Cytotoxic Arylnaphthalide Lignan Glycosides from the Aerial Parts of *Phyllanthus taxodiifolius*

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Abstract

The arylnaphthalide lignan glycosides, taxodiifoloside (**1**), cleistanthoside A (**2**), cleistanthin A (**3**) and cleistanthin A methyl ether (**4**), together with a triterpene, glochidone (**5**), have been isolated from the aerial parts of *Phyllanthus taxodiifolius.* The structures were established using spectral and chemical methods. Compounds **3** and **4**, as well as the derivatives **2a** and **3a** exhibited potent cytotoxic activities with GI_{50} values in the range of 10^{-7} – 10^{-9} M in five cultured mammalian cancer cell lines while the new compound **1** showed moderate activity (GI_{50} in the order of 10^{-6} M). Compounds **2** and **5** were inactive in all tested cell lines.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Phyllanthus taxodiifolius Beille (Euphorbiaceae), a shrub found in the central and Northeastern parts of Thailand [1], is used in Thai traditional medicine as a diuretic. In our ongoing search for anticancer agents from plants, an investigation of the cytotoxic EtOAc fraction obtained from the partitioning of the crude MeOH extract of the aerial parts of this plant has led to the isolation of a new arylnaphthalide lignan glycoside, taxodiifoloside (**1**), along with three known arylnaphthalide lignan glycosides, cleistanthoside A (**2**) [2], cleistanthin A (**3**) [3], [4], [5], cleistanthin A methyl ether (**4**) [3], [4] and a known triterpene, glochidone (**5**) [6], [7]. Compound **4**, previously prepared from natural **3**, was isolated for the first time as a natural product. NMR assignments of **1** are reported (Table **1**) while the revised data of other glycosides are included in the Supporting Information [8]. Both natural and modified compounds **1**, **2**, **2a**, **3a**, **4** and **5** were evaluated for cytotoxic effects against five cultured mammalian cancer cell lines for the first time.

Taxodiifoloside (**1**) ($C_{36}H_{40}O_{17}$) displayed typical ^{1}H-NMR signals of a substituted arylnaphthalene nucleus and a disaccharide portion (Table **1**). The doubling of some signals, due to restricted rotation around the aryl-naphthalene bond [9], is indicated by an asterik (*). The presence of signals corresponding to two aromatic protons, a 1,3,4-trisubstituted phenyl moiety, a lactone methylene, a methylenedioxy group and two aromatic methoxy groups suggested that **1** was a diphyllin analogue. The sugars in the disaccharide unit were identified as 3,4-di-*O*-methyl-*β*-D-xylopyranose and 6-acetoxy-*β*-D-glucopyranose by analyses of the *J* values, ^{13}C-NMR (Table **1**) and 2D-NMR data (Table **1** in Supporting Information). The locations of the methoxy groups at C-3″, C-4″ and the acetoxy group at C-6‴ were confirmed with HMBC correlations. The connectivities of the two sugars and to the diphyllin

Table **1** ^{1}H- and ^{13}C-NMR data of compound **1** in $CDCl_3$

Position	δ_H[a]	δ_C[b]
1	–	136.42 (C)
1a	–	130.76, 130.70* (C)
2	–	119.13 (C)
2a	–	169.74 (C)
3	–	130.81 (C)
3a	5.557, 5.556* (d each, 15, Ha), 5.424, 5.418* (d each, 15, Hb)	67.28 (CH_2)
4	–	143.72 (C)
4a	–	126.86 (C)
5	7.932, 7.927* (s each)	101.52 (CH)
6	–	151.95 (C)
7	–	150.29 (C)
8	7.08 (s)	106.21 (CH)
1′	–	128.32 (C)
2′	6.84, 6.82* (d each, 1.6)	110.71, 110.61* (CH)
3′ and 4′	–	147.53 (C)
5′	6.96 (d, 8.0)	108.19 (CH)
6′	6.79 (dd, 8.0, 1.6)	123.61, 123.50* (CH)
7′	6.099, 6.097* (d each, 1.4, Ha), 6.055, 6.053* (d each, 1.4, Hb)	101.22 (CH_2)
1″	4.996, 4.993* (d each, 7.0)	103.27 (CH)
2″	3.937, 3.934* (dd each, 8.3, 7.0)	80.99, 80.92* (CH)
3″	3.43 (t, 8.3)	83.08 (CH)
4″	3.46 (obsc.)	79.56 (CH)
5″	4.037, 4.034* (dd each, 12.1, 4.6, Ha), 3.132, 3.131* (dd each, 12.1, 8.8, Hb)	62.54 (CH_2)
1‴	4.68 (d, 7.6)	105.91, 105.87* (CH)
2‴	3.48 (dd, 9.1, 7.6)	75.32 (CH)
3‴	3.64 (t, 9.1)	75.74 (CH)
4‴	3.40 (t, 9.1)	69.61 (CH)
5‴	3.51 (ddd, 9.1, 4.7, 2.0)	74.76 (CH)
6‴	4.38 (dd, 12.3, 4.7, Ha), 4.09 (obsc., Hb)	63.06 (CH_2)
6-OMe	4.11 (s)	56.53 (CH_3)
7-OMe	3.81 (s)	55.81 (CH_3)
3″-OMe	3.74 (s)	60.78 (CH_3)
4″-OMe	3.47 (s)	58.23 (CH_3)
6‴-OCO*Me*	1.79 (s)	20.20 (CH_3)
6‴-O*CO*Me	–	171.54 (C = O)
OH	4.69 (br), 3.34 (br), 3.29 (br)	–

[a] Recorded at 500 MHz; chemical shift given in ppm using TMS as internal reference; multiplicities and *J* values (Hz) are given in parentheses; obsc. = obscured signal.

[b] Recorded at 125 MHz; chemical shift given in ppm using $CDCl_3$ signal at δ_C = 77.00 as reference.

* A doubling of signals was observed due to restricted rotation.

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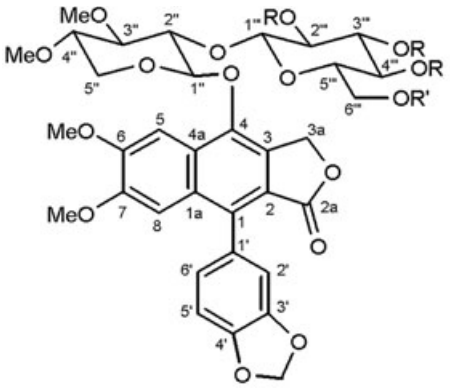
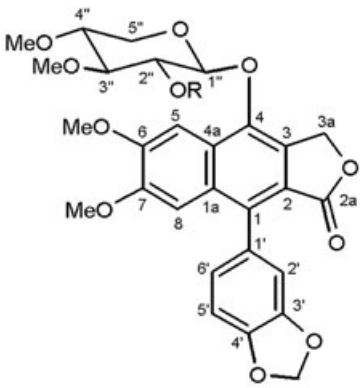


1 R = H, R' = Ac
2 R = R' = H
2a R = R' = Ac



3 R = H
3a R = Ac
4 R = Me

moiety were deduced from the NOESY and HMBC correlations. Therefore, the structure of **1** was established as 4-*O*-[6-acetoxy-β-D-glucopyranosyl(1→2)-3,4-di-*O*-methyl-β-D-xylopyranosyl]-diphyllin. The stereochemistry within the sugars was confirmed by conversion to the tetraacetate **2a**, which was found identical to the compound derived from acetylation of **2**.

Cleistanthoside A (**2**), previously isolated from *Cleistanthus patulus* [2], was characterized as its tetraacetate **2a**. Cleistanthin A (**3**) [10], [11] was identical to that reported from *Cleistanthus collinus* [3], [4], [5]. The preparation of acetate **3a** [3], [4] further confirmed the structure **3** and provided material for biological evaluation. Methylation of **3** gave **4**, which was identical to natural **4**. Glochidone (**5**) was identified by comparison of the physical and spectral data to those reported for the substance isolated from *Glochidion hohenackeri* [6] and *Glochidion heyneanum* [7].

The MeOH extract, fractions and pure compounds were screened for cytotoxicity against five cultured mammalian cancer cell lines according to an established protocol [12]. Ellipticine was used as a positive control (Table **2** in Supporting Information). Compounds **2a**, **3**, **3a** and **4** exhibited potent activities with GI_{50} values in the range of 10^{-7} – 10^{-9} M. Compound **1** was less active with a GI_{50} value in the order of 10^{-6} M. Compounds **2** and **5** were inactive in all tested cell lines. It is noteworthy that **2a** was very active (3.4 nM in P-388, KB and Col-2, 4.6 nM in MCF-7 and 46.0 nM in Lu-1) while its precursor **2** was inactive. The cytotoxic values of **3a** are comparable to those of **3** and **4**. Overall, the present work represents a significant contribution to the chemical and biological aspects of arylnaphthalide lignan glycosides.

Materials and Methods

Melting points (°C): uncorrected; NMR spectra: Bruker AV 500 spectrometer; HR-TOF-MS: Micromass model VQ-Tof2. CC: silica gel 60 (63 – 200 μm); PLC silica gel 60 PF_{254} (5 – 40 μm, 0.5 mm). Plant materials: collected in January 2002 from Amnartchareon, Thailand. Voucher specimen (BKF no. 127614): deposited at the Forest Herbarium in Bangkok.

Dried, powdered aerial parts (14.3 kg) were extracted with MeOH (6 × 33 L) to give a crude MeOH extract (835 g). After suspension in H_2O (2.5 L), it was sequentially partitioned with hexane (5 × 4 L), EtOAc (5 × 4 L) and *n*-BuOH (4 × 3 L), Removal of solvents yielded the hexane, EtOAc, *n*-BuOH and H_2O fractions in 230, 159, 140 and 283 g, respectively. The cytotoxic EtOAc fraction (158 g) was subjected to CC (SiO_2, 1.5 kg), eluting with CH_2Cl_2-hexane (0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100%, 4 L each), followed by MeOH-CH_2Cl_2 (1, 2, 3, 5, 7, 10, 15, 20, 30, 50, 70 and 100%, 6 L each). Frs. (500 mL each) were combined on the basis of TLC behaviour to give frs. A1 – A12. Fr. A4 (14.0 g, eluted with 30 – 50% CH_2Cl_2-hexane), after recrystallization gave **5** {8.8 g, colourless needles, m.p. 166.0 – 166.6 °C (CH_2Cl_2 – MeOH), $[\alpha]_{589}^{30}$: + 72.38° (*c* 0.1 $CHCl_3$)}.

Purification of fr. A9 (22.2 g, eluted with 2 – 5% MeOH-CH_2Cl_2) by CC (SiO_2, 230 g) afforded frs. B1 - B3. Fr. B1 (13.0 g, eluted with 0 – 30% Me_2CO-hexane) was separated by CC (SiO_2, 325 g) to give frs. C1 – C5. Further separation of fr. C1 (2.6 g, eluted with 0 – 15% Me_2CO-hexane) by CC (SiO_2, 60 g), yielded frs. D1 – D8. Fr. D7 (173.2 mg, eluted with 40 – 50% EtOAc-hexane) afforded **4** {11.3 mg, white powder, m.p. 211 – 215 °C (EtOH), $[\alpha]_{589}^{27}$: –50° (*c* 1.0, $CHCl_3$)} after PLC (40% EtOAc-hexane as eluent).

Fr. B2 (7.0 g, eluted with 30 – 35% Me_2CO-hexane) yielded **3** {2.9 g, white powder, m.p. 140.0 – 141.3 °C (EtOH), $[\alpha]_{589}^{27}$: –69.6° (*c* 1.02, $CHCl_3$)} after recrystallization.

Fr. A10 (13.5 g, eluted with 5 – 7% MeOH-CH_2Cl_2) was purified by CC (SiO_2, 170 g) to provide frs. E1 - E3. Separation of Fr. E2 (4.7 g, eluted with 1 – 15% MeOH-CH_2Cl_2) by CC (SiO_2, 226 g) gave frs. F1 – F7. Fr. F4 (1.5 g, eluted with 6% MeOH-CH_2Cl_2) was separated by CC (Sephadex LH20, 4.8 g, MeOH as eluent) to afford frs. G1 - G4. Further separation of fr. G2 (834.5 mg) by CC (SiO_2, 20 g) gave frs. H1 and H2. Fr. H2 (402.3 mg, eluted with 60 – 100% EtOAc-hexane), after PLC (SiO_2, 2% MeOH-EtOAc, 3 elutions) and recrystallization (EtOAc-hexane), afforded **1** (27.2 mg).

Fr. F5 (410 mg, eluted with 6 – 20% MeOH-CH_2Cl_2) was further purified by CC (SiO_2, 26 g) to give frs. I1 – I4. Fr. I4 (60.3 mg, eluted with 1 – 20% Me_2CO-MeOH), after recrystallization, afforded **2**

{14.7 mg, white powder, m.p. 237 – 240 °C (EtOH), $[\alpha]_{589}^{26}$: –5.7° (MeOH, *c* 1.0)}.

Fr. A11 (25.9 g, eluted with 7 – 10% MeOH-CH_2Cl_2) after separation on CC (SiO_2, 500 g) yielded frs. J1 – J4. Fr. J2 (6.13 g, eluted with 4 – 8% MeOH-CH_2Cl_2) was separated by CC (SiO_2, 200 g) to provide frs. K1 - K3. Separation of fr. K2 (1.95 g, eluted with 1 – 20% MeOH-CH_2Cl_2) by CC (SiO_2, 62 g) gave frs. L1 – L3. Fr. L2 (336.5 mg, eluted with 6 – 9% MeOH-CH_2Cl_2) was separated by PLC (SiO_2, 0.5% MeOH-EtOAc, 3 elutions) to give **2** (150.1 mg).

Fr. J3 (14.34 g, eluted with 8 – 10% MeOH-CH_2Cl_2) was separated by CC (SiO_2, 320 g) to yield frs. M1 – M3. Fr. M3 (9.47 g, eluted with 3% MeOH-CH_2Cl_2) crystallized in EtOH to give **2** (5.62 g).

Taxodiifoloside (**1**): White powder, m.p. 142.3 – 143.1 °C (EtOAc-hexane); $[\alpha]_{589}^{27}$: –34° ($CHCl_3$, *c* 0.5); UV (EtOH): λ_{max} (log ε) = 223 (3.92), 258 (4.31), 294 (3.67), 314 (3.70), 349 (3.46) nm; CD (5.38×10^{-5} M, EtOH): λ_{max} ($\Delta\varepsilon$) = 315 (–2. 37), 263 (–9.08) nm; IR (KBr): ν_{max} = 3419, 1756, 1742, 1507, 930 cm^{-1}; EI-MS: *m/z* (rel. int.) = 744 $[M]^+$ (0.5), 394 (100), 203 (29), 97 (24); HR-TOF-MS (ESI positive): *m/z* = 767.2163 (calcd. for $C_{36}H_{40}O_{17}Na$: 767.2163).

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