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SIX FLAVONOSTILBENES FROM *GNETUM AFRICANUM* AND *GNETUM GNEMON*

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Abstract- Six new flavonostilbenes (gnetoflavanols A, B, C, D, E and F) were isolated from the stem of *Gnetum africanum* and the root of *G. gnemon*. The structures of the compounds were determined by spectroscopic analysis. The antioxidant activity of the compounds on lipid peroxide inhibition and superoxide scavenging activity were also investigated.

A number of stilbenoids have been isolated from the family of Gnetaceae which have a variety of skeletons resulting from oxidative coupling of several types of monomeric stilbenes such as isorhapontigenin, oxyresveratrol, piceatannol and resveratrol units, in addition to several stilbene glucosides.¹⁻⁵ In continuation of our phytochemical studies of the *Gnetum* we report in this paper the isolation and structure determination of six new flavonostilbenes isolated from two *Gnetum* species (*G. africanum* and *G. gnemon*).

The compounds were considered to be formed by oxidative coupling of a flavan-3-ol (afzelechin)⁶ with a stilbene monomer (isorhapontigenin, piceatannol and resveratrol unit). Gnetoflavanols A (**1**), B (**2**), C (**3**) and D (**4**) were isolated from the stem of *G. africanum*. Gnetoflavanols E (**5**) and F (**6**) were obtained

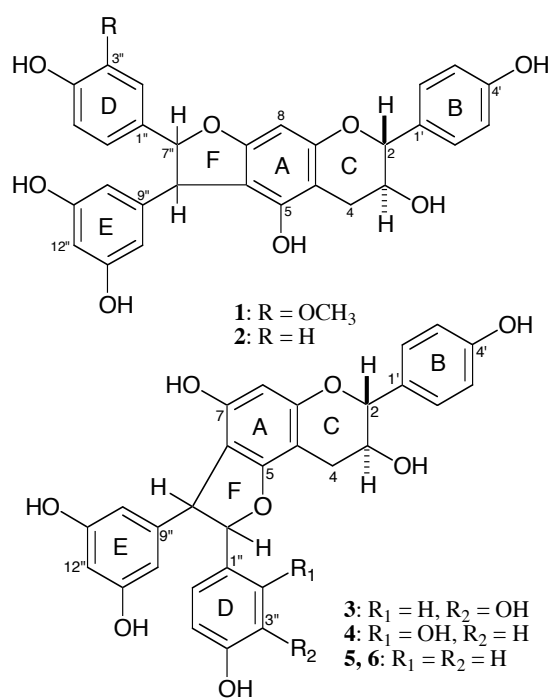


Figure 1

from the root of *G. gnemon*. The structures of the compounds were established by spectroscopic methods. The bioactivity of the compounds on lipid peroxide inhibition activity and scavenging activity for super oxide in a xanthine-xanthine oxidase system were evaluated. Compounds (**1**, **5** and **6**) exhibited a considerable antioxidant activity.

Gnetoflavanol A (**1**), a white amorphous powder, showed positive reaction to Gibbs reagent. The HR-FAB-MS [M-H]⁻ ion at *m/z* 529.1509 and the FAB-MS at *m/z* 529 are in consistence with the molecular formula of C₃₀H₂₆O₉. The UV absorptions (227 and 282 nm) showed the presence of aromatic ring. The ¹H NMR spectrum (Table 1) exhibited the presence of a set of aromatic protons on a 4-hydroxyphenyl group [δ 6.84 (2H, d, *J* = 8.5 Hz, H-3', 5'), 7.28 (2H, d, *J* = 8.5 Hz, H-2', 6')], a set on a 1,3,4-trisubstituted benzene ring [δ 6.82 (1H, d, *J* = 8.5 Hz, H-5''), 6.82 (1H, dd, *J* = 2.0, 8.5 Hz, H-6''), 6.96 (1H, d, *J* = 2.0 Hz, H-2'')] and a set on a 3,5-dihydroxyphenyl group [δ 6.16 (2H, d, *J* = 2.0 Hz, H-10'', 14''), 6.24 (1H, t, *J* = 2.0 Hz, H-12'')] were observed. An aromatic proton on a *penta*-substituted benzene

Table 1. ¹H NMR Spectral Data of Compounds (**1** - **6**)

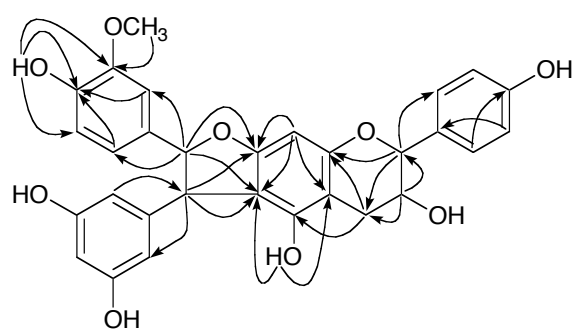
No.	1 ^a	2 ^b	3 ^a	4 ^b	5 ^b	6 ^b
2	4.64 d (7.5)	4.64 d (8.4)	4.68 d (7.8)	4.66 d (8.8)	4.71 d (7.8)	4.70 d (8.3)
3	4.04 m	4.01 m	4.10 m	4.10(m)	4.11 m	4.11 m
4	2.96 dd (6.0, 16.0) 2.56 dd (9.0, 16.0)	2.98 dd (5.4, 16.0) 2.56 d, (8.8, 16.0)	2.99 dd 6.0, 16.5) 2.65 dd (9.6, 16.5)	3.01 dd (6.4, 15.6) 2.69 dd (8.8, 16.4)	2.99 dd (5.4, 15.6) 2.64 dd (8.8, 15.6)	3.01 dd (5.1, 16.1) 2.68 dd (8.8, 16.1)
8	6.01 br s	6.10 br s	5.94 s	5.90 s	5.96 s	5.96 s
2'(6')	7.28 d (8.5)	7.29 d (8.4)	7.29 d (8.4)	7.30 d (8.8)	7.28 d (8.3)	7.30 d (8.3)
3'(5')	6.84 d (8.5)	6.85 d (8.4)	6.82 d (8.4)	6.84 d (8.8)	6.85 d (8.3)	6.85 d (8.3)
2''	6.96 d (2.0)	7.10 d (8.4)	6.87 d (2.1)		7.19 d (8.8)	7.23 d (8.3)
3''		6.84 d (8.4)		6.45 d (2.5)	6.85 d (8.8)	6.85 d (8.3)
5''	6.82 d (8.5)	6.84 d (8.4)	6.85 d (8.4)	6.32 dd (2.5, 8.4)	6.85 d (8.8)	6.85 d (8.3)
6''	6.82 dd (2.0, 8.5)	7.10 d (8.4)	6.72 dd (2.1, 8.4)	7.02 d (8.4)	7.19 d (8.8)	7.23 d (8.3)
7''	5.30 d (5.0)	5.18 d (4.4)	5.33 d (4.8)	5.76 d (3.6)	5.40 d (4.9)	5.40 d (5.2)
8''	4.42 d (5.0)	4.39 d (4.4)	4.35 d (4.8)	4.38 d (3.6)	4.35 d (4.9)	4.37 d (5.2)
10''(14'')	6.16 d (2.0)	6.15 d (2.0)	6.17 d (2.1)	6.26 d (2.0)	6.19 d (2.0)	6.19 d (2.0)
12''	6.24 t (2.0)	6.30 d (2.0)	6.23 t (2.1)	6.19 t (2.0)	6.25 t (2.0)	6.25 t (2.0)
OCH ₃ -3''	3.82 s					

All measured in (CD₃)₂CO. a: 300 MHz and b: 400 MHz

Table 2. ^{13}C NMR Spectral Data of Compounds (1 - 6)

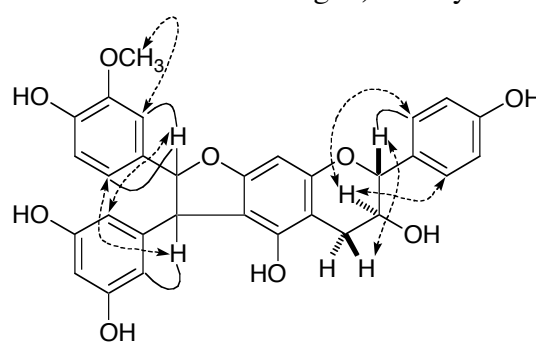
No.	1 ^a	2 ^b	3 ^a	4 ^b	5 ^b	6 ^b
2	82.7	83.2	83.1	83.3	82.7	83.1
3	69.2	68.6	68.0	68.3	67.9	68.1
4	29.5	29.3	29.2	28.9	28.8	29.4
4a	102.1	102.4	98.5	96.7	96.3	96.6
5	152.7	153.1	160.7	160.1	160.4	160.7
6	108.1	108.4	107.6	108.4	107.4	107.7
7	161.0	161.5	153.8	154.1	153.7	153.9
8	90.5	90.9	96.6	96.7	96.5	96.8
8a	157.2	157.6	157.1	157.0	156.9	157.2
1'	131.2	131.6	131.3	131.5	131.0	131.3
2'(6)'	129.6	130.1	129.7	129.9	129.5	129.8
3'(5)'	115.8	116.1	115.8	116.0	115.7	115.9
4'	158.1	158.47	158.1	159.1	157.9	158.18
1''	134.4	134.5	135.0	120.8	133.9	134.2
2''	110.2	128.1	113.4	156.3	127.5	127.9
3''	148.3	116.5	145.9	103.7	116.1	116.3
4''	147.3	158.51	146.1	158.3	157.9	158.22
5''	115.8	116.5	116.1	107.5	116.1	116.3
6''	119.2	128.1	118.0	128.0	127.5	127.9
7''	93.9	94.0	94.2	89.8	94.0	94.3
8''	55.6	55.9	56.2	54.6	55.9	56.2
9''	146.7	146.8	146.9	147.5	146.6	146.8
10''(14'')	106.8	107.0	106.7	107.2	106.6	106.8
11''(13'')	159.6	160.0	159.5	159.4	159.3	159.6
12''	102.1	102.6	101.8	101.8	101.8	102.0
OCH ₃ -3''	56.3					

All measured in (CD₃)₂CO. a: 75 MHz and b: 100 MHz



Correlations in the HMBC spectrum

Figure 2



Correlations in the ^1H - ^1H long range
COSY spectrum
Correlations in NOESY spectrum

Figure 3

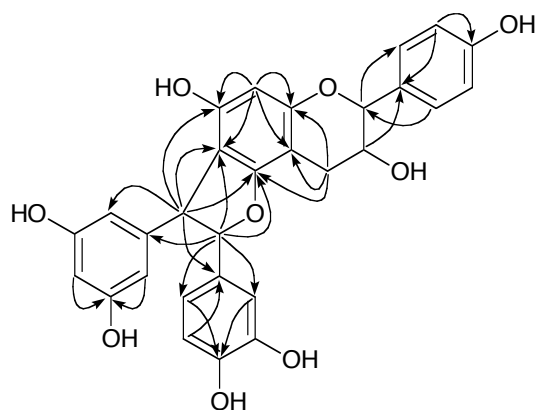
ring [δ 6.01 (1H, br s, H-8)] and four aliphatic protons coupled successively in the order CH₂-CH(OH)-CH(O) [δ 2.56 (1H, dd, J = 9.0, 16.0 Hz, H-4_{ax}), 2.96 (1H, dd, J = 6.0, 16.0 Hz, H-4_{eq}), 4.04 (1H, m, H-3), 4.64 (1H, d, J = 7.5 Hz, H-2)] were also shown in the spectrum. The spectrum further exhibited a set of mutually coupled methine protons [δ 4.42 (1H, d, J = 5.0 Hz, H-8''), 5.30 (1H, d, J = 5.0 Hz, H-7'')], a OCH₃ protons [δ 3.82 (3H, br s, OCH₃-3''), five phenolic and an alcoholic hydroxyl groups. The ^{13}C NMR (30 carbon atoms, Table 2) and the above results showed that **1** is composed of a flavan-3-ol (rings A, B and C) and an isorhapontigenin (ring D-7''-8''-ring E). Analysis of HMQC

and HMBC spectra (Figure 2) enabled the complete assignment of all protonated and quaternary carbons in **1**. In the ^1H - ^1H long range COSY spectrum (Figure 3), the correlations between H-2''(6'')/H-7'', H-10''(14'')/H-8'' and H-2'(6')/H-2 substantiated the respective connections of C-1'/C-7'', C-9''/C-8'' and C-1'/C-2. In the HMBC spectrum the cross peak correlations observed between H-7''/C-6(7), H-8''/C-6(7),

H-2/C-8a and H-4/C-5(8a) revealed the linkages of C-7"/C-7, C-8"/C-6, C-2/C-8a and C-4/C-4a, respectively. The correlations between OH-5/C-4a(6) in the HMBC showed that a hydroxyl group is attached at C-5 on the ring A. The correlation observed between OCH₃ proton and C-3" in the HMBC revealed that a methoxyl group is attached to C-3" and was further confirmed by NOESY experiment (Figure 3). The *trans* orientation of hydrogens on the dihydrofuran ring was established by the correlations of H-7"/H-10"(14") and H-8"/H-2"(6") in NOESY experiment. The *trans* configuration of H-2/3 was also deduced by the results of NOESY experiment and found to be similar to daphnodorin.⁷ However, no correlation was observed in the NOESY spectrum between the dihydrofuran protons and the pyran protons to allowed the complete relative structure of **1** to be drawn.

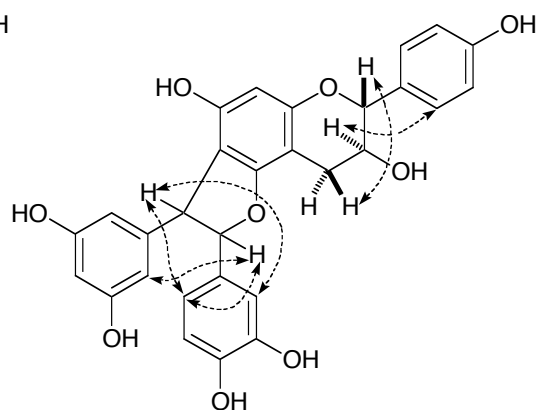
Gnetoflavanol B (**2**), a white amorphous powder. The negative FAB-MS exhibited an [M-H]⁻ ion peak at 499 and the molecular formula of C₂₉H₂₄O₈ was deduced by HR-FAB-MS (*m/z* 499.1385). The ¹H-NMR spectrum (Table 1) showed the presence of two sets of aromatic protons on 4-hydroxyphenyl groups (rings B and D), a set on a 3,5-dihydroxyphenyl group (ring E) and an aromatic proton on a *penta*-substituted benzene ring (ring A). The spectrum also exhibited the presence of four coupled aliphatic protons [CH₂-CH(OH)-CH(O)], a set of mutually coupled methine protons H-7"/8", five phenolic and an alcoholic hydroxyl groups. The ¹H and ¹³C NMR spectra (Tables 1 and 2) of **2** showed a closed resemblance to those of **1** except the disappearance of a methoxyl signal and the appearance of a 4-hydroxyphenyl group (ring D) in **2** in placed of a 1,3,5-trisubstituted benzene ring. Analysis of the ¹H-¹H long range COSY revealed that **2** is composed of a resveratrol unit (ring D-7"-8"-ring E) and a flavan-3-ol (rings A, B and C). All quaternary carbons and the relative structure of **2** were assigned with respect to **1**.

Gnetoflavanol C (**3**), a white amorphous powder, showed [M-H]⁻ ion peak at *m/z* 515 in the negative FAB-MS and *m/z* 515.1348 in the negative HR-FAB-MS which corresponded to the molecular formula of C₂₉H₂₄O₉. The ¹H NMR spectrum (Table 1) exhibited the presence of eleven aromatic protons assigned to a set on a 4-hydroxyphenyl group (ring B), a set on a 1,3,4-trisubstituted benzene ring (ring D) and a set on a 3,5-dihydroxyphenyl group (ring E). A proton signal on a *penta*-substituted benzene ring (ring A), four aliphatic coupled protons [□ 2.65 (1H, dd, *J*= 9.6, 16.5 Hz, H-4_{ax}), 2.99 (1H, dd, *J*= 6.0, 16.5 Hz, H-4_{eq}), 4.10 (1H, m, H-3), 4.68 (1H, d, *J*= 7.8 Hz, H-2)] and a set of mutually coupled methine protons [□ 4.35 (1H, d, *J*= 4.8 Hz, H-8"), 5.33 (1H, d, *J*= 4.8 Hz, H-7")] were also shown in the spectrum. Analysis of the ¹H NMR and ¹³C NMR spectral data (Tables 1 and 2) of **3** revealed that it is also composed of a monomeric stilbene and a flavan-3-ol. In the HMBC spectrum (Figure 4), the correlation observed between H-2/C-2'(6'), H-4/C-5(8a), H-7"/C-2"(6") and H-8"/C-10"(14") revealed the respective linkages of C-2/C-1', C-4/C-4a, C-7"/C-1" and C-8"/C-9". The correlations between H-7"/C-5 and H-8"/C-6(7) observed in HMBC spectrum revealed the linkages of C-7"/C-5 and C-8"/C-6



Correlations in the HMBC spectrum

Figure 4

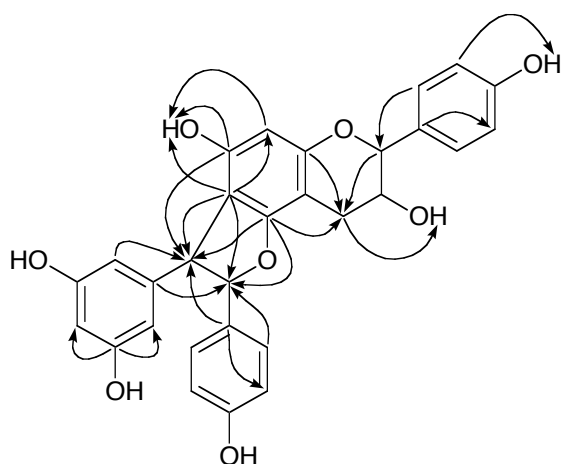


Correlations in the NOESY spectrum

Figure 5

respectively and subsequently established the position of the oxidative linkage of a monomeric stilbene, piceatannol unit⁸ (ring D-7''-8''-ring E) to the flavan-3-ol (rings A, B and C) at position C-5 and C-6, which is different from that of **1** (C-6 and C-7). The *trans* configuration of the dihydrofuran ring and the relative stereochemistry of H-2/3 were drawn by the NOESY experiment (Figure 5).

Gnetoflavanol D (**4**), a white amorphous powder, gave blue color to Gibbs reagent. The molecular formula $C_{29}H_{24}O_9$ was deduced from the HR-FAB-MS $[M-H]^-$ ion peak at m/z 515.1348. Analysis of the 1H , ^{13}C NMR spectra (Tables 1 and 2) and 1H - 1H long range COSY spectral data of **4** revealed the presence of four aromatic rings (rings A, B, D and E), a dihydrofuran ring (ring F), a pyran (ring C), two mutually coupled methine protons and four aliphatic coupled protons. The spectral data of **4** showed a



Correlations in the COLOC spectrum

Figure 6

closed resemblance to those of **3** except the appearance of an OH group at C-2'' in **4** in placed of C-3'' in **3**, which resulted in the lower field chemical shift of H-7'' (0.43 ppm), and higher field shift of C-7'' (4.4 ppm) and C-1'' (14.2 ppm). These results indicated that **4** is composed of an oxyresveratrol⁹ (ring D-7''-8''-ring E) and a flavan-3-ol. The *trans* orientations of the dihydrofuran ring (H-7''/8'') and the pyran ring (H-2/3) protons were established by comparison with **3**.

Gnetoflavanol E (**5**), $[\alpha]_D = +12^\circ$ and gnetoflavanol F (**6**), $[\alpha]_D = -7^\circ$, showed positive reaction to Gibbs reagent. The molecular formulas of $C_{29}H_{24}O_8$ of the two compounds were deduced by the HR-FAB-MS $[M-H]^-$ ion peak at m/z 499.1234. The 1H and ^{13}C NMR spectral data of **5** and **6** (Tables 1 and 2) were similar to those of **3** except that they both showed the appearance of a 4-hydroxyphenyl group (ring D) in placed of the 1,3,4-trisubstituted benzene ring as observed in **3**. All

protonated and quaternary carbons in **5** and **6** were assigned with the aid of COLOC experiments (Figure 6). Although the ^1H and ^{13}C NMR spectral data of **5** and **6** are similar, the difference in the optical rotation and retention time observed in HPLC revealed that the compounds are diastereoisomer. Considering the orientation of the pyran hydrogens (H-2/3) in all the compounds to be fixed, the difference between the two compounds might be due to opposite *trans* orientation of the dihydrofuran hydrogen (H-7"/8").

Compounds (**1**, **5** and **6**) showed 32, 90 and 40 μM inhibition in lipid peroxide (IC_{50}),^{8,9} and **1** exhibited 34 μM scavenging activity for super oxide in xanthine-xanthine oxidase system (IC_{50}).^{10,11} as compared to 2100 μM scavenging activity for super oxide and 1000 μM inhibition in lipid peroxide exhibited by vitamin E.

EXPERIMENTAL

General Method

^1H and ^{13}C NMR spectra were measured on JNM EX-400 and AL-300 (JOEL) spectrometers. Chemical shifts were shown as δ values with trimethylsilane (TMS) as an internal reference. Peak multiplicities were quoted in Hz. Negative FAB-MS and HR-FAB-MS were recorded on JMS-DX-300 spectrometer equipped with JMA 3500 data analysis (JEOL). UV spectra were recorded on UV-2200 spectrophotometer (Shimadzu) and optical rotations were measured on P-1020 (Jasco) polarimeter. Silica gel 60 (70-230 mesh, Merck), Sephadex LH-20, Fuji Silysia Chemical ODS (100-200 mesh), ODS Sep-Pak C18 and tC18 Cartridges (Waters), were used for column chromatography. Kiesel-gel 60 F₂₅₄ (Merck) was used analytical and preparative TLC. HPLC Tri-ROTAR-V pump, 870-UV detector (JASCO) and Migthylsil RP-18 GP 150-46 (5 μm) column were used for analysis.

Plant Material

Stem of *Gnetum africanum* was obtained in March 2001 at Nsukka, Nigeria and root of *G. gnemon* was collected in April 2000 at Bogor Botanical Garden, Indonesia.

Extraction and Isolation

The dried stem of *Gnetum africanum* (1.3 kg) and the root of *G. gnemon* (2.0 kg) were powdered and extracted successively with acetone (3L, weekly x 3) and methanol (3L x weekly x 3) at rt. The acetone extract (35 g) of *G. africanum* was subjected to chromatography on silica gel eluted with a mixture of CHCl_3 - CH_3OH of increasing concentration to yield 26 fractions. Fraction 11 [CHCl_3 - CH_3OH (10 : 1)] was further subjected to chromatography on Sephadex LH-20 eluted with CH_3OH to give seven sub-fractions (Fr. 11a–11g). Compounds (**1**) (30 mg) and (**2**) (8 mg) were obtained from Fr. 11c and 11d,

respectively. Fraction 14 was also subjected to same chromatography as Fr. 11 mentioned above to give 10 sub-fractions (Fr. 14a –14j). Further purification of Fr. 14d by preparative TLC developed with AcOEt-CHCl₃-CH₃OH-H₂O (15 : 8 : 4 : 1) gave **3** (15 mg) and **4** (5 mg).

The acetone extract of *G. gnemon* (60 g) was chromatographed on silica gel eluted with a mixture of CHCl₃-CH₃OH by increasing polarity to give 11 fractions (Fr. A-K). Compounds (**5**) (70 mg) and (**6**) (90 mg) were isolated as a mixture from Fr. C and purified with CH₃OH-H₂O (2 : 8) on Sek-Pak tC₁₈ Cartridges, each fraction about 10 ml was collected and checked on HPLC for purity, eluted with CH₃OH-H₂O (40 : 60) at a flow rate of 0.8 mL/min. The retention time of 8.2 and 9.7 minutes was observed for **5** and **6** respectively.

Gnetoflavanol A (**1**): A white amorphous powder; Negative ion HR-FAB-MS: ([M-H]⁻ *m/z* 529.1509 (Calcd 529.1499 for C₃₀H₂₅O₉); Negative ion FAB-MS: [M-H]⁻ *m/z* 529; UV: 227, 282; [α]_D²⁰ = + 22° (*c* = 0.10, MeOH); The ¹H and ¹³C NMR are listed in Tables 1 and 2.

Gnetoflavanol B (**2**): A white amorphous powder; Negative ion HR-FAB-MS: ([M-H]⁻ *m/z* 499.1385 (Calcd 499.1392 for C₂₉H₂₃O₈); Negative ion FAB-MS: [M-H]⁻ *m/z* 499; UV: 207, 278; [α]_D²⁰ = + 21° (*c* = 0.10, MeOH); The ¹H and ¹³C NMR are shown in Tables 1 and 2.

Gnetoflavanol C (**3**): A white amorphous powder; Negative ion HR-FAB-MS: ([M-H]⁻ *m/z* 515.1348 (Calcd 515.1342 for C₂₉H₂₃O₉); Negative ion FAB-MS: [M-H]⁻ *m/z* 515; UV: 208, 281; [α]_D²⁰ = - 18° (*c* = 0.18, MeOH); The ¹H and ¹³C NMR are listed in Tables 1 and 2.

Gnetoflavanol D (**4**): A white amorphous powder; Negative ion HR-FAB-MS: ([M-H]⁻ *m/z* 515.1348 (Calcd 515.1342 for C₂₉H₂₃O₉); Negative ion FAB-MS: [M-H]⁻ *m/z* 515; UV: 206, 278, 281; [α]_D²⁰ = + 8° (*c* = 0.10, MeOH); The ¹H and ¹³C NMR are listed in Tables 1 and 2.

Gnetoflavanol E (**5**): A white amorphous powder; Negative ion HR-FAB-MS: ([M-H]⁻ *m/z* 499.1234 (Calcd 499.1242 for C₂₉H₂₃O₈); Negative ion FAB-MS: [M-H]⁻ *m/z* 499; UV: 213, 259, 277; [α]_D²⁰ = + 12° (*c* = 0.15, MeOH); The ¹H and ¹³C NMR are listed in Tables 1 and 2.

Gnetoflavanol F (**6**): A white amorphous powder; Negative ion HR-FAB-MS: ([M-H]⁻ *m/z* 499.1234 (Calcd 499.1242 for C₂₉H₂₃O₈); Negative ion FAB-MS: [M-H]⁻ *m/z* 499; UV: 227, 282; [α]_D²⁰ = - 7° (*c* = 0.20, MeOH); The ¹H and ¹³C NMR are listed in Tables 1 and 2.

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