HETEROCYCLES, Vol. 57, No. 8, 2002, pp. 1507 - 1512, Received, 6th June, 2002

FOUR DIMERIC STILBENES IN STEM LIANAS OF *GNETUM AFRICANUM*

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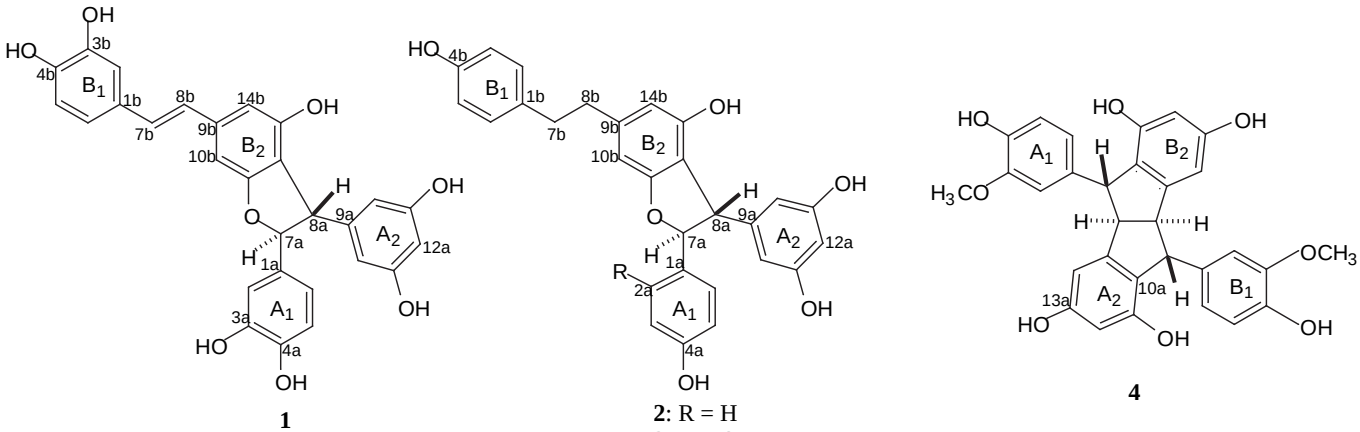
Abstract – From the stem lianas of *Gnetum africanum* Welw., four new stilbenoid dimers (gneafricanins C, D, E and F) were isolated along with four known compounds. The structures were established by spectroscopic analyses and chemical evidence. In addition the antioxidant activities of the compounds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



1

2: R = H
3: R = OH

4

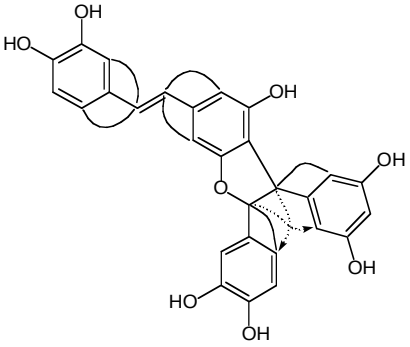
Figure 1

Table1 ^{1}H and ^{13}C 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 CD_3COCD_3. *400 MHz (^{1}H) and 100 MHz (^{13}C). **300 MHz (^{1}H) and 75 MHz (^{13}C).

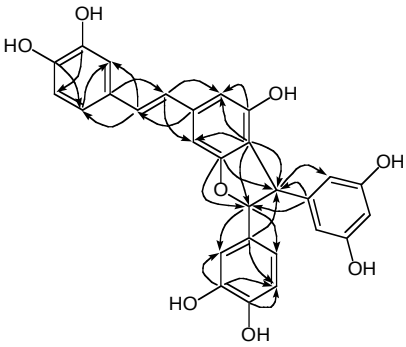
high resolution (HR) FAB-MS showed the molecular formula to be $C_{28}H_{22}O_8$. The ^{1}H NMR spectrum (Table 1) exhibited the presence of two sets of aromatic protons on 1,2,4-trisubstituted benzene rings [δ 6.71 (1H, dd, *J*= 2.0, 8.6 Hz, H-6a), 6.81 (1H, d, *J*= 8.6 Hz, H-5a), 6.83 (1H, d, *J*= 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* olefenic 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



^{1}H-^{1}H long range COSY

DIFNOE

Figure 2

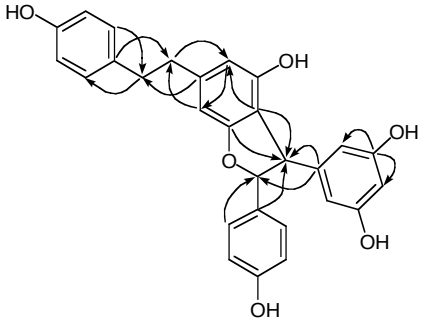


Correlation in COLOC

Figure 3

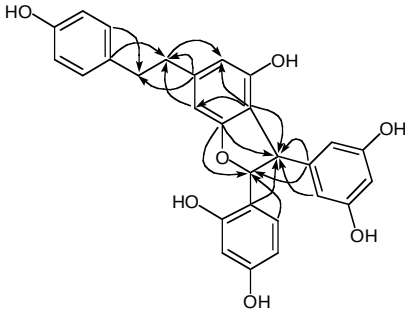
(1H, d, *J*= 4.6 Hz, H-7a)] were also observed in the spectrum. The ^{13}C NMR spectrum (Table 1) showed 28 carbon signals. The ^{1}H and ^{13}C NMR spectral data revealed that **1** is a dimeric stilbene composed of two piceatanol units.[2] In the COLOC spectrum (Figure 3), the correlations observed 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 A_1-7a-8a-ring A_2) is connected to another piceatanol unit B (ring B_1-7b-8b-ring B_2) 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 gneafricanin C relative structure was drawn as **1**.

Gneafricanin D (**2**), $[\alpha]_D$ -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 $C_{28}H_{24}O_6$. The ^{1}H NMR spectrum (Table 1) showed the presence of two 4-hydroxyphenyl groups (rings $A_1$and B_1), a set of *meta* coupled protons on a 1,2,3,5-tetrasubstituted benzene ring (ring B_2) and a set on a 3,5-dihydroxyphenyl ring (ring A_2). The spectrum also exhibited the presence of a pair of mutually coupled methine protons (H-7a/8a) and aliphatic protons of a CH_2-CH_2 moiety [δ 2.75 (2H, m, H-7b), 2.76 (2H, m, H-8b)] in addition to five hydroxyl groups. The



Correlation in COLOC

Figure 4



Correlation in COLOC

Figure 5

C-H COSY and COLOC spectra (Figure 4) allowed the assignment of all protonated and quaternary carbons in **2**. Analysis of the ^{1}H and ^{13}C 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 olefenic 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.

Gneafricanin E (**3**), $[\alpha]_D$ –10°, a white amorphous powder, reacted positively to Gibbs reagent and

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 in consistent with the molecular formula $C_{28}H_{24}O_7$. The ^1H NMR spectrum (Table 1) showed a closed 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 closed resemblance to gnetin D[3] except for the signals due to aliphatic protons CH_2-CH_2 in place olefenic 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 Welwitschia[5] and the possibility of having a common descent.

Gneafricanin F (**4**), $[\alpha]_D$ −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 $C_{30}H_{26}O_8$. The ^1H NMR spectrum (Table 1) showed the presence of two sets 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 due to two methoxyl groups [δ 3.78 (6H, br s, OCH_3) 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 A_1 and B_1) 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 OCH_3 (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 respectively. The relative structure of **4** was established by the result of NOESY experiment and comparison with pallidol.

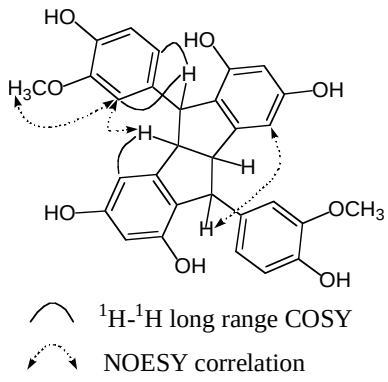


Figure 6

Table 2. Antioxidant Activities of Stilbenoids isolated from *Gnetum africanum*

	Lipid Peroxide Inhibition [IC50 (μM)]	scavenging activity of superoxide [IC50 (μM)]
gneafricanin C (**1**)	13	10
gneafricanin D (**2**)	50	33
gneafricanin E (**3**)	32	30
gnetin F	29	26
longusol A	15	16
biisorhapontigenin	45	29
gneafricanin A	34	20

Gnetin F,[5] scirpusin A,[6] gnetin D and gnetuhainin A[7] 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] (IC_{50}) and SOD[10,11] (IC_{50}) 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 gneafricanin A,[1] which were also isolated from *G. africanum* were examined and the results are listed in Table 2.

EXPERIMENTAL

General Methods

^{1}H and ^{13}CNMR 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 F_{254} (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 $CHCl_3$-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-$CHCl_3$-MeOH-H_2O (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 H_2 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).

Gneafricanin C (**1**): A white amorphous powder; Negative HR-FAB-MS: $[M-H]^-$ *m/z* 485.1245 (Calcd 485.1237 for $C_{28}H_{21}O_8$); Negative FAB-MS: $[M-H]^-$ *m/z* 485; UV λ (nm) 210, 266, 285, 329; $[\alpha]_D$ +7° (*c*= 0.10, MeOH); The ^{1}H and ^{13}C NMR spectral data are shown in Table 1.

Gneafricanin D (**2**): A white amorphous powder; Negative HR-EI-MS: M^+ *m/z* 456.1564 (Calcd 456.1573. for $C_{28}H_{24}O_7$); Negative EI-MS: M^+ *m/z* 456; $[\alpha]_D$ -16° (*c*= 0.10, MeOH); UV λ (nm) 229, 278; The ^{1}H and ^{13}C NMR spectral data are listed in Table 1.

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

471.1435 for $C_{28}H_{23}O_7$); Negative FAB-MS: $[M-H]^-$ *m/z* 471; $[\alpha]_D$ -10° (*c*= 0.10, MeOH); UV λ (nm) 214, 279; The ^1H and ^{13}C NMR spectral data are shown in Table 1.

Gneafricanin F (**4**): A white amorphous powder; Negative HR-FAB-MS: $[M-H]^-$ *m/z* 513.1559 (Calcd 513.1550 for $C_{30}H_{25}O_8$); Negative FAB-MS: $[M-H]^-$ *m/z* 513; $[\alpha]_D$ -18° (*c*= 0.05, MeOH); UV λ (nm) 208, 284; The ^1H and ^{13}C NMR spectral data are listed in Table 1.

ACKNOWNLEGEMENT

The authors are grateful to Prof. C. O. Akueshi (Faculty of Natural Sciences, University of Jos, Nigeria) and Prof. C. O. C. Agwu (Dept. of Botany, University of Nigeria, Nsukka, Nigeria) for their assistance of identification and collection of the plant material.

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