Three New Lignan Derivatives from *Lindera glauca* (SIEBOLD et ZUCC.) BLUME

by **Won Se Suh**[a]), **Ki Hyun Kim**[a]), **Ho Kyung Kim**[a]), **Sang Un Choi**[b]), and **Kang Ro Lee***[a])

[a]) Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea (phone: +82-31-2907710; fax: +82-31-2907730; e-mail: krlee@skku.ac.kr)

[b]) Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea

Two new aryl-tetralin lignan glycosides, linderanosides A and B (**1** and **2**, resp.), and a new dihydrobenzofuran neolignan glycoside, linderanoside C (**3**), together with five known lignan derivatives (**4**–**8**) were isolated from the trunk of *Lindera glauca*. The structures of these new compounds were determined through spectroscopic analyses, including extensive 2D-NMR data and acid hydrolysis. The absolute configurations of the compounds were clarified by circular dichroism (CD) spectroscopic studies. Compounds **1**–**8** were evaluated for their cytotoxicity against A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), A498 (human kidney epithelial cells), and HCT-15 (colon cancer cells) human tumor cell lines using sulforhodamine B assays *in vitro*.

Introduction. – *Lindera glauca* (SIEBOLD et ZUCC.) BLUME is a deciduous shrub belonging to the Lauraceae family and widely distributed in Korea, China, and Japan [1]. *L. glauca* has been used in Korean traditional medicine to treat diverse diseases such as paralysis, pain, extravasation, and cancer without any side effect [2]. Previous phytochemical investigations on *L. glauca* reported the isolation of alkaloids, butanolides, terpenoids, and phenolic compounds [3–8].

The nitrogen-containing compounds and monoterpenes from *L. glauca* were reported to have anti-tumor activities [9]. As part of our efforts to search for bioactive constituents of Korean medicinal plants with anti-tumor activity, we found that the MeOH extract of the twigs of *L. glauca* had excellent cytotoxic activity against human cancer cells using the sulforhodamine B (SRB) bioassay.

Our earlier phytochemical investigation on *L. glauca* resulted in the isolation of anti-inflammatory lignans from $CHCl_3$-soluble fraction [8]. In the process of our continuing efforts to study this source, we further isolated eight lignans (**1**–**8**), including three new lignan glycoside derivatives, named linderanosides A–C (**1**–**3**, resp.) from the AcOEt soluble fraction. We describe the isolation, and structural determination of compounds **1**–**8**, and the cytotoxic activities of the isolates.

Results and Discussion. – A MeOH extract of twigs of *L. glauca* was partitioned successively with hexane, $CHCl_3$, AcOEt, and BuOH. Repeated chromatographic purification of the AcOEt-soluble fraction afforded three new lignan glycosides (**1**–**3**), together with five known lignan derivatives (**4**–**8**; *Fig. 1*).

Compound **1** was obtained as an amorphous gum. The molecular formula of **1** was determined to be $C_{27}H_{36}O_{12}$ on the basis of a $[M+Na]^+$ peak (*m*/*z* 575.2104 ($C_{27}H_{36}NaO_{12}^+$; calc. 575.2104)) in positive-ion high-resolution fast-atom bombardment

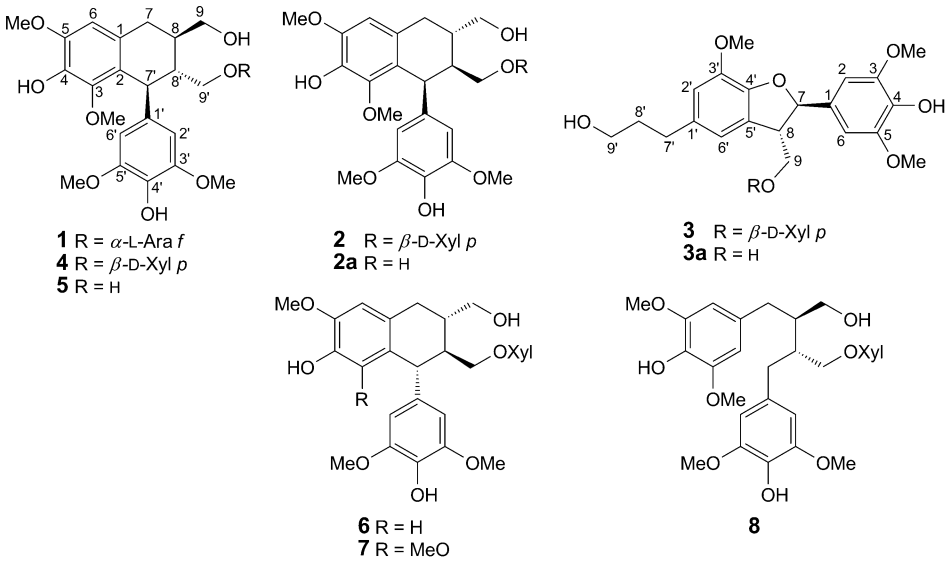

Fig. 1. *Chemical structures of compounds* **1**–**8**

mass spectrometry (HR-FAB-MS). The ^{1}H-NMR spectrum showed three aromatic H-atoms δ(H) 6.61 (*s*, H–C(6)), 6.40 (*s*, H–C(2′,6′)) and four aromatic MeO groups δ(H) 3.38 (*s*, MeO–C(3)), 3.76 (*s*, MeO–C(3′,5′)), 3.89 (*s*, MeO–C(5); *Table*). ^{1}H- and ^{13}C-NMR spectral data of **1** were similar to those of lyoniside (**4**) [10][11] isolated from this source, except the signals assigned to the sugar unit (**1**: δ(H) 4.92 (*d*, $J = 1.7$, H–C(1″)); δ(C) 109.8, 85.5, 83.9, 78.8, and 63.0; **4**: δ(H) 4.30 (*d*, $J = 7.5$, H–C(1″)); δ(C) 105.2, 78.2, 75.2, 71.4, and 67.2), indicating that **1** had an arabinofuranose moiety [12] instead of the xylopyranose moiety in **4**. This structure was confirmed by analysis of the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra (*Fig. 2*). The coupling constant ($J = 1.7$) of H–C(1″) suggested the α-configuration of the arabinose [12][13]. The arabinose unit was placed at C(9′) by the observation of an HMBC from H–C(1″) to C(9′). Acid hydrolysis of **1** afforded the aglycone, lyoniresinol (**5**), which was identified by comparison of its ^{1}H-NMR data [14], together with L-arabinose, which was identified by Co-TLC analysis with an authentic sample (R_f of arabinose ($CHCl_3$/MeOH/H_2O 6 : 4 : 1) 0.55), and GC analysis [15]. Finally, the positions of the four MeO groups were confirmed to be at C(3), C(5), C(3′), and C(5′) respectively, by the HMBC cross-peaks of MeO–C(3)/C(3), MeO–C(5)/C(5), MeO–C(3′)/C(3′), and MeO–C(5′)/C(5′) (*Fig. 2*).

The absolute configuration of **1** was established on the basis of the examination of the CD spectrum of **1** in combination with the NOESY experiment. The observed NOESY correlations (*Fig. 3*) of H–C(7′)/H–C(9′), H–C(2′)/H–C(8′) and H–C(7′)/H–C(8) indicated the relative configuration as (7′*S**,8*R**,8′*R**). The CD spectrum of **1** showed positive *Cotton* effects at 242 and 272 nm consistent with those of the reported compound, (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside [15]. Consequently, the absolute configuration of **1** was determined to be (7′*S*,8*R*,8′*R*). Thus, the structure of **1** was

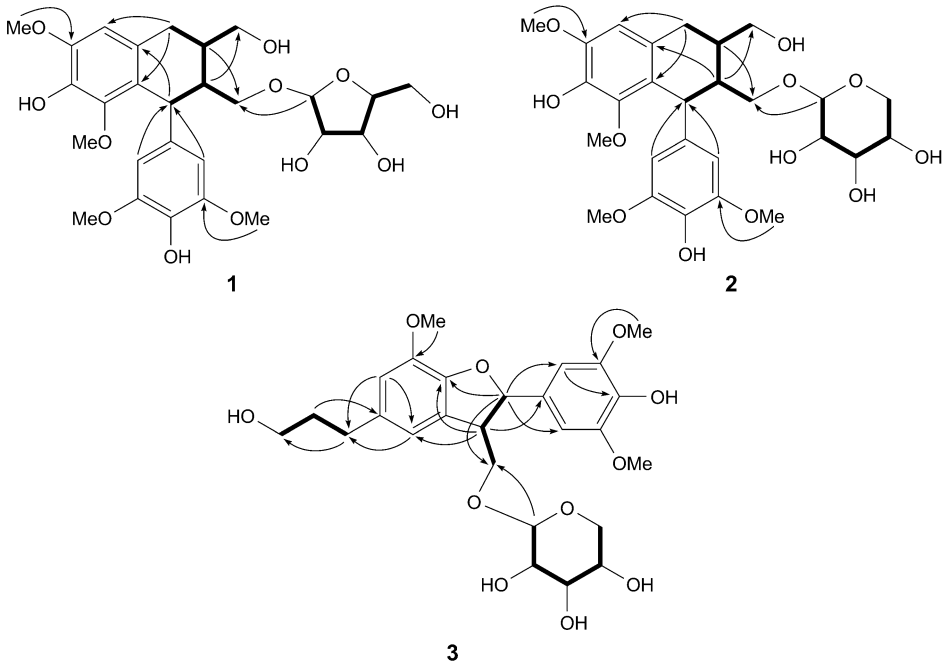

Fig. 2. *Key HMBC* (H → C) *and* $^1H,^1H$*-COSY* (—) *correlations of* **1**, **2**, *and* **3**

established as (+)-(7′*S*,8*R*,8′*R*)-lyociresinol 9′-*O*-*α*-L-arabinofuranoside, and named linderanoside A.

Compound **2** was isolated as amorphous gum with the molecular formula of $C_{27}H_{36}O_{12}$ based on the positive-ion HR-FAB-MS data (*m*/*z* 575.2104 ($[M+Na]^+$, $C_{27}H_{36}NaO_{12}^+$; calc. 575.2104)). The ^{1}H-NMR spectrum showed two sets of aromatic H-atoms at δ(H) 6.61 (*s*, H–C(6)), 6.48 (*s*, H–C(2′,6′)), and four aromatic MeO groups at δ(H) 3.22 (*s*, MeO–C(3)), 3.78 (*s*, MeO–C(3′,5′)), 3.87 (*s*, MeO–C(5)) (*Table*). ^{1}H- and ^{13}C-NMR spectral data of **2** closely resembled those of nudiposide (**7**) [10][11], but with differences being the chemical shifts of C(1), C(2), C(8), C(1′), C(7′), and C(8′) (**2**: δ(C) 127.7, 128.1, 35.2, 135.1, 41.9, 42.6; **7**: δ(C) 126.7, 129.9, 40.6, 139.5, 43.4, 46.7, resp.), indicating that compound **2** was a stereoisomer of **7** at C(7′), C(8), and C(8′). The ^{1}H,^{1}H-COSY, HMQC, and HMBC correlations confirmed the planar structure of **2** (*Fig. 2*). The coupling constant ($J = 7.5$ Hz) of the H–C(1″) of D-xylose suggested that it was the β-form [11][16]. Acid hydrolysis of **2** gave polystachyol (**2a**) by comparison of its ^{1}H-NMR spectrum data [17], as well as D-xylose, which was identified by Co-TLC analysis with an authentic sample (R_f of xylose ($CHCl_3$/MeOH/H_2O 8 : 5 : 1) 0.56), and GC analysis [14]. The absolute configuration of **2** was assigned on the basis of the examination of the CD spectrum of **2** in combination with the NOESY experiment. The small coupling constant ($J = 4.5$) between H–C(7′) and H–C(8′), as opposed to the large coupling constant ($J = 7.1$) between H–C(7′) and H–C(8′) in **7**, established that H–C(7′) and H–C(8′) are in the same orientation [11][18]. Also, the

Table. *^{1}H-NMR* (700 MHz) *and ^{13}C-NMR* (175 MHz) *Data* (CD_3OD) *of Compounds* **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		126.4		127.7		134.1
2		130.4		128.1	6.62 (*s*)	104.3
3		147.8		146.4		149.4
4		148.8		149.3		136.4
5		139.1		138.6		149.4
6	6.61 (*s*)	107.9	6.61 (*s*)	107.8	6.62 (*s*)	104.3
7	2.64 (*dd*, J = 15.0, 11.8), 2.75 (*dd*, J = 15.0, 4.7)	33.9	2.75 (*dd*, J = 17.0, 11.3), 3.01 (*dd*, J = 17.0, 5.7)	34.1	5.50 (*d*, J = 6.2)	89.1
8	1.71–1.66 (*m*)	40.9	2.07–2.01 (*m*)	35.2	3.54–3.51 (*m*)	53.7
9	3.57–3.53 (*m*), 3.67–3.63 (*m*)	66.4	3.61–3.56 (*m*)	65.4	4.02–3.98 (*m*, H_a), 3.70–3.67 (*m*, H_b)	72.6
1′		139.5		135.1		137.2
2′	6.40 (*s*)	106.9	6.48 (*s*)	109.4	6.64 (*s*)	114.4
3′		149.2		148.8		145.4
4′		134.7		135.1		147.6
5′		149.2		148.8		129.6
6′	6.40 (*s*)	106.9	6.48 (*s*)	109.4	6.65 (*s*)	118.2
7′	4.37 (*d*, J = 6.0)	43.2	4.65 (*d*, J = 4.5)	41.9	2.52 (*t*, J = 7.6)	33.0
8′	2.10–2.06 (*m*)	46.7	2.13–2.08 (*m*)	42.6	1.74–1.68 (*m*)	36.0
9′	3.37–3.34 (*m*), 3.74–3.71 (*m*)	69.6	3.37–3.40 (*m*), 3.93–3.90 (*m*)	70.8	3.46 (*t*, J = 6.5)	62.4
1″	4.92 (*d*, J = 1.7)	109.8	4.30 (*d*, J = 7.5)	105.2	4.22 (*d*, J = 7.5)	105.6
2″	4.08–4.06 (*m*)	83.9	3.31–3.27 (*m*)	75.2	3.14–3.11 (*m*)	75.2
3″	3.90–3.86 (*m*)	78.8	3.36–3.32 (*m*)	78.2	3.23–3.20 (*m*)	78.1
4″	3.98–3.95 (*m*)	85.5	3.55–3.50 (*m*)	71.4	3.40–3.36 (*m*)	71.4
5″	3.67–3.63 (*m*), 3.77–3.74 (*m*)	63.0	3.25–3.20 (*m*), 3.93–3.90 (*m*)	67.2	3.12–3.09 (*m*), 3.79–3.76 (*m*)	67.2
*Me*O–C(3)	3.38 (*s*)	60.6	3.22 (*s*)	60.1	3.72 (*s*)	56.9
*Me*O–C(5)	3.89 (*s*)	56.8	3.87 (*s*)	56.6	3.72 (*s*)	56.9
*Me*O–C(3′)	3.76 (*s*)	56.8	3.78 (*s*)	57.1	3.77 (*s*)	53.7
*Me*O–C(5′)	3.76 (*s*)	56.8	3.78 (*s*)	57.1		

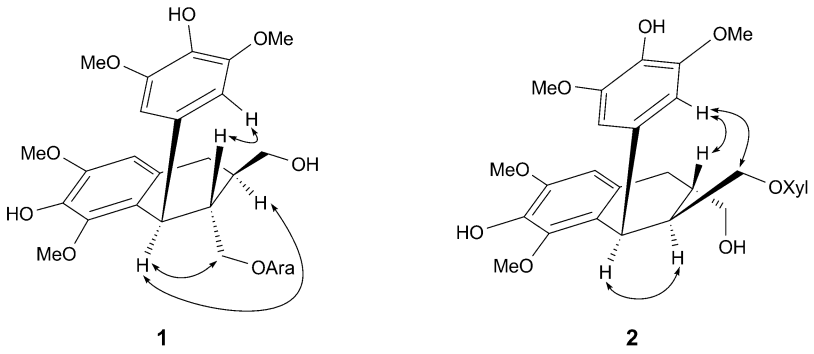

Fig. 3. *Key NOESY* (H ↔ H) *correlations of* **1** *and* **2**

NOESY correlations of H–C(8)/H–C(2′), H–C(2′)/H–C(9′), and H–C(7′)/H–C(8′) confirmed the relative configuration of **2** to be (7′*S**,8*S**,8′*S**) (*Fig. 3*). In the CD spectrum, positive *Cotton* effects at 247 and 272 nm indicated that **2** had (7′*S*,8*S*,8′*S*) configuration [11][15][19]. On the basis of above data, compound **2** was determined as (+)-(7′*S*,8*S*,8′*S*)-lyociresinol 9′-*O*-*β*-D-xylopyranoside, and named linderanoside B.

Compound **3** was obtained as amorphous gum. The HR-FAB-MS displayed a molecular ion peak (*m*/*z* 545.1998 ($[M+Na]^+$, $C_{26}H_{34}NaO_{11}^+$; calc. 545.1999)), consistent with a molecular formula of $C_{26}H_{34}O_{11}$. The ^{1}H-NMR spectrum showed the presence of four aromatic H-atoms at δ(H) 6.62 (*s*, H–C(2,6)), 6.64 (*s*, H–C(2′)), 6.65 (*s*, H–C(6′)), two CH_2 groups at δ(H) 2.52 (*t*, $J = 7.6$, CH_2(7′)), and 1.74 – 1.68 (*m*, CH_2(8′)), two CH_2O groups at δ(H) 4.02 – 3.98 (*m*, H_a–C(9)), 3.70 – 3.67 (*m*, H_b–C(9)), and 3.46 (*t*, $J = 6.5$, H–C(9′)), two CH H-atoms at δ(H) 5.50 (*d*, $J = 6.2$, H–C(7)), and 3.54 – 3.51 (*m*, H–C(8)), and three aromatic MeO groups at δ(H) 3.72 (*s*, MeO–C(3,5)), and 3.77 (MeO–C(3′); *Table*). ^{1}H- and ^{13}C-NMR spectral data were quite similar with data for *rel*-(7*R*,8*S*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol 9-*β*-D-glucopyranoside, which was isolated from *Selaginella moellendorffii* [20], except for the signals attributable to a sugar unit (**3**: δ(H) 4.22 (*d*, $J = 7.5$); δ(C) 105.6, 78.1, 75.2, 71.4, and 67.2), indicating that **3** had a xylopyranose moiety instead of the glucopyranose moiety in *rel*-(7*R*,8*S*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol 9-*β*-D-glucopyranoside. This structure was confirmed by analysis of the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra (*Fig. 2*). The xylose unit was linked at C(9′) which was proved by the detection of an HMBC from H–C(1″) to C(9′). The coupling constant ($J = 7.5$) of the H–C(1″) of D-xylose suggested that it was the *β*-form [11][16]. Acid hydrolysis of **3** yielded the aglycone (**3a**) which was identified as (7*R*,8*S*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol [20] by comparing its ^{1}H-NMR spectrum data, together with D-xylose, which was identified by Co-TLC with an authentic sample (R_f of xylose ($CHCl_3$/MeOH/H_2O 8 : 5 : 1) 0.56), and GC analysis [14]. The absolute configuration at C(7) and C(8) were identified to be (7*R*) and (8*S*), respectively, based on the coupling constants ($J = 6.2$) between H–C(7) and H–C(8) in ^{1}H-NMR spectrum of **3** [20] and the CD spectrum showing a positive *Cotton* effect at 216 nm and a negative *Cotton* effect at 233 nm [21]. Thus, the structure of **3** was

determined to be (7*R*,8*S*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol 9-*β*-D-xylopyranoside, and named linderanoside C.

The five known lignans were identified as lyoniside (**4**) [10][11], lyoniresinol (**5**) [14], 5-methoxy-9-*β*-D-xylopyranosyl-(−)-isolariciresinol (**6**) [22], nudiposide (**7**) [10][11], and ssioriside (**8**) [23][24] by comparing their spectroscopic data with the reported data in the literature.

To evaluate compounds **1**–**8** as cytotoxic agents, we evaluated their anti-proliferative activities against the A549, SK-OV-3, A498, and HCT-15 cell lines using the SRB bioassay [25]. Doxorubicin was used as a positive control. The cytotoxicities of doxorubicin against the A549, SK-OV-3, A498, and HCT-15 cell lines showed IC_{50} values of 0.076 ± 0.003, 0.114 ± 0.026, 0.043 ± 0.007, and 1.124 ± 0.064 μM, respectively. Compounds **1**–**4**, and **7** had selective cytotoxicity against A498 cells, with IC_{50} values of 20.86 ± 0.94, 21.85 ± 0.61, 22.67 ± 1.16, 18.95 ± 0.55, and 28.42 ± 0.80 μM, respectively. However, both compounds were inactive against the other cell lines ($IC_{50} > 30$ μM). The other compounds were inactive against the four tested cell lines ($IC_{50} > 30$ μM).

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Experimental Part

General. TLC: silica gel *60 F_{254}* and *RP-18 F_{254s}* silica gel plates (*Merck*, Germmay); detection under UV light and by spraying with 10% aq. H_2SO_4 soln., followed by heating at 120° for 1 min. Column chromatography (CC): silica gel (SiO_2, 230–400 mesh; *Merck*, Germany), *Lichroprep RP$_{18}$* gel (40–60 μm; *Merck*, Darmstadt, Germany), and *Sephadex LH-20* (*Amersham Pharmacia Biotech*, UK). HPLC: prep. HPLC *Gilson 306 pump*, *Gilson-101 RI* detector, *Phenomenex-Luna-C18-(2)* column (5 μm, 250 mm × 10.00 mm i.d.); t_R in min. Optical rotation: *JASCO P-1020* polarimeter (*JASCO*, Japan). UV Spectra: *Shimadzu UV-1601 UV-Visible* spectrophotometer (*Shimadzu*, Japan) using MeOH as a solvent; λ_{max} (log ε) in nm. CD Spectra: *Jasco J-715* spectropolarimeter (*JASCO*, Japan) using MeOH as a solvent; λ_{max} ($\Delta\varepsilon$) in nm. IR Spectra: *Bruker IFS-66/S* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AVANCEIII 700 NMR* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. FAB-MS and HR-FAB-MS: *JEOL JMS-700* (*Jeol*, Japan) mass spectrometer; in *m*/*z*.

Plant Material. The twigs of *L. glauca* were purchased from Hongcheon, Chungcheongbuk-do, Korea, in March 2010. The plant was identified by one of the authors (*K. R. L.*). A voucher specimen (SKKU 2010-3B) has been deposited with the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The twigs of *L. glauca* (6 kg) were extracted with 80% MeOH two times under reflux (MeOH/H_2O 80:20; 2 × 10 l). The MeOH extract (120 g) was suspended in dist. H_2O (1 l) and then successively partitioned with hexane (3 × 800 ml), $CHCl_3$ (3 × 800 ml), AcOEt (3 × 800 ml), and BuOH (3 × 800 ml), yielding 2.5, 13.3, 6.4, and 17.5 g of residues, resp. The AcOEt soluble fraction (6.0 g) was separated by CC (SiO_2 (35 g), $CHCl_3$/MeOH 50:1 → 1:1 (300 ml each)) to give 13 fractions, *Frs. A–M*. *Fr. D* (425 mg) was subjected to CC (*Sephadex LH-20* (100 g); CH_2Cl_2/MeOH 1:1) and further separated by semi-prep. HPLC (*RP-C_{18}*; MeOH/H_2O 35:65; 2 ml/min) to yield **5** (t_R 36.2 min;12 mg). *Fr. G* (417 mg) was subjected to CC (*RP-C_{18}* (15 g); MeOH/H_2O 40:60): *Frs. G1–G8*. *Fr. G3* (32 mg) was purified by prep. HPLC (*RP-C_{18}*; MeOH/H_2O 85:15; 2 ml/min): **1** (t_R 27.1 min; 5 mg) and **2** (t_R 33.2 min; 5 mg). *Fr. G6* (47 mg) was purified by prep. HPLC (*RP-C_{18}*; MeOH/H_2O 40:60; 2 ml/min): **3** (t_R 34.2 min; 3 mg). *Fr. H* (635 mg) was subjected to CC (*RP-C_{18}* (15 g); MeOH/H_2O 40:60): *Frs. H1–H8*. *Fr. H2* (263 mg) was purified by prep. HPLC (*RP-C_{18}*; MeOH/H_2O 38:62; 2 ml/

min): **4** (t_R 19.0 min; 90 mg) and **7** (t_R 21.2 min; 73 mg). *Fr. H4* (29 mg) was purified by prep. HPLC (*RP-C_{18}*; MeOH/H_2O 40:60; 2 ml/min): **6** (t_R 19.2 min; 7 mg) and **8** (t_R 26.1 min; 8 mg).

Linderanoside A (=*(+)-(7′*S*,8*R*,8′*R*)-Lyociresinol 9′*-O-α-L-*Arabinofuranoside*; =*[(1*S*,2*R*,3*R*)-1,2,3,4-Tetrahydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-3-(hydroxymethyl)-6,8-dimethoxy-2-naphthalenyl]methyl α-*L*-Arabinofuranoside*; **1**). Amorphous gum. $[\alpha]_D^{25} = +20.0$ ($c = 0.30$, MeOH). UV (MeOH): 228 (4.1), 284 (3.2). CD (MeOH): 242 (+32.5), 272 (+5.9), 287 (−2.2). IR (KBr): 3385, 2924, 1611, 1513, 1462, 1221, 1113, 670. ^{1}H- (700 MHz) and ^{13}C-NMR (175 MHz): see *Table*. HR-FAB-MS: 575.2104 ($[M+Na]^+$, $C_{27}H_{36}NaO_{12}^+$; calc. 575.2104).

Linderanoside B (=*(+)-(7′*S*,8*S*,8′*S*)-Lyociresinol 9′*-O-β-D-*Xylopyranoside*; =*[(1*S*,2*S*,3*S*)-1,2,3,4-Tetrahydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-3-(hydroxymethyl)-6,8-dimethoxy-2-naphthalenyl]methyl β-*D*-Xylopyranoside*; **2**). Amorphous gum. $[\alpha]_D^{25} = +108.0$ ($c = 0.35$, MeOH). UV (MeOH): 225 (4.0), 284 (3.1). CD (MeOH): 247 (+100.1), 272 (+44.2), 287 (−4.8). IR (KBr): 3423, 2924, 1641, 1548, 1501, 1218, 1113, 673. ^{1}H- (700 MHz) and ^{13}C-NMR (175 MHz): see *Table*. HR-FAB-MS: 575.2104 ($[M+Na]^+$, $C_{27}H_{36}NaO_{12}^+$; calc. 575.2104).

Linderanoside C (=*(7*R*,8*S*)-3,3′,5-Trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol 9-β-D-Xylopyranoside*; =*[(2*R*,3*S*)-2,3-Dihydro-2-(4-hydroxy-3,5-dimethoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofuranyl]methyl β-*D*-Xylopyranoside*; **3**). Amorphous gum. $[\alpha]_D^{25} = -6.0$ ($c = 0.30$, MeOH). UV (MeOH): 210 (4.3), 288 (3.2). CD (MeOH): 216 (+6.1), 233 (−6.2), 291 (−2.9). IR (KBr): 3385, 2924, 1611, 1548, 1501, 1462, 1216, 1117, 1033, 673. ^{1}H- (700 MHz) and ^{13}C-NMR (175 MHz): see *Table*. HR-FAB-MS: 545.1998 ($[M+Na]^+$, $C_{26}H_{34}NaO_{11}^+$; calc. 545.1999).

Acid Hydrolysis of Compound **1**–**3**. Compounds **1**–**3** (each 1 mg) were hydrolyzed by 1N HCl (dioxane/H_2O 1:1, 2 ml) under reflux for 2 h. After cooling, the mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ was removed under reduced pressure to give lyoniresinol (**5**), polystachyol (**2a**), and (7*R*,8*S*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol (**3a**). The structures were identified by ^{1}H-NMR and comparing these data with those reported in the literature [15][17][19].

Lyoniresinol (=*(6*R*,7*R*,8*S*)-5,6,7,8-Tetrahydro-8-(4-hydroxy-3,5-dimethoxyphenyl)-6,7-bis(hydroxymethyl)-1,3-dimethoxynaphthalen-2-ol*; **5**). Amorphous gum. ^{1}H-NMR (CD_3OD, 700 MHz): 6.60 (*s*, H–C(2′)); 6.41 (*s*, H–C(2,6)); 4.32 (*d*, $J = 5.5$, $CH_2(7)$); 3.87 (*s*, MeO–C(3′)); 3.75 (*s*, MeO–C(3,5)); 3.61 (*dd*, $J = 10.0$, 5.0, H_a–C(9′)); 3.50 (overlap, H_b–C(9′)); 3.50 (*d*, $J = 5.0$, $CH_2(9)$); 3.40 (*s*, MeO–C(5′)); 2.72 (*dd*, $J = 15.0$, 5.0, H_a–C(7′)); 2.59 (*dd*, $J = 15.0$, 11.0, H_b–C(7′)); 2.00–1.98 (*m*, H–C(8)); 1.66–1.62 (*m*, H–C(8′)).

Polystachyol (=*(6*S*,7*S*,8*S*)-5,6,7,8-Tetrahydro-8-(4-hydroxy-3,5-dimethoxyphenyl)-6,7-bis(hydroxymethyl)-1,3-dimethoxynaphthalen-2-ol*; **2a**). Amorphous gum. ^{1}H-NMR (CD_3OD, 700 MHz): 6.60 (*s*, H–C(2′)); 6.41 (*s*, H–C(2,6)); 4.60 (*d*, $J = 4.4$, $CH_2(7)$); 3.87 (*s*, MeO–C(3′)); 3.76 (*s*, MeO–C(3,5)); 3.61 (*dd*, $J = 10.0$, 5.0, H_a–C(9′); 3.62–3.58 (*m*, H_b–C(9′)); 3.50 (*d*, $J = 5.0$, $CH_2(9)$); 3.27 (*s*, MeO–C(5′)); 3.00 (*dd*, $J =$ 17.0, 5.7, H_a–C(7′)); 2.67 (*dd*, $J =$ 17.0, 11.3, H_b–C(7′)); 2.04–2.00 (*m*, H–C(8)); 2.01–1.98 (*m*, H–C(8′)).

*(7*R*,8*S*)-3,3′,5-Trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol* (=*4-[(2*R*,3*S*)-2,3-Dihydro-3-(hydroxymethyl)-5-(3-hydroxypropyl)-7-methoxy-1-benzofuran-2-yl]-2,6-dimethoxyphenol*; **3a**). Amorphous gum. ^{1}H-NMR (CD_3OD, 700 MHz): 6.76 (*s*, H–C(6′)); 6.75 (*s*, H–C(2′)); 6.70 (*s*, H–C(2,6)); 5.53 (*d*, $J = 6.2$, H–C(7)); 3.89 (*s*, MeO–C(3′)); 3.89–3.85 (*m*, H_a–C(9)); 3.84 (*s*, MeO–C(3,5)); 3.85–3.82 (*m*, H_b–C(9)) 3.59 (*t*, $J = 6.5$, $CH_2(9')$); 3.51–3.48 (*m*, H–C(8)); 2.65 (*t*, $J = 7.7$, $CH_2(7')$); 1.86–1.82 (*m*, $CH_2(8')$).

Determination of the Sugars of Compounds **1**–**3**. Each layer was neutralized by passage through an *Amberlite IRA-67* column and was evaporated under reduced pressure to give the sugar fraction. The sugars obtained from hydrolysis were dissolved in anh. pyridine (0.5 ml) followed by adding of L-cysteine methyl ester hydrochloride (2 mg; *Sigma*, St. Louis, MO). The mixture was stirred at 60° for 1.5 h. After the mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 ml; *Sigma*, St. Louis, MO) for 2 h. The mixture was partitioned between hexane and H_2O (1 ml, each), and the org. layer (1 µl) was analyzed by gas chromatography (GC) [26]. Identification of L-arabinose and D-xylose for **1**, **2**, and **3** was performed in each case by co-injection of the hydrolysate with derivatized standard sugars, giving single peaks at L-arabinose (5.39 min) for **1** and D-xylose for **2** and **3** (5.55 and

5.54 min, resp.). t_R Values of authentic D-xylose and L-arabinose samples that were treated in the same way were 5.53 and 5.40 min., resp.

Cytotoxicity Assay. A sulforhodamine B bioassay (SRB) was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines [25]. The cell lines (National Cancer Institute, Bethesda, MD, USA) used were A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), A498 (human kidney epithelial cells), and HCT-15 (colon cancer cells). Doxorubicin (*Sigma Chemical Co.*, ≥98%) was used as a positive control. Tested compounds were demonstrated to be pure as evidenced by NMR and HPLC analysis (purity ≥95%). All experiments were performed in triplicate, and all the 50% cell growth inhibitory concentration (IC_{50}) were expressed as mean ± SEM.

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