Stilbene Derivatives from Two Species of Gnetaceae

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Five new stilbene oligomers (gnemonols A, B and C, gnemonoside E and gnetal) were isolated together with 2b-hydroxyampelopsin F and gnetin E from *Gnetum gnemon* and *G. gnemonoides*. The structures of the compounds were elucidated on the basis of spectral evidence.

Key words *Gnetum gnemon*; *Gnetum gnemonoides*; Gnetaceae; stilbenoid; resveratrol oligomer

The family of Gnetaceae is known to contain stilbenoids.[1,2] Various species in the family have been used as folk medicine for the treatment of arthritis, bronchitis and asthma. The leaves and the fruits are also used as food in many parts of the tropics.[3,4] In continuation of our studies of the chemical constituents of the Gnetaceous plants, we report in this paper the structures of five new stilbenoids isolated from two species of Gnetaceace. Gnemonols A (**1**) and B (**2**) were obtained from the root of *Gnetum gnemon*. Gnemonol C (**3**), gnemonoside E (**4**) and gnetal (**5**) were isolated from the stem of *G. gnemonoides*, along with 2b-hydroxyampelopsin F (**7**). Gnetin E (**6**) was also isolated from both species. The structures of the stilbenoids were elucidated on the basis of spectral evidence.

Gnemonol A (**1**), obtained as a white amorphous powder, showed a positive reaction to Gibbs reagent. The negative FAB-MS exhibited an $[M-H]^-$ ion peak at *m*/*z* 695, indicating the molecular weight to be 696. The molecular formula of $C_{42}H_{32}O_{10}$ was supported by the high resolution (HR) negative FAB-MS (*m*/*z* 695.1919). The ^{1}H-NMR spectrum showed the presence of two sets of *ortho*-coupled aromatic protons assigned to 4-hydroxyphenyl groups [δ 6.79 (2H, d, J=8.8 Hz, H-3a, 5a), 7.16 (2H, d, J=8.8 Hz, H-2a, 6a); 6.75 (2H, d, J=8.8 Hz, H-3c, 5c), 7.15 (2H, d, J=8.8 Hz, H-2c, 6c)], and the presence of coupled aromatic protons based on the ABX spin system on a 1,2,4-trisubstituted benzene ring [δ 6.15 (1H, d, J=1.8 Hz, H-3b), 6.21 (1H, dd, J=1.8, 8.5 Hz, H-5b), 6.74 (1H, br d, J=8.5 Hz, H-6b)]. The spectrum also exhibited a set of *meta*-coupled aromatic protons on a 1,2,3,5-tetrasubstituted benzene ring [δ 6.36 (1H, d, J=2.0 Hz, H-14a), 6.37 (1H, d, J=2.0 Hz, H-12a)], a set on a 3,5-dihydroxyphenyl group in an A_2B spin system [δ 6.23 (1H, t, J=2.0 Hz, H-12c), 6.29 (2H, d, J=2.0 Hz, H-10c, 14c)], and a signal of an aromatic proton on a penta-substituted benzene ring at δ 6.26 (1H, br s, H-12b). Three sets of aliphatic protons, coupled successively in the order [δ 2.92, 3.05 (1H each, dd, J=3.9, 14.2 Hz, H-8b), 4.92 (1H, t, J=3.9 Hz, H-7b); 4.62 (1H, d, J=11.3 Hz, H-8a), 5.92 (1H, d, J=11.3 Hz, H-7a); 4.29 (1H, d, J=3.9 Hz, H-8c), 5.41 (1H, d, J=3.9 Hz, H-7c)], and eight signals of phenolic hydroxyl groups [δ 8.20 (1H, br s, OH-4b), 8.23 (2H, br s, OH-11c, 13c), 8.24—8.83 (OH×5)] were also shown in the spectrum. Analysis of ^{13}C–^{1}H shift correlation spectroscopy (^{13}C–^{1}H COSY) and correlation spectroscopy involving long range coupling (COLOC) spectra enabled the assignment of all protonated and quaternary carbons in **1**. Clear cross peak correlations in the ^{1}H–^{1}H long range COSY spectrum (Fig. 2) were observed between the aromatic protons and the methine protons as follows: H-2a(6a)/H-7a, H-14a/H-8a, H-6b/H-7b, H-2c(6c)/H-7c and H-10c(14c)/H-8c, revealing the respective connections of C-1a/C-7a, C-9a/C-8a, C-1b/C-7b, C-1c/C-7c and C-9c/C-8c. In the COLOC spectrum (Fig. 2), the cross peak correlations observed between C-9b/H-8b and C-10b(14b)/H-8b established the connection between C-9b/C-8b. The correlation between C-9a(11a)/H-7b and C-10a/H-7b observed in the COLOC spectrum revealed the linkage between C-10a and C-7b. Significant correlations between C-10b/H-8a, C-13b/H-7c and C-14b/H-8c, also observed in the COLOC spectrum, revealed the respective connections between C-10b/C-8a, C-13b/C-7c and C-14b/C-8c. The above results showed that the resveratrol units A (ring A_1-7a-8a-ring A_2) and C (ring C_1-7c-8c-ring C_2) form a dihydrofuran ring with an oxyresveratrol unit B (ring B_1-7b-8b-ring B_2) at the positions of C-10b, C-11b and C-13b, C-14b respectively. Thereby, the planar structure of **1** can be drawn as in Fig. 1, a congener of vitisin E[3] but different from vitisin E at ring B_1. The 4-hydroxyphenyl ring (ring B_1) in vitisin E is replaced with a 2,4-dihydroxyphenyl ring in **1**. The partial relative structure of **1** was deduced by the results of an nuclear Overhauser effect (NOE) experiment (Fig. 2). The differential NOE observed between H-7a/H-14a, H-8a/H-2a(6a) and H-7c/H-10c(14c), H-8c/H-2c(6c) revealed the orientation of the two dihydrofuran rings to be *trans*. The relative configuration of H-7a(α), H-8a(β) and H-7b(α) was deduced by the NOE between H-8a/H-2a and H-8a/H-6b observed in the difference NOE experiment, which allowed the partial relative structure of **1** to be drawn as in Fig. 1. However, no NOE was observed between the protons of the dihydrofuran ring at H-7c(8c) and H-7a(8a), H-7b(8b) to allow the complete relative structure to be drawn.

Gnemonol B (**2**), a white amorphous powder, showed a positive reaction to Gibbs reagent. The UV spectrum (λ_{max} 327 nm) revealed the presence of a strong conjugated system in the molecule. The negative FAB-MS exhibited an

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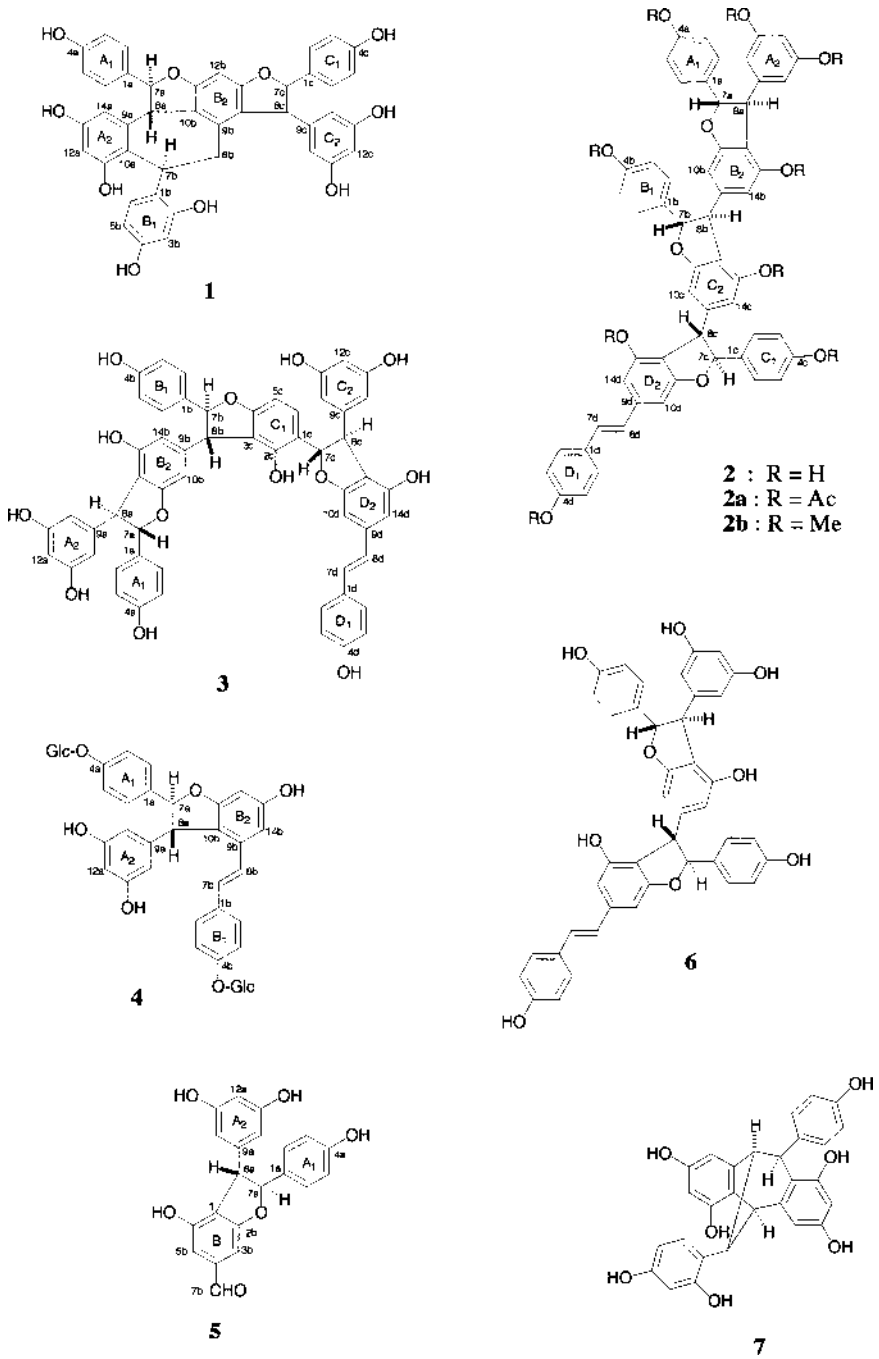

Fig. 1

$[M-H]^-$ ion peak at *m/z* 905, and the molecular formula of $C_{56}H_{42}O_{12}$ was deduced from HR-FAB-MS *m/z* 905.2614 $[M-H]^-$. The ^{1}H-NMR spectrum showed the presence of four 4-hydroxy phenyl groups (ring A_1, B_1, C_1, D_1), three 1,3,4,5-tetrasubstituted benzene rings (ring B_2, C_2, D_2) and a 3,5-dihydroxyphenyl group (ring A_2). A set of *trans*-coupled olefinic protons (H-7d/8d) and three sets of mutually coupled aliphatic methine protons (H-7a/8a, H-7b/8b H-7c/8c) were also observed in the spectrum in addition to nine phenolic hydroxyl groups. The number of the hydroxyl group was confirmed by the results of acetylation (**2a**) and methylation (**2b**) experiments. The molecular formula ($C_{56}H_{42}O_{12}$) and ^{1}H-, ^{13}C-NMR spectral data (Table) revealed that **2** is a resveratrol tetramer. All protonated carbons were assigned with the help of a ^{13}C–^{1}H COSY spectrum. The correlations between H-2a(6a)/H-7a, H-10a(14a)/H-8a, H-2b(6b)/H-7b, H-10b(14b)/H-8b, H-2c(6c)/H-7c, H-10c(14c)/H-8c, H-2d(6d)/H-7d and H-10d(14d)/H-8d observed in ^{1}H–^{1}H long range COSY spectrum (Fig. 3) revealed the following respective connections: C-1a/C-7a, C-9a/C-8a, C-1b/C-7b, C-9b/C-8b, C-1c/C-7c, C-9c/C-8c, C-1d/C-7d and C-9d/C-8d. Furthermore, the cross peak correlations between C-2a(6a)/H-7a, C-10a(14a)/H-8a, C-2b(6b)/H-7b, C-10b(14b)/-8b, C-2c(6c)/H-7c, C-10c(14c)/H-8c, C-2d(6d)/H-7d and C-10d(14d)/H-8d in the COLOC spectrum (Fig. 4) substantiated the assignment of all the aliphatic and olefinic protons to their respective resveratrol units. The correlations between C-12b/H-8a, C-11c(12c)/H-8b and C-11d(12d)/H-8c in the

COLOC spectrum showed the connectivity pattern of the four resveratrol units A—D (Fig. 4). Although no correlations were observed between C-11b/H-7a, C-11c/H-7b and C-11d/H-7c for the three ether linkages (C-7a-O-C-11b, C-7b-O-C-11c and C-7c-O-C-11d) in the COLOC spectrum, the chemical shift differences of about 7 ppm (δ_C) downfield were observed at C-11b (δ 162.7), C-11c (δ 162.8) and C-11d (δ 163.1), compared to C-13b (δ 155.3), C-13c (δ 155.4) and C-13d (δ 155.5), supporting the replacement of hydroxyl groups with ether linkages at these respective positions. Furthermore, out of the 36 degrees of unsaturation (according to the molecular formula), 33 were allotted to eight benzene rings and an olefinic moiety. The remaining must be fulfilled by the three dihydrofuran rings [H-7a(8a)-ring B_2, H-7b(8b)-ring C_2 and H-7c(8c)-ring D_2]. The *trans* orientations of the hydrogen atoms on the dihydrofuran rings were deduced by the result of a NOESY experiment (Fig. 3). The relative stereochemistry of chiral centers was assigned by comparison with the chemical shift values of the corresponding dihydrofuran rings of gnetin E[6] in the ^{1}H- and ^{13}C-NMR spectra. Thus, **2** was found to be a tetramer formed as a result of oxidative coupling of gnetin E with a resveratrol unit.

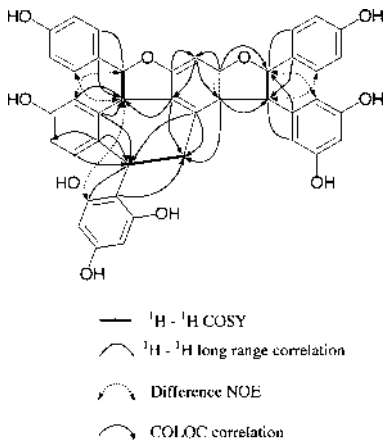

Fig. 2

Gnemonol C (**3**) was obtained as an amorphous white powder and showed an $[M-H]^-$ ion peak at *m/z* 921 in negative FAB-MS and an *m/z* 921.2540 in negative HR-FAB-MS, indicating the molecular formula to be $C_{56}H_{42}O_{13}$. The ^{1}H-NMR spectrum showed the presence of three 4-hydroxyphenyl groups (ring A_1, B_1, D_1) and a set on a 1,2,3,4-tetrasubstituted benzene ring (ring C_1). The presence of two 1,3,4,5-tetrasubstituted benzene rings (ring B_2, D_2) and two 3,5-dihydroxyphenyl groups (ring A_2, C_2) were observed in the spectrum. The spectrum also exhibited a pair of olefinic protons (H-7d/8d) and three sets of coupled benzylic protons (H-7a/8a, H-7b/8b, H-7c/8c), in addition to ten phenolic hydroxyl groups [δ 8.00—8.50 (OH×10)]. The following cross peak correlations in the ^{1}H–^{1}H long range COSY spectrum (Fig. 5) were observed between H-2a(6a)/H-7a, H-10a(14a)/H-8a, H-2b(6b)/H-7b, H-10b(14b)/H-8b, H-6c/H-7c, H-10c(14c)/H-8c, H-2d(6d)/H-7d and H-10d(14d)/H-8d, which helped to establish the connections of C-1a/C-7a, C-9a/C-8a, C-1b/C-7b, C-9b/C-8b, C-1c/C-7c, C-9c/C-8c, C-1d/C-7d and C-9d/C-8d, respectively. Similarly, the correlations observed in the ^{1}H detected multiple quantum coherence (HMBC) spectrum between H-7a/C-2a(6a), H-8a/C-10a(14a), H-7b/C-2b(6b), H-8b/C-10b(14b), H-7c/C-6c, H-8c/C-10c(14c), H-7d/C-2d(6d) and H-8d/C-10d(14d) (Fig. 6) further substantiate the linkages between the methine and

Table. 1. ^{13}C-NMR Spectral Data of **1**—**4**

	1[a]	**2**[a]	**3**[a]	**4**[b]		**1**	**2**	**3**	**4**
1a	131.5	133.6	133.9	134.6	9c	147.0	146.6	146.1	
2a(6a)	129.8	128.0	127.3	126.9	10c	106.8	101.1	107.0	
3a(5a)	116.1	116.1	116.4	116.3	11c	159.9	162.8	159.5	
4a	158.4	158.2	157.9	157.2	12c	102.1	114.3	101.9	
7a	88.3	93.8	93.6	91.9	13c	159.9	155.4	159.5	
8a	49.2	56.0	56.1	55.1	14c	106.8	108.3	107.0	
9a	142.9	145.9	146.2	145.9	1d		129.9	131.2	
10a	120.3	106.8	106.8	105.4	2d(6d)		128.7	128.8	
11a	155.5	159.5	159.9	158.7	3d(5d)		116.3	116.1	
12a	101.6	102.0	101.9	101.1	4d		158.0	158.2	
13a	157.3	159.5	159.9	158.7	7d		129.2	129.1	
14a	105.1	106.8	106.8	105.4	8d		126.7	127.0	
1b	120.1	133.7	134.0	130.7	9d		141.2	141.4	
2b	156.3	127.8	128.1	127.5	10d		99.2	99.3	
3b	103.6	116.1	116.3	116.4	11d		163.1	163.3	
4b	157.7	158.2	158.2	157.1	12d		115.1	115.6	
5b	107.1	116.1	116.3	116.4	13d		155.5	155.6	
6b	128.4	127.8	128.1	127.5	14d		108.0	108.0	
7b	31.9	93.7	93.9	128.6	Glc-1a				100.5
8b	31.5	55.9	56.3	123.5	1b				100.4
9b	135.0	146.4	146.6	134.6	2a(b)				73.23
10b	120.2	101.1	101.0	118.5					73.19
11b	160.4	162.7	163.0	160.7	3a(b)				76.61
12b	90.3	114.1	114.3	96.3					76.56
13b	161.2	155.3	155.4	158.6	4a(b)				69.7
14b	120.6	108.4	108.3	103.6					69.7
1c	134.3	134.0	115.7		5a(b)				77.0
2c	128.4	127.7	155.4						77.0
3c	116.0	116.1	115.7		6a(b)				60.7
4c	158.0	158.2	159.8						60.7
5c	116.0	116.1	109.4						
6c	128.4	127.7	128.8						
7c	93.9	93.6	89.7						
8c	56.9	55.9	54.4						

a) acetone-d_6. *b*) DMSO-d_6. All carbons were assigned by C–H COSY, HMQC, COLOC and HMBC spectra.

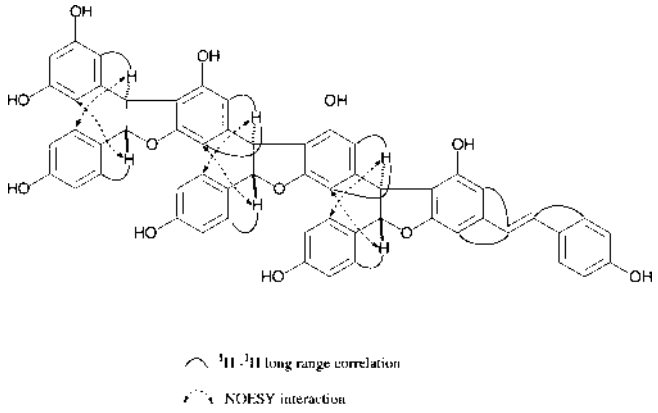

Fig. 3

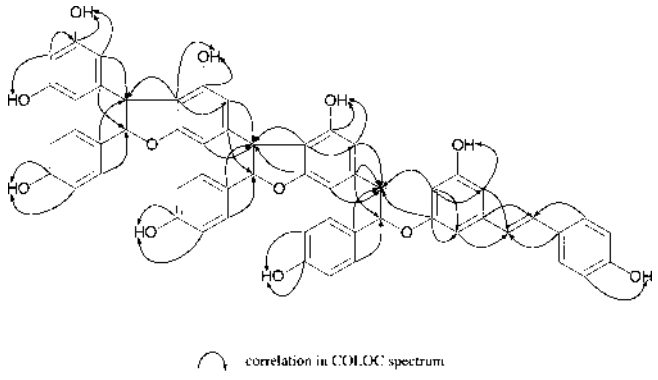

Fig. 4

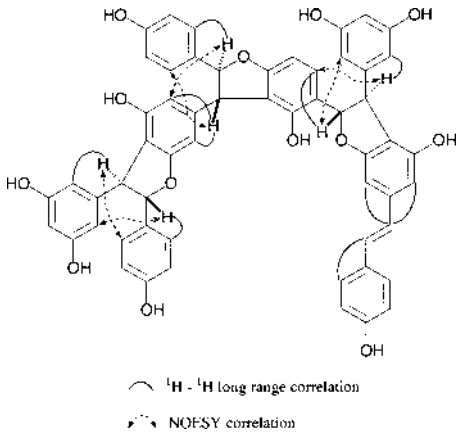

Fig. 5

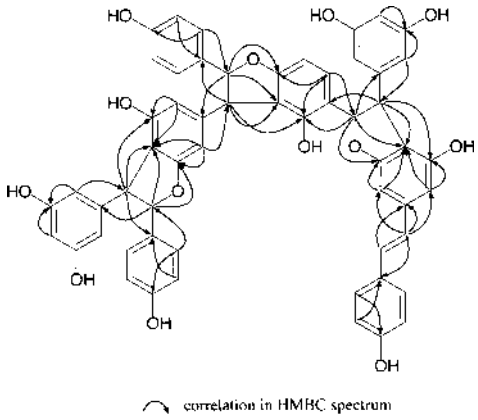

Fig. 6

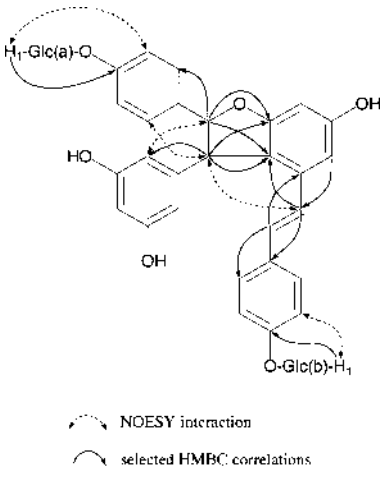

Fig. 7

olefinic protons to their respective aromatic rings. The linkages between C-8a/C-12b, C-8b/C-3c and C-8c/C-12d were deduced from the correlations between H-8a/C-12b(13b), H-8b/C-2c(3c) and H-8c/C-12d(13d) observed in the HMBC. A correlation also observed in HMBC between H-7a/C-11b, H-7b/C-4c and H-7c/C-11d established the presence of three dihydrofuran rings in **3**. A significant ^3J correlation between H-7c/C-2c observed in the HMBC spectrum revealed that C-7c is connected to C-1c located at the *ortho* position to C-2c. The chemical shift value of δ 155.4 (C-2c) indicated that C-2c must be a quaternary carbon with a hydroxyl group similar to C-13b (δ 155.4) and C-13d (δ 155.6). Finally, the planar structure of **3** was determined as in Fig. 1. The *trans* orientation of the dihydrofuran rings was drawn from the results of a NOESY experiment (Fig. 5), and the relative structure was also deduced by comparison with the chemical shift values of the corresponding dihydrofuran rings of gnetin E.

Gnemonoside E (**4**), a brown amorphous powder, gave a positive reaction to Gibbs reagent. It exhibited an $[M-H]^-$ ion peak at *m*/*z* 777 in negative FAB-MS and *m*/*z* 777.2385 in negative HR-FAB-MS, which suggested that the molecular formula is $C_{40}H_{42}O_{16}$. The ^{1}H-NMR spectrum revealed the presence of two 4-hydroxyphenyl groups (rings A_1, B_1), a 1,2,3,5-tetrasubstituted benzene ring (ring B_2) and a 3,5-dihydroxyphenyl group (ring A_2). A pair of *trans*-olefinic protons (H-7b/8b), a set of mutually coupled methine protons (H-7a/8a) and signals of two anomeric protons [δ 4.86 (2H, d, J=7.7 Hz, H-Glc(a, b)-H-1)] were also exhibited, along with signals of three phenolic hydroxyl groups. These results indicated that **4** is a dimeric stilbene glucoside. Analysis of the ^{1}H–^{1}H long range COSY spectrum (Fig. 7) revealed the following cross peak correlations between H-2a(6a)/H-7a, H-10a(14a)/H-8a, H-2b(6b)/H-7b, H-14b/H-8b, which helped to establish the connections between C-1a/C-7a, C-9a/C-8a, C-1b/C-7b and C-9b/C-8b, respectively. Significant correlations in the HMBC experiment (Fig. 7) observed between H-7a/C-10b(11b) and H-8a/C-10b(11b) indicated that a resvera-

trol unit (ring A1-7a-8a-ring A2) formed a dihydrofuran ring with another resveratrol unit B at C-10b and C-11b. These results led to the determination of the structure of the aglycone unit to be ε-viniferin.[7] The positions of the glucose molecules were determined from the results of nuclear Overhauser effect spectroscopy (NOESY) and heteronuclear multiple bond correlation (HMBC) experiments; in the NOESY experiment (Fig 7), cross peak correlations were observed between Glc(a)-H-1/H-3a(5a) and Glc(b)-H-1/H-3b(5b). Similarly, in the HMBC experiment (Fig. 7), two anomeric protons at δ 4.84 exhibited a cross peak correlation with an aromatic carbon at C-4a and C-4b, respectively, confirming the positions of the glucose molecules. The *trans* configuration of the dihydrofuran ring was deduced from the results of NOESY experiment, and subsequently led to the characterization of **4** as ε-viniferin 4a, 4b-*O*-β-diglucopyranoside.[8]

Gnetal (**5**) was obtained as a white amorphous powder, showed a positive reaction to Gibbs reagent, and gave an $[M-H]^-$ ion peak at 364.0928 in the HR-EI-MS corresponding to the molecular formula of $C_{21}H_{16}O_6$. The ^{1}H-NMR spectral data showed the presence of a 4-hydroxylphenyl group (ring A_1), a 1,2,4,6-tetrasubstituted benzene ring (ring B_1) and a 3,5-dihydroxyphenyl group (ring A_2). Two mutually coupled aliphatic protons (H-7a/8a) and a signal due to an aldehyde group at δ 9.22 (1H, br s, H-7b) were also observed in the spectrum, in addition to four phenolic hydroxyl groups. In the ^{13}C-NMR spectrum of **5**, 21 carbon atoms were observed. The signal at δ 192.3 observed in ^{13}C-NMR was assigned to the aldehyde moiety. The correlation in ^{1}H–^{1}H long range COSY observed between H-7a/H-2a(6a) and H-8a/H-10a(14a) supported the planar structure of **5**. All the quaternary carbon atoms and the relative structure of **5** were assigned by comparison with the spectral data of gnetin C.[7]

Gnetin E (**6**) was obtained in the two species, whereas 2b-hydroxyampelopsin F (**7**)[9] was only isolated from the stem of *G. gnemonoides*. The structures of the known compounds were identified by spectral analysis and comparison with authentic samples.

Compounds **2** and **3** are the first instance of a stilbene tetramer reported from the family of Gnetaceae.

Experimental

General Methods ^{1}H- and ^{13}C-NMR spectra were recorded on LA 400 (JEOL), α-500 and GE-Omega 600 spectrometers. Chemical shift values are shown as δ values with tetramethylsilane (TMS) as an internal reference. Peak multiplicities were quoted in Hz. Negative ion FAB-MS were measured on a JMS-DX 300 spectrometer equipped with a JMA 3500 data analysis system (JEOL), and optical rotations were recorded on a P-1020 (JASCO) polarimeter. UV spectra were recorded on a UV 2200 spectrometer (Shimadzu). Silica gel 60 (70—230 mesh, Merk), Sephadex L-H 20 (Pharmacia), ODS (100—200 mesh, Fuji Silysia Chemical) and an ODS Sep-Pak C_{18} Cartridge (Merck) were used for column chromatography. Kiesel-gel 60_{254} (Merck) was used for analytical and preparative TLC.

Extraction and Isolation The dried Root of *Gnetum gnemon* (2.0 kg) and the stem lianas of *G. gnemonoides* (1.0 kg), collected in April 2001 at Bogor Botanical Garden, Indonesia, were powdered and separately extracted, successively, with acetone and methanol. The acetone extract of *Gnetum gnemon* (60 g) was chromatographed on silica gel and eluted with a mixture of $CHCl_3$–MeOH of increasing polarity to give 11 fractions. Compound **6** (1.5 g) was obtained from fraction 3 and was purified by VLC on ODS and eluted with MeOH : H_2O (1 : 1). Fraction 4 was subjected to VLC on ODS and eluted with MeOH–H_2O (1 : 1) to give 10 fractions (fr. A—J). Further purification of fraction F by PTLC [benzene : EtOAc : acetone : H_2O (40 : 30 : 30 : 1)] gave **1** (20 mg). Compound **2** (97 mg) was obtained from fraction J in pure form. The acetone extract of *G. gnemonoides* (27 g) was also subjected to chromatograhy on silica gel and eluted with a mixture of $CHCl_3$–MeOH of increasing polarity to give 33 fractions. Fractions 12, 13, 14 and 15 were separately chromatographed on sephadex LH-20 and eluted several times with methanol to yield compounds **6** (34.0 mg), **7** (30 mg), **5** (3.0 mg) and **3** (24.0 mg), respectively. Compound **4** (23.0 mg) was obtained from fraction 23 by repeated chromatography of the fraction on an ODS open column eluted with a mixture of MeOH–H_2O (4 : 6).

Gnemonol A (**1**): A white amorhous powder; negative ion HR-FAB-MS $[M-H]^-$ *m/z*: 695.1919, (Calcd for $C_{42}H_{31}O_{10}$: 695.1917); negative ion FAB-MS $[M-H]^-$ *m/z*: 695; UV λ_{max} (MeOH) nm: 218, 284, 323; $[\alpha]_D$ −205.0° (*c*=0.102, MeOH); ^{1}H-NMR [400 MHz, acetone-d_6] δ: 2.92, 3.05 (1H each, dd, *J*=3.9, 14.2 Hz, H-8b), 4.29 (1H, d, *J*=3.9 Hz, H-8c), 4.62 (1H, d, *J*=11.3 Hz, H-8a), 4.92 (1H, t, *J*=3.9 Hz, H-7b), 5.41 (1H, d, *J*=3.9 Hz, H-7c), 5.92 (1H, d, *J*=11.3 Hz, H-7a), 6.15 (1H, d, *J*=1.8 Hz, H-3b), 6.21 (1H, dd, *J*=1.8, 8.5 Hz, H-5b), 6.23 (1H, t, *J*=2.0 Hz, H-12c), 6.26 (1H, br s, H-12b), 6.29 (2H, d, *J*=2.0 Hz, H-10c,14c), 6.36 (1H, d, *J*=2.0 Hz, H-14a), 6.37 (1H, d, *J*=2.0 Hz, H-12a), 6.74 (1H, br d, *J*=8.5 Hz, H-6b), 6.75 (2H, d, *J*=8.8 Hz, H-3c,5c), 6.79 (2H, d, *J*=8.8 Hz, H-3a,5a), 7.15 (2H, d, *J*=8.8 Hz, H-2c,6c), 7.16 (2H, d, *J*=8.8 Hz, H-2a,6a), 8.20 (1H, br s, OH-4b), 8.23 (2H, br s, OH-11c,13c), 8.24—8.83 (OH×5). The ^{13}C-NMR spectral data are listed shown in the Table.

Gnemonol B (**2**): A white amorphous powder; negative ion HR-FAB-MS $[M-H]^-$ *m/z*: 905.2614 (Calcd for $C_{56}H_{41}O_{12}$: 905.2596); UV λ_{max} (MeOH) nm: 225, 286, 327; $[\alpha]_D$ +8.0° (*c*=0.34, MeOH); ^{1}H-NMR [400 MHz, acetone-d_6] δ 4.38 (1H, d, *J*=5.4 Hz, H-8a), 4.47 (1H, d, *J*=5.4 Hz, H-8b), 4.48 (1H, d, *J*=4.4 Hz, H-8c), 5.35 (1H, d, *J*=5.4 Hz, H-7a), 5.45 (1H, d, *J*=5.4 Hz, H-7b), 5.46 (1H, d, *J*=4.4 Hz, H-7c), 6.17 (2H, d, *J*=2.0 Hz, H-10a,14a), 6.22 (1H, t, *J*=2.0 Hz, H-12a), 6.25 (1H, br s, H-14c), 6.27 (1H, br s, H-10b), 6.28 (1H, br s, H-14b), 6.34 (1H, br s, H-10c), 6.61 (1H, br s, H-14d), 6.71 (1H, br s, H-10d), 6.82 (2H, d, *J*=8.8 Hz, H-3a,5a), 6.83 (4H, d, *J*=8.8 Hz, H-3b,5b,3d,5d), 6.84 (2H, d, *J*=8.3 Hz, H-3c,5c), 6.94 (1H, d, *J*=16.6 Hz, H-8d), 7.08 (1H, d, *J*=16.6 Hz, H-7d), 7.18 (2H, d, *J*=8.8 Hz, H-2a,6a), 7.22 (2H, d, *J*=8.8 Hz, H-2b,6b), 7.24 (2H, d, *J*=8.3 Hz, H-2c,6c), 7.42 (2H, d, *J*=8.8 Hz, H-2d,6d), 8.07 (1H, br s, OH-13c), 8.20 (2H, br s, OH-11a,13a), 8.22 (1H, br s, OH-13b), 8.34 (1H, br s, OH-13d), 8.53 (3H, br s, OH-4a,4b,4c), 8.59 (1H, br s, OH-4d). The ^{13}C-NMR spectral data are listed in the Table.

Acetylation of 2 Fifteen milligrams of **2** was dissolved in a mixture of pyridine (0.5 ml) and acetic anhydride (0.5 ml). The mixture was stirred at rt for 24 h and treated in the usual manner. The crude product (18 mg) was purified by PTLC (hexane–acetone 1 : 1) to afford **2a** as an amorphous white solid (14.5 mg). ^{1}H-NMR (400 MHz, acetone-d_6) δ: 1.81, 1.89, 2.24, 2.26, (OAc×9). 4.55, 4.56, 4.58 (1H each, d, *J*=7.8 Hz, H-8a,8b,8c), 5.66 (1H, d, *J*=7.8 Hz, H-7a), 5.74, 5.75 (1H each, d, *J*=7.8 Hz, H-7b,7c), 6.54** (2H, br s, H-10c,10b), 6.75** (1H, br s, H-10d), 6.76** (1H, br s, H-14b), 6.85 (2H, d, *J*=1.9 Hz, H-10a,14a), 6.86** (1H, br s, H-14c), 6.91** (1H, br s, H-14d), 6.93 (1H, t, *J*=1.9 Hz, H-12a), 7.12* (2H, d, *J*=8.3 Hz, H-3b,5b), 7.13* (2H, d, *J*=8.3 Hz, H-3c,5c), 7.15* (2H, d, *J*=8.3 Hz, H-3d,5d), 7.16 (2H, d, *J*=8.8 Hz, H-3a,5a), 7.21 (1H, d, *J*=16.1 Hz, H-8d), 7.30 (1H, d, *J*=16.1 Hz, H-7d), 7.39 (2H, d, *J*=8.8 Hz, H-2a,6a), 7.43 (2H, d, *J*=8.3 Hz, H-2b,6b), 7.46 (2H, d, *J*=8.3 Hz, H-2c,6c), 7.64 (2H, d, *J*=8.3 Hz, H-2d,6d), *, ** are interchangeable.

Methylation 2 Ten milligrams of **2** was refluxed with K_2CO_3 (100 mg) and MeI (20 mg) in dry acetone (1.5 ml) for 4 h. The reaction mixture was treated in the usual manner and the crude product (13.7 mg) was purified by PTLC (hexane–acetone 1 : 1) to afford **2b** as an amorphous pale yellow solid (9.3 mg). FAB-MS $[M-H]^-$ *m/z*: 1031; ^{1}H-NMR (400 MHz, acetone-d_6) δ: 3.60, 3.65, 3.73×3, 3.75, 3.79×2, 3.81, (OMe), 4.45 (1H, d, *J*=5.4 Hz, H-8a), 4.53 (2H, d, *J*=4.9 Hz, H-8b, H-8c), 5.47 (1H, d, *J*=5.4 Hz, H-7a), 5.56, 5.59 (1H each, d, *J*=4.9 Hz, H-7b,7c), 6.33 (2H, d, *J*=1.9 Hz, H-10a,14a), 6.38 (1H, t, *J*=1.9 Hz, H-12a) 6.39, 6.40, 6.41, 6.46, 6.79, 6.84, (1H each, br s, H-10b,10c,10d,14b,14c,14d,), 6.90** (2H, d, *J*=9.2 Hz, H-3b,5b), 6.91** (2H, d, *J*=9.2 Hz, H-3c, 5c), 6.92* (2H, d, *J*=8.8 Hz, H-3a,5a), 6.93* (2H, d, *J*=8.8 Hz, H-3d,5d), 7.12 (1H, d, *J*=16.6 Hz, H-8d), 7.22 (1H, d, *J*=16.6 Hz, H-7d), 7.26 (2H, d, *J*=8.8 Hz, H-2a,6a), 7.30 (2H, d, *J*=9.2 Hz, H-2b,6b), 7.33 (2H, d, *J*=9.2 Hz, H-2c,6c), 7.53 (2H, d, *J*=8.8 Hz, H-2d,6d), *,** are interchangeable.

Gnemonol C (**3**): A white amorphous powder; negative ion HR-FAB-MS $[M-H]^-$ *m/z*: 921.2540 (Calcd for $C_{56}H_{41}O_{13}$: 921.2547); UV λ_{max} (MeOH) nm: 216, 285, 297, 310, 319; $[\alpha]_D$ +24.0° (*c*=0.10, MeOH); ^{1}H-NMR [600 MHz, acetone-d_6] δ: 4.40 (1H, d, *J*=5.4 Hz, H-8b), 4.49 (1H, d, *J*=4.2 Hz, H-8a), 4.68 (1H, d, *J*=4.8 Hz, H-8c), 5.38 (1H, d, *J*=5.4 Hz, H-

7b), 5.57 (1H, d, J=4.2 Hz, H-7a), 5.69 (1H, d, J=4.8 Hz, H-7c), 6.19 (2H, d, J=1.8 Hz, H-10a,14a), 6.23 (2H, t, J=1.8 Hz, H-12a,12c), 6.25 (2H, d, J=1.8 Hz, H-10c,14c), 6.26 (1H, br s, H-14b), 6.36 (1H, br s, H-10b), 6.43 (1H, d, J=8.4 Hz, H-5c), 6.59 (1H, br s, H-14d), 6.71 (1H, br s, H-10d), 6.84 (2H, d, J=8.4 Hz, H-3a,5a), 6.85 (4H, d, J=8.4 Hz, H-3b,5b,3d,5d), 6.97 (1H, d, J=16.2 Hz, H-8d), 7.10 (1H, d, J=16.2 Hz, H-7d), 7.13 (1H, d, J=8.4 Hz, H-6c), 7.20 (2H, d, J=8.4 Hz, H-2a,6a), 7.21 (2H, d, J=8.4 Hz, H-2b,6b), 7.44 (2H, d, J=8.4 Hz, H-2d,6d), 8.00—8.50 (OH×10). The ^{13}C-NMR spectral data are listed in the Table.

Gnemonoside E (**4**): A brown amorphous powder; negative ion HR-FAB-MS: $[M-H]^-$ m/z: 777.2385 (Calcd for $C_{40}H_{41}O_{16}$: 777.2394); UV λ_{max} (MeOH) nm: 213, 318; $[\alpha]_D$ −75° (c=0.10, MeOH); ^{1}H-NMR [500 MHz DMSO-d_6] δ: 4.48 (1H, d, J=5.0 Hz, H-8a), 5.43 (1H, d, J=5.0 Hz, H-7a), 6.07 (1H, t, J=2.0 Hz, H-12a), 6.10 (2H, d, J=2.0 Hz, H-10a,14a), 6.32 (1H, d, J=1.8 Hz, H-12b), 6.61 (1H, d, J=1.8 Hz, H-14b), 6.70 (1H, d, J=16.0 Hz, H-8b), 6.92 (1H, d, J=16.0 Hz, H-7b), 6.96 (2H, d, J=8.8 Hz, H-3b,5b), 7.05 (2H, d, J=8.8 Hz, 3a,5a), 7.27 (2H, d, J=8.8 Hz, H-2a,6a), 7.28 (2H, d, J=8.8 Hz, H-2b,6b), 9.19 (2H, br s, OH-11a,13a), 9.23 (1H, br s, OH-13b), 3.18—3.27 [8H, m, Glc(a,b)-H-2,3,4,5], 3.47, 3.75 [2H each, m, Glc(a,b)-H-6], 4.86 [2H, d, J=7.7 Hz, Glc(a,b)-H-1]. The ^{13}C-NMR spectral data are shown in the Table.

Gnetal (**5**): A white amorphous powder; EI-MS: m/z: 364, HR-EI-MS: m/z: 364.0928 (Calcd for $C_{21}H_{16}O_6$: 364.0947); UV λ_{max} (MeOH) nm: 225, 285; ^{1}H-NMR [600 MHz acetone-d_6] δ: 4.48 (1H, d, J=4.8 Hz, H-8a), 5.49 (1H, d, J=4.8 Hz, H-7a), 6.16 (2H, d, J=2.0 Hz, H-10a,14a), 6.23 (1H, t, J=2.0 Hz, H-12a), 6.98 (1H, br s, H-5b), 7.00 (1H, br s, H-3b), 7.00 (2H, d, J=8.8 Hz, H-3a,5a), 7.22 (2H, d, J=8.8 Hz, H-2a,6a), 8.18 (2H, br s, OH-11a,13a), 8.47 (1H, br s, OH-6b), 8.74 (1H, br s, OH-4a), 9.22 (1H, br s, H-7b); ^{13}C-NMR (acetone-d_6) δ: 55.2 (C-8a), 94.6 (C-7a), 102.2 (C-12a,3b), 106.7 (C-10a,14a), 108.0 (C-5b), 116.2 (C-1b), 116.3 (C-3a,5a), 127.9 (C-2a,6a), 133.3 (C-1a), 137.8 (C-4b), 146.7 (C-9a), 158.4 (C-4a), 159.6 (C-6b), 159.9 (C-11a,13a), 164.4 (C-2b), 192.3 (C-7b).

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