Lignans from Bark of *Larix olgensis* var. *koreana*

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Six new lignans (**2**−**7**) were isolated from the bark of *Larix olgensis* var. *koreana*, and their structures were determined on the basis of their spectroscopic data. Seven known lignans were also obtained and identified as (+)-lariciresinol 9′-*p*-coumarate (**1**), (+)-lariciresinol, (−)-secoisolariciresinol, (+)-isolariciresinol, vladinol D, sesquipinsapol B, and ehletianol C. Compound **1** showed weak inhibition against K562, SHG44, HCT-8, A549, and PC-3M tumor cells with IC_{50} values of 2.9, 21.4, 32.9, 33.8, and 28.0 μg/mL, respectively.

Larix olgensis Henry var. *koreana* Nakai (*Pinaceae*) is distributed in the Changbai Mountain area of Jilin Province and the drainage area of the Mudan River of Heilongjiang Province in China, and its stem bark has been used in the Chinese leather industry for a long time.[1] Bark extracts of related species have been used in the food, pharmaceutical, cosmetic, and chemical industries in many European countries. Chemical investigation of the title plant has not been reported previously. We have systematically investigated the chemical constituents of the bark of *L. olgensis* and obtained six new lignans, (−)-7-hydroxylariciresinol 9′-*p*-coumarate (**2**), (+)-lariciresinol 9′-caffeinate (**3**), (+)-isolariciresnol 9′-*p*-coumarate (**4**), (7*R*,8*S*)-3′-*O*-methylcedrusin 9-*p*-coumarate (**5**), larixnaphthaone (**6**), and larixsin (**7**), and seven known ones including lariciresinol 9′-*p*-coumarate (**1**). Herein we report the isolation and structural elucidation of these new compounds, along with the results of preliminary tests for antitumor activity.

Results and Discussion

The molecular formula of compound **1** was deduced to be $C_{29}H_{30}O_8$ as the HRESIMS spectrum showed the $[M + Na]^+$ ion at *m/z* 529.1840 (calcd for $[C_{29}H_{30}O_8+Na]^+$: 529.1838). The IR spectrum showed absorption bands for hydroxyl (3409 cm^{-1}), carbonyl (1707 cm^{-1}), and aromatic rings (1604, 1515 cm^{-1}). In the ^1H NMR spectrum, signals at δ 7.40 (1H, d, J = 16 Hz, H-7″) and 6.15 (1H, d, J = 16 Hz, H-8″) indicated the presence of a *trans*-substituted double bond. Signals at δ 7.35 (2H, d, J = 8.0 Hz, H-2″, 6″) and 6.80 (2H, d, J = 8.0 Hz, H-3″, 5″) in the ^1H NMR spectrum suggested the presence of a *p*-substituted aromatic ring, as did signals at δ 131.5 (C-2″, 6″) and 117.2 (C-3″, 5″) in the ^{13}C NMR (DEPT) spectrum. In the HMBC spectrum, long-range correlations from the olefinic proton (δ = 7.40, H-7″) to the carbonyl carbon (δ = 169.2, C-9″) and the methine carbons (δ = 131.5, C-2″ and 6″) were observed, indicating the presence of a *p*-coumaroyl group. The other signals in the NMR spectra were very similar to those of (+)-lariciresinol.[2] The molecular weight of **1**, 506, was 146 units above that of lariciresinol, also indicating that there was a *p*-coumaroyl group in **1**, and the NMR indicated that it was linked to C-9′. Thus, compound **1** was a *p*-coumaric acid ester of lariciresinol, a conclusion confirmed further by correlations between the methylene protons at δ 4.22 and 4.42 (H-9′) and the carbonyl carbon at δ 169.2 in the HMBC spectra. The absolute configuration of **1** should be the same as (+)-lariciresinol, as deduced from its optical rotation and CD spectrum. Therefore, compound **1** was identical to (+)-lariciresnol 9′-*p*-coumarate reported in 1976 [3]. The spectroscopic data of **1** are reported here in detail for the first time.

Compound **2** was obtained as white power, $[\alpha]^{20}_D$ −23.0° (*c* 2.03, MeOH). The molecular formula of **2** was established as $C_{29}H_{30}O_9$ by HRESIMS. The ^1H NMR spectrum was very similar to that of **1**, only the H-7 (δ = 4.62) signal was shifted downfield by 2.0 ppm, indicating that C-7 was substituted with an oxygen group in **2**. The molecular weight, 16 units higher than that of **1**, suggested that C-7 was substituted with an OH group. The relative configurations of 8,8′-*cis* and 7′,8′-*trans* were confirmed by the NOESY correlations from H-8 to H-8′ and from H-8′ to H-2′ and H-6′ in the NOESY spectrum, the same as those of **1**. Thus, **2** was established as (−)-7-hydroxylariciresinol 9′-*p*-coumarate.

The HRESIMS of compound **3** exhibited an $[M + Na]^+$ ion at *m/z* = 545.1792, corresponding to a molecular formula of $C_{29}H_{30}O_9$. Its molecular weight was 16 units higher than that of compound **1**. The ^{13}C NMR spectrum was very similar to that of **1**, except for the replacement of the hydroxyl group by a downfield shift of the C-3″ signal by 29.6 ppm and upfield shifts of the C-2″ and C-4″ signals by 16.1 and 12.1 ppm, respectively. Thus, **3** had one hydroxyl group more than **1** at C-3″. The absolute configuration of **3** was also the same as that of **1**, as deduced from its optical rotation and CD spectrum. Therefore, compound **3** was elucidated as (+)-lariciresinol 9′-caffeinate.

Compound **4** was obtained as white powder, and the HRESIMS gave an $[M + Na]^+$ ion at *m/z* = 529.1837, corresponding to a molecular formula of $C_{29}H_{30}O_8$. In the NMR spectra, signals corresponding to a *p*-coumaroyl group were also found, while the other signals were similar to those of isolariciresinol.[2] The molecular weight of **4** was 146 units higher than that of isolariciresinol, and the upfield shift of C-8′ by 3.3 ppm and downfield shift of C-9′ by 2.8 ppm suggested that the *p*-coumaroyl group was connected at C-9′. The optical rotation and CD spectrum suggested that the absolute configuration of **4** was the same as that of (+)-isolariciresinol. Thus, compound **4** was established as (+)-isolariciresnol 9′-*p*-coumarate.

The molecular formula of compound **5** was $C_{29}H_{30}O_8$ (HRESIMS). The IR spectrum exhibited absorption bands

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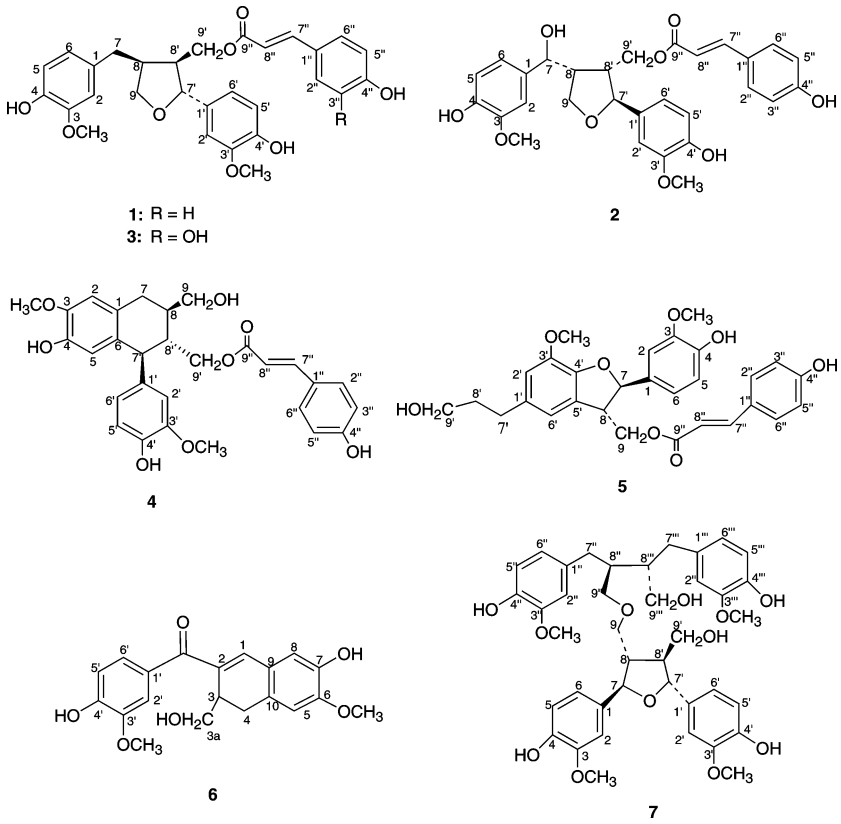
Chart 1



of hydroxyl (3424 cm^{-1}), carbonyl (1699 cm^{-1}), and aromatic rings (1604, 1515 cm^{-1}). Signals identical to a *p*-coumaroyl group were also found in the NMR spectra. In addition to the protons due to the *p*-coumaroyl group, signals of two methoxy groups (δ = 3.85, 3H, s; 3.77, 3H, s), one ABX coupling system of protons (δ = 6.98, 1H, d, J = 1.9 Hz, H-2; 6.92, 1H, dd, J = 8.1 Hz,1.9 Hz, H-6; 6.77, 1H, d, J = 8.0 Hz, H-5), and one AB coupling system of protons (δ = 6.75, 1H, d, J = 2.0 Hz, H-2′; 6.74, 1H, d, J = 2.0 Hz, H-6′) were observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum showed 18 carbon signals, other than those of the *p*-coumaroyl group and two methoxy carbons. Two C_6-C_3 units, elucidated as Ph−CH_2−CH_2−CH_2−O− and Ph−CH(OR)−CH−CH_2−O−, were deduced from analyses of its 2D NMR (HMQC, HMBC, ^{1}H−^{1}H COSY) spectra, implying that **5** was a lignan. In the HMBC spectrum, correlations from H-8 (δ = 3.80, m) to C-5′ (δ = 128.9), C-4′ (δ = 147.6), and C-6′ (δ = 117.7) and from H-7 (δ = 5.49, 1H, d, J = 7.1 Hz) to C-4′ suggested that **5** was a dihydrobenzofuran lignan. The *p*-coumaroyl group linked to C-9 was confirmed by the HMBC correlation from H-9 to a carbonyl carbon. The CD spectrum of **5** showed a negative cotton effect, $[\theta]_{243.0}$ −6421, $[\theta]_{309.0}$ −7278, suggesting C-7 and C-8 to be *R* and *S*, respectively.[4,5] The structure of **5** was thus designated as (7*R*,8*S*)-9-*p*-coumaroyl-oxo-methyl-7,8-dihydro-1′-(8-hydroxypropyl)-3′-methoxy-7-4-hydroxy-3′-methoxyl)phenylbenzofuran, named (7*R*,8*S*)-3′-*O*-methylcedrusin 9-*p*-coumarate.

Compound **6** had the molecular formula $C_{20}H_{20}O_6$ (HRESIMS). The IR spectrum exhibited absorption bands of a hydroxyl group (3423 cm^{-1}), a carbonyl group (1689 cm^{-1}), and double bonds (1565 cm^{-1}). The ^{1}H NMR spectrum showed signals of a 1,3,4-trisubstituted aromatic ring (δ = 6.88, 1H, d, J = 8 Hz; 7.27, 1H, dd, J = 2, 8 Hz; 7.43, 1H, d, J = 2 Hz) and a 1,2,4,5-tetrasubstituted aromatic ring (δ = 6.68, 1H, s; 6.85, 1H, s). The signal at δ = 7.07 (1H, s) was due to the olefinic proton. Its ^{13}C NMR and DEPT spectrum displayed 20 carbon signals (2×CH_3, 2×CH_2, 7×CH, 9×C). In the HMBC, correlations from all of the ABX system protons to the carbonyl carbon suggested that the carbonyl was linked to C-1 of the 1,3,4-trisubstituted aromatic ring directly, while C-3 and C-4 were determined to be linked to OH and OCH_3 groups, respectively. Correlations from the olefinic proton (δ = 7.07) to three carbons of the 1,2,4,5-tetrasubstituted aromatic ring and the carbonyl carbon revealed that the methine carbon (δ = 141.9) of the double bond was linked to the 1,2,4,5-tetrasubstituted aromatic ring directly and that the quaternary carbon of the double bond was linked to carbonyl. The 3,4-dihydronaphthalene moiety of **6** was confirmed by correlations from H-3 (δ = 3.15, 1H, m) to C-2 (δ = 129.1) and C-1 (δ = 141.9) and from H-4 (δ = 3.12, 1H, dd, J = 16.0 Hz, 1.0 Hz) to C-2 (δ = 129.1) and C-10 (δ = 136.4) in the HMBC spectrum. In addition, correlations from H-5 (δ = 6.85, 1H, s) to C-6 (δ = 150.9) and from the protons of the methoxy group to C-6 indicated that C-6 rather than C-7 was substituted by OCH_3. Compound **6** was thus elucidated as (−)-(3,4-dihydro-7-hydroxy-3-hydroxymethyl-6-methoxylnaphthalen)-2-yl-(4′-hydroxy-3′-methoxyphenyl)one, named larixnaphthaone.

The molecular formula of compound **7**, $C_{40}H_{48}O_{12}$, was established by HRESIMS. The IR spectrum exhibited absorption bands of a hydroxyl group (3413 cm^{-1}) and an aromatic ring (1604, 1515, and 1464 cm^{-1}). The ^{13}C NMR showed 36 carbons signals, except for those of four OCH_3 groups. Four C_6-C_3 units were elucidated as two Ph−CH(OR)−CH−CH_2O− moieties and two Ph−CH_2−CH−CH_2O− moieties by analyzing 2D NMR (HMQC, HMBC, ^{1}H−^{1}H COSY) spectra. All information above implied that compound **7** was a dimeric lignan. The NMR data of one lignan moiety were identical to those of neo-olivil,[6] and the other to secoisolariciresinol,[2] indicating that **7** was composed of neo-olivil and secoisolariciresinol. In the neo-olivil moiety, signals of C-8′ and C-9′ were shifted by −3.5 and +9.7 ppm

Table 1. ^{1}H NMR Data of Compounds **1**−**4** in CD_3OD (500 MHz)

no.	1	2	3	4
2	6.78, d (2.0)	7.00, d (2.0)	6.79	6.70, s
5	6.70, d (8.0)	6.79,d (2.0)	6.72, d (8.0)	6.20, s
6	6.58, dd (2.0, 8.0)	6.82, dd (2.0, 8.0)	6.66, dd (2.0,8.0)	
7	2.48, dd (11.0, 14.0)	4.62, d (2.0)	2.55, dd (11.0, 13.0)	2.86, d (7.4)
	2.81, dd (6.0, 14.0)		2.88, dd (6.0,13.0)	
8	2.65, m	2.58, m	2.80, m	2.10, m
9	3.68, dd (6.0, 8.0)	3.75, dd (8.0, 9.0)	3.72, dd (6.0,8.0)	3.61, dd (6.3, 12.0)
	3.98, dd (7.0, 8.0)	3.85, dd (5.0, 9.0)	4.15, dd (6.0,8.0)	3.71, dd (3.7, 12.1)
2′	6.90, d (2.0)	6.91, d (2.0)	6.92, d (2.0)	6.67, d (1.6)
5′	6.80	6.75, d (7.0)	6.78, d (8.0)	6.76, d (8.0)
6′	6.80	6.81, dd (2.0, 8.0)	6.81, dd (2.0, 8.0)	6.58, dd (6.8, 8.1)
7′	4.75, d (7.0)	4.55, d (8.0)	4.80, d (8.0)	3.90, d (10.1)
8′	2.60, m	2.62, m	2.65, m	2.05, m
9′	4.22, dd (8.0, 12.0)	4.21, dd (8.0, 11.0)	4.28, dd (8.0, 11.0)	4.03, dd (3.0, 11.3)
	4.42, dd (6.0, 12.0)	4.41, dd (4.0, 11.0)	4.48, dd (7.0, 11.0)	4.25, dd (3.2, 11.3)
2″	7.35, d (8.0)	7.36, d (7.0)	7.05, d (2.0)	7.46, d (8.6)
3″	6.80, d (8.0)	6.80, d (7.0)		6.80, d (8.6)
5″	6.80, d (8.0)	6.80, d (7.0)	6.78, d (8)	6.86, d (8.6)
6″	7.35, d (8.0)	7.36, d (7.0)	7.00, dd (2.8)	7.46, d (8.6)
7″	7.40, d (16.0)	7.28, d (16.0)	7.30, d (16)	7.55, d (16.0)
8″	6.15, d (16.0)	6.12, d (16.0)	6.10, d (16)	6.33, d (16.0)
3-OCH_3	3.75, s	3.75, s	3.81, s	3.83, s
3′-OCH_3	3.75, s	3.85, s	3.81, s	3.75, s

compared to the literature,[2] while in the secoisolariciresinol moiety signals of C-8″ and C-9″ were shifted by −2.9 and +6.3 ppm,[6] suggesting that the neo-olivil moiety was connected with the secoisolariciresinol moiety through $C_{9'}$−O−$C_{9''}$. The two lignans were linked only by an ether bond, which was unique in lignan structure. No attempt was made to determine the absolute configuration of **7**, but $J_{7,8}$ = 8.8 Hz and cross-peaks between H-8′ and H-2′ and H-6′ in the NOESY spectrum stuggested a 7,8-*trans* and 7′,8′-*trans* assignment. All these data were in agreement with the all-*trans* configuration of neo-olivil.[6] Therefore, **7** was established as (−)-neo-olivil-(9-*O*-9″)-seco-isolariciresinol, named larixsin.

The results of preliminary cytotoxicity tests revealed that only **1** showed weak inhibitory activity, with IC_{50} values of 2.9, 21.4, 32.9, 33.8, and 28.0 μg/mL against K562, SHG44, HCT-8, A549, and PC-3M tumor cells, respectively.

Experimental Section

General Experimental Procedures. All melting points were determined on a RY-2 electric hot-stage melting point apparatus and are uncorrected. Optical rotations were measured at λ = 598 nm and 20 °C on a Perkin-Elmer 343 polarimeter in MeOH. CD spectra were recorded on a JASCO J-810 spectropolarimeter. IR spectra were obtained on a Bruker Vector-22 IR spectrophotometer in KBr pellets. NMR spectra were acquired on a Bruker DRX-500 spectrometer in CD_3OD with TMS as internal standard, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ESIMS data were obtained on a Q-Tof micro mass spectrometer. Silica gel H (10−40 μm) for column chromatography and HPTLC plates precoated with silica gel HF_{254} (5−7 μm) were supplied by Zhifu Huangwu Silica Gel D & R Plant, Yantai, China. Sephadex LH-20 and ODS were purchased from Pharmacia and Merck, respectively. Spots on TLC were developed by heating after spraying with 15% H_2SO_4 in alcohol (v/v).

Biological Material. The stem bark of *Larix olgensis* Henry var. *koreana* Nakai was collected in Jilin Province, China, in September 2002 and identified by Professor Hanchen Zheng, Department of Pharmacognosy of this college, where a voucher specimen (20020910) is deposited.

Materials and Methods. The air-dried and powdered stem bark (35 kg) was extracted with 80% EtOH (600 L). After removal of EtOH by evaporation under reduced pressure, the remaining aqueous solution (50 L) was partitioned successively with $CHCl_3$ (3 × 50 L) and EtOAc (6 × 50 L). The $CHCl_3$ fraction (460 g) was chromatographed on silica gel (ϕ 9 × 100 cm, 2.5 kg), eluting with petroleum ether−EtOAc (30:1, 10:1, 8:1, 5:1, 3:1, 1:1) and $CHCl_3$−MeOH (5:1, 1:1), to afford fractions 1−200. After concentration, crystals of (+)-lariciresinol,[2] (−)-secoisolariciresinol,[2] and (+)-isolariciresinol[2] were obtained in fractions 134−140, 146−149, and 173−175, respectively. Crystals of (+)-lariciresinol and (−)-secoisolariciresinol were recrystallized from petroleum ether−EtOAc (1:1), respectively, and (+)-isolariciresinol was recrystalized from $CHCl_3$−MeOH (5:1), yielding 6, 2, and 4 g, respectively. Fractions 1−200 were combined to form fractions I−VI. Fraction IV (70 g) was rechromatographed on silica gel (ϕ 9 × 100 cm, 2 kg), eluting with petroleum ether−EtOAc (3:1, 1:1), to yield impure **1**, which was subjected to CC on Sephadex LH-20 (200 mL) using $CHCl_3$−MeOH (3:1) as eluent to give pure **1** (222 mg). Fraction V (32 g) was separated on silica gel (ϕ 6 × 80 cm, 1 kg), eluting with petroleum ether−EtOAc (2:1, 1:1), to afford fractions V-1−V-8. Fraction V-2 (5 g) was further separated on an ODS (100 g) column, eluting with 60% MeOH, to afford impure compounds **2**−**7**, which were further purified on a Sephadex LH-20 (100 mL) column respectively using 60% MeOH as eluent to yield compounds **2** (12 mg), **3** (16 mg), **4** (10.8 mg), **5** (9.4 mg), vladinol D[8] (219 mg), and **6** (20 mg). Fraction VI (37 g) was chromatographed on silica gel (ϕ 6 × 80 cm, 1 kg), eluting with $CHCl_3$−MeOH (20:1 to 5:1), to afford fractions V-1 (400 mg) and VI-2 (300 mg). Fraction VI-1 on ODS (60 g), using 50% MeOH as eluent, yielded sesquipinsapol B[9] (86 mg) and **7** (9 mg). Fraction VI-2 using the same method yielded ehletianol C[10] (200 mg).

Compound 1: white powder (MeOH); mp 120−121 °C; $[\alpha]^{20}_D$ +47.4° (*c* 0.51, MeOH); CD (*c* 0.69 mM, MeOH) $[\theta]_{219}$ 0, $[\theta]_{234}$ −8460, $[\theta]_{256}$ 0, $[\theta]_{313}$ +4581, $[\theta]_{344}$ 0; IR (Nujol) ν_{max} 3409, 2940, 1707, 1604, 1515, 1447, 1271, 1167, 1032 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Tables 1 and 2; ESIMS *m/z* 529.14 [M + Na]$^+$, 1035.39 [2M + Na]$^+$; HRESIMS *m/z* 529.1840 [M + Na]$^+$ (calcd for [$C_{29}H_{30}O_8$+Na]$^+$, 529.1838).

Compound 2: white powder (MeOH); mp 106−152 °C; $[\alpha]^{20}_D$ −23.0° (*c* 2.03, MeOH); CD (*c* 0.61 mM, MeOH) $[\theta]_{218}$ 0, $[\theta]_{234}$ +13767, $[\theta]_{246}$ 0, $[\theta]_{305}$ −7283, $[\theta]_{374}$ 0; IR (Nujol) ν_{max} 3421, 1686, 1604, 1515, 1434, 1373, 1271, 1166 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Tables 1 and 2; ESIMS *m/z* 545.20 [M + Na]$^+$, 1067.41 [2M + Na]$^+$; HRESIMS *m/z* 545.1775 [M + Na]$^+$ (calcd for [$C_{29}H_{30}O_9$+Na]$^+$, 545.1788).

Compound 3: white powder (MeOH); mp 105−107 °C; $[\alpha]^{20}_D$ +49.5° (*c* 0.53, MeOH); CD (*c* 0.61 mM, MeOH) $[\theta]_{224}$ 0, $[\theta]_{229}$ −6675, $[\theta]_{255}$ 0, $[\theta]_{312}$ +3727, $[\theta]_{363}$ 0; IR (Nujol) ν_{max} 3431, 2942, 1690, 1604, 1516, 1273 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Tables 1 and 2; ESIMS *m/z* 545.19 [M + Na]$^+$, 1067.40

Table 2. ^{13}C NMR Data of Compounds **1**−**4** in CD_3OD (125 MHz)

no.	1	2	3	4
1	133.4	136.3	133.1	133.9
2	113.7	111.4	113.4	112.6
3	149.3	148.9	149.0	147.4
4	146.2	147.2	145.9	145.4
5	116. 6	117.0	116.3	117.2
6	122.5	120.6	122.1	129.0
7	34.4	77.1	34.1	33.5
8	44.5	51.0	44.2	39.8
9	74.0	70.8	73.7	65.2
1′	135. 6	133.8	135.1	137.8
2′	111.4	111.7	111.0	113.9
3′	149.3	149.0	149.0	149.1
4′	147.5	147.4	147.3	146.2
5′	116.4	116.0	116.5	116.2
6′	120.5	121.1	120.2	123.1
7′	85.5	87.7	85.2	48.8
8′	50.7	51.0	50.5	44.8
9′	64.2	66.4	63.9	64.9
1″	127.4	127.1	127.6	127.1
2″	131.5	131.2	115.1	131.2
3″	117.2	116.8	146.8	116.9
4″	161.6	161.8	149.7	161.4
5″	117.2	116.8	116.1	116.9
6″	131.5	131.2	123.1	131.2
7″	147.0	146.6	147.1	146.6
8″	115.2	114.9	114.8	115.1
9″	169.2	169.0	169.0	169.4
3-OCH_3	56.7	56.4	56.4	56.5
3′-OCH_3	56.7	56.4	56.4	56.4

$[2M + Na]^+$; HRESIMS *m/z* 545.1792 $[M + Na]^+$ (calcd for $[C_{29}H_{30}O_9+Na]^+$, 545.1788).

Compound 4: white powder (MeOH); mp 125−127 °C; $[\alpha]^{20}_D$ +86.5° (*c* 0.48, MeOH); CD (*c* 0.69 mM, MeOH) $[\theta]_{232}$ 0, $[\theta]_{235}$ +41024, $[\theta]_{247}$ 0, $[\theta]_{272}$ +19852, $[\theta]_{282}$ 0, $[\theta]_{289}$ −16453, $[\theta]_{295}$ 0, $[\theta]_{309}$ +32911, $[\theta]_{350}$ 0; IR (Nujol) ν_{max} 3399, 2936, 1685, 1604, 1513, 1448, 1266, 1203, 1167, 1030, 832 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Tables 1 and 2; ESIMS *m/z* 529.27 [M + Na]$^+$, 1035.56 [M + Na]$^+$; HRESIMS *m/z* 529.1837 [M + Na]$^+$ (calcd for $[C_{29}H_{30}O_8+Na]^+$, 529.1838).

Compound 5: white powder (MeOH); mp 103−105 °C; $[\alpha]^{20}_D$ −50.0° (*c* 0.24, MeOH); CD (*c* 0.59 mM, MeOH) $[\theta]_{192}$ 0, $[\theta]_{243.0}$ −6421, $[\theta]_{294.0}$ −10585, $[\theta]_{309.0}$ −7278, $[\theta]_{350}$ 0; IR (Nujol) ν_{max} 3424, 2939, 1699, 1604, 1515, 1454, 1270, 1206, 1165, 1033, 832 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 7.49 (1H, d, J = 15.9 Hz, H-7″), 7.34 (2H, d, J = 8.6 Hz, H-2″, H-6″), 6.98 (1H, d, J = 1.9 Hz, H-2), 6.92 (1H, dd, J = 8.1 Hz,1.9 Hz, H-6), 6.78 (2H, d, J = 8.6 Hz, H-3″, H-5″), 6.77(1H, d, J = 8.0 Hz, H-5), 6.75 (1H, d, J = 2.0 Hz, H-2′), 6.74 (1H, d, J = 2.0 Hz, H-6′), 6.25 (1H, d, J = 15.9 Hz, H-8″), 5.49 (1H, d, J = 7.1 Hz, H-7), 4.56 (1H, dd, J = 11.1 Hz, 5.1 Hz, H-9), 4.39 (1H, dd, J = 11.1 Hz, 7.9 Hz, H-9), 3.85 (3H, s, 3′-OCH_3), 3.80 (1H, m, H-8), 3.77 (3H, s, 3-OCH_3), 3.55 (2H, t, J = 6.5 Hz, H-9′), 2.64 (2H, t, J = 7.5 Hz, H-7′), 2.30 (2H, m, H-8′); ^{13}C NMR (CD_3OD, 125 MHz) δ 168.9 (C, C-9″), 161.4 (C, C-4″), 149.1 (C, C-3), 147.8 (C, C-4), 147.6 (C, C-4′), 147.0 (CH, C-7″), 145.4 (C, C-3′), 137.3 (C, C-1′), 134.0 (C, C-1), 131.2 (CH, C-2″), 131.2 (CH, C-6″), 128.9 (C, C-5′), 127.1 (C, C-1″), 120.1 (CH, C-6), 117.7 (CH, C-6′), 116.9 (CH, C-3″), 116.9 (CH, C-5″), 116.3 (CH, C-5), 114.8 (CH, C-8″), 114.7 (CH, C-2′), 111.0 (CH, C-2), 90.0 (CH, C-7), 66.8 (CH_2, C-9), 62.2 (CH_2, C-9′), 56.8 (CH_3, 3′-OCH_3), 56.5 (CH_3, 3-OCH_3), 52.1 (CH, C-8), 35.7 (CH_2, C-8′), 32.9 (CH_2, C-7′); ESIMS *m/z* 529.28 [M + Na]$^+$, 1035.58 [2M + Na]$^+$; HRESIMS *m/z* 529.1838 [M + Na]$^+$ (calcd for $[C_{29}H_{30}O_8+Na]^+$, 545.1838).

Compound 6: amorphours powder (MeOH); mp 114−115 °C; $[\alpha]^{20}_D$ −56.1° (*c* 0.66, MeOH); CD (*c* 0.89 mM, MeOH) $[\theta]_{223}$ 0, $[\theta]_{238}$ +14741, $[\theta]_{259}$ +18529, $[\theta]_{298}$ 0, $[\theta]_{307}$ −4339, $[\theta]_{320}$ 0, $[\theta]_{331}$ +7100, $[\theta]_{352}$ 0; IR (Nujol) ν_{max} 3423, 2937, 1689, 1565, 1511, 1276, 1127, 1024 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 7.43 (1H, d, J = 2.0 Hz, H-2′), 7.27 (1H, dd, J = 2.0 Hz, 8.0 Hz, H-6′), 7.07 (1H, s, H-1), 6.85 (1H, s, H-5), 6.88 (1H, d, J = 8.0 Hz, H-5′), 6.68 (1H, s, H-8), 3.90 (6H, s, 2 × OCH_3), 3.60 (1H, dd, J = 10.0 Hz, 5.0 Hz, H-3a), 3.27 (1H, t, J = 10.0 Hz, H-3a), 3.15 (1H, m, H-3), 3.12 (1H, dd, J = 16.0 Hz, 1.0 Hz, H-4), 2.97 (1H, dd, J = 16.0 Hz, 7.0 Hz, H-4); ^{13}C NMR (CD_3OD, 125 MHz) δ 198.5 (C, C═O), 152.3 (C, C-4′), 150.9 (C, C-6), 149.0 (C, C-3′), 146.2 (C, C-7), 141.9 (CH, C-1), 136.4 (C, C-10), 131.3 (C, C-1′), 129.1 (C, C-2), 126.1 (C, C-9), 125.6 (CH, C-6′), 116.7 (CH, C-8), 115.5 (CH, C-5′), 113.6 (CH, C-2′), 113.3 (CH, C-5), 62.4 (CH_2, C-3a), 56.4 (CH_3, OCH_3), 37.7 (CH, C-3), 29.9 (CH_2, C-4); ESIMS *m/z* 379.13 $[M + Na]^+$, 735.30 $[2M + Na]^+$; HRESIMS *m/z* 379.1159 $[M + Na]^+$ (calcd for $[C_{20}H_{20}O_6+Na]^+$, 379.1159.

Compound (7): white powder (MeOH); mp 109−121 °C; $[\alpha]^{20}_D$ −19.1° (*c* 0.68, MeOH); CD (*c* 0.63 mM, MeOH) $[\theta]_{230}$ 0, $[\theta]_{237}$ +8737, $[\theta]_{245}$ 0, $[\theta]_{276}$ +2445, $[\theta]_{285}$ 0, $[\theta]_{290}$ −12158, $[\theta]_{310}$ 0; IR (Nujol) ν_{max} 3413, 2935, 1604, 1515, 1464, 1430, 1367, 1272, 1236, 1154, 1123, 1032, 857 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 6.93 (1H, d, J = 1.5 Hz, H-2′), 6.83 (1H, dd, J = 8.1 Hz, 1.5 Hz, H-6′), 6.78 (1H, d, J = 8.1 Hz, H-5), 6.77 (1H, dd, J = 8.1 Hz, 1.7 Hz, H-6), 6.76 (1H, d, J = 1.7 Hz, H-2), 6.75 (1H, d, J = 8.1 Hz, H-5′), 6.68 (1H, d, J = 8.0 Hz, H-5″), 6.65 (1H, d, J = 8.0 Hz, H-5‴), 6.54 (1H, d, J = 1.5 Hz, H-2‴), 6.53 (1H, dd, J = 8.0 Hz, 1.5 Hz, H-6‴), 6.52 (1H, d, J = 1.5 Hz, H-2″), 6.49 (1H, dd, J = 8.0 Hz, 1.5 Hz, H-6″), 4.67 (1H, d, J = 7.2 Hz, H-7′), 4.33 (1H, dd, J = 8.8 Hz, 4.4 Hz, H-9), 4.03 (1H, d, J = 8.8 Hz, H-7), 3.99 (1H, dd, J = 8.5 Hz, 7.5 Hz, H-9), 3.82 (3H, s, 3-OCH_3), 3.77 (3H, s, 3′-OCH_3), 3.72 (3H, s, 3″-OCH_3), 3.68 (3H, s, 3‴-OCH_3), 3.61 (1H, dd, J = 11.0 Hz, 4.8 Hz, H-9‴), 3.50 (1H, dd, J = 11.0 Hz, 6.0 Hz, H-9‴), 3.27 (1H, dd, J = 11.0 Hz, 4.1 Hz, H-9′), 3.26 (1H, dd, J = 7.2 Hz, 3.4 Hz, H-9″), 3.18 (1H, dd, J = 7.2 Hz, 5.9 Hz, H-9″), 3.17 (1H, dd, J = 11.0 Hz, 6.0 Hz, H-9′), 2.57 (1H, m, H-7‴), 2.55 (2H, m, H-7″), 2.55 (1H, m, H-8), 2.52 (1H, m, H-7‴), 1.98 (1H, m, H-8″), 1.98 (1H, m, H-8‴), 1.90 (1H, m, H-8′); ^{13}C NMR (CD_3OD, 125 MHz) δ 149.1 (C, C-3), 149.0 (C, C-3′), 148.9 (C, C-3‴), 148.8 (C, C-3″), 147.5 (C, C-4), 147.1 (C, C-4′), 145.5 (C, C-4″), 145.5 (C, C-4‴), 134.8 (C, C-1′), 133.9 (C, C-1‴), 133.8 (C, C-1″), 133.0 (C, C-1), 122.7 (CH, C-6‴), 122.6 (CH, C-6″), 121.8 (CH, C-6), 120.2 (CH, C-6′), 116.1 (CH, C-5), 116.0 (CH, C-5′), 115.9 (CH, C-5″), 115.9 (CH, C-5‴), 113.5 (CH, C-2‴), 113.4 (CH, C-2″), 111.8 (CH, C-2), 111.0 (CH, C-2′), 85.4 (CH, C-7), 84.8 (CH, C-7′), 71.9 (CH_2, C-9), 69.2 (CH_2, C-9″), 62.9 (CH_2, C-9‴), 62.2 (CH_2, C-9′), 56.5 (CH_3, 3′-OCH_3), 56.4 (CH_3, 3-OCH_3), 56.3 (CH_3, 3″-OCH_3), 56.3 (CH_3, 3‴-OCH_3), 53.5 (CH, C-8′), 50.0 (CH, C-8), 44.7 (CH, C-8‴), 41.8 (CH, C-8″), 36.3 (CH_2, C-7″), 35.9 (CH_2, C-7‴); ESIMS *m/z* 743.27 [M + Na]$^+$, 464.59 [2M + Na]$^+$; HRESIMS *m/z* 743.3042 $[M + Na]^+$ (calcd for $[C_{40}H_{48}O_{12}+Na]^+$, 743.3043).

Evaluation of Cytotoxicity. Cytotoxicities to human leukemia cells (K562), human lung cancer cells (A549), human prostate cells (PC-3M), human intestine cancer cells (HCT-8), and human pectin cancer cells (SHG44) were determined in vitro by the MTT assay according to a previously described procedure with minor modification.[7] Cell suspensions of (4−5) × 10^4 cell/mL by 100 μL/well were used. After a 24-hour incubation at 37 °C in a 5% CO_2 atmosphere, test compounds (10^{-2}−10^2 μg/mL) were added to the microplates (10 μL amounts). Then, the tumor cell lines were exposed to the test compounds for another 72 h. The absorbance was read on a Wellscan reader (MK-2, Labsystems, Finland) at 570 nm. Topotecan (purchased from Nanjing Tianzun Zezhong Chemical Co. Ltd., P. R. China) was used as positive control with concentrations of 10^{-3}−10^2 μg/mL. Inhibition concentrations (IC_{50}) to cells were computed.

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