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DIMERIC STILBENES FROM STEM LIANAS OF *GNETUM AFRICANUM*

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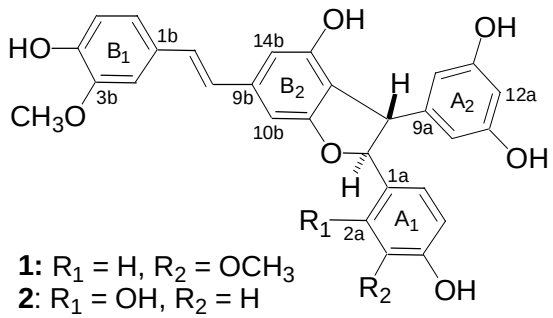
Abstract – Two new stilbene dimers; gneafricanins A, B and bisisorhapontigenin B were isolated from the stem lianas of *Gnetum africanum* (Gnetaceae) along with eight known stilbenoids; longusol A, gnetin C, gnetin D, gnetin E, gnetofolin E, gnetol, isorhapontigenin and resveratrol. The structures of the new compounds were determined by spectral analysis.

Gnetum africanum Welw. is one of the two species of Gnetaceae distributed in Africa and little phytochemical study of the plant has been reported.[1] The plant grows widely in the tropical rain forest of South Eastern Nigeria and Western Cameroon. The plant is popularly known as afang or okazi in Nigeria, the leaves are not only eaten finely shredded for soup or made up into condiments, but also used for the treatment of enlarged spleen and sore throat.[2] Previously we reported, the occurrence of oligostilbenes in the stem lianas of *Gnetum parvifolium*[3] and *G. gnemonoides*.[4] In this paper we describe the structure elucidation of new stilbene dimers named gneafricanins A (**2**) and B (**3**), isolated from the stem lianas of *G. africanum* along with bisisorhapontigenin B[5] (**1**) as a naturally occuring stilbene dimer and eight known stilbenoids.



1: R_1 = H, R_2 = OCH_3
2: R_1 = OH, R_2 = H
3: R_1 = H, R_2 = OH

Figure 1

Bisisorhaphontigenin B (**1**) obtained as a white amorphous powder showed positive reaction to Gibbs reagent. The absorption band at 210, 320, 390 nm in the UV spectrum exhibited the presence of an aromatic ring and a strong conjugation in the system. The $[M-H]^-$ ion peak at *m/z* 513.1541 in the high resolution (HR) FAB-MS corresponds to the molecular formula of $C_{30}H_{26}H_8$. The ^1H NMR spectrum showed the presence of two sets of protons on a 1,3,4-trisubstituted benzene ring [δ 6.83 (1H, d, *J*= 8.7 Hz, H-5a), 6.83 (1H, dd, *J*= 2.0, 8.7 Hz, H-6a), 7.00 (1H, d, *J*= 2.0 Hz, H-2a); 6.84 (1H, d, *J*= 8.7 Hz, H-5b), 7.04 (1H, dd, *J*= 2.0, 8.7 Hz, H-6b), 7.24 (1H, d, *J*= 2.0 Hz,

Table 1 ^{1}H- and ^{13}C-NMR Spectral Data of Compounds (**1** - **3**)

No.	**1***		**2***		**3****	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a		134.3		120.7		135.1
2a	7.00 (d, 2.0)	110.1		156.1	6.83 (d, 2.0)	113.3
3a		148.4	6.42 (d, 2.2)	103.5		146.0
4a		146.3		158.9		145.9
5a	6.83 (d, 8.7)	115.9	6.25 (dd, 2.2, 8.2)	107.3	6.83 (d, 8.4)	116.1
6a	6.83 (dd, 2.0, 8.7)	119.3	6.98 (d, 8.2)	127.8	6.73 (dd, 2.0, 8.4)	118.1
7a	5.38 (d, 5.4)	93.8	5.70 (d, 3.7)	89.1	5.33 (d, 4.5)	93.7
8a	4.42 (d, 5.4)	56.0	4.39 (d, 3.7)	54.3	4.37 (d, 4.5)	56.0
9a		146.1		146.8		146.5
10a (14a)	6.18 (d, 2.0)	106.8	6.22 (d, 2.4)	106.8	6.16 (d, 2.0)	106.7
11a (13a)		159.6		159.3		159.6
12a	6.24 (t, 2.0)	102.0	6.17 (t, 2.4)	101.8	6.23 (t, 2.0)	102.0
1b		129.5		130.0		130.5
2b	7.24 (d, 2.0)	110.1	7.19 (d, 2.0)	110.2	7.26 (d, 2.0)	110.2
3b		148.6		148.6		148.5
4b		146.5		147.5		147.6
5b	6.84 (d, 8.7)	115.8	6.78 (d, 8.2)	121.1	6.85 (d, 8.4)	115.9
6b	7.04 (dd, 2.0, 8.7)	121.2	7.00 (dd, 2.0, 8.2)	115.8	7.05 (dd, 2.0, 8.4)	121.2
7b	7.11 (d, 16.0)	130.5	7.06 (d, 16.0)	129.3	7.13 (d, 16.2)	129.6
8b	7.01 (d, 16.0)	127.0	6.97 (d, 16.0)	127.1	7.04 (d, 16.2)	126.9
9b		141.2		140.9		141.2
10b	6.70 (br s)	99.2	6.68 (br s)	99.2	6.70 (d, 1.0)	99.1
11b		163.0		163.4		163.3
12b		115.1		116.0		115.1
13b		155.4		155.6		155.5
14b	6.60 (br s)	108.1	6.52 (br s)	107.9	6.59 (d, 1.0)	108.1
OCH_3-3a	3.83 (s)	56.29				
OCH_3-3b	3.90 (s)	56.28	3.85 (s)	56.3	3.90 (s)	56.2

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

H-2b)], a set of *meta*-coupled aromatic protons on a 1,3,4,5-tetrasubstituted benzene ring [δ 6.60 (1H, br s, H-14b), 6.70 (1H, br s, H-10b)] and a set on a 3,5-dihydroxyphenyl group [δ 6.18 (2H, d, *J*= 2.0 Hz, H-10a, 14a), 6.24 (1H, t, *J*= 2.0 Hz, H-12a)]. The presence of a pair of olefinic protons [δ 7.01 (1H, d, *J*= 16.0 Hz, H-8b), 7.11 (1H, d, *J*= 16.0 Hz, H-7b)], a pair of mutually coupled aliphatic methine protons [δ 4.42 (1H, d, *J*= 5.4 Hz, H-8a), 5.38 (1H, d, *J*= 5.4 Hz, H-7a)] and two signals of methoxyl groups (δ 3.83 and 3.90) were also exhibited in the spectrum. These results indicated that **1** is a stilbene dimer composed

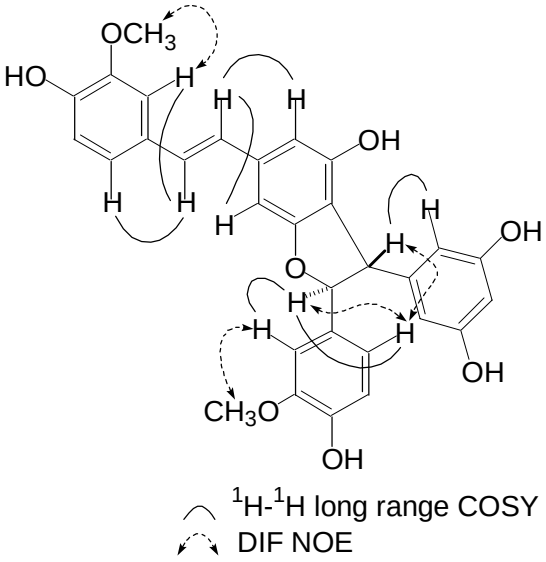


Figure 2

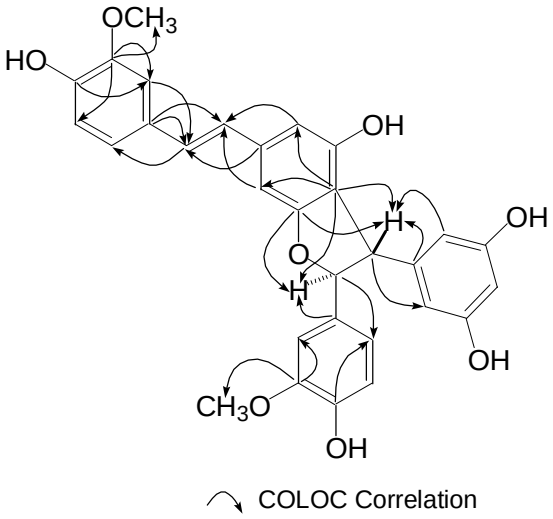


Figure 3

of two isorhapontigenin units. In the ^{1}H-^{1}H long rage COSY spectrum (Figure 2), the following correlations were observed between H-2a(6a)/H-7a, H-10a(14a)/H-8a, H-2b(6b)/H-7b and H-10b(14b)/H-8b, which revealed the respective connections of C-1a/C-7a, C-9a/C-8a, C-1b/C-7b and C-9b/8b. The linkage of isorhapontigenin unit A (ring A_1-7a-8a-ring A_2) and isorhapontigenin unit B (ring B_1-7b-8b-ring B_2) through a dihydrofuran ring was deduced by the correlations between C-11b/H-7a(8a) and C-12b/H-7a(8a) in the COLOC spectrum (Figure 3). The positions of the methoxyl groups were determined by the differential NOE experiment (Figure 2). Irradiation of the methoxyl groups at δ 3.83 and 3.90, significant NOEs' were observed at δ 7.00 (H-2a) and 7.24 (H-2b), respectively, revealing that the positions of the methoxyl groups are at C-3a and C-3b, respectively. The *trans* orientation of the dihydrofuran ring was deduced by the DIFNOE experiment and the structure of **1** was drawn as in Figure 1. Compound (**1**) has been previously synthesized by oxidative coupling of two isorhapontigenins,[5] however, this is the first isolation of **1** as a natural product.

Gneafricanin A (**2**), a white amorphous powder, reacted positively to Gibbs reagent. The negative FAB-MS exhibited an $[M-H]^-$ ion peak at *m/z* 499 and the HR-FAB-MS (*m/z* 499.1401) supported the molecular formula to be $C_{29}H_{24}O_8$. The ^{1}H NMR spectrum showed the presence of an aromatic protons on a 1,2,4-trisubstituted benzene ring [δ 6.25 (1H, dd, *J*= 2.2, 8.2 Hz, H-5a), 6.42 (1H, d, *J*= 2.2 Hz, H-3a), 6.98 (1H, d, *J*= 8.2 Hz, H-6a)], a set on a 1,3,4-trisubstituted benzene [6.78 (1H, d, *J*= 8.2 Hz, H-5b), 7.00 (1H, dd, *J*= 2.0, 8.2 Hz, H-6b), 7.19 (1H, d, *J*= 2.0 Hz, H-2b)] and a set of *meta*-coupled aromatic protons on a 1,3,4,5-tetrasubstituted benzene ring [6.52 (1H, br s, H-14b), 6.68 (1H, br s, H-10b)]. A set of aromatic protons in an A_2X spin system on a 3,5-dihydroxyphenyl group [6.17 (1H, t, *J*= 2.4 Hz, H-12a), 6.22 (2H, d, *J*= 2.4 Hz, H-10a, 14a)], a pair of mutually coupled methine protons [4.39 (1H, d, *J*= 3.7 Hz, H-8a), 5.70 (1H, d, *J*= 3.7 Hz, H-7a)] and a pair of olefinic protons [6.97 (1H, d, *J*= 16.0 Hz, H-8b), 7.06 (1H, d, *J*= 16.0 Hz, H-7b)] were also exhibited in the spectrum in addition to a signal of a methoxyl group at δ 3.85. The correlations observed between H-6a/H-7a, H-10a(14a)/H-8a, H-2b(6b)/H-7b and H-10b(14b)/H-8b in the ^{1}H-^{1}H long range COSY spectrum (Figure 4) revealed the linkages of the methine protons and the olefinic protons to their respective aromatic rings. The

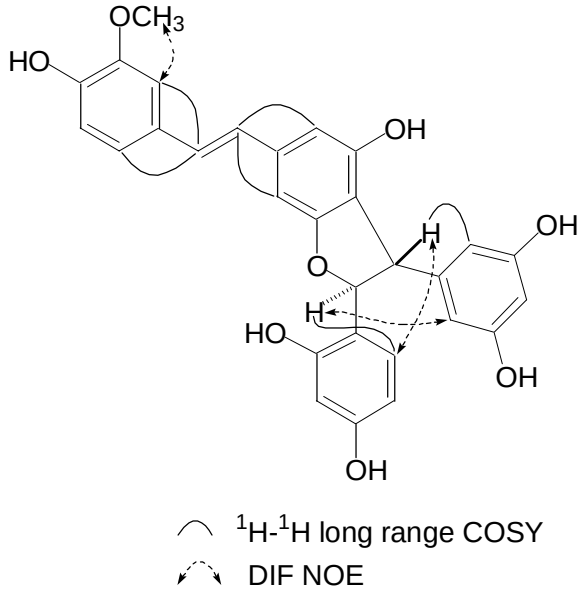


Figure 4

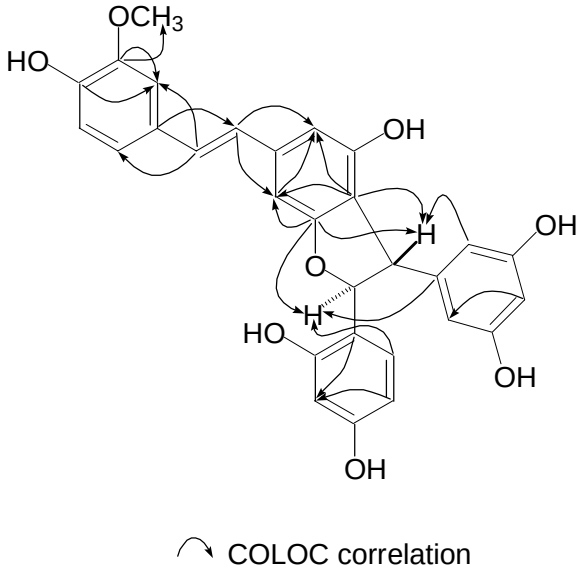


Figure 5

correlations between C-11b/H-7a(8a) and C-12b/H-8a observed in the COLOC spectrum (Figure 5) revealed the connection between C-7a/C-11b and C-8a/C-12b. The position of the methoxyl group at C-3b was determined by the results of DIFNOE (Figure 4) and COLOC (Figure 5) experiments. When the methoxyl group at δ 3.85 was irradiated, a significant NOE was observed at δ 7.19 (H-2b). Similarly a clear cross peak correlation was observed between δ 148.6 (C-3b) and δ 3.85 (OCH_3-3b) in the COLOC spectrum. The *trans* orientation of the dihydrofuran ring was determined by the DIFNOE experiment, thereby allowing the structure of **2** to be drawn as in Figure 1. The structure is composed of an oxyresveratrol unit (ring A_1-7a-8a-ring A_2)[7] and an isorhapontigenin unit (ring B_1-7b-8b-ring B_2).

Gneafricanin B (**3**) was obtained as a white amorphous powder. An $[M-H]^-$ ion peak at *m/z* 499 was exhibited in the negative FAB-MS and its molecular formula of $C_{29}H_{24}O_8$ was deduced by HR-FAB-MS (*m/z* 499.1400). The presence of two sets of aromatic protons on a 1,3,4-trisubstituted benzene ring [δ 6.73 (1H, dd, *J*= 2.0, 8.4Hz, H-6a), 6.83 (1H, d, *J*= 8.4 Hz, H-5a), 6.83 (1H, d, *J*= 2.0 Hz, H-2a); 6.85 (1H, d, *J*= 8.4 Hz, H-5b), 7.05 (1H, dd, *J*= 2.0, 8.4 Hz, H-6b), 7.26 (1H, d, *J*= 2.0 Hz, H-2b)], a set of *meta* coupled protons on a 1,3,4,5- tetrasubstituted benzene ring [δ 6.59 (1H, d, *J*= 1.0 Hz, H-14b), 6.70 (1H, d, *J*= 1.0 Hz, H-10b)] and a set on a 3,5-dihydroxyphenyl group in an A_2X spin system [δ 6.16 (2H, d, *J*= 2.0 Hz, H-10a, 14a), 6.23 (1H, t, *J*= 2.0 Hz, H-12a)] was observed in the ^1H NMR spectrum. A pair of olefinic protons [δ 7.04 (1H, d, *J*= 16.2 Hz, H-8b), 7.13 (1H, d, *J*= 16.2 Hz, H-7b)] and a pair of mutually coupled methine protons [δ 4.37 (1H, d, *J*= 4.5 Hz, H-8a), 5.33 (1H, d, *J*= 4.5 Hz, H-7a)] were also exhibited in addition to a signal due to a methoxyl group at δ 3.90 in the spectrum. These results showed that **3** is a dimeric stilbene similar to **1** except that **3** showed the presence of a hydroxyl group in place of a methoxyl group at C-3a (ring A_1). The C-H COSY and COLOC experiments allowed the assignment of all protonated and quartenary carbons in **3**. The position of the methoxyl group was determined by NOESY (Figure 6) and HMBC (Figure 7) experiments. The *trans* orientation of the dihydrofuran ring was deduced by the results of NOESY experiment and the structure of **3** was characterized as in Figure 1.

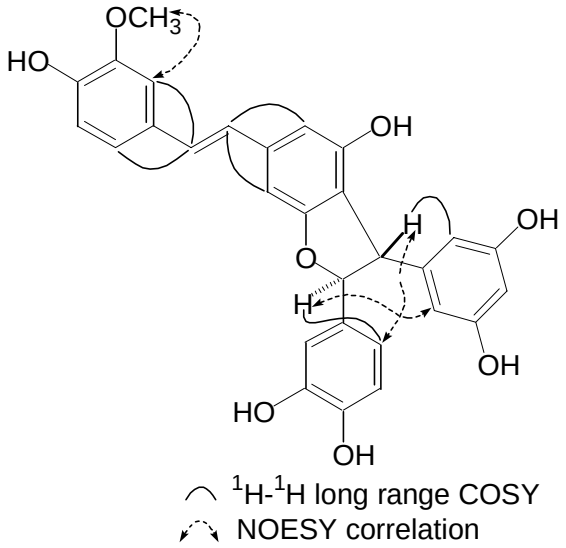


Figure 6

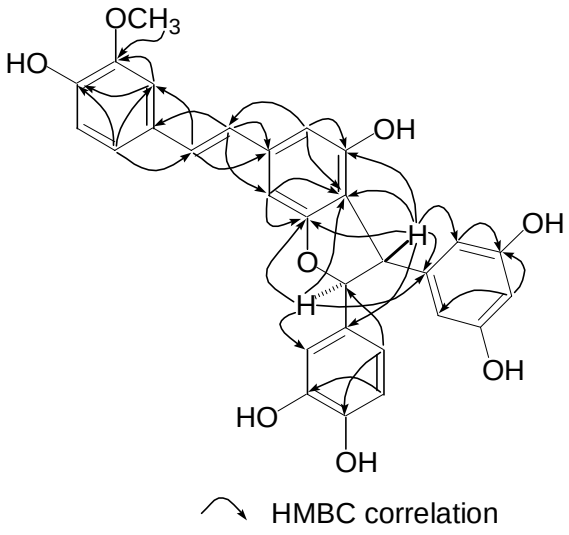


Figure 7

Longusol A,[8] gnetins C, D and E,[7] gnetofolin E, gnetol, isorhapontigenin and resveratrol[9] were isolated from the stem of lianas and the structures were identified by spectral analysis and comparison with the authentic samples.

Further investigation of much polar fraction of the acetone extract of stem lianas of *G. africanum* is in progress.

EXPERIMENTAL

General Method

^{1}H and ^{13}C NMR spectra were measured on JNM EX-400 and AL-300 (JEOL) spectrometers. Chemical shift values were shown as δ values with tetramethylsilane (TMS) as an internal reference. Peak multiplicities are quoted in Hz. Negative FAB-MS were measured on JMS-DX 300 spectrometer equipped with JMA 3500 data analysis system (JEOL). UV spectra were recorded on a UV-2200 spectrophotometer (Shimidzu) and optical rotation were measured on P-1020 (Jasco) Polarimeter. Silica gel 60 (70-230 mesh, Merck), Sephadex LH 20 and Sep-Pak C-18 Cartridges were used for column chromatography. Kielsel-gel 60 F_{254} (Merck) was used for analytical and preparative TLC.

Plant Material

Stems of *Gnetum africanum* were collected in March 2001 at Nsukka, Nigeria.

Extraction and Isolation

The stem lianas of *G. africanum* (1.30 kg) was air dried, powdered and extracted with acetone (3L x 3), MeOH (3L x weekly x 3) and 70% MeOH (3L x weekly x 3) successively at rt. The acetone extract (35g) was subjected to chromatograhy on silica gel eluted with $CHCl_3$-MeOH increasing polarity to give 26 fractions. Repeated chromatography of fraction 7 on Sephadex LH 20 eluted with acetone yielded isorhapontigenin (37 mg), resveratrol (32 mg), gnetol (12 mg) and **1** (35 mg). Compound gnetofolin E

(53 mg) was obtained in pure form from fraction 14 by reverse phase chromatography on ODS eluted with H_2O-MeOH (7 : 3). Fractions 8-12 were combined and subjected to chromatography on Sephadex LH 20 eluted with acetone yielded 10 fractions (A-J). Compounds gnetin C (20 mg), longusol A (41 mg), gnetin D (17 mg) and gnetin E (35 mg) were obtained in pure form respectively from fractions C, E, F and G. Compounds **3** (18 mg) and **2** (22 mg) were obtained from fraction D by PPTLC using EtOAc : $CHCl_3$: MeOH : H_2O (15 : 10 : 4 : 1).

Bisisorhapontigenin B (**1**): A white amorphous powder; Negative HR-FAB-MS: $[M-H]^-$ *m/z* 513.1541 (Calcd 513.1549 for $C_{30}H_{25}O_8$); Negative FAB-MS: $[M-H]^-$ *m/z* 513; UV λ (nm) 210, 320, 390; $[\alpha]_D$ + 4^o (*c*= 0.1, MeOH); ^1H and ^{13}C NMR spectral data are shown in Table 1.

Gneafricanin A (2): A white amorphous powder; Negative HR-FAB-MS: $[M-H]^-$ *m/z* 499.1401 (Calcd 499.1393 for $C_{29}H_{23}O_8$); Negative FAB-MS: $[M-H]^-$ *m/z* 499; UV λ (nm) 213, 286, 330, 390; $[\alpha]_D$ + 23^o (*c*= 0.1, MeOH); ^1H and ^{13}C NMR spectral data are listed in Table 1.

Gneafricanin B (3): A white amorphous powder; Negative HR-FAB-MS: $[M-H]^-$ m/z 499.1400 (Calcd 499.1393 for $C_{29}H_{23}O_8$); Negative FAB-MS: $[M-H]^-$ *m/z* 499; UV λ (nm) 215, 285, 330, 403; $[\alpha]_D$ – 44 ^{o} (*c*= 0.1, MeOH); ^1H and ^{13}C NMR spectral data are shown in Table 1.

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REFERENCES

1. J. W. Wallace, P. L. Porter, E. Besson, and J. Chopin, *Phytochemistry*, 1982, **21**, 482.
2. H. M. Burkill, “The Useful Plants of West Africa”, 1994, **2**, 168.
3. T. Tanaka, I. Iliya, T. Ito, M. Furasawa, K. Nakaya, M. Iinuma, Y. Shirataki, N. Matsuura, F. Simozono, and K. Hirai, *Chem. Pharm. Bull*., 2001, **49**, 858.
4. I. Iliya, T. Tanaka, M. Furasawa, Z. Ali, K. Nakaya, M. Iinuma, Y. Shirataki, J. Murata, and D. Darnaedi, *Heterocyles*, 2001, **55**, 2123.
5. L.-X. Zhou and M. Lin, *Acta Pharmaceutica Sinica*, 2000, **35**, 669.
6. J.-B. Li, M. Lin, S.-Z. Li, and W.-Z. Song, *Acta Pharm. Sinica*, 1991, **26**, 437.
7. A. P Lins, M. N. De Rebeiro, O. R Gottlieb, and H. E. Gottlieb, *J. Nat. Prod.*, 1982, **45**, 754.
8. H. Matsuda, T. Morikawa, F.-W. Xu, and M. Yoshikawa, Abstract Papers of the 48th Annual Meeting of the Japanese Society of Pharmacognosy, 2001, 152.
9. M. Lin, J.-B. Li, S.-Z. Li, D.-Q. Yu, and X.-T. Liang, *Phytochemistry*, 1992, **31**, 633.