

FOUR DIMERIC STILBENES IN STEM LIANAS OF *GNETUM AFRICANUM*

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Abstract – From the stem lianas of *Gnetum africanum* Welw., four new stilbenoid dimers (gneafricanins C, D, E and F) were isolated along with four known compounds. The structures were established by spectroscopic analyses and chemical evidence. In addition the antioxidant activities of the compounds were also investigated.

Previously, we reported the isolation of dimeric stilbenes from stem lianas of *Gnetum africanum* (Gnetaceae).¹ Further investigation resulted in the isolation of four new stilbene dimers named gneafricanins C, D, E and F along with gnetin F, scirpussin A, gnetin D and gnetuhainin A. The structures of new compounds were elucidated to be gneafricanin C (**1**), gneafricanin D (**2**), gneafricanin E (**3**) and gneafricanin F (**4**) by spectroscopic analyses and chemical evidence. The bioactivity of the isolates on lipid peroxide inhibition and super oxide scavenging activity were also examined.

Gneafricanin C (**1**), $[\alpha]_D +7^\circ$, a white amorphous powder, showed positive reaction to Gibbs reagent. The negative FAB-MS exhibited an $[M-H]^-$ ion peak at m/z 485. The $[M-H]^-$ ion peak at m/z 485.1245 in the

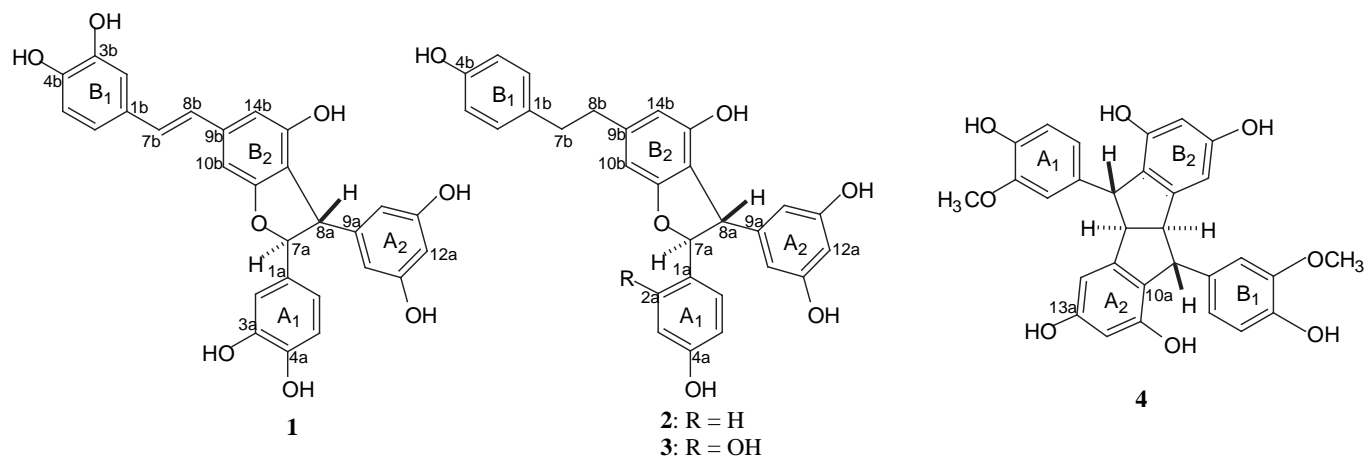


Figure 1

Table 1 ¹H and ¹³C NMR Spectral Data of Compounds (1 - 4)

	1*		2*		3*		4**	
	δ H	δ C	δ H	δ C	δ H	δ C	δ H	δ C
1a		135.1		134.1		121.0		138.1
2a	6.83 d (2.0)	113.3	7.14 d (8.6)	127.7		156.2	6.83 d (2.0)	111.6
3a		146.0	6.80 d (8.6)	116.1	6.46 d (2.4)	103.7		147.8
4a		145.9		158.1		159.0		145.3
5a	6.81 d (8.6)	116.1	6.80 d (8.6)	116.1	6.30 dd (2.4, 8.6)	107.4	6.68 d (8.0)	115.3
6a	6.71 dd (2.0, 8.6)	118.0	7.14 d (8.6)	127.7	7.00 d (8.6)	128.0	6.55 dd (2.0, 8.0)	120.1
7a	5.32 d (4.6)	93.6	5.30 d (4.8)	93.0	5.72 d (3.4)	89.1	4.57 br s	54.4
8a	4.36 d (4.6)	56.0	4.30 d (4.8)	55.9	4.40 d (3.4)	54.3	3.86 br s	60.6
9a		146.2		146.4		147.2		150.2
10a	6.16 d (2.0)	106.7	6.12 d (2.0)	106.7	6.26 d (2.4)	107.2		122.9
11a		159.6		159.5		159.4		155.1
12a	6.23 t (2.0)	102.0	6.19 t (2.0)	101.9	6.21 t (2.4)	101.8	6.20 d (2.0)	102.3
13a		159.6		159.5		159.4		159.1
14a	6.16 d (2.0)	106.7	6.12 d (2.0)	106.70	6.26 d (2.4)	107.2	6.64 d (2.0)	103.3
1b		130.7		133.4		133.2		138.1
2b	7.10 d (2.0)	113.3	7.02 d (8.8)	130.1	7.06 d (8.6)	130.5	6.83 d (2.0)	111.6
3b		146.4	6.71 d (8.8)	115.9	6.77 d (8.6)	116.1		147.8
4b		146.2		156.40		156.5		145.3
5b	6.82 d (8.6)	116.3	6.71 d (8.8)	115.9	6.77 d (8.6)	116.1	6.68 d (8.0)	115.3
6b	6.92 dd (2.0, 8.6)	120.0	7.02 d (8.8)	130.5	7.06 d (8.6)	130.5	6.55 dd (2.0, 8.0)	120.1
7b	7.04 d (16.2)	129.5	2.75 m	39.2	2.79 m	39.5	4.57 br s	54.4
8b	6.91 d (16.2)	126.8	2.76 m	37.7	2.81 m	37.9	3.86 br s	60.6
9b		141.2		145.7		145.7		150.2
10b	6.78 br s	99.1	6.28 br s	101.9	6.72 br s	102.2		122.9
11b		163.2		162.7		163.2		155.1
12b		115.0		113.2		114.0	6.20 d (2.0)	102.3
13b		155.4		155.2		155.4		159.1
14b	6.57 br s	108.0	6.25 br s	109.4	6.34 br s	109.5	6.64 d (2.0)	103.3
OCH ₃ -3a(3b)							3.78 br s	56.1

Measured in CD₃COCD₃. *400 MHz (¹H) and 100 MHz (¹³C). **300 MHz (¹H) and 75 MHz (¹³C).

high resolution (HR) FAB-MS showed the molecular formula to be C₂₈H₂₂O₈. The ¹H NMR spectrum (Table 1) exhibited the presence of two sets of aromatic protons on 1,2,4-trisubstituted benzene rings [δ 6.71 (1H, dd, *J*= 2.0, 8.6 Hz, H-6a), 6.81 (1H, d, *J*= 8.6 Hz, H-5a), 6.83 (1H, d, *J*= 2.0 Hz, H-2a); 6.82 (1H, d, *J*= 8.6 Hz, H-5b), 6.92 (1H, dd, *J*= 2.0, 8.6 Hz, H-6b), 7.10 (1H, d, *J*= 2.0 Hz, H-2b)], a set of *meta*-coupled aromatic protons on a 1,2,3,5-tetrasubstituted benzene ring [δ 6.57 (1H, br s, H-14b), 6.78 (1H, br s, H-10b)] and a set on a 3,5-dihydroxyphenyl group [δ 6.16 (2H, d, *J*= 2.0 Hz, H-10a, 14a), 6.23 (1H, t, *J*= 2.0 Hz, H-12a)]. A pair of *trans* olefinic protons [δ 6.91 (1H, d, *J*= 16.2 Hz, H-8b), 7.04 (1H, d, *J*=16.2 Hz, H-7a)] and a pair of mutually coupled methine protons [δ 4.36 (1H, d, *J*= 4.6 Hz, H-8a), 5.32

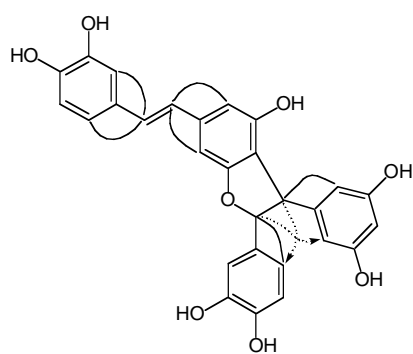


Figure 2
 1H-1H long range COSY
 DIFNOE

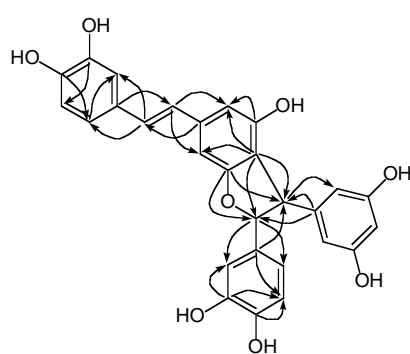


Figure 3
 Correlation in COLOC

the respective connections between C-7a/C-1a, C-8a/C-9a, C-7b/C-1a and C-8b/C-9b. Significant correlations between C-11b/H-7a(8a) and C-12b/H-7a(8a) also in the COLOC spectrum showed the connections between C-7a/C-11b and C-8a/C-12b respectively. It also showed that piceatanol unit A (ring A₁-7a-8a-ring A₂) is connected to another piceatanol unit B (ring B₁-7b-8b-ring B₂) at C-11b and C-12b and subsequently allowed the planar structure of **1** to be drawn as in Figure 1. The *trans* orientation of the protons on the dihydrofuran ring was deduced from DIFNOE experiment (Figure 2). Irradiation of H-7a and H-8a resulted in significant NOE at H-10a(14a) and H-2a(6a), respectively. Thus gneaffricanin C relative structure was drawn as **1**.

Gneaffricanin D (**2**), [α]_D -16°, a white amorphous powder, gave positive reaction to Gibbs reagent and showed absorption band at 229, 278 nm in the UV spectrum. The HR-EI-MS exhibited an M⁺ ion peak at m/z 456.1564 corresponding to the molecular formula C₂₈H₂₄O₆. The ¹H NMR spectrum (Table 1) showed the presence of two 4-hydroxyphenyl groups (rings A₁ and B₁), a set of *meta* coupled protons on a 1,2,3,5-tetrasubstituted benzene ring (ring B₂) and a set on a 3,5-dihydroxyphenyl ring (ring A₂). The spectrum also exhibited the presence of a pair of mutually coupled methine protons (H-7a/8a) and aliphatic protons of a CH₂-CH₂ moiety [δ 2.75 (2H, m, H-7b), 2.76 (2H, m, H-8b)] in addition to five hydroxyl groups. The

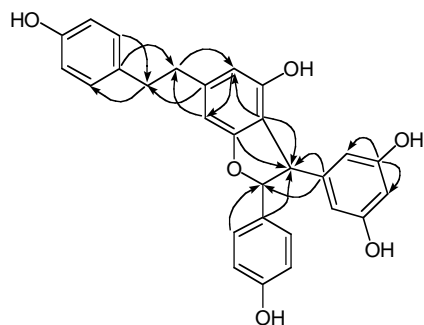


Figure 4
 Correlation in COLOC

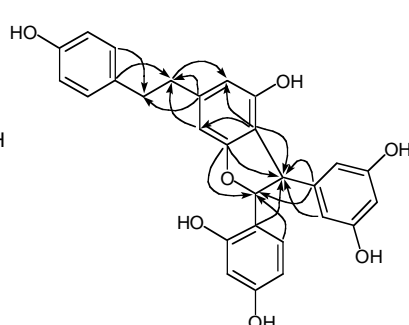


Figure 5
 Correlation in COLOC

C-H COSY and COLOC spectra (Figure 4) allowed the assignment of all protonated and quaternary carbons in **2**. Analysis of the ¹H and ¹³C NMR spectral data revealed a closed resemblance to those of gnetin C,³ except the appearance of signals due to aliphatic protons (δ 2.75 H-7b and 2.76 H-8b) in a relatively higher field in place of olefinic proton signals, which suggested that **2** is a 7b/8b dihydro derivative of gnetin C. The structure of **2** was further confirmed by catalytic hydrogenation of gnetin C. Treatment of gnetin C with Pd/C under hydrogen for 12 h gave **2a**, which was found to be identical to **2** and thus allowed the relative structure of **2** to be drawn as in Figure 1.

Gneaffricanin E (**3**), [α]_D -10°, a white amorphous powder, reacted positively to Gibbs reagent and

(1H, d, *J* = 4.6 Hz, H-7a)] were also observed in the spectrum. The ¹³C NMR spectrum (Table 1) showed 28 carbon signals. The ¹H and ¹³C NMR spectral data revealed that **1** is a dimeric stilbene composed of two piceatanol units.² In the COLOC spectrum (Figure 3), the correlations observed between C-7a/H-2a(6a), C-8a/H-10a(14a), C-7b/H-2b(6b) and C-8b/H-10b(14b) revealed

the respective connections between C-7a/C-1a, C-8a/C-9a, C-7b/C-1a and C-8b/C-9b. Significant correlations between C-11b/H-7a(8a) and C-12b/H-7a(8a) also in the COLOC spectrum showed the connections between C-7a/C-11b and C-8a/C-12b respectively. It also showed that piceatanol unit A (ring A₁-7a-8a-ring A₂) is connected to another piceatanol unit B (ring B₁-7b-8b-ring B₂) at C-11b and C-12b and subsequently allowed the planar structure of **1** to be drawn as in Figure 1. The *trans* orientation of the protons on the dihydrofuran ring was deduced from DIFNOE experiment (Figure 2). Irradiation of H-7a and H-8a resulted in significant NOE at H-10a(14a) and H-2a(6a), respectively. Thus gneaffricanin C relative structure was drawn as **1**.

showed absorption bands at 214, 279 nm in the UV spectrum. The HR-FAB-MS showed the [M-H]⁻ ion peak at m/z 471.1434, which is consistent with the molecular formula C₂₈H₂₄O₇. The ¹H NMR spectrum (Table 1) showed a close similarity to that of **2**, except the appearance of a 1,2,5-trisubstituted benzene ring in **3** in place of a 4-hydroxyphenyl group in **2**. It also showed close resemblance to gnetin D³ except for the signals due to aliphatic protons CH₂-CH₂ in place of olefinic protons CH=CH. These results indicated that **3** is a dihydro derivative of gnetin D.³ Catalytic hydrogenation of gnetin D resulted in **3a** which was found to be same as **3**. The C-H COSY and COLOC experiments (Figure 5) allowed the complete assignment of all protonated and quaternary carbons in **3**. Subsequently the relative structure of **3** is drawn as in Figure 1.

The occurrence of dihydrostilbene derivatives; compounds (**2**, **3**) and gnetin F in the family of Gnetaceae is of chemotaxonomical importance as to the similarity of chemical compounds with the family of Welwitschia⁵ and the possibility of having a common descent.

Gneaffricanin F (**4**), [α]_D -18°, a white amorphous powder, showed strong reaction to Gibbs reagent. The [M-H]⁻ ion peak at m/z at 513.1559 in the HR-FAB-MS corresponded to the molecular formula C₃₀H₂₆O₈. The ¹H NMR spectrum (Table 1) showed the presence of two sets of protons on two 1,2,4-trisubstituted benzene rings [δ 6.55 (2H, dd, J = 2.0, 8.0 Hz, H-6a, 6b), 6.68 (2H, d, J = 8.0 Hz, H-5a, 5b), 6.83 (2H, d, J = 2.0 Hz, H-2a, 2b)], two sets of *meta* coupled protons on a 1,2,3,5-tetrasubstituted benzene ring [δ 6.20 (2H, d, J = 2.0 Hz, H-12a, 12b), 6.64 (2H, d, J = 2.0 Hz, H-14a, 14b)] and two sets of methine coupled protons [δ 3.86 (2H, br s, H-8a, 8b), 4.57 (2H, br s, H-7a, 7b)]. The spectrum also exhibited the signals

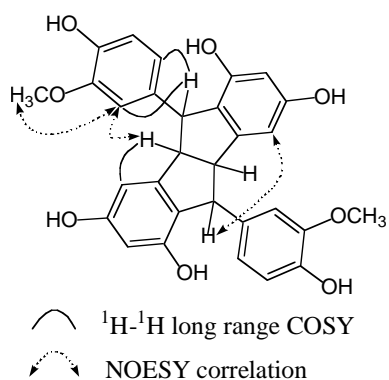


Figure 6

due to two methoxyl groups [δ 3.78 (6H, br s, OCH₃) and six hydroxyl groups signals. Analysis of the ¹H-¹H-long range COSY (Figure 6) revealed that **4** is a dimeric stilbene with close resemblance to pallidol,⁴ however, the presence of two methoxyl groups and the appearance of two sets of a 1,3,4-trisubstituted benzene rings (ring A₁ and B₁) in **4** were different from pallidol (4-hydroxyphenyl ring). These results suggested that **4** is a stilbenoid composed of two isorhapontigenin units in a symmetrical form. The appearance of the protons at H-7a, 8a, 7b and 8b as singlets also similar to pallidol indicated that the dihedral angles of the methines protons are at 90°.

The positions of the methoxyl groups were determined by NOESY experiment (Figure 6), a cross peak correlations observed between OCH₃ (δ 3.78) and H-2a and H-2b (δ 6.83) in the NOESY spectra established the positions of the methoxyl groups at C-3a and C-3b

	Antioxidant Activities of Stilbenoids isolated from <i>Gnetum africanum</i>	
	Lipid Peroxide Inhibition [IC50 (μM)]	scavenging activity of superoxide [IC50 (μM)]
gneaffricanin C (1)	13	10
gneaffricanin D (2)	50	33
gneaffricanin E (3)	32	30
gnetin F	29	26
longusol A	15	16
biisorhapontigenin	45	29
gneaffricanin A	34	20

respectively. The relative structure of **4** was established by the result of NOESY experiment and comparison with pallidol. Gnetin F,⁵ scirpusin A,⁶ gnetin D and gnetuhainin A⁷ were also obtained from stem of *G. africanum* and the structures

were identified by spectroscopic analysis and comparison with authentic samples.

The antioxidant activity of the compounds was conducted in lipid peroxide inhibition^{8, 9} (IC₅₀) and SOD^{10,11} (IC₅₀) like antioxidant activity, the result showed all the compounds exhibited a considerable degree of activity. Compounds (**1**, **2** and **3**) showed 13, 50 and 32 (μM) inhibition in lipid peroxide, and 10, 34 and 30 (μM) scavenging activity of super oxide. The results of the other stilbenoids; longusol A, biisorhapontigenin and gneaffricanin A,¹ which were also isolated from *G. africanum* were examined and the results are listed in Table 2.

EXPERIMENTAL

General Methods

¹H and ¹³C NMR spectra were recorded on EX-400 and AL 300 spectrometers (JEOL). Chemical shifts were shown as δ values with tetramethylsilane (TMS) as an internal reference. Peak multiplicities were quoted in Hz. Negative ion FAB-MS, HR-FAB-MS, EI and HR-EI-MS were recorded on JMS-DA spectrometer equipped with a JMA 3500 data analysis system (JEOL). UV spectra was recorded on a UV 2200 spectrophotometer (Shimadzu) and optical rotation were measured on P-1020 (JASCO) Polarimeter. Silica gel 60 (70-230 mesh, Merck), Sephadex LH-20 and Sek-Pak C-18 Cartridges were used for column chromatography. Kiesel-gel 60 F₂₅₄ (Merck) was used for analytical and preparative TLC.

Plant Material

Stem of *Gnetum africanum* was collected in March, 2001 at Nsukka, Nigeria.

Extraction and isolation

Dried stem of *G. africanum* (1.3 kg) was powdered and extracted with acetone and methanol (3L x 3) at rt. The acetone extract (35 g) was chromatographed on silica gel eluted with CHCl₃-MeOH increasing the concentration of MeOH to give 26 fractions. Fractions 8-12 were combined and subjected to chromatography on Sephadex LH-20 eluted with acetone yielded 10 fractions (Fr. A-J). Compound **1** (26 mg) was obtained from Fr. H. Further purification of Fr. B by reversed phase chromatography on Sek-Pak C-18 gave five fractions (B1-B5). **4** (2 mg) was obtained from Fr. B5. **2** (8 mg) and **3** (11 mg) were separated from Fr. B3 by PTLC developed with EtOAc-CHCl₃-MeOH-H₂O (15 : 12 : 4 : 1).

Catalytic hydrogenation of gnetins C and D: A solution of gnetin C (20 mg) in dry EtOH (200 mL) was stirred over 10% Pd-C (10 gm) under H₂ atmosphere for 12 h. Usual work up afforded **2a** (17 mg). Gnetin D (20 mg) was hydrogenated in same manners to give **3a** (19 mg).

Gneaffricanin C (**1**): A white amorphous powder; Negative HR-FAB-MS: [M-H]⁻ *m/z* 485.1245 (Calcd 485.1237 for C₂₈H₂₁O₈); Negative FAB-MS: [M-H]⁻ *m/z* 485; UV λ (nm) 210, 266, 285, 329; [α]_D +7° (c= 0.10, MeOH); The ¹H and ¹³C NMR spectral data are shown in Table 1.

Gneaffricanin D (**2**): A white amorphous powder; Negative HR-EI-MS: M⁺ *m/z* 456.1564 (Calcd 456.1573 for C₂₈H₂₄O₇); Negative EI-MS: M⁺ *m/z* 456; [α]_D -16° (c= 0.10, MeOH); UV λ (nm) 229, 278; The ¹H and ¹³C NMR spectral data are listed in Table 1.

Gneaffricanin E (**3**): A white amorphous powder; Negative HR-FAB-MS: [M-H]⁻ *m/z* 471.1434 (Calcd

471.1435 for C₂₈H₂₃O₇); Negative FAB-MS: [M-H]⁻ *m/z* 471; [α]_D -10° (*c*= 0.10, MeOH); UV λ (nm) 214, 279; The ¹H and ¹³C NMR spectral data are shown in Table 1.

Gneaffricanin F (**4**): A white amorphous powder; Negative HR-FAB-MS: [M-H]⁻ *m/z* 513.1559 (Calcd 513.1550 for C₃₀H₂₅O₈); Negative FAB-MS: [M-H]⁻ *m/z* 513; [α]_D -18° (*c*= 0.05, MeOH); UV λ (nm) 208, 284; The ¹H and ¹³C NMR spectral data are listed in Table 1.

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