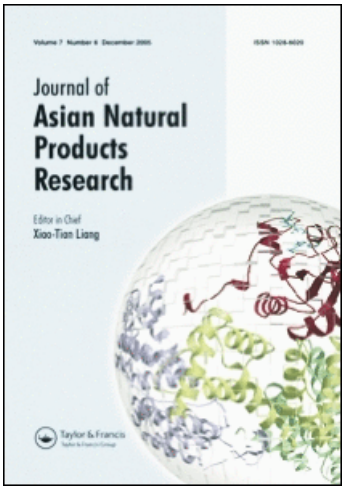

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Stilbene derivatives from Gnetum cleistostachyum

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To cite this Article Yao, Chun-Suo , Lin, Mao , Liu, Xin and Wang, Ying-Hong(2005) 'Stilbene derivatives from Gnetum cleistostachyum', Journal of Asian Natural Products Research, 7: 2, 131 — 137
To link to this Article: DOI: 10.1080/10286020310001625102
URL: http://dx.doi.org/10.1080/10286020310001625102

Journal of Asian Natural Products Research, Vol. 7, No. 2, April 2005, 131–137

Stilbene derivatives from *Gnetum cleistostachyum*

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(Received 6 June 2003; revised 8 July 2003; in final form 15 July 2003)

Three new stilbene derivatives, named gnetucleistol A (**1**), B (**2**) and C (**3**), together with four known compounds, gnetifolin A (**4**), *p*-hydroxycinnamic acid (**5**), piceatannol (**6**) and resveratrol (**7**), were isolated from *Gnetum cleistostachyum* C.Y. Cheng (Gnetaceae). Their structures were elucidated on the basis of spectroscopic evidence (EI-MS, UV, IR, NOE, ^{1}H, ^{13}C and 2D NMR).

Keywords: *Gnetum cleistostachyum*; Gnetaceae; Gnetucleistol A; Gnetucleistol B; Gnetucleistol C; Stilbene

1. Introduction

Gnetum cleistostachyum C.Y. Cheng grows in the southern part of Yunnan province of China, and is not recorded in folk medicine. However, some *Gnetum* species, such as *G. parvifolium* and *G. montanum*, have been used to treat rheumatic arthritis and bronchitis in folk medicine [1]. Previously, the constituents of five *Gnetum* species have been studied by our research group, which revealed that they were rich in various stilbenes and oligostilbenes. Some of them were found to have multiple bioactivities, such as antioxidation, anti-inflammatory, antitumor and so on [2]. In particular, isorhapontigenin and resveratrol showed potent inhibition on biosynthesis and receptor antagonist of leukotriene [3]. To expand the medicinal sources of *G. parvifolium* and find more active components, the lianas of *Gnetum cleistostachyum* C.Y. Cheng have been investigated, resulting in the isolation of three new compounds, gnetucleistol A (**1**), B (**2**) and C (**3**), together with four known compounds: gnetifolin A (**4**) [4,5], *p*-hydroxycinnamic acid (**5**), piceatannol (**6**) [6] and resveratrol (**7**) [7]. This paper deals with the isolation and structure elucidation of the three new compounds (figure 1).

2. Results and discussion

Gnetucleistol A (**1**) was isolated as an amorphous powder, with an optical rotation $[\alpha]_{D}^{25} = +13.3$ (*c* 0.09, MeOH). The HREI-MS showed a molecular ion peak at 396.1195

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2005 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020310001625102

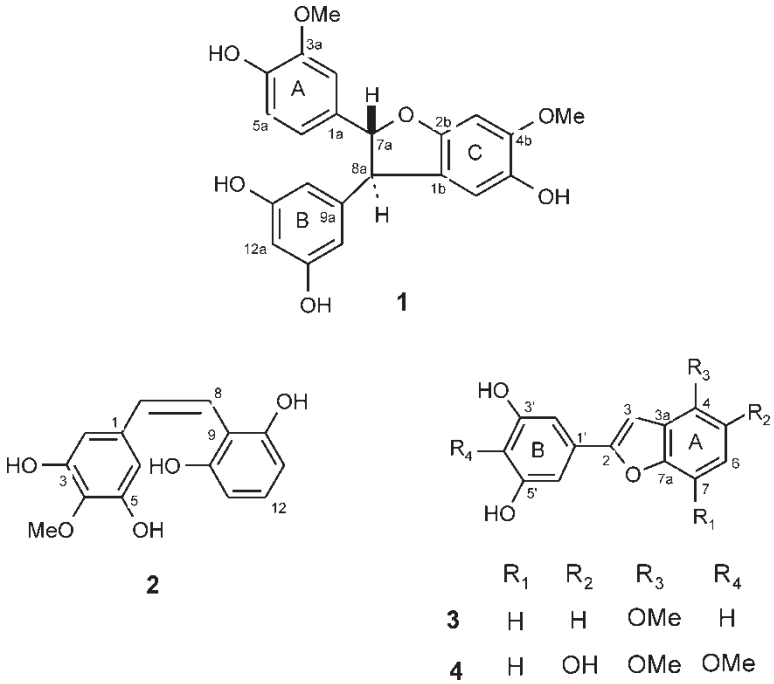

Figure 1. Structures of **1**–**4**.

(M^+, *m/z*), which combined with its ^1H and ^{13}C NMR spectra gave the molecular formula of $C_{22}H_{20}O_7$. The UV spectrum of **1** showed an absorption band at λ_{max} 285 nm, suggesting the absence of a *trans* stilbene conjugated system [8]. The IR spectrum revealed hydroxyl (3350 cm^{-1}) and aromatic groups (1603, 1550, 1500, 1458 cm^{-1}). The ^1H NMR spectrum in acetone-d_6 of **1** displayed signals at δ 6.24 (1H, t, $J = 1.2$ Hz, H-12a), 6.15 (2H, d, $J = 1.2$ Hz, H-10a, H-14a) for one set of AB_2 type (3,5-disubstituted) *meta*-coupled aromatic hydrogens, at δ 6.80 (2H, d, $J = 0.9$ Hz, H-5a, H-6a), 6.98 (1H, brs, H-2a) for one set of ABX type aromatic hydrogens, at δ 6.47 (1H, s, H-6b), 6.57 (1H, s, H-3b) for two aromatic proton singlets, and at δ 3.85 (3H, s, OCH_3), 3.81 (3H, s, OCH_3) for two methoxyls (table 1).

Table 1. ^1H and ^{13}C NMR data of compound **1***.

Position	*^1H*	*^{13}C*	*Position*	*^1H*	*^{13}C*
1a		133.0	11a		159.3
2a	6.98 brs	110.4	12a	6.24 t, 1.2	101.9
3a		148.2	13a		159.3
4a		147.2	14a	6.15 d, 1.2	107.0
5a	6.80 brs	115.3	1b		121.3
6a	6.80 brs	119.7	2b		153.7
7a	5.31 d, 8.4	93.6	3b	6.57 s	95.1
8a	4.38 d, 8.4	58.0	4b		148.0
9a		145.3	5b		141.5
10a	6.15, d, 1.2	107.0	6b	6.47 s	111.8
OCH_3	3.85, s	56.3	OCH_3	3.81 s	56.0
OH	7.60 brs, 1 × OH 8.15 brs, 2 × OH		OH	6.98 brs, 1 × OH	

* Measured in CD_3COCD_3 at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR, with assignments confirmed by ^1H–^1H COSY, HMQC, HMBC and NOE.

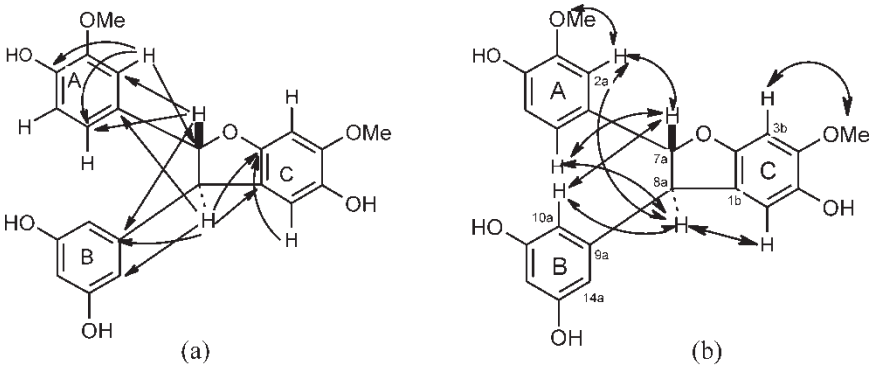

Figure 2. Important HMBC (a) and NOE (b) correlations of **1**.

Upon comparison with the signal patterns of the ^{1}H and ^{13}C NMR spectra of shegansu B [9], the signals at δ 5.31 (1H, d, $J = 8.4$ Hz, H-7a) and 4.38 (1H, d, $J = 8.4$ Hz, H-8a) in ^{1}H NMR and δ 93.6 and 58.0 in ^{13}C NMR for two connected methines suggest the presence of one dihydrobenzofuran moiety. In addition, the ^{13}C NMR spectrum of **1** also revealed signals for two methoxyl carbons at δ 56.0, 56.3 and eighteen aromatic carbons. These evidences, combined with the consideration of biogenesis, indicated that **1** was a stilbene derivative coupled by an isorhapontigenin and a phenol with a dihydrobenzofuran ring. In the HMBC spectrum, the correlations between H-7a and C-2a, C-6a, C-9a; H-8a and C-1a, C-9a, C-10(14)a, C-1b, C-2b further confirmed this assumption. Thus, compound **1** was characterized as shown in figure 1.

To clarify the stereochemistry of **1**, an NOE (figure 2) experiment was carried out. Irradiation of H-7a showed enhancement of the signals of H-2a, H-6a and H-10(14)a. Irradiation of H-8a showed enhancement of the signals of H-2a, H-6a and H-10(14)a. These data suggest a *trans* orientation of H-7a and H-8a. In addition, the NOEs between the protons of the methoxyl (δ 3.85) and H-2a, between the protons of the methoxyl (δ 3.81) and H-3b reveal that the two methoxyls are at C-3a and C-4b respectively. Therefore, the stereochemistry of **1** was elucidated to be as shown in figure 1.

Gnetucleistol B (**2**) was obtained as an amorphous powder, which exhibited a visible darkened zone under UV light at 254 nm. The HREI-MS showed a molecular ion peak at *m/z* 274.0837, corresponding to the molecular formula $C_{15}H_{14}O_5$. The UV spectrum of **2** shows an absorption band at λ_{max} 284 nm, suggesting a *cis* stilbene conjugated system [10]. The IR spectrum displays the absorption of hydroxyls (3400 cm^{-1}), olefinic bond and aromatic groups (1610, 1585, 1510, 1464, 766, 730 cm^{-1}). The ^{1}H NMR spectrum of **2** shows signals assignable to an AB_2 system at δ 6.95 (1H, t, $J = 8.0$ Hz, H-12) and 6.38 (2H, d, $J = 8.0$ Hz, H-11, H-13), two *cis* olefinic protons at δ 6.50 (1H, d, $J = 12.0$ Hz, H-7), and 6.30 (1H, d, $J = 12.0$, H-8), one singlet represented two protons at δ 6.36 (2H, s, H-2, H-6) and one methoxyl at δ 3.79 (3H, s, OCH_3). The ^{13}C NMR spectrum of **2** showed signals for 14 carbons at δ 107–160 except for the signals due to one methoxyl carbons at δ 60.18 (OCH_3). These data suggest that compound **2** is a *cis* stilbene with a similar skeleton as combretastatin A-4 [10]. The HMBC spectrum (table 2) shows long-range correlations between H-8 and C-10(14); H-7 and C-2(6), C-9, which further support the conclusion. In the NOE experiment, the absence of NOEs between methoxyl and any other protons indicate that the methoxyl was situated at C-4, and the two hydroxyls were at C-3 and C-5. Accordingly,

Table 2. HMBC of compounds **1** and **2**.

		1		*2*	
^{1}H	*Cross peaks to ^{13}C*	*^{1}H*	*Cross peaks to ^{13}C*	*^{1}H*	*Cross peaks to ^{13}C*
H-2a	C-4a, C-6a, C-7a	H-12a	C-11(13)a	H-7	C-2(6), C-9
H-5a	C-1a, C-3a		C-10(14)a	H-8	C-10(14)
H-6a	C-2a, C-7a	H-10(14)a	C-12a, C-11(13)a	H-2(6)	C-3(5)
H-7a	C-2a, C-6a, C-9a	H-3b	C-1b, C-2b, C-4b, C-5b	H-11(13)	C-9, C-10(14), C-11(13)
H-8a	C-1b, C-2b, C-1a, C-9a	H-6b	C-2b, C-4b, C-5b	H-12	C-10(14)

compound **2** was elucidated as *cis*-3,5,10,14-tetrahydroxy-4-methoxylstilbene. It is the first *cis* stilbene from the *Gnetum* genus.

Gnetucleistol C (**3**) was also obtained as an amorphous powder; the molecular ion peak of **3** in HREI-MS at *m/z* 256.0733 is compatible with the molecular formula $C_{15}H_{12}O_4$. The UV spectrum absorption bands at λ_{max} 297 (4.24), 306 (4.23) nm suggest a conjugated system. The IR spectrum indicates the existence of hydroxyls (3300 cm^{-1}) and aromatic groups (1604, 1550, 1491, 1464 cm^{-1}). The ^{1}H NMR spectrum in acetone-d_6 of **3** (table 3) exhibits signals attributable to an AB_2 system at δ 6.41 (1H, t, $J = 2.1$ Hz, H-4′), 6.93 (2H, d, $J = 2.1$ Hz, H-2′, H-6′), an ABX system at δ 6.78 (1H, d, $J = 8.1$ Hz, H-5), 7.13 (1H, dd, $J = 8.1$, 0.9 Hz, H-7), 7.24 (1H, t, $J = 8.1$ Hz, H-6), and a doublet at δ 7.15 (1H, d, $J = 0.9$ Hz, H-3), which shows a long-range coupling with the signal of H-7, closely similar to those of gnetifolin A [4,5]. Upon comparison with the ^{1}H and ^{13}C NMR data of gnetifolin A [5], **3** was deduced to have a benzofuran skeleton. The ^{13}C NMR spectrum of **3** shows fourteen carbon signals at δ 98–160, except for one methoxyl carbon at δ 55.3 (OCH_3), which further confirms the assumption. The presence of ABX type (3,4,5-trisubstituted) aromatic hydrogens, together with the long-range coupling between H-3 and H-7, suggests that the methoxyl is probably at C-4. In the NOE experiment, irradiation of the methoxyl signals at δ 3.96 (3H, s) enhanced the signal of H-5, illustrating that the methoxyl group

Table 3. ^{1}H and ^{13}C NMR data of compounds **2** and **3**∗.

	2			*3*	
Position	*^{1}H*	*^{13}C*	*Position*	*^{1}H*	*^{13}C*
1		133.6	2		155.9
2	6.36 brs	108.2	3	7.15 d (0.9)	98.9
3		150.5	3a		125.5
4		135.5	4		153.8
5		150.5	5	6.78 d (8.1)	103.8
6	6.36 brs	108.2	6	7.24 t (8.1)	125.5
7	6.50 d (12.0)	133.1	7	7.13 dd (8.1, 0.9)	104.2
8	6.30 d (12.0)	120.8	7a		155.1
9		112.7	1′		132.2
10		155.8	2′	6.93 d (2.1)	103.4
11	6.38 d (8.0)	107.4	3′		159.4
12	6.95 t (8.0)	128.9	4′	6.41 t (2.1)	103.5
13	6.38 d (8.0)	107.4	5′		159.4
14		155.8	6′	6.93 d (2.1)	103.4
OMe	3.79 s	60.18	OMe	3.96 s	55.3
OH	7.84 brs, 2 × OH				
	7.49 brs, 2 × OH				

∗ Measured in CD_3COCD_3 at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, with assignments confirmed by ^{1}H–^{1}H COSY, HMQC, HMBC and NOE.

is at C-4. Therefore, the structure of **3** was established as 2-(3′,5′-dihydroxyphenyl)-4-methoxybenzofuran (figure 1).

3. Experimental

3.1 General experimental procedures

The optical rotation was determined on a Perkin-Elmer digital polarimeter. UV spectra were taken on a Shimadzu UV-300 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 683 infrared spectrometer as KBr pellets. NMR spectra were recorded on a Bruker AM-500 NMR spectrometer using TMS as internal standard. EI-MS were obtained using an Autospec-Ulma-T of mass spectrometer.

3.2 Plant material

The lianas of *Gnetum cleistostachyum* C.Y. Cheng (Gnetaceae) were collected in Wenshan county of Yunnan province in October 2001, and authenticated by Dr Y.M. Shui at the Kunming Institute of Botany, Academia Sinica, Kunming, Yunnan, PR, China, where a voucher specimen has been deposited.

3.3 Extraction and isolation

The dried, powdered lianas of *Gnetum cleistostachyum* (35 kg) were extracted with 65% EtOH under reflux, and the crude extract (3.0 kg) obtained after removing solvent *in vacuo* was further extracted with EtOAc to provide a residue (185 g) after evaporation. The residue was subjected to silica-gel column chromatography (100–200 mesh), eluted with a $CHCl_3$–MeOH gradient of increasing MeOH to provide seven fractions (A–G). Fraction F was further subjected to silica-gel column chromatography (140–180 mesh) eluted with cyclohexane–acetone of increasing acetone to give seven fractions (F1–F7). Fraction F3 was then further divided into four fractions I–IV by silica gel chromatography [140–180 mesh, cyclohexane–acetone (4:1–2:1)]. Fraction I was applied to a Rp-18 column eluted with MeOH–H_2O (4:6) to afford compounds **3** (100 mg) and **5** (46 mg). Compound **6** (13 mg) was obtained from Fraction II by the same method. Fraction III was subjected to silica-gel column chromatography eluted with cyclohexane–acetone (3:1) to provide compound **4** (320 mg). F5 was then further subjected to silica-gel column chromatography, eluted with cyclohexane–acetone (3:1–1:1), to give Fr1–Fr8, Fr1 was subjected to an Rp-18 column eluted with MeOH–H_2O (4:6) to provide compound **7** (200 mg). Compounds **2** (2.1 mg) and **1** (4 mg) were obtained from Fr2 through silica-gel chromatography (140–180 mesh), eluting with $CHCl_3$–MeOH (25:1).

3.3.1 Gnetucleistol A (1). Amorphous powder, EIMS (*m/z*) 396 (M^+); HREI-MS *m/z* 396.1195 (calcd for $C_{22}H_{20}O_7$, 396.1209); $[\alpha]_D^{25} = +13.3$ (*c* 0.09, MeOH); UV(MeOH) λ_{max} (log ε) (nm): 285 (3.80), 305 (3.79); IR (KBr) ν_{max} (cm^{-1}): 3350, 2954, 1603, 1550, 1500, 1458, 1377, 1271, 1157; ^1H and ^{13}C NMR see table 1.

3.3.2 Gnetucleistol B (2). Amorphous powder, EIMS *m/z* 274 (M^+), HREI-MS *m/z* 274.0837 (calcd for $C_{15}H_{14}O_5$, 274.0841); UV (MeOH) λ_{max} (log ε): 284 (3.81) nm; IR (KBr) ν_{max} (cm^{-1}): 3400, 2919, 1610, 1585, 1510, 1464, 1377, 1275, 1200, 1012, 766, 730; ^1H and ^{13}C NMR see table 3.

3.3.3 Gnetucleistol C (3). Amorphous powder, EI-MS (*m/z*): 256 (M^+), 241, 149, 128; HREI-MS *m/z* 256.0733 (calcd for $C_{15}H_{12}O_4$, 256.0736); UV (MeOH) λ_{max} (log ε) (nm): 297 (4.24), 306 (4.23); IR (KBr) ν_{max} (cm^{-1}): 3300, 2924, 1604, 1550, 1491, 1464, 1377, 1275, 1155, 1093, 953; ^1H and ^{13}C NMR see table 3.

3.3.4 Gnetifolin A (4). EI-MS (*m/z*): 302 (M^+), 301, 286, 271, 258, 343; UV (MeOH) λ_{max} (nm): 218, 306; ^1H NMR (300 MHz, CD_3COCD_3) δ (ppm): 3.85 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 6.86 (1H, d, $J = 9.0$ Hz, H-6), 6.99 (2H, s, H-2′, H-6′), 7.09 (1H, d, $J = 9.0$ Hz, H-7), 7.22 (1H, s, H-3), 7.55 (1H, brs, OH), 8.21 (2H, brs, $2 \times$ OH); ^{13}C NMR (300 MHz, CD_3COCD_3) δ (ppm): 155.8 (C-5), 151.2 (C-3′, C-5′), 150.0 (C-7a), 144.2 (C-2), 139.3 (C-4), 136.4 (C-4′), 126.3 (C-1′), 122.1 (C-3a), 113.5 (C-7), 105.6 (C-6), 104.5 (C-2′, C-6′), 98.7 (C-3), 60.1 (OCH_3), 59.9 (OCH_3).

3.3.5 *p*-Hydroxycinnamic acid (5). Amorphous powder, mp 210–212°C; ^1H NMR (300 MHz, CD_3COCD_3) δ (ppm): 6.31 (1H, d, $J = 16.2$ Hz), 6.86 (2H, d, $J = 8.1$ Hz), 7.51 (2H, d, $J = 8.1$ Hz), 7.62 (1H, d, $J = 16.2$ Hz).

3.3.6 Piceatannol (6). Amorphous powder, mp 220–222°C; ^1H NMR (300 MHz, CD_3OD) δ (ppm): 6.12 (1H, t, $J = 2.1$ Hz, H-12), 6.42 (2H, d, $J = 2.1$ Hz, H-11, H-13), 6.75 (1H, dd, $J = 8.7$, 2.7 Hz, H-5), 6.86 (1H, d, $J = 8.7$ Hz, H-6), 6.92 (H, d, $J = 16.5$ Hz, H-8), 7.08 (H, d, $J = 2.7$ Hz, H-2), 7.37 (H, d, $J = 16.5$ Hz, H-7).

3.3.7 Resveratrol (7). Colorless needles, mp 254–255°C; ^1H NMR (300 MHz, CD_3OD) δ (ppm): 6.26 (1H, t, $J = 2.1$ Hz, H-12), 6.53 (2H, d, $J = 2.1$ Hz, H-11, H-13), 6.84 (2H, d, $J = 8.7$ Hz, H-3, H-5), 6.89 (1H, d, $J = 16.5$ Hz, H-7), 7.00 (1H, d, $J = 16.5$ Hz, H-8), 7.42 (2H, d, $J = 8.7$ Hz, H-2, H-6).

Acknowledgements

This research program was supported by the National Natural Science Foundation of China (No. 30070889). We thank the Department of Instrumental Analysis of our institute for the measurement of UV, IR and MS spectra and thank also Y.M. Shui (Kunming Institute of Botany, Chinese Academy of Sciences) for the collection and identification of the plant materials.

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