Cuspidans A and B, Two New Stilbenoids from the Bark of *Gnetum cuspidatum*

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Cuspidan A (1), a new stilbene sestermer consisting of a resveratrol, an oxyresveratrol, and a 3,5-dihydroxyphenylmethanol constituent units and cuspidan B (2), a new tri-cyclic stilbene monomer were isolated from the bark of *Gnetum cuspidatum*. The structures and configurations of 1 and 2 were elucidated on the basis of 2D-NMR correlations.

Key words stilbenoid; *Gnetum cuspidatum*; cuspidan A; cuspidan B; cytotoxicity

Gnetum species (Gnetaceae) produce a number of structurally diverse stilbenoids consisting of monomeric stilbenes such as resveratrol, oxyresveratrol, piceatannol, gnetol, and isorhapontigenin.[1–5] Several monomeric and oligomeric stilbenes showed various biological activities such as hepatoprotective,[3] antioxidant,[6] antimicrobial,[7] and inhibition of tyrosinase,[4] lipase,[7] α-amylase,[7] and beta-secretase 1 (BACE1)[8] activities. Recently, we have reported the isolation of two new oxyresveratrol trimers, gneyulins A and B, from the bark of *G. gnemonoides* showed the inhibitory activity against Na^+-glucose cotransporter (SGLT).[9]

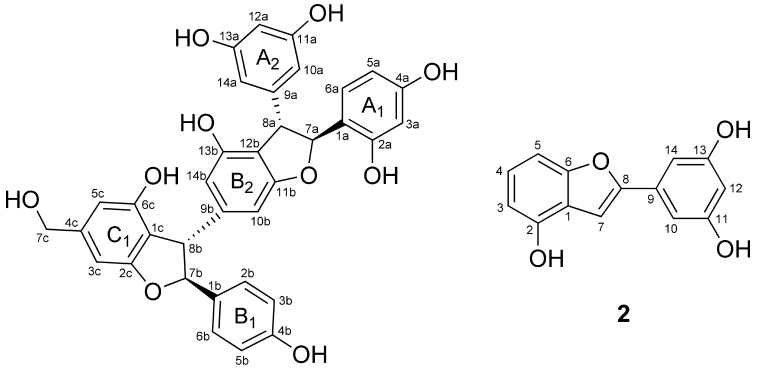
In our search for structurally and biological interesting compounds from tropical plants found in Malaysia,[10,11] two new stilbenoids, cuspidans A (**1**) and B (**2**) were isolated from the bark of *Gnetum cuspidatum*. We report here the isolation and structure elucidation of cuspidans A (**1**) and B (**2**) as well as their *in vitro* cell growth inhibitory activities against human promyelocytic leukemia (HL-60) cells.

Results and Discussion

The dried bark of *G. cuspidatum* was extracted with methanol, and the extract was partitioned with *n*-hexane and water. Then, the aqueous layer was extracted with EtOAc. The EtOAc extract was subjected to an LH-20 column ($CHCl_3$/MeOH) followed by octadecyl silica (ODS) HPLC (MeOH aq.) to afford cupidans A (**1**, 0.004% yield) and B (**2**, 0.002%) together with resveratrol.

Cuspidan A (**1**), yellowish amorphous powder, had a molecular formula $C_{35}H_{28}O_{10}$, by high resolution electron spray ionization time of flight mass spectrum (HR-ESI-TOF-MS) [*m*/*z* 609.1769 $(M+H)^+$, Δ 1.4 mmu]. The ^{1}H-NMR spectrum exhibited the presence of a set of two dihydrobenzofuran peaks of H-7a and H-7b (δ_H 5.62, 5.38) and H-8a and H-8b (δ_H 4.37, 4.40), and oxymethylene protons, H_2-7c (δ_H 4.53, 2H). The presence of a 1,2,4-trisubstituted benzene moiety (ring A_1: δ_H 6.21, 6.31, 6.92), a symmetrical 1,3,5-trisubstituted benzene unit (ring A_2: δ_H 6.11, 6.19), a *p*-substituted benzene unit (ring B_1: δ_H 6.77, 7.16), and a set of two *meta*-coupled protons (ring B_2: δ_H 6.17, 6.20, ring C_1: δ_H 6.41, 6.46) were also indicated. All protonated carbons were assigned by the hetero-nuclear multiple quantum coherence (HMQC) spectrum.

The presence of an oxyresveratrol unit (rings A_1 and A_2, and C-7a, 8a) was elucidated by hetero-nuclear multiple bond connectivity (HMBC) correlations of H-3a/C-1a (δ_C 120.7), 2a (δ_C 156.9), and 5a (δ_C 107.2), H-5a/C-4a (δ_C 159.3), H-7a/C-6a (δ_C 128.3), H-8a/C-14a (δ_C 107.3), H-10a/C-11a (δ_C 159.3), H-12a/C-11a, and H-14a/C-12a (δ_C 101.7). And, the presence of a resveratrol unit (rings B_1 and B_2, and C-7b, 8b) was elucidated by HMBC correlations of H-2b/C-4b (δ_C 158.6), H-3b/C-1b (δ_C 134.4), H-7b/C-2b (δ_C 128.0), H-8b/C-9b (δ_C 146.7) and



1 **2**

Chart 1

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C-14b (δ_C 108.5), H-10b/C-12b (δ_C 115.1), and H-14b/C-12b and C-13b (δ_C 155.8). The connectivity between C-8a and C-12b was deduced from the HMBC correlations of H-8a/C-12b and C-13b, and that of C-7a and C-11b through an oxygen atom was indicated by the correlation of H-7a/C-11b (δ_C 163.4). On the other hand, the structure of 3,5-dihydroxyphenylmethanol unit which constitutes the second dihydrobenzofuran ring was elucidated by HMBC correlations of H-7b to C-1c (δ_C 115.1) and C-2c (δ_C 163.2), H-8b/C-6c (δ_C 155.8), H-3c/C-7c (δ_C 65.3), H-5c/C-1c, C-3c (δ_C 100.6), C-6c, and C-7c, and H_2-7c to C-4c (δ_C 145.4). Since all the unsaturations of cuspidan A were accounted for, **1** was inferred to possess 8 hydroxyl groups at C-2a, C-4a, C-11a, C-13a, C-4b, C-13b, C-6c, and C-7c.

Thus, the gross structure of **1** was elucidated as a new stilbene sestermer consisting of a resveratrol, an oxyresveratrol, and a 3,5-dihydroxyphenylmethanol constituent units as shown in Fig. 1. The nuclear Overhauser effect spectroscopy (NOESY) correlations for H-6a/H-8a, H-7a/H-10a and H-14a, H-7b/H-10b and H-14b, and H-8b/H-2b and H-6b revealed the *trans*-configuration of the two dihydrofuran rings.

Cuspidan B (**2**), yellowish amorphous powder, had a molecular formula $C_{14}H_{10}O_4$, by HR-ESI-TOF-MS [*m/z* 243.0656 $(M+H)^+$, Δ 0.4 mmu]. The ^{1}H-NMR spectrum exhibited the presence of a 1,2,3-trisubstituted benzene moiety (C-1–C-6: δ_H 6.57, 6.95, 7.06), a symmetrical 1,3,5-trisubstituted benzene unit (C-9–C-14: δ_H 6.25, 6.78), and a singlet aromatic proton (H-7: δ_H 7.09). Since 9 out of 10 elements of unsaturation implied by the molecular formula were accounted for, **2** was inferred to possess 3 rings. The gross structure of **2** was deduced from extensive analyses of the two-dimensional NMR data, including the ^{1}H–^{1}H correlation spectroscopy (COSY), HMQC, and HMBC spectra in CD_3OD (Fig. 2). The ^{1}H–^{1}H COSY and HMQC spectra revealed connectivities of C-3–C-5 as shown in Fig. 2. The connection between C-8 and C-9 was elucidated by HMBC correlations of H-10 and H-14 to C-8. Furthermore, the presence of a benzofuran-4-ol moiety was deduced from HMBC correlations of H-3 and H-5/C-1, H-4/C-2 and C-6, and H-7/C-2 and C-8. Thus, the structure of **2** was elucidated as a new stilbene monomer, which was demethylated compound of gnetucleistol C.[12)]

Cuspidan A (**1**) might be generated through oxidative bond cleavage between C-7 and C-8 of a heterotrimer consisting of resveratrol and/or oxyresveratrol. Biogenetically, oxidative coupling between a hydroxy group (6-OH) and a double bond (C-8) from gnetol to form furan ring might give cuspidan B (**2**).

Cuspidan A (**1**) showed no cytotoxicity (IC_{50} >100 μM), and cuspidan B (**2**) had moderate cell growth inhibitory activities against HL-60 cells (IC_{50} 33.5 μM).

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Shimadzu UVmini-1240 spectrophotometer and IR spectra on a JASCO FT/IR-4100 spectrophotometer. Circular dichroism (CD) spectra were recorded on a JASCO J-820 polarimeter. Mass spectra were obtained using a Waters ZQ-2000 and an LTQ Orbitrap XL (Thermo Scientific) spectrometers. 1D and 2D NMR spectra were recorded on JEOL ECA 600 and Bruker AV 400 spectrometers, and chemical shifts were referenced to the residual solvent peaks (δ_H 3.31 and δ_C 49.0 for CD_3OD). Standard pulse sequences were employed for the 2D-NMR experiments. HPLC was performed on a CAPCELL PAK C_{18} MG-II, 5 μm (φ 10×250 mm).

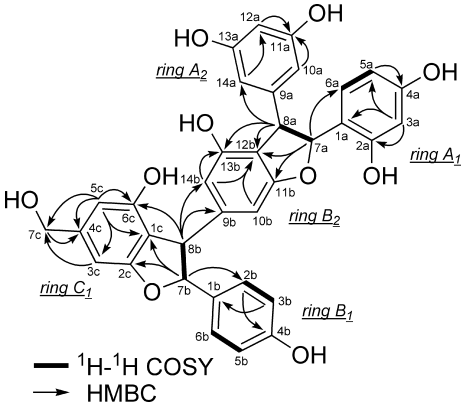

Fig. 1. Selected 2D-NMR Correlations for Cuspidan A (**1**)

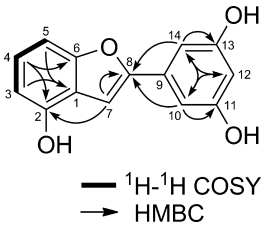

Fig. 2. Selected 2D-NMR Correlations for Cuspidan B (**2**).

Plant Material Bark of *G. cuspidatum* was collected in Mersing, Malaysia in 2008. The botanical identification was made by Mr. Teo Leong Eng, Faculty of Science, University of Malaya.

Extraction and Purification The dried bark of *G. cuspidatum* (15 g) was extracted with methanol, and the extract (1.34 g) was partitioned with *n*-hexane and water. Then, the aqueous layer was extracted with EtOAc. The EtOAc extract (210.5 mg) was subjected to an LH-20 column in $CHCl_3$/MeOH (1 : 1) to yield 18 fractions. The fraction 12 (3.4 mg) was separated by an ODS HPLC (60% MeOH aq., 2.0 mL/min, 254 nm) to afford cuspidan B (**2**, 0.3 mg, 0.002%, t_R=18.5 min). The fraction 13 (5.9 mg) was separated by an ODS HPLC (50% MeOH aq., 2.4 mL/min, 254 nm) to afford cuspidan A (**1**, 0.6 mg, 0.004%, t_R=24.0 min). The fraction 10 (2.7 mg) was resveratrol.

Cuspidan A (**1**): Yellowish amorphous powder; $[\alpha]_D^{26}$ −146 (c=0.3, MeOH); UV (MeOH) λ_{max} (log ε) 205 (5.17) and 281 (4.16) nm; CD (MeOH) λ_{max} 208 (θ −44000), 217 (6300), 235 (−80000), 256 (−8900), 264 (−4500), 274 (−12000), and 290 (4000) nm; IR (KBr) ν_{max} 3397, 1614, 1149, and 691 cm^{-1}; ^{1}H- and ^{13}C-NMR data (Table 1); ESI-MS *m/z* 607 $(M-H)^-$; HR-ESI-TOF-MS *m/z* 609.1769 (M+H; Calcd for $C_{35}H_{29}O_{10}$, 609.1755).

Cuspidan B (**2**): Yellowish amorphous powder; UV (MeOH) λ_{max} (log ε) 209 (4.23), 225 (4.18), and 298 (4.17) nm; IR (KBr) ν_{max} 3436, 1605, 1039, and 806 cm^{-1}; ^{1}H- and ^{13}C-NMR data (Table 1); ESI-MS *m/z* 241 $(M-H)^-$; HR-ESI-TOF-MS *m/z* 243.0656 (M+H; Calcd for $C_{14}H_{11}O_4$, 243.0652).

Cytotoxicity HL-60, human promyelocytic leukemia cells were maintained in RPMI-1640 medium. The growth medium was supplemented with 10% fetal calf serum and

Table 1. ^{1}H- (J, Hz) and ^{13}C-NMR Data of Cuspidans A (**1**) and B (**2**) in CD_3OD at 300 K

Position	**1**		Position	**2**	
	δ_H	δ_C		δ_H	δ_C
1a		120.7	1		120.0
2a		156.9	2		153.2
3a	6.31 (d, 2.2)	103.4	3	6.57 (d, 7.9)	109.0
4a		159.3	4	7.06 (dd, 8.3, 7.9)	126.2
5a	6.21 (dd, 8.2, 2.2)	107.2	5	6.95 (d, 8.3)	103.1
6a	6.92 (d, 8.2)	128.3	6		157.7
7a	5.62 (d, 4.1)	90.1	7	7.09 s	99.7
8a	4.37 (d, 4.1)	54.7	8		155.5
9a		147.3	9		133.6
10a (14a)	6.19 (d, 2.0)	107.3	10 (14)	6.78 (d, 2.1)	104.1
11a (13a)		159.3	11 (13)		160.2
12a	6.11 (dd, 2.0, 2.0)	101.7	12	6.25 (dd, 2.1, 2.1)	103.9
1b		134.4			
2b (6b)	7.16 (d, 8.6)	128.0			
3b (5b)	6.77 (d, 8.6)	116.3			
4b		158.6			
7b	5.38 (d, 4.9)	94.5			
8b	4.40 (d, 4.9)	56.6			
9b		146.7			
10b	6.20 s	101.0			
11b		163.4			
12b		115.1			
13b		155.8			
14b	6.17 s	108.5			
1c		115.1			
2c		163.2			
3c	6.46 s	100.6			
4c		145.4			
5c	6.41 s	108.2			
6c		155.8			
7c	4.53 s	65.3			

1% penicillin–streptomycin. The cells (5×10^3 cells/well) were cultured in Nunc disposable 96-well plates containing 90 μL of growth medium per well and were incubated at 37°C in a humidified incubator of 5% CO_2. Ten microliter of serially diluted samples (50–1.56 μM) were added to the cultures at 24 h of incubation. After 48 h of incubation with the samples, 15 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) were added to each of the wells. The cultures were incubated for another 3 h before the cells supernatant are removed. After the removal of the cells supernatant, 50 μL of dimethyl sulfoxide (DMSO) was added to each well. The formed formazan crystal was dissolved by re-suspension by pipette. The optical density was measured using a microplate reader (Bio-Rad) at 550 nm with reference wavelength at 700 nm. In all experiments, three replicates were used. Cisplatin was used as positive control (IC_{50}: 2.4 μM for HL-60).

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