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STILBENOIDS FROM THE LIANAS OF *GNETUM PENDULUM*

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The first new isorhapontigenin dimer glucoside gnetupendin D (**1**) and a stilbene dimer coupled by a resveratrol and an oxyresveratrol with a *cis*-dihydrobenzofuran were obtained from the lianas of *Gnetum pendulum* C. Y. Cheng (Gnetaceae), along with nine known compounds. Their structures and stereochemistry were elucidated by chemical and spectral evidence, especially 2D NMR spectroscopic techniques. Pharmacological activities on anti-inflammation have been tested.

Keywords: *Gnetum pendulum*; Gnetaceae; Gnetupendin D; Stilbenoid

INTRODUCTION

In recent years, various types of oligostilbenes showing potent inhibition of anti-inflammatory activities have been found from three Chinese *Gnetum* species, *G. parvifolium*, *G. montanum* and *G. hainanense* [1]. To find leading compounds for drug development, we further investigated another *Gnetum* species, *G. pendulum*, from which a new stilbenoid, gnetupendin D (**1**), and gnetupendin C (**3**), a stilbene dimer coupled by a resveratrol and an oxyresveratrol with a *cis*-dihydrobenzofuran, were obtained along with nine known compounds, namely isorhapontigenin, resveratrol, daucosterol, shegansu B (**2**), gnetupendin A and B [2], gnetulin, gnetin D (**4**), and isorhapontigenin-3-*O*-β-D-glucoside. Compound **1** is an isorhapontigenin dimer glucoside, in which the aglycone part is shegansu B [3] and the sugar part is a D-glucose. This is an isorhapontigenin dimer glucoside obtained from nature for the first time. Compound **3** is a stilbene dimer coupled by a resveratrol and an oxyresveratrol with a *cis*-dihydrobenzofuran [4], which is different from **4**, first obtained from *G. leyboldii* Tull. [5]. This paper mainly deals with the isolation and structural determination of the new stilbenoid **1** and **3** by chemical and spectral evidences. The pharmacological activities of **3** and **4** are also evaluated.

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RESULTS AND DISCUSSION

Gnetupendin D (**1**) was obtained as a brown amorphous powder; $[\alpha]_D^{17}$ −32.2 (*c* 0.09, MeOH); exhibiting strong blue fluorescence under UV light at 254 nm. The IR and UV spectra were similar to those of compound **2**. Its FAB-MS spectrum showed a molecular ion peak at *m/z* 676 $[M + H]^+$ and significant fragment peaks at *m/z* 515 [aglycone + H]$^+$ and 163 [M + H-aglycone]$^+$, indicating that **1** was a glucoside of **2** (the ^{1}H and ^{13}C NMR data are given in Table I). Therefore, the molecular formula of **1** was deduced as $C_{36}H_{36}O_{13}$, which was supported by HR-FABMS. The ^{1}H NMR spectrum of **1** was similar to that of **2** except for the additional aliphatic protons signals of glucose at high field, especially the obvious appearance of the anomeric proton at δ 4.94 (1H, d, $J = 7.8$ Hz). The ^{1}H NMR spectrum showed two methoxy groups, one ABX system for ring A_1, one AB_2 system for ring A_2, a pair of *meta*-coupled olefinic doublets for ring B_1, two coupled doublets of *trans* olefinic protons, two signals of dihydrobenzofuran moiety, and three signals without symmetry characteristic of an AB_2 system for ring B_2 appearing at δ 6.81 (1H, br s, H-10b),

TABLE I ^{1}H and ^{13}C NMR data for compounds **1*** and **2**†

	1			***2***	
Position	^1H	^{13}C	*HMBC*	^1H	^{13}C
1a		132.7			132.7
2a	7.01 d (1.8)		3a, 4a, 6a, 7a	7.02 d (2.0)	110.9
3a		148.1			148.4
4a		147.1			147.6
5a	6.82 d (7.8)	115.4	3a	6.81 d (8.0)	116.6
6a	6.85 dd (7.8, 1.8)	119.9	1a	6.83 dd (8.0, 2.0)	120.1
7a	5.45 d (7.8)	94.2	2a, 6a, 8a, 9a	5.54 d (8.6)	94.4
8a	4.52 d (7.8)	57.8	1a, 7a, 9a, 10(14)a, 5b	4.48 d (8.6)	58.1
9a		144.6			144.9
10(14)a	6.21 d (1.8)	107.2	8a, 11(13)a, 12a	6.20 d (2.2)	107.5
11(13)a		159.4		159.7	
12a	6.28 t (1.8)	102.1	10(14)a, 11(13)a	6.23 t (2.2)	102.6
1b		132.1		132.6	
2b	7.17 br s	111.5	3b, 4b, 6b, 7b	7.14 d (1.1)	111.6
3b		145.1		145.4	
4b		148.9		149.1	
5b		132.4		133.0	
6b	6.84 d (1.8)	116.4	8a, 2b, 4b, 5b, 7b	6.79 d (1.1)	115.7
7b	7.12 d (16.5)	129.6	2b, 6b, 8b, 9b	6.89 d (16.3)	129.3
8b	6.96 d (16.5)	126.8	1b, 7b, 10b, 14b	7.00 d (16.3)	127.5
9b		140.4		140.6	
10b	6.81 br s	106.2	8b, 11b, 12b	6.51 d (2.2)	105.6
11b		159.9			159.5
12b	6.46 q (1.8)	103.7	10b, 11b, 13b, 14b	6.26 t (2.2)	102.1
13b		159.0			159.5
14b	6.69 br s	107.9	8b, 10b, 12b, 13b	6.51 d (2.2)	105.6
3a-OCH_3	3.83 s	56.0	3a	3.83 s	56.3
3b-OCH_3	3.93 s	56.1	3b	3.92 s	56.5
1′	4.94 d (7.8)	101.5	11b		
2′		74.4			
3′		77.4			
4′	3.11–3.94 (7H, overlap)	71.0			
5′		77.7			
6′		62.4			

* Measured in CD_3COCD_3 at 300 MHz for ^{1}H and 75 MHz for ^{13}C NMR, respectively, with assignments confirmed by ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra.
† Measured in DMSO-d_6 at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR, respectively, with assignments confirmed by DEPT, ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra.

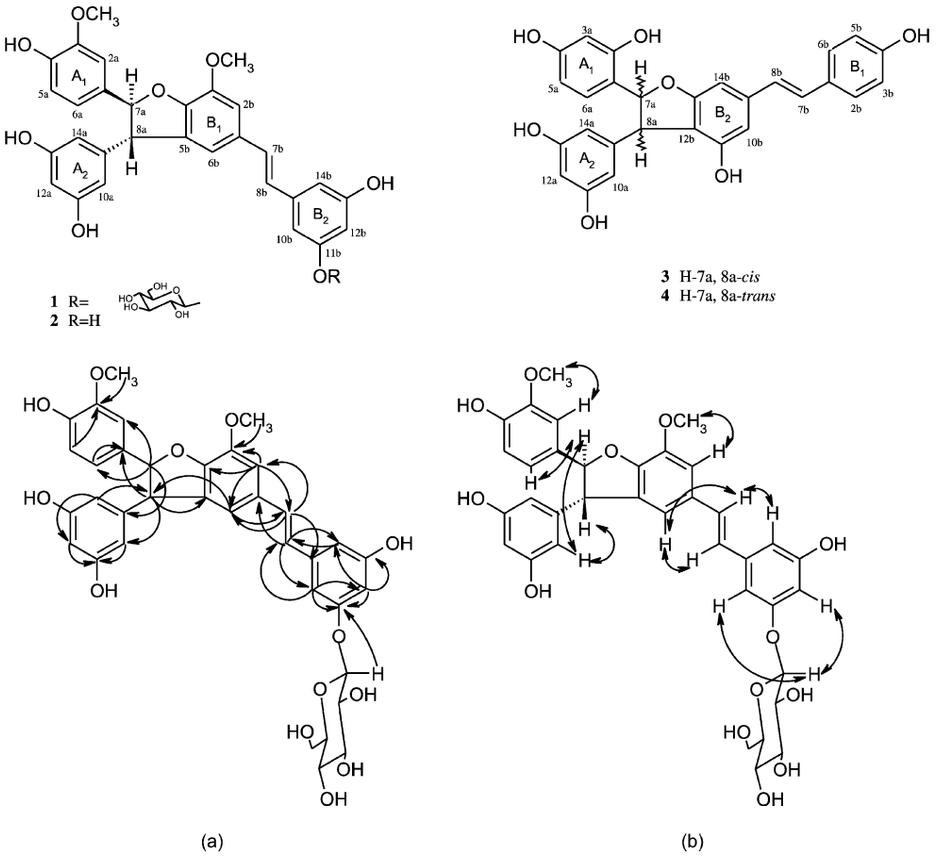

FIGURE 1 Important $^{13}C-^{1}H$ long-range correlations in the HMBC spectrum (a) and NOE interactions in the NOESY spectrum (b) of **1**.

6.46 (1H, q, $J = 1.8$, 1.8 Hz, H-12b) and 6.69 (1H, br s, H-14b) respectively, which indicated the substituted position of glucose was located on a hydroxyl of 3,5-dihydroxy substituted benzene ring (B_2). The ^{13}C NMR spectrum of **1** was also similar to that of **2**, except for six additional carbons between δ 62.4 and 101.5. The important signals at δ 101.5 indicated an anomeric carbon signal of glucose in the molecule. By comparison of the ^1H and ^{13}C NMR spectral data with **2**, one β-glucosidic unit should be attached to ring B_2. This was further confirmed by the NOE interactions (Fig. 1b) between H-1′/H-10b, H-1′/H-12b and the significant C–H long-range correlations between H-1′/C-11b in the HMBC spectrum of **1** (Fig. 1a). The structure of compound **1** was further confirmed by acid hydrolysis. Two spots identical to authentic samples of shegansu B and glucose were observed by hydrolysis with HCl gas on a TLC plate, developed with $CHCl_3$–MeOH–H_2O (8:2:1) and *n*-BuOH–HOAc–H_2O (4:1:5), respectively. Thus, from the above chemical reaction and spectroscopic data, **1** was characterized as shegansu B-11b-*O*-*β*-D-glucopyranoside. From the biogenesis viewpoint, **1** may be formed from one isorhapontigenin and one isorhapontigenin-3-*O*-*β*-D-glucopyranoside by tail–tail linkage with a dihydrobenzofuran.

Gnetupendin C (**3**) was obtained as a yellowish amorphous powder, exhibiting strong blue–violet fluorescence under UV light at 254 nm; $[\alpha]_D^{18}$ −220.0 (*c* 0.10, MeOH). The high resolution EIMS *m*/*z* 470.1344 agreed with a molecular formula of $C_{28}H_{22}O_7$, corresponding to a dimer of resveratrol and oxyresveratrol (^1H and ^{13}C NMR spectral data are given in Table II). The IR spectrum of **3** suggested the presence of hydroxy (3350 cm^{-1}) and aromatic moieties (1604, 1514 cm^{-1}). The UV spectrum (λ_{max} 327 nm) revealed the presence of a conjugated system which is attributed to a stilbene dimer with a five-membered oxygen heterocyclic ring [6] in the structure. The ^1H NMR spectrum of **3** displayed one set of ABX system for ring A_1, one set of AB_2 system for ring A_2, one set of A_2B_2 system for ring B_1, two *meta*-coupled protons for ring B_2, two coupled doublets for two olefinic protons and two methoxyl groups; all aromatic and olefinic protons signals were similar to those of **4**. However, two coupled doublets at δ 6.03 and 4.66 (each 1H) for a dihydrobenzofuran moiety with a coupling constant of $J = 8.1$ Hz were quite different from **4**. These patterns, and especially their different optical rotation data, suggested compound **3** is an isomer of **4**. The ^{13}C NMR spectrum of **3** revealed the presence of two aliphatic carbons at δ 86.1 and 49.8 shifted 2.9 and 4.4 ppm upfield compared to **4**; besides 26 aromatic and olefinic carbons between δ 99.3 and 162.8, all the carbons were assigned from the HMQC and HMBC spectra analysis. The HMBC spectrum of **3** (Fig. 2a), showed significant CH long-range correlations between H-6a/C-7a; H-7a/C-2a, 6a, 9a; H-8a/C-10 (14)a, 11b, 13b; H-10b/C-12b and H-14b/C-12b, 13b, which suggested that a resveratrol unit and an oxyresveratrol unit were connected by a dihydrobenzofuran ring (B_2). In the NOESY spectrum of **3** (Fig. 2b), the NOEs between H-7a/H-8a and H-6a/H-10(14)a indicated a *cis* orientation of H-7a and H-8a along with ring A_1 and A_2, respectively. Thus, the relative configuration of **3** was determined to be *rel*-(7a*R*,8a*S*), as shown in **3**; it is a diastereoisomer of **4**.

The pharmacological activities of compounds **3** and **4** have been tested. The inhibitory rates of TNF-α for compounds **3** and **4** at concentrations of 10^{-5} mol l^{-1} were 59.50% ($P < 0.05$) and 67.23% ($P < 0.05$), respectively. It suggested that compounds **3** and **4** may have anti-inflammatory activity.

EXPERIMENTAL

General Procedures

Optical rotations were measured on a Perkin–Elmer digital polarimeter. UV spectra were recorded on a Shimadzu UV-260 spectrophotometer. IR spectra were determined on

TABLE II ^{1}H and ^{13}C NMR data for compounds **3*** and **4***

	3			***4***	
Position	^{1}H	^{13}C	*HMBC*	^{1}H	^{13}C
1a		116.2			120.6
2a		155.1			156.0
3a	6.27 d (2.1)	102.4	1a, 2a, 4a, 5a	6.45 d (2.4)	103.5
4a		158.0			158.8
5a	6.09 dd (8.4, 2.1)	106.8	1a, 3a, 4a	6.28 dd (8.4, 2.4)	107.2
6a	6.89 d (8.4)	128.6	1a, 2a, 4a, 7a	7.01 d (8.4)	127.8
7a	6.03 d (8.1)	86.1	2a, 6a, 8a, 9a	5.73 d (3.6)	89.0
8a	4.66 d (8.1)	49.8	7a, 9a, 10(14)a, 11b, 12b, 13b	4.41 d (3.6)	54.2
9a		142.6			146.8
10(14)a	5.81 d (2.1)	108.1	8a, 11(13)a, 12a	6.25 d (2.1)	106.9
11(13)a		158.3			159.2
12a	5.95 t (2.1)	101.4	10(14)a, 11(13)a	6.19 t (2.1)	101.7
1b		129.9			130.0
2(6)b	7.45 d (8.4)	128.6	3(5)b, 4b, 7b	7.44 d (8.7)	128.6
3(5)b	6.85 d (8.4)	116.3	1b	6.84 d (8.7)	116.3
4b		158.0			158.0
7b	7.13 d (16.2)	128.9	2(6)b, 8b, 9b	7.11 d (16.8)	128.9
8b	6.99 d (16.2)	126.8	1b, 7b, 9b, 10b, 14b	6.97 d (16.8)	126.8
9b		140.5			140.9
10b	6.60 d (1.5)	108.1	8b, 11b, 12b, 14b	6.56 d (1.2)	107.9
11b		155.1			155.4
12b		117.4			115.7
13b		162.8			163.3
14b	6.75 br s	99.3	8b, 10b, 12b, 13b	6.73 d (1.2)	99.1
OH	8.47 (1H)[†]			8.58 (1H)[‡]	
	8.44 (1H)[†]			8.47 (1H)[‡]	
	8.16 (1H)[†]			8.22 (1H)[‡]	
	7.97 (1H)[†]			8.07 (1H)[‡]	
	7.78 (2H)[†]			8.06 (2H)[‡]	

* Measured in CD_3COCD_3 at 300 MHz for ^{1}H and 75 MHz for ^{13}C NMR, respectively, with assignments confirmed by $^{1}H-^{1}H$ COSY, HMQC, HMBC and NOESY spectra.
[†,‡] Each was a sharp singlet, the signals cannot be assigned unambiguously by the spectra; hence they are interchangeable.

a Perkin–Elmer 683 infrared spectrometer as KBr pellets. NMR spectra were carried out on a Varian Mercury-300 NMR spectrometer using TMS as internal standard. FABMS were obtained using a Zabspec Tofspe Platform-ESI mass spectrometer, EIMS and HREIMS were obtained using an Autospec-UltimaTof mass spectrometer. TCL was performed on silica gel GF_{254}. HPLC was performed on a Waters Model 510 instrument equipped with a Waters 2487 dual λ absorbance UV detector.

Plant Material

The lianas of *G. pendulum* C. Y. Cheng (Gnetaceae) were collected at Menghai in Xishuangbanna County of Yunnan Province, China in July 1999, and were identified by Professor W.Z. Song, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medial College. A voucher specimen has been deposited in the herbarium of this institute.

Extraction and Isolation

The lianas of *G. pendulum* (10 kg) were extracted with 60% EtOH, and the crude extract (1.5 kg) was further extracted with EtOAc to provide a EtOAc soluble residue (90 g), which was subjected to silica gel column chromatography (140–180 mesh, 10 × 150 cm) to provide

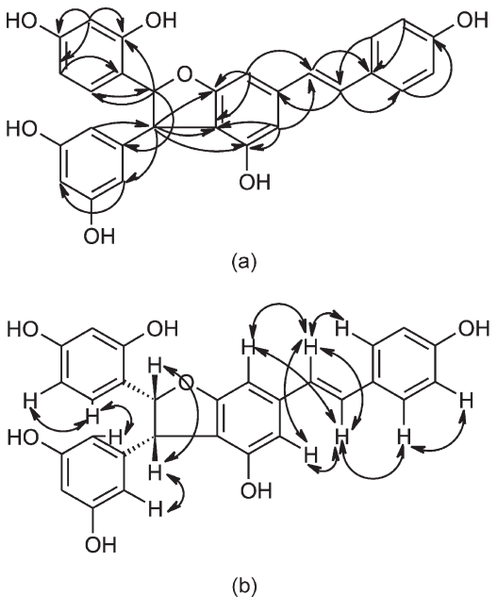

FIGURE 2 Important $^{13}C-^{1}H$ long-range correlations in the HMBC spectrum (a) and NOE interactions in the NOESY spectrum (b) of **3**.

five fractions (A–E). Isorhapontigenin, resveratrol isolated from fraction A and gnetupendin A and B from fraction B were elucidated in previous paper [2]. Fraction C (35 g) was subjected to gradient silica gel column chromatography (140–180 mesh, 5 × 100 cm) eluted with cyclohexane–acetone 10:1 to 6:4 (increasing acetone) to yield C_1-C_3. Fraction C_2 (3.5 g) was subjected to ODS column chromatography (RP-18, 35–75 μm, 4 × 35 cm) eluted with MeOH–H_2O (4.5:5.5) to give crude gnetulin (0.33 g), crude **4** (1.01 g) and pure **3** (130 mg). The crude sample of gnetulin was further purified by medium-pressure liquid chromatography (Lobar column, RP-18, 43–63 μm, 2.5 × 31 cm) with MeOH–H_2O of 3.5:6.5, and the crude sample of **4** was purified by MPLC with MeOH–H_2O (4:6); pure gnetulin (120 mg) and pure **4** (410 mg) were obtained. Fraction D (9.0 g) was subjected to silica gel column chromatography (140–180 mesh, 5 × 100 cm) eluted with cyclohexane–acetone (gradient 6:4 to 1:1) to provide fractions D_1-D_4. Fraction D_2 (5.9 g) was subjected to ODS column chromatography (RP-18, 35–75 μm, 4 × 35 cm) eluted with a MeOH–H_2O (4.5:5.5) system to afford isorhapontigenin-3-*O*-D-glucopyranoside (3.0 g); fraction D_3 was subjected to ODS column chromatography (RP-18, 35–75 μm, 2.8 × 21 cm) eluted with MeOH–H_2O (3:7) to provide **4** (35 mg).

Gnetupendin D (1)

Brown amorphous powder; $[\alpha]_D^{17}$ −32.2 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ): 286 (4.47), 310 (4.51), 327 (4.57) nm; IR (KBr) ν_{max}: 3408, 2937, 1604, 1516, 1452, 1340, 1271, 1215, 1159, 1074, 1030, 843 cm^{-1}; ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data see Table I; FAB-MS *m/z* (%): 676 $[M]^+$ (31), 515 (34), 391 (16), 313 (14), 282 (34), 223 (26), 181 (39), 163 (23), 137 (87), 115 (100), 69 (76); HR-FABMS *m/z*: 676.2236 $[M]^+$ (calcd for $C_{36}H_{36}O_{13}$, 676.2156).

Acid Hydrolysis of Compound (1)

A Me_2CO-H_2O (1:1, 2 mL) solution of compound **2** (5 mg) in 5% H_2SO_4 (1 mL) was refluxed for 9 h. After cooling, the reaction mixture was diluted with H_2O and then extracted with EtOAc. The EtOAc solution was evaporated to dryness and gave **2** which was identified by TLC ($CHCl_3-MeOH-H_2O$, 8:2:1, R_f 0.34) analysis by comparison with an authentic sample. A sample of **2** was applied to a TLC plate, placed in a container filled with HCl gas and heated for 8 h at 60°C. The plate was then developed with *n*-BuOH–HOAc–H_2O (4:1:5) to gave a spot of R_f 0.48, the same as the authentic sample of glucose.

Shegansu B (2)

Yellowish amorphous powder; $[\alpha]_D^{25}$ +21.0 (*c* 0.05, EtOH); UV (EtOH) λ_{max} (log ϵ): 285 (sh) (4.21), 328 (4.48) nm; IR (KBr) ν_{max}: 3302, 1603, 1514, 1462, 1340, 1279, 1155, 1005, 837 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectral data see Table I; FAB-MS *m/z* 515 $[M+1]^+$ (70%).

Gnetupendin C (3)

Yellowish amorphous powder; $[\alpha]_D^{18}$ −220.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ): 222 (4.47), 288 (sh), 310 (sh), 327(4.42) nm; IR (KBr) ν_{max}: 3350, 1697, 1622, 1604, 1514, 1450, 1433, 1365, 1234, 1157, 1103, 1061, 999, 974, 835 cm^{-1}; ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectral data see Table II; LR-EIMS *m/z* (%): 470 $[M]^+$ (52), 453 (5), 360 (9), 258 (13.5), 242 (100), 228 (58), 181 (14), 157 (9), 115 (11), 77 (7); HR-EIMS *m/z*: 470.1344 $[M]^+$ (calcd for $C_{28}H_{22}O_7$, 470.1366).

Gnetin D (4)

Pale yellow amorphous powder; $[\alpha]_D^{17}$ +65.9 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ): 207 (4.90), 220 (4.74), 286 (4.44), 310 (4.60), 327 (4.62) nm; IR (KBr) ν_{max}: 3315, 2918, 1695, 1622, 1604, 1514, 1429, 1363, 1234, 1159, 1103, 999, 976, 835 cm^{-1}; ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectral data see Table II; EI-MS *m/z*: 470 $[M]^+$, 470, 331, 272, 258, 228, 181, 149, 83, 55, 43.

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