.8$, 12.1, 1 H), 3.73 (*dd*, $J = 6.1$, 12.1, 1 H)). The ^{13}C-NMR (DEPT) spectra of **2** showed 28 C-atom signals, including one CO C-atom (δ(C) 165.7 (C(7‴))), 18 aromatic C-atoms (δ(C) 139.5 (C(1)), 108.2 (C(2)), 158.7 (C(3)), 109.5 (C(4)), 159.2 (C(5)), 106.7 (C(6)), 130.9 (C(1′)), 128.8 (C(2′ and 6′)), 115.9 (C(3′ and 5′)), 157.9 (C(4′)), 122.6 (C(1‴)), 132.7 (C(2‴ and 6‴)), 115.6 (C(3‴ and 5‴)), and 162.5 (C(4‴))), two olefinic C-atoms (δ(C) 129.7 (C(α)) and 127.1 (C(β))), one Me C-atom (δ(C) 8.7 (C(7))), and a glucopyranosyl moiety (δ(C) 101.3 (C(1″)), 77.8 (C(2″)), 75.1 (C(3″)), 71.5 (C(4″)), 77.1 (C(5″)), 62.9 (C(6″))) (*Table*).

From the ^{1}H,^{1}H-COSY spectrum and the above data, the presence of three benzene rings was inferred, in two 1,4-disubstituted and one 1,3,4,5-tetrasubstituted forms. The presence of one *trans* C=C bond from the analysis of the HMBC spectrum was consistent with a stilbene moiety in compound **2**. From the coupling pattern of these two benzene rings and the presence of β-glucopyranosyl moiety, compound **2** was postulated as being a derivative of piceid 2′-*O*-*p*-hydroxybenzoate [10]. The ^{13}C-NMR spectrum of **2** was very similar to that of piceid 2′-*O*-*p*-hydroxybenzoate except for one Me group, which was present in **2** at the C(4) position. In the HMBC spectrum, distinct correlations were observed between H–C(7)/C(3), C(4), and C(5) (*Fig. 2*), confirming the Me group at C(4). Correlations of H–C(2″), H–C(2‴), and H–C(6‴) with the CO C-atom at C(7‴) revealed that C(2″) of the glucose is substituted with a *p*-hydroxybenzoyl group. Furthermore, the D-configuration of the glucose unit was determined by acid hydrolysis of **2** followed by HPLC analysis. Additionally, the HMBC of H–C(1″)/C(3) indicated β-D-glucopyranosyl to be at C(3) of (*E*)-resveratrol moiety, which was also in agreement with the NOESY correlations of H–C(2)/

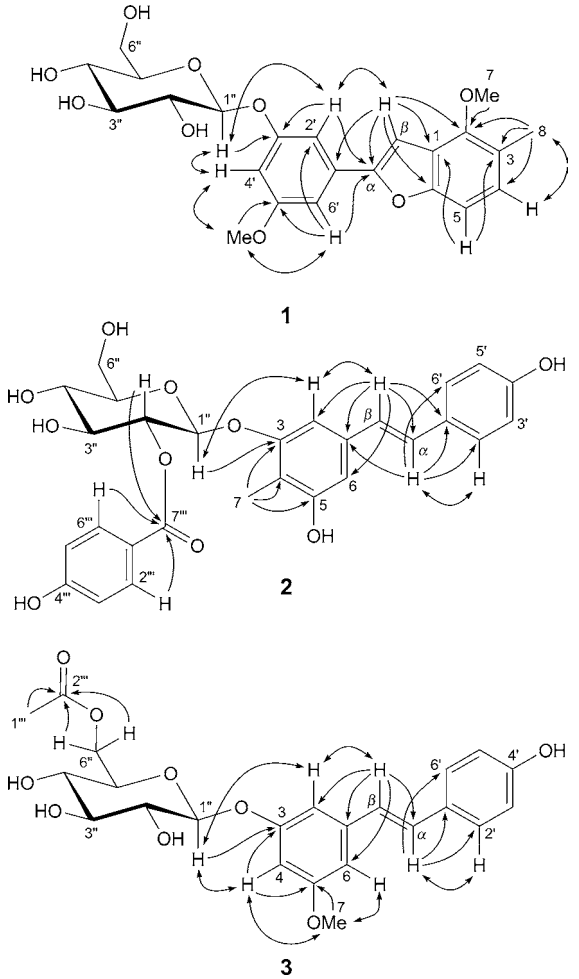

Fig. 2. *Major HMBC* (H → C) *and NOESY* (H ↔ H) *correlations for compounds* **1**, **2**, *and* **3**

H–C(1″). Detailed analysis of DEPT, HMBC, NOESY spectra, and comparison with literature data [9][10], confirmed the structure of the new (*E*)-resveratrol-type stilbenoid **2** as 4-methyl-(*E*)-resveratrol 3-(2″-*p*-hydroxybenzoyl)-*O*-*β*-D-glucopyranoside.

Compound **3** was obtained as white amorphous powder. The molecular formula was determined to be $C_{23}H_{26}O_9$, corresponding to eleven degrees of unsaturation, from the HR-ESI-MS at *m*/*z* exhibited the 469.1468 $[M + Na]^+$ (calc. 469.1475). The UV spectrum of **3** exhibited absorptions at 320, 306, and 216 nm, which indicated the presence of a conjugated aromatic system. The IR spectrum showed OH (3380 cm^{-1}), CO (1727 cm^{-1}), and aromatic group (1596, 1511 cm^{-1}) absorptions. The ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **2** (*Table*). The main differences were the presence of signals for an AcO group (*δ*(H) 2.12 (*s*), corresponding to *δ*(C) 172.1

(C(2‴)) and 20.8 (C(1‴))) and a MeO group (δ(H) 3.81 (*s*), corresponding to δ(C) 55.9 (C(7))) in **3**, while the absence of a Me and a *p*-hydroxybenzoyl groups in **3**. The NMR spectral data (*Table*) of **3** were very similar to those of 5-*O*-methyl-(*E*)-resveratrol 3-*O*-β-D-glucopyranoside [11][12] except for one AcO group, which was present in **3** at the C(6″) position. The presence of a downfield signal at δ(C) 64.9 (C(6″)) also indicated the attachment of the AcO group at C(6″) of glucose. Similarly, the D-configuration of the glucose unit was determined by acid hydrolysis of **3** followed by HPLC analysis. The locations of the glucosyl, MeO, and AcO groups, were also identified by the observed HMBC and NOESY correlations (*Fig. 2*). On the basis of the above spectroscopic studies, the structure of compound **3** was determined as 5-*O*-methyl-(*E*)-resveratrol 3-(6″-acetyl)-*O*-β-D-glucopyranoside, which is also a new (*E*)-resveratrol-type stilbenoid.

By spectroscopic data comparison with literature values, the other six known compounds were identified as acetovanillone 1-(6′-*O*-vanilloyl)-*O*-β-D-glucopyranoside [13], eugenyl-*O*-β-D-glucopyranoside [14], α-(3′-hydroxy-5′-methoxy-2′-methylphenyl)-2-hydroxybenzofuran [7][15], α-(3′-hydroxy-5′-methoxyphenyl)-2-hydroxybenzofuran [16], pinosilvin 3-*O*-β-D-glucopyranoside [17], and (*E*)-resveratrol 3-(6″-galloyl)-*O*-β-D-glucopyranoside [18], respectively. They were isolated for the first time from this plant.

The new compounds were evaluated for their *in vitro* cytotoxicities against human cancer cell A549 by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay with camptothecin as a positive control. The result indicated that compound **2** and **3** exhibited weak cytotoxicity against A549 cell line with IC_{50} values of 4.87 and 5.63 μM, respectively, while compound **1** was inactive against the growth of A549 cell line.

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Experimental Part

General. Column chromatography (CC): Silica gel *H* (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Industry*), *Sephadex LH-20* gel (*Pharmacia*). Gel permeation chromatography (GPC): *H2001* and *H2002* (*Shodex*, *GS-310 2G*; *Asahipak*). TLC: Silica-gel GF_{254} (*Qingdao Marine Chemical Industry*). M.p.: *X-4* digital micro-melting point apparatus; uncorrected. Optical rotations: *PerkinElmer 341* digital polarimeter. UV Spectra: *Shimadzu UV-2401* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Shimadzu 8400S FTIR* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AMX-500* instruments; at 500 (^1H) or 125 MHz (^{13}C) in D_2O at r.t.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS (pos.): *Bruker APEX II FT-ICRMS* mass spectrometer; in *m*/*z*. HPLC: *Shimadzu LC-10AD* high performance liquid chromatograph, *SPDS-10A* ultraviolet detector, C_{18} column (3.9 mm × 300 mm, 5 μm).

Plant Material. The stem barks of *A. brachypus* were collected in October 2013 from the Ziwuling mountains (Gansu Province, P. R. China), and identified by Prof. *Guo Xiao-Qiang* working at College of Life-Science and Technology of Longdong University. A voucher specimen (No. 201010732) was deposited with the Herbarium of College of Life-Science and Technology, Longdong University, Qingyang 745000, P. R. China. The stem barks were washed and dried in the shade then powdered using grinder.

Extraction and Isolation. The air-dried and crushed stem bark of *A. brachypus* (15.0 kg) were extracted three times with 75% aq. EtOH (15 l × 3) for 48 h at r.t., and then the combined extracts were concentrated under reduced pressure at 60° to yield 735 g of a brown viscous residue. The EtOH extract was suspended in dist. H_2O (1500 ml) and partitioned successively with Et_2O (750 ml), AcOEt (750 ml), and BuOH (sat. with H_2O, 750 ml). The concentrated AcOEt-soluble extract (384 g) was subjected to SiO_2 CC with different solvents of increasing polarity (hexane/AcOEt, AcOEt/MeOH) to give 24 fractions (*1–24*). *Fr. 6* (214 mg) was subjected to GPC (AcOEt) to give acetovanillone 1-(6′-vanilloyl)-*O*-*β*-D-glucopyranoside (37 mg). *Fr. 9* (1.5 g) was separated using GPC (MeOH) and SiO_2 CC ($CHCl_3$/MeOH, 8:2, and MeOH) to give eugenyl-*O*-*β*-D-glucopyranoside (18 mg). *Fr. 12* (7.2 g) was applied to *Sephadex LH-20* (MeOH) to give three subfractions (*12.1–12.3*). *Subfr. 12.1* was separated by SiO_2 CC ($CHCl_3$/MeOH, 9:1 → 8:2, and MeOH) and GPC (MeOH) to furnish *α*-(3′-hydroxy-5′-methoxy-2′-methylphenyl)-2-hydroxybenzofuran (52 mg) and *α*-(3′-hydroxy-5′-methoxyphenyl)-2-hydroxybenzofuran (45 mg), resp. *Subfr. 12.3* was subjected to SiO_2 CC ($CHCl_3$/MeOH, 7:3, and MeOH) and further purified by prep. TLC ($CHCl_3$/MeOH, 3:2) to afford **1** (14 mg). *Fr. 14* (5.5 g) was applied to SiO_2 CC ($CHCl_3$/MeOH, 8:2, and MeOH), and further recrystallized with MeOH to provide pinosilvin 3-*O*-*β*-D-glucopyranoside (9 mg). *Fr. 15* (3.8 g) was applied to SiO_2 CC ($CHCl_3$/MeOH, 8:2 → 7:3, and MeOH) to obtain (*E*)-resveratrol 3-(6″-galloyl)-*O*-*β*-D-glucopyranoside (20 mg). *Fr. 17* (1.8 g) was separated by SiO_2 CC ($CHCl_3$/MeOH, 8:2 → 7:3, and MeOH) and GPC (MeOH) to afford **2** (16 mg). *Fr. 22* (2.1 g) was separated by SiO_2 CC ($CHCl_3$/MeOH, 8:2 to 7:3, and MeOH) and GPC (MeOH) to yield **3** (12 mg).

*3-Methoxy-5-(4-methoxy-5-methyl-1-benzofuran-2-yl)phenyl α-*D*-glucopyranoside* (**1**). White amorphous powder (MeOH). M.p.: 173–175°. $[\alpha]_D^{20} = -16.2$ ($c = 0.20$, MeOH). UV (MeOH): 324 (3.37), 306 (3.68), 296 (3.76), 218 (4.40). IR: 3425, 1615, 1601, 1516, 1454, 1334, 1152, 1065, 964, 843. ^{1}H- and ^{13}C-NMR: see *Table.* HR-ESI-MS: 469.1481 ($[M + Na]^+$, $C_{23}H_{26}NaO_9^+$; calc. 469.1475).

*3-Hydroxy-5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]-2-methylphenyl 2-*O*-(4-Hydroxybenzoyl)-β-*D*-glucopyranoside* (**2**). White amorphous powder (MeOH). M.p.: 165–168°. $[\alpha]_D^{20} = -13.7$ ($c = 0.12$, MeOH). UV (MeOH): 326 (4.47), 305 (4.38), 298 (4.30), 218 (4.41). IR: 3368, 1691, 1624, 1602, 1510, 1450, 1334, 1241, 1065, 964, 832. ^{1}H- and ^{13}C-NMR: see *Table.* HR-ESI-MS: 547.1573 ($[M + Na]^+$, $C_{28}H_{28}NaO_{10}^+$; calc. 547.1580).

*3-[(*E*)-2-(4-Hydroxyphenyl)ethenyl]-5-methoxyphenyl 6-*O*-Acetyl-β-*D*-glucopyranoside* **3**). White amorphous powder (MeOH). M.p.: 168–171°. $[\alpha]_D^{20} = -30.5$ ($c = 0.20$, MeOH). UV (MeOH): 320 (4.42), 306 (4.26), 216 (4.37). IR: 3380, 2923, 1727, 1596, 1511, 1446, 1342, 1164, 1073, 965. ^{1}H- and ^{13}C-NMR: see *Table.* HR-ESI-MS: 469.1468 ($[M + Na]^+$, $C_{23}H_{26}NaO_9^+$; calc. 469.1475).

Acid Hydrolysis and Determination of Sugar Components. The absolute configurations of sugar units were assigned by HPLC and optical rotation analysis after total acid hydrolysis of each compound. The sugars were compared with those of the authentic samples prepared in a modified manner [19][20]. In brief, compounds **1**, **2**, and **3** (each 10 mg) were dissolved in 1.0 ml of 2N HCl and then refluxed in a H_2O bath at 90° for 30 h, resp. After cooling, the mixtures were evaporated *in vacuo* and the residues were dissolved in H_2O and extracted with AcOEt. The aq. layers were neutralized with Ag_2CO_3 powder and then filtered to remove the inorganic materials. Each product, obtained by evaporation of the solvent from the filtrate *in vacuo*, was analyzed by HPLC under the following conditions: column, carbohydrate analysis column (3.9 mm × 300 mm, 5 μm); solvent, MeCN/H_2O (17:3); flow rate, 1.0 ml/min; detection, *RI*. The absolute configuration of each sugar was identified by the comparison of retention time and optical rotation: t_R 7.21 min (D-glucose, positive polarity).

Cytotoxicity Assay. The cell growth inhibitory activities of compounds **1**, **2**, and **3** against human cell line A549 were determined using the MTT assay as described in [21]. Briefly, cancer cell line was cultured in *RPMI 1640* medium (*Thermo Fisher*) supplemented with 10% fetal bovine serum in humidified atmosphere of 5% CO_2 at 37°. Then, cell suspension (100 μl) was placed in 96-well microtiter plates to a final concentration of 2×10^3 cells per well and incubated for 12 h. Following incubation, 50 μl of the test compound solns. (in DMSO) at various concentrations were added to each well. After the exposure to compounds **1**, **2** and **3** for 48 h, 50 μl of MTT soln. (1 mg/ml in PBS) was added to each well, and the plates were incubated for 4 h at 37°. Then, 200 μl of DMSO were added to each well. The absorbance caused by formazan crystallization was determined at 550 nm using a microplate reader (*Bio-Rad*, model

550). To calculate the cell viability, the following equation was used: cell viability [%] = (the average $A_{550\,nm}$ of the treated group/the average $A_{550\,nm}$ of the untreated group) × 100%.

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