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Three new cytotoxic aryltetralin lignans from *Sinopodophyllum emodi*

Yan-Jun Sun [a,b], Zhan-Lin Li [a,b], Hong Chen [c,d,*], Xiao-Qiu Liu [a], Wei Zhou [a,b], Hui-Ming Hua [a,b,*]

[a] Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, PR China
[b] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, PR China
[c] Pharmacognosy Division, Medical College of Chinese People's Armed Police Force, Tianjin 300162, PR China
[d] Tianjin Key Laboratory for Biomarkers of Occupational and Environmental Hazard, Tianjin 300162, PR China

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ABSTRACT

Three new aryltetralin lignans, 4-acetyl-4-demethyl-podophyllotoxin (**1**) and sinolignans A, B (**2**–**3**), and two new natural products (**4**–**5**), were isolated from the roots and rhizomes of *Sinopodophyllum emodi* together with twelve known lignans (**6**–**17**). Their structures and stereochemistry were elucidated on the basis of spectroscopic evidence, and circular dichroism (CD) method. The cytotoxic activities of all isolated compounds were evaluated against HeLa and KB cell lines. Compared with etoposide, compounds **1**, **6**–**9**, and **13** showed more potent cytotoxicities against two tumor cell lines. On the basis of IC_{50} values, deoxypodophyllotoxin (**7**) was about 579 and 1123 times more toxic than etoposide in HeLa and KB cell lines, respectively. The preliminary SAR study indicated that an oxygenated group at C-7′ might decrease cytotoxicity against two cell lines, which was different from most previous studies. However, this needs to be systematically verified by extensive pharmacological experiments.

The aryltetralin lactone podophyllotoxin occupies a unique position among lignan natural products since its glucopyranoside derivatives, etoposide and teniposide, have been developed as clinically useful anticancer medicines. Although they are active in the treatment of many cancers and are widely used in the therapy, it presents several limitations, such as moderate potency, poor water solubility, development of drug resistance, metabolic inactivation, and toxic effects.[1,2] To improve its clinical efficacy and reduce side effects, extensive structural modification of podophyllotoxin and an ongoing search for new podophyllotoxin derivatives from natural medicines have been conducted in numerous laboratories.[3–7] *Sinopodophyllum emodi* (Wall.) Ying is a folk-medicine used for treating cancer, and various verrucosis in the southwest of China.[3] Previous chemical and pharmacological investigations on *S. emodi* revealed the presence of bioactive aryltetralin lignans,[3–6] and cytotoxic and radioprotective properties.[8] In a continuation of our search for cytotoxic natural products, we herein described the isolation, structure elucidation and evaluation of cytotoxic activity of seventeen aryltetralin lignans (Fig. 1) along with their preliminary structure–activity relationships.

The 95% EtOH extract of the roots and rhizomes of *S. emodi* (18.8 kg) was partitioned between petroleum ether (PE), $CHCl_3$, *n*-BuOH and water, respectively. The PE, $CHCl_3$ and *n*-BuOH layers were fractionated and purified by repeated column chromatography, allowing the isolation of three new lignans (**1**–**3**), two new natural products (**4**–**5**), and twelve known lignans.

Compound **1** was obtained as white needles and possessed a molecular formula $C_{23}H_{22}O_9$, as revealed from its TOF-ESI-MS analysis (*m*/*z* 465.1163 $[M+Na]^+$, calcd for 465.1162).[9] The ^1H NMR spectrum showed signals of two methoxyl groups at δ 3.70 (6H, s), four aromatic protons at δ 7.11 (1H, s), 6.51 (1H, s), 6.41 (2H, s), methylenedioxy group protons at 5.97 (1H, s), 5.96 (1H, s), and a methyl proton at δ 2.30 (3H, s). The ^{13}C NMR spectrum revealed a skeleton of aryltetralin lactone lignan including one carbonyl group at δ 174.5, twelve aromatic carbons and five aliphatic carbons, besides two methoxyl groups at δ 56.3 (×2), one acetyl at δ 169.1, 20.6, one methylenedioxy group at δ 101.6.[10] A careful comparison of the NMR spectra of **1** with 4-demethylpodophyllotoxin indicated that compound **1** was an acetylation derivative of 4-demethyl-podophyllotoxin, which was confirmed by detailed analysis of the HSQC and HMBC spectra. The HMBC correlation between the signal at δ 127.9 (C-4) and δ 2.31 ($COCH_3$), indicated that acetoxyl was located at C-4 of 4-demethyl-podophyllotoxin.

Establishment of the relative configuration was based on the ^1H coupling constants (*J* values) and NOESY experiment. The $J_{H\text{-}8/H\text{-}8'}$ (14.2 Hz) and $J_{H\text{-}8'/H\text{-}7'}$ (9.0 Hz) values indicated the *trans*-form of H-8/H-8′ and H-8′/H-7′.[6,11–13] The $J_{H\text{-}7/H\text{-}8}$ value was 3–5 Hz for *cis*-configuration of H-7/H-8 and 7–9 Hz for the *trans*-configuration.[4–6] Compound **1** gave $J_{H\text{-}7/H\text{-}8}$ = 4.4 Hz, indicating *cis*-configuration of H-7/H-8. The relative configuration was further confirmed by NOESY experiment. NOE of H-7/H-7′, H-7/H-8, H-8/H-7′ indicated H-7, H-8, H-7′ were at the same face. Studies on the ORD

* Corresponding authors. Tel./fax: +86 22 60578193 (H.C.); tel./fax: +86 24 23986465 (H.-M.H.).
E-mail addresses: Chenhongtian06@yahoo.com.cn (H. Chen), huimhua@163.com (H.-M. Hua).

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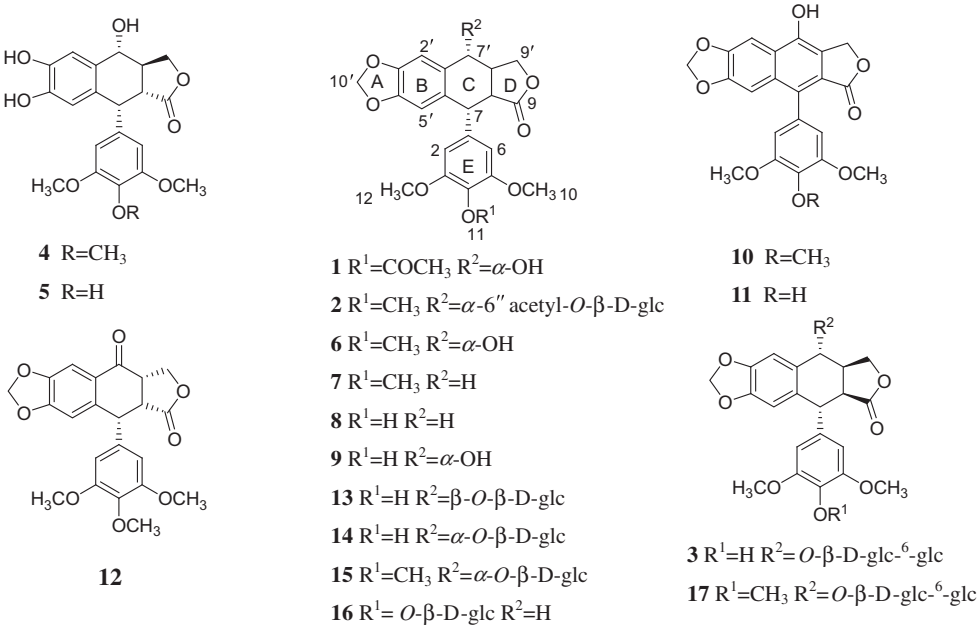

Figure 1. Structures of compounds **1**–**17** from *S. emodi*.

and CD curves of 7-aryltetralin lignans showed that all 7β (*S*)-aryl compounds gave negative Cotton effects at around 280–290 nm, while all 7α (*R*)-aryl compounds gave positive Cotton effect.[5] The CD spectrum of compound **1** exhibited positive Cotton effect at 288 nm. Consequently, the absolute configuration of C-7 was determined to be *R*. Thus, compound **1** was established as 4-acetyl-4-demethyl-podophyllotoxin.

Compound **2** was obtained as amorphous powder and possessed a molecular formula $C_{30}H_{34}O_{14}$, as revealed from its QFT-ESI-MS analysis (*m*/*z* 641.1845 [M+Na]$^+$, calcd for 641.1846).[14] The ^{1}H NMR spectrum showed proton signals of three methoxyl groups at δ 3.62 (6H, s), 3.61 (3H, s), four aromatic protons at δ 7.35 (1H, s), 6.54 (1H, s), and 6.30 (2H, s), methylenedioxy group protons at 6.00 (1H, s), 5.98 (1H, s), methyl protons at δ 1.63 (3H, s). The ^{13}C NMR spectrum revealed a skeleton of aryltetralin lactone lignan including one carbonyl group at δ 174.6, twelve aromatic carbons and five aliphatic carbons, besides three methoxyl groups at δ 55.9 (×2), 60.0, 6-acetyl-glucopyranosyl at δ 100.8, 73.4, 76.4, 70.4, 74.0, 63.8, 170.1, 20.0, and one methylenedioxy group at δ 101.2.[10] A careful comparison of the NMR spectra of **2** with podophyllotoxin-4-*O*-β-D-glucopyranoside suggested compound **2** to be an acetylation derivative of podophyllotoxin-4-*O*-β-D-glucopyranoside. This assignment was confirmed by detailed analysis of the HSQC and HMBC spectra. The HMBC correlations between the signal at δ 170.1 (C-7″) and δ 4.31, 3.95 (H-6″) indicated that acetoxyl was located at C-6 of glucopyranosyl group. The $J_{H\text{-}8/H\text{-}8'}$ (14.5 Hz), $J_{H\text{-}8'/H7'}$ (10.2 Hz) and $J_{H\text{-}7/H\text{-}8}$ (4.9 Hz) values and NOE correlation of H-7/H-7′, H-8/H-7′ indicated that **2** possessed the same relative configuration as podophyllotoxin.[2–5] The CD spectrum of compound **2** gave positive Cotton effect at 289 nm, so the absolute configuration of C-7 was determined to be *R*. Thus, compound **2** was deduced as 6″-acetyl-podophyllotoxin-7′-*O*-β-D-glucopyranoside, and named sinolignan A.

Compound **3** was obtained as amorphous powder and its molecular formula was determined as $C_{33}H_{40}O_{18}$ on the basis of its QFT-ESI-MS (*m*/*z* 747.2096 [M+Na]$^+$, calcd for 747.2112).[15] The ^{1}H NMR spectrum showed proton signals of two methoxyl groups at δ 3.74 (6H, s), four aromatic protons at δ 7.22 (1H, s), 5.96 (1H, s), 6.58 (2H, s), methylenedioxy group proton signals at 5.95 (1H, s), 5.84 (1H, s). The ^{13}C NMR spectrum exhibited one carbonyl group at δ 174.6, twelve aromatic carbons and five aliphatic carbons, besides two methoxyl groups at δ 56.1 (×2), one methylenedioxy group at δ 100.7, two sets of glucopyranosyl groups at δ 103.7, 73.8, 77.0, 70.4, 76.8, 68.1, 103.2, 73.5, 76.4, 70.0, 76.4, 61.0. The aglycone was identified as picropodophyllotoxin by comparison of its NMR data with those reported in the literature,[16] combined with correlations observed in the NOESY, HSQC and HMBC spectra. The ^{13}C NMR chemical shifts and spin-spin coupling constants (7.6, 8.1 Hz) of two anomeric protons allowed the identification of two β-glucopyranosyl moieties. The absolute configuration of glucose was determined by a microhydrolysis method and GC analysis.[17] The cross peaks of the anomeric proton at δ 4.49 (H-1″) with C-7′ (δ 76.4) and the other anomeric proton δ 4.07 (H-1‴) with C-6″ at δ 68.1, indicated that one glucopyranosyl was at C-7′ of the aglycone and the another was substituted at C-6″ of the inner glucose.

The $J_{H\text{-}7/H\text{-}8}$ (8.2 Hz), $J_{H\text{-}8'/H7'}$ (9.7 Hz) and $J_{H\text{-}8/H\text{-}8'}$ (9.7 Hz) values combined with NOE correlation of H-7/H-7′ and H-8/H-8′ determined the relative configuration of 7,8-*trans*-8′,7′-*trans*-8,8′-*cis*. Compound **3** gave positive Cotton effects at 288 nm, indicating the absolute configuration of C-7 to be *R*. Thus, compound **3** was deduced as 4-demethyl-picropodophyllotoxin-7′-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, and named sinolignan B.

By comparing physical and spectroscopic data with literatures, the fourteen known constituents were identified as 3′,4′-demethylene-podophyllotoxin (**4**),[18,19] 3′,4′-demethylene-4-demethyl-podophyllotoxin (**5**),[18,19] podophyllotoxin (**6**),[6] deoxypodophyllotoxin (**7**),[6] 4-demethyl-deoxypodophyllotoxin (**8**),[6] 4-demethyl-podophyllotoxin (**9**),[6] dehydropodophyllotoxin (**10**),[20] 4-demethyl-dehydropodophyllotoxin (**11**),[20] isopicropodophyllone (**12**),[6] 4-demethyl-epipodophyllotoxin-7′-*O*-β-D-glucopyranoside (**13**),[3] 4-demethyl-podophyllotoxin-7′-*O*-β-D-glucopyranoside (**14**),[4] podophyllotoxin-7′-*O*-β-D-glucopyranoside (**15**),[4] 4-demethyl-deoxypodophyllotoxin-4-*O*-β-D-glucopyranoside (**16**),[21] and picropodophyllotoxin-7′-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**17**).[5]

All isolated compounds were tested for their in vitro cytotoxic activity against HeLa and KB cell lines using MTT assay (Table 2).[22] Compound **7** showed the highest cytotoxicity against HeLa cell line

Table 1
^{1}H NMR and ^{13}C NMR spectroscopic data for compounds **1**–**3**

Position	**1**[a]		**2**[b]		**3**[b]	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	138.5		136.5		132.1	
2	107.9	6.41, s	108.4	6.30, s	106.6	6.58, s
3	151.6		152.1		148.0	
4	127.9		136.7		134.2	
5	151.6		152.1		148.0	
6	107.9	6.41, s	108.4	6.30, s	106.6	6.58, s
7	44.3	4.61, d, 4.4	43.3	4.53, d, 4.9	44.3	3.85, d, 8.2
8	45.4	2.85, dd, 4.4, 14.2	44.6	3.17, dd, 4.9, 14.5	44.3	3.43, dd, 9.7, 8.2
9	174.7		174.6		178.0	
1′	130.9		130.9		132.3	
2′	106.6	7.11, s	107.9	7.35, s	105.8	7.22, s
3′	147.8		146.8		145.5	
4′	147.8		147.1		146.1	
5′	109.9	6.51, s	109.4	6.54, s	107.5	5.96, s
6′	133.5		132.3		132.8	
7′	72.7	4.73, d, 9.0	78.6	4.97, d, 10.2	76.4	4.62, d, 10.0
8′	40.7	2.78, m	38.3	2.80, m	42.1	2.76, m
9′	71.6	4.56, dd, 9.0, 7.1; 4.03, dd, 9.6, 9.0	71.2	4.46, dd, 8.5, 7.6; 4.15, dd, 10.3, 8.5	68.9	4.57, dd, 9.5, 1.1; 4.42, dd, 9.5, 6.7
10	56.1	3.70, s	55.9	3.62, s	56.1	3.74, s
11			60.0	3.61, s,		
12	56.3	3.70, s	55.9	3.62, s	56.1	3.74, s
10′	101.6	5.96, s; 5.97, s	101.2	5.98, s; 6.00, s	100.7	5.84, s; 5.95, s
1″			100.8	4.28, d, 7.5	103.7	4.49, d, 7.6
2″			73.4	3.10, m	73.8	3.19, m
3″			76.4	3.15, m	77.0	3.24, m
4″			70.4	3.07, m	70.4	3.07, m
5″			74.0	3.24, m	76.8	3.41, m
6″			63.8	4.31, dd, 1.9, 11.2; 3.95, dd, 8.3, 11.2	68.1	3.93, br d,11.3; 3.52, m
1‴					103.2	4.07, d, 7.6
2‴					73.5	2.84, m
3‴					76.4	2.54, m
4‴					70.0	2.94, m
5‴					76.4	2.69, m
6‴	169.0		170.1		61.0	3.56, m; 3.35, dd, 5.7, 11.7
$COCH_3$	20.6	2.31, s	20.0	1.63, s		

[a] NMR spectroscopic data were recorded in $CDCl_3$ at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR).
[b] NMR spectroscopic data were recorded in DMSO-d_6 at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR).

Table 2
Cytotoxic activities of compounds **1**–**17** against HeLa and KB cell lines

Compound	IC_{50}[c] (μM)		Compound	IC_{50}[c] (μM)	
	HeLa	KB		HeLa	KB
1	0.076 ± 0.005	0.05 ± 0.003	**10**	8.28 ± 0.79	9.79 ± 0.9
2	3.77 ± 0.32	8.76 ± 0.75	**11**	>100	84.7 ± 8.1
3	>100	>100	**12**	32.1 ± 3.11	>100
4	>100	>100	**13**	0.048 ± 0.004	<0.01
5	79.6 ± 6.8	>100	**14**	5.33 ± 0.42[e]	9.51 ± 0.7[e]
6	0.069 ± 0.005[d]	0.056 ± 0.004[d]	**15**	6.65 ± 0.58[d]	9.25 ± 0.8[d]
7	0.0069 ± 0.0005	0.0089 ± 0.0006	**16**	0.87 ± 0.06	2.09 ± 0.2
8	0.074 ± 0.006	0.0078 ± 0.0006	**17**	>100	>100
9	0.08 ± 0.005	0.064 ± 0.005[e]	Etoposide	4.0 ± 0.3	10.0 ± 0.9

[c] IC_{50} is defined as the concentration of drug required to inhibit cell growth by 50% compared with untreated control; It is expressed as mean ± SD of at least three determinations.
[d] Significant differences are indicated as: p <0.05 compared with compound **7**.
[e] Significant differences are indicated as: p <0.05 compared with compound **8**.

and compound **8** was the most active against KB cell line. The 7′α-hydroxy derivatives **6**, **15**, and **9**, **14** exhibited significantly lower activity compared to compounds **7** and **8** without 7′-substitution. The glycosylation of the 7′α-hydroxy group (**9** and **6**) with a glucose moiety (**14** and **15**) strongly reduced the activity. The configuration at C-7′ is important, as 7′β-glucosyl derivative **13** showed more potent activity than 7′α-glucosyl compounds **14** and **15**. Compounds **6** and **9** containing a non-aromatized ring C showed to have more cytotoxic activity than aromatized compounds **10** and **11**, indicating that a non-aromatized ring C was structurally required for the cytotoxicity against HeLa and KB cells lines. The ring A-opened compounds (**4** and **5**) were found to be less potent than the methylenedioxy-bearing analogues (**6** and **9**). Methylation and acetylation of a free hydroxyl at C-4 did not significantly influence the cytotoxic activity, for example, compared **6** and **1** to **9**, and **15** to **14**.

The remarkable biological activity makes podophyllotoxin an important starting product for the development of new therapeutic agents.[7] Extensive structural modifications of podophyllotoxin have been undertaken to obtain more active and less toxic antitu-

mor agents. On the basis of the published SAR studies related podophyllotoxin analogues, the substitution and configuration at C-7′, a methylenedioxy at ring A, non-aromatized ring C, and a *trans*-fusion between the tetraline and lactone, play a very important role in maintaining cytotoxicity for this series of compounds.[7,23–27] C-7′ position was the only molecular area tolerable to significant structural diversification and resulted in a number of promising candidates. Most of research efforts have been focused on exploring different 7′-substituted podophyllotoxin analogues.[7] Position 7′ was very important in antimitotic activity.[28] However, disappearance of the free hydroxyl group at C-7′ did not elicit significant variations in the antitumor activity.[28] Deoxypodophyllotoxin (**7**) showed stronger cytotoxicity than podophyllotoxin (**6**) against GLC4 cell line.[23] Podophyllotoxin analogues (**6**, **15** and **9**, **14**) with 7′α oxygenated group exhibited significantly lower activity than corresponding analogues (**7** and **8**) without 7′-substitution, which suggested that 7′-substitution may be not essential for cytotoxicity in podophyllotoxin-type compounds. Novel analogues with no substitution at C-7′ would be necessarily designed and synthesized to obtain new antitumor agents.

The continuous search of natural podophyllotoxin analogues from medicinal plants or their tissue cultures was carried out by natural products chemists. More than 40 aryltetralin lignans have been isolated from the plants of genus *Sinopodophyllum*, *Podophyllum*, *Dysosma*, *Diphylleia* (Berberidaceae), *Linum* (Linaceae), *Libocedrus* (Cupressaceae), *Bursera* (Burseraceae), etc.[3–6,13,20,21,29–34] A few of them were tested for the cytotoxic activity in tumor cell lines.[30–34] The phytochemical studies on *S. emodi* resulted in the isolation of 17 aryltetralin lignans including three new compounds (**1**–**3**), two new natural products (**4**–**5**). Compounds **4** and **5** represent the first report of ring A-opened podophyllotoxin analogues from *Sinopodophyllum*. Most compounds showed moderate to high activity against HeLa and KB cell lines. Compounds **7** and **8** were the most interesting of the isolated compounds based upon their IC_{50} values. Based on these preliminary results obtained by us, further studies on compound **7** are necessary to explore antitumor mechanism and cytotoxicities in normal cells.

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Supplementary data

Supplementary data (spectra of compounds **1**–**3** along with the general experimental details, extraction and isolation, acid hydrolysis and sugar determination, and in vitro cytotoxicity assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.036.

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14. *Sinolignan A* (**2**): amorphous powder; $[\alpha]_D^{25}$ −79.1 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 208 (1.7), 288 (0.2); CD (MeOH) λ_{max} (Δε) 234 (+74.1), 266 (−17.8), 289 (+2.9) nm; IR (KBr) ν_{max} 3440, 2936, 1772, 1738, 1590, 1506, 1239, 1127, 1037, 931 cm^{-1}; NMR data (DMSO-d_6), see Table 1; ESI-MS *m*/*z* 641 $[M+Na]^+$, 657 $[M+K]^+$, 653 $[M+Cl]^-$, 663 $[M+HCOO^-]^+$; QFT-HR-ESI-MS *m*/*z* 641.1845 $[M+Na]^+$ (calcd for $C_{30}H_{34}O_{14}Na$, 641.1846).
15. *Sinolignan B* (**3**): Amorphous powder(MeOH); $[\alpha]_D^{25}$ -40.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 208 (2.1), 284 (0.3); CD (MeOH) λ_{max} (Δε) 224 (+2.2), 264 (−1.1), 288 (+1.0) nm; IR (KBr) ν_{max} 3425, 2919, 1772, 1769, 1618, 1523, 1265, 1120, 1035, 929 cm^{-1}; NMR data (DMSO-d_6), see Table 1; ESI-MS: *m*/*z* 747 $[M+Na]^+$, 763 $[M+K]^+$, 723 $[M-H]^-$; QFT-HR-ESI-MS *m*/*z* 747.2096 $[M+Na]^+$, (calcd for $C_{33}H_{40}O_{18}Na$, 747.2107).
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