Available online at www.sciencedirect.com
SCIENCE DIRECT®

PERGAMON

Phytochemistry 62 (2003) 601–606

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Stilbene derivatives from *Gnetum gnemon* Linn.

Ibrahim Iliya[a], Zulfiqar Ali[b], Toshiyuki Tanaka[b,*], Munekazu Iinuma[a], Miyuki Furusawa[b], Ken-ichi Nakaya[b], Jin Murata[c], Dedy Darnaedi[d], Nobuyasu Matsuura[e], Makoto Ubukata[e]

[a]*Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502-8585, Japan*
[b]*Gifu Prefectural Institute of Health and Environmental Sciences, 1-1 Naka-fudogaoka, Kakamigahara 504-0838, Japan*
[c]*Botanical Gardens, Koishikawa, Graduate School of Science, University of Tokyo, 3-7-1, Hakusan, Bunkyo-Ku, Tokyo, 112-0001, Japan*
[d]*Indonesian Institute of Sciences, Jalan Ir. H. Juanda 13, Bogor 16122, Indonesia*
[e]*Toyama Prefectural University, 5180 Kosugi-machi, Izumi-gun, Toyama 939-0398, Japan*

Received 26 August 2002; received in revised form 31 October 2002

Abstract

Four stilbene derivatives, gnemonols K and L (resveratrol trimers), M (isorhapontigenin dimer), and gnemonoside K (glucoside of resveratrol trimer) together with eleven known stilbenoids and a lignan were isolated from the acetone, methanol and 70% methanol soluble parts of the root of *Gnetum gnemon* (Gnetaceae). The structures of the isolates were determined by spectral analysis. The antioxidant activity of the stilbenoids on lipid peroxide inhibition and super oxide scavenging activity were also investigated.

Keywords: Gnetum gnemon; Gnetaceae; Gnemonols; Gnemonoside; Antioxidant activity

1. Introduction

The interesting biological activities recently found in stilbene derivatives such as induction of apoptosis in colon cancer (Ito et al., 2002) and blood sugar reduction (Huang et al., 2001) revealed the importance of plants containing stilbenoids as resources for the development of new drugs. The genus *Gnetum* (Gnetaceae) is known to contain abundant stilbene derivatives (Huang et al., 2001, 2000a,b; Li et al., 2001). In continuation of our phytochemical studies of *Gnetum* plants (Tanaka et al., 2001; Iliya et al., 2001, 2002a,b,c,d,e), we report in this paper the isolation and structure elucidation of a known lignan and fifteen stilbene derivatives including four new compounds, gnemonols K–M (**1**–**3**) and gnemonoside K (**4**), from the acetone, methanol and 70% methanol extracts of the root of *Gnetum gnemon* Linn. This species is commonly found in south east Asia and is used as food in Indonesia. The known constituents comprised (−)-ε-viniferin, gnetol, isorhapontigenin, gnetifolin E, and isorhapontigenin-3-*O*-β-D-glucopyranoside (Lin et al., 1992), resveratrol (Ingham, 1976), latifolol (Iliya et al., 2002d), gnemonosides A, and B (Iliya et al., 2001), gnetifolin K (Xu and Lin, 1997), gnemonoside F (Iliya et al., 2002c), and (+)-lirioresinol B (Briggs et al., 1968). The compounds were isolated by column chromatography (silica gel, Sephadex LH-20, and ODS) and prep. TLC and their structures were established on the basis of their spectral data. The new as well as known compounds were tested for their potential antioxidant activities. Some of the isolates exhibited considerable inhibitory activity on lipid peroxidation and superoxide scavenging activity in a xanthine–xanthine oxidase system whereas others had no antioxidant activity.

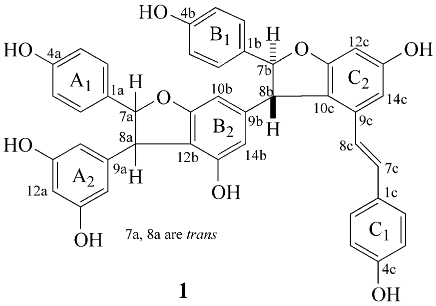
2. Results and discussion

Gnemonol K (**1**), a colorless amorphous powder, showed a positive reaction to Gibbs reagent. The negative FAB-MS exhibited an $[M–H]^-$ ion peak at *m/z* 679 and the molecular formula, $C_{42}H_{32}O_9$, was deduced from the HRFAB-MS *m/z* 679.1962 $[M–H]^-$. The ^1H NMR spectrum exhibited signals of three sets of *ortho*-

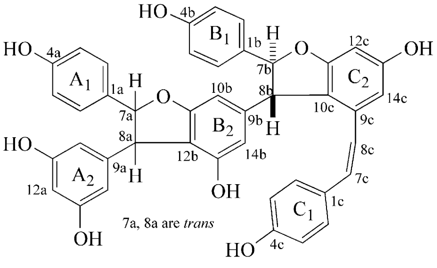
---

* Corresponding author. Tel.: +81-583-80-2120; fax: +81-583-71-5016.
E-mail address: yhy06063@nifty.ne.jp (T. Tanaka).

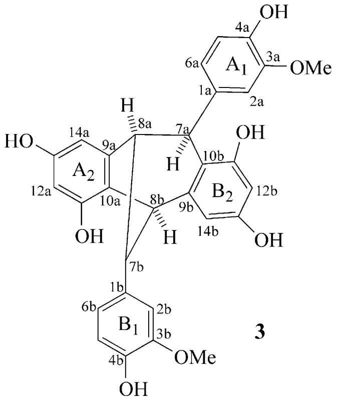
0031-9422/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(02)00670-2



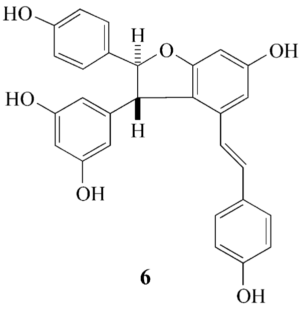
1



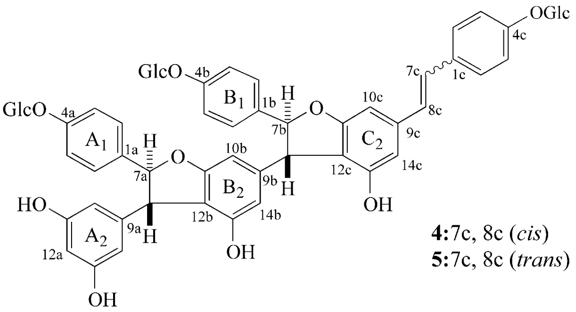
2



3



6



4:7c, 8c (*cis*)
5:7c, 8c (*trans*)

coupled H-atoms in A_2B_2 systems on *p*-substituted phenyl moieties [δ 7.18 (2H, *d*, J=8.8 Hz, H-2a,6a)/6.80 (2H, *d*, J=8.8 Hz, H-3a,5a); δ 7.25 (2H, *d*, J=8.8 Hz, H-2b,6b)/6.80 (2H, *d*, J=8.8 Hz, H-3b,5b); δ 7.24 (2H, *d*, J=8.8 Hz, H-2c,6c)/6.85 (2H, *d*, J=8.8 Hz, H-3c,5c)]. The spectrum also showed two sets of *meta*-coupled H-atoms in AB systems on tetra-substituted benzene rings [δ 6.55 (1H, *br. s*, H-10b)/6.21 (1H, *br. s*, H-14b); δ 6.75 (1H, *d*, J=2.0 Hz, H-14c)/6.37 (1H, *d*, J=2.0 Hz, H-12c)], and a set of A_2B H-atoms on a 1,3,5-trisubstituted benzene ring [δ 6.26 (1H, *t*, J=2.4 Hz, H-12a)/6.22 (2H, *d*, J=2.4 Hz, H-10a,14a)]. Furthermore, a set of *trans*-coupled olefinic H-atoms [δ 6.94 (1H, *d*, J=16.6 Hz, H-7c)/6.79 (1H, *d*, J=16.6 Hz, H-8c)], and two sets of mutually coupled methines [δ 5.42 (1H, *d*, J=5.4 Hz, H-7a)/4.35 (1H, *d*, J=5.4 Hz, H-8a); δ 5.52 (1H, *d*, J=4.9 Hz, H-7b)/4.61 (1H, *d*, J=4.9 Hz, H-8b)] together with seven phenolic OH H-atoms [δ 8.46 (7H, *br. s*)] were observed. Considering the molecular formula ($C_{42}H_{32}O_9$), and the ^1H and ^{13}C NMR spectral data (Tables 1 and 2), **1** could be assigned as a trimer of resveratrol units. The chemical shift values were assigned with the aid of C–H COSY, COLOC and long range ^1H–^1H COSY experiments. The correlations between C-2a(6a)/H-7a, C-10a(14a)/H-8a, C-2b(6b)/H-7b, C-10b(14b)/H-8b, C-2c(6c)/H-7c, C-10c(14c)/H-8c in the COLOC spectrum (Fig. 1) and 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-14c/H-8c in the long range ^1H–^1H COSY spectrum (Fig. 2) revealed the linkages of C-1a/C-7a, C-9a/C-8a, C-1b/C-7b, C-9b/C-8b, C-1c/C-7c and C-9c/C-8c, respectively. The correlations between C-11b(12b)/H-7a(8a) and C-10c(11c)/H-7b(8b) showed that a resveratrol (unit A) is connected to another resveratrol (unit B) through C-7a/C-11b and C-8a/C-12b which in turn is connected to a third resveratrol (unit C) through C-7b/C-11c and C-8b/C-10c. The presence of two dihydrofuran rings (7a-8a-12b-11b-O) and (7b-8b-10c-11c-O) was deduced by considering the degrees of unsaturation and confirmed by the cross-peaks observed

Table 1
^{1}H NMR spectral data of compounds **1–4**

H-atom	**1**[a] (mult., *J* in Hz)	**2**[a] (mult., *J* in Hz)	**3**[a] (mult., *J* in Hz)	**4**[b] (mult., *J* in Hz)
H-2a	7.18 (*d*, 8.8)	7.03 (*d*, 8.8)	6.96 (*d*, 2.0)	7.20 (*d*, 8.8)
H-3a	6.80 (*d*, 8.8)[c]	6.70 (*d*, 8.8)	–	7.00 (*d*, 8.8)
H-5a	6.80 (*d*, 8.8)[c]	6.70 (*d*, 8.8)	6.73 (*d*, 8.4)	7.00 (*d*, 8.8)
H-6a	7.18 (*d*, 8.8)	7.03 (*d*, 8.8)	6.67 (*dd*, 2.0, 8.4)	7.20 (*d*, 8.8)
H-7a	5.42 (*d*, 5.4)	5.21 (*d*, 5.4)	4.21 (*br. s*)	5.32 (*d*, 5.6)
H-8a	4.35 (*d*, 5.4)	4.21 (*d*, 5.4)	3.45 (*br. s*)	4.21 (*d*, 5.6)
H-10a	6.22 (*d*, 2.4)	6.04 (*d*, 2.0)	–	5.97 (*d*, 2.0)
H-12a	6.26 (*t*, 2.4)	6.10 (*t*, 2.0)	6.07 (*d*, 2.0)	6.05 (*t*, 2.0)
H-14a	6.22 (*d*, 2.4)	6.04 (*d*, 2.0)	6.55 (*d*, 2.0)	5.97 (*d*, 2.0)
H-2b	7.25 (*d*, 8.8)	6.97 (*d*, 8.8)	6.61 (*d*, 2.0)	7.23 (*d*, 8.8)
H-3b	6.80 (*d*, 8.8)[c]	6.67 (*d*, 8.8)	–	7.02 (*d*, 8.8)
H-5b	6.80 (*d*, 8.8)[c]	6.67 (*d*, 8.8)	6.56 (*d*, 8.4)	7.02 (*d*, 8.8)
H-6b	7.25 (*d*, 8.8)	6.97 (*d*, 8.8)	6.40 (*dd*, 2.0, 8.4)	7.23 (*d*, 8.8)
H-7b	5.52 (*d*, 4.9)	5.26 (*d*, 5.4)	3.70 (*br. s*)	5.43 (*d*, 3.9)
H-8b	4.61 (*d*, 4.9)	3.97 (*d*, 5.4)	4.14 (*br. s*)	4.31 (*d*, 3.9)
H-10b	6.55 (*br*. s)	6.06 (br. *s*)	–	6.20 (*br. s*)
H-12b	–	–	6.16 (*d*, 2.4)	–
H-14b	6.21 (*br*. s)	5.97 (br. *s*)	6.44 (*d*, 2.4)	6.17 (*br. s*)
H-2c	7.24 (*d*, 8.8)	6.88 (*d*, 8.8)	–	7.23 (*d*, 8.8)
H-3c	6.85 (*d*, 8.8)	6.52 (*d*, 8.8)	–	6.92 (*d*, 8.8)
H-5c	6.85 (*d*, 8.8)	6.52 (*d*, 8.8)	–	6.92 (*d*, 8.8)
H-6c	7.24 (*d*, 8.8)	6.88 (*d*, 8.8)	–	7.23 (*d*, 8.8)
H-7c	6.94 (*d*, 16.6)	6.16 (*d*, 12.2)	–	6.48 (*d*, 12.2)
H-8c	6.79 (*d*, 16.6)	5.95 (*d*, 12.2)	–	6.42 (*d*, 12.2)
H-10c	–	–	–	6.28 (*br*. s)
H-12c	6.37 (*d*, 2.0)	6.18 (*d*, 2.0)[c]	–	–
H-14c	6.75 (*d*, 2.0)	6.18 (*d*, 2.0)[c]	–	6.37 (*br*. s)
OMe-3a	–	–	3.80 (*s*)	–
OMe-3b	–	–	3.65 (*s*)	–
Glc-4a,4b,4c				
H-1′(1″,1‴)	–	–	–	4.86 (*d*, 8.0)
H-2′(2″,2‴)	–	–	–	3.24 (*m*)
H-3′ (3″,3‴)	–	–	–	3.28 (*m*)
H-4′ (4″,4‴)	–	–	–	3.22 (*m*)
H-5′ (5″,5‴)	–	–	–	3.30 (*m*)
2H-6′ (6″,6‴)	–	–	–	3.43–3.69 (*m*)

[a] Measured in CD_3COCD_3 at 400 MHz.
[b] Measured in CD_3SOCD_3 at 400 MHz.
[c] Overlapped.

Table 2
^{13}C NMR spectral data of compounds **1–4**

C-atom	**1**[a]	**2**[a]	**3**[a]	**4**[b]
C(1a)	133.5	133.8	139.0	135.5
C(2a)	127.4	127.9	113.0	127.2
C(3a)	116.2	116.1	147.6[d]	116.7
C(4a)	158.0[c]	158.1[c]	145.5[d]	157.6[d]
C(5a)	116.2	116.1	115.19	116.7
C(6a)	127.4	127.9	121.4	127.2
C(7a)	93.4	93.7	47.5	92.3
C(8a)	55.9	55.9	57.8	54.9
C(9a)	145.8	146.1	148.0[c]	144.9
C(10a)	106.5	106.9	127.8	105.7
C(11a)	159.2	159.4	158.6[d]	158.8
C(12a)	101.7	102.0	101.9	101.4
C(13a)	159.2	159.4	158.6[d]	158.8
C(14a)	106.5	106.9	104.2	105.7
C(1b)	133.5	133.7	136.2	135.1
C(2b)	127.6	128.1	112.3	126.9
C(3b)	115.92	116.1	147.6[d]	116.8
C(4b)	157.8[c]	158.1[c]	145.5[d]	157.6[d]
C(5b)	115.92	116.1	115.24	116.8
C(6b)	127.6	128.1	120.9	126.9
C(7b)	93.5	94.0	51.1	91.9
C(8b)	56.7	56.9	50.1	54.8
C(9b)	147.2	147.2	147.2[c]	145.2
C(10b)	101.2	101.4	113.4	99.7
C(11b)	162.6	162.8	157.9	161.4[d]
C(12b)	114.0	114.1	101.9	113.9
C(13b)	155.6	155.4	157.2	155.0
C(14b)	107.9	108.4	105.7	107.7
C(1c)	129.5	129.4	–	130.6
C(2c)	128.4.0	130.9	–	130.2
C(3c)	115.88	115.8	–	116.2
C(4c)	157.9[c]	157.6[c]	–	156.9
C(5c)	115.88	115.8	–	116.2
C(6c)	128.4	130.9	–	130.2
C(7c)	130.1	130.3	–	129.6
C(8c)	123.1	126.1	–	129.4
C(9c)	136.1	137.1	–	139.8
C(10c)	119.5	120.2	–	100.7
C(11c)	162.0	162.4	–	161.4[d]
C(12c)	96.6	96.7	–	113.4
C(13c)	159.3	159.3	–	155.1
C(14c)	104.1	108.5	–	108.9
OMe-C(3a)	–	–	56.3	–
OMe-C(3b)	–	–	56.0	–
Glc-C(4a,4b,4c)				
C(1′)	–	–	–	100.5[c]
C(1″)	–	–	–	100.7[c]
C(1‴)	–	–	–	100.8[c]
C(2′,2″,2‴)	–	–	–	73.4
C(3′,3″,3‴)	–	–	–	77.0
C(4′,4″,4‴)	–	–	–	70.1
C(5′,5″,5‴)	–	–	–	77.4
C(6′,6″,6‴)	–	–	–	61.0

[a] Measured in CD_3COCD_3 at 100 MHz.
[b] Measured in CD_3SOCD_3 at 100 MHz.
[c] Interchangeable.
[d] Overlapped.

between C-11b(12b)/H-7a(8a) and C-10c(11c)/H-7b(8b) in the COLOC spectrum. The planar structure of **1** might be formed by the oxidative coupling of a resveratrol and ϵ-viniferin (**6**) unit. The relative stereochemistry of chiral centers at the two dihydrofuran rings was deduced to be *trans* by the interactions between H-7a/H-10a(14a), H-8a/H-2a(6a) and H-7b/H-10b(14b), H-8b/H-2b(6b) in the NOESY experiment (Fig. 2). However, no interactions between either H-7b/H-7a(8a) or H-8b/H-7a(8a) were observed in the NOESY experiment due to which, we were unable to assign the complete relative stereochemistry of **1**.

Gnemonol L (**2**), a colorless amorphous powder, showed a positive reaction to Gibbs reagent. The negative FAB-MS *m*/*z* 679 $[M–H]^-$ and the HRFAB-MS *m*/*z* 679.1964 supported the molecular formula $C_{42}H_{32}O_9$. The ^{1}H and ^{13}C NMR spectra of **2** showed a close resemblance to **1**. However, the relatively higher field chemical shifts and the smaller coupling constants exhibited by the olefinic H-atoms [δ 6.16 (1H, *d*, $J=12.2$ Hz, H-7c)/5.95 (1H, *d*, $J=12.2$ Hz, H-8c)] revealed that **2** was a *cis* isomer of **1**. The chemical shift values in **2** were assigned with the aid of C–H COSY, COLOC (Fig. 3) and long-range ^{1}H–^{1}H COSY (Fig. 4) experiments. The relative configurations at the two dihydrofuran rings were established similarly to **1**, with again the stereochemistry at H-7a and H-8a being unresolved. Compound **1** was

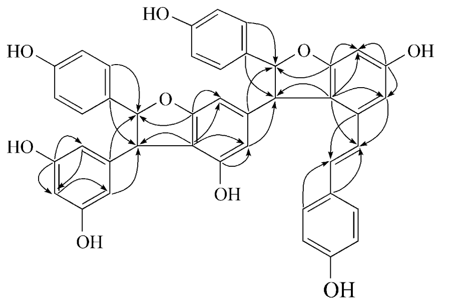


Fig. 1. Correlations in the COLOC spectrum of **1**.

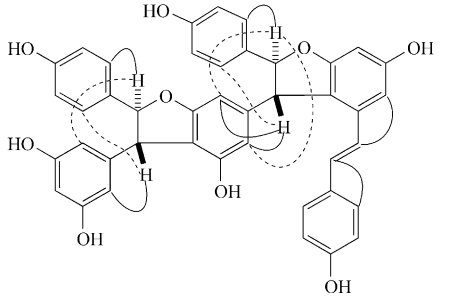


Fig. 2. —, ^{1}H–^{1}H long range correlations; - - - -, NOESY interactions for **1**.

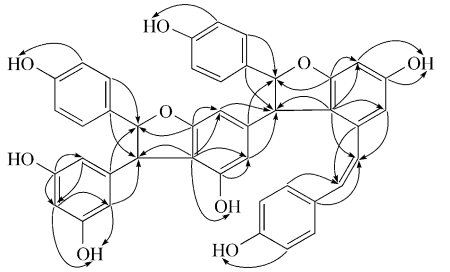


Fig. 3. Correlations in the COLOC spectrum of **2**.

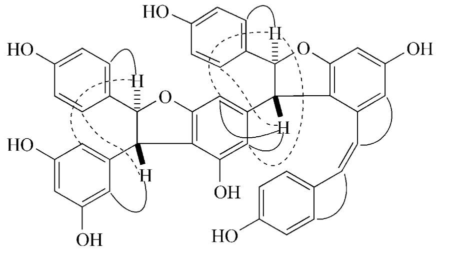


Fig. 4. —, ^{1}H–^{1}H long range correlations; - - - -, NOESY interactions for **2**.

found to be stable whereas slight interconversion of **2** to more stable *trans* isomer (**1**) was observed under storage conditions in the cold and in the dark.

Gnemonol M (**3**), a colorless amorphous powder, showed a positive reaction to Gibbs reagent. The [M] ion peak at *m*/*z* 514 in the EI-MS and the [M–H]$^{-}$ ion peak in the FAB-MS at *m*/*z* 513 showed that **3** has the molecular formula of $C_{30}H_{26}O_8$, which was established from the HREI-MS (*m*/*z* 514.1638 [M]) spectral analysis. The ^{1}H NMR spectrum showed the presence of two sets of H-atoms in ABX systems on 1,2,4-trisubstituted benzene rings [δ 6.96 (1H, *d*, *J* = 2.0 Hz, H-2a)/6.73 (1H, *d*, *J* = 8.4 Hz, H-5a)/6.67 (1H, *dd*, *J* = 2.0, 8.4 Hz, H-6a); δ 6.61 (1H, *d*, *J* = 2.0 Hz, H-2b)/6.56 (1H, *d*, *J* = 8.4 Hz, H-5b)/6.40 (1H, *dd*, *J* = 2.0 Hz, 8.4, H-6b)]. The spectrum also showed two sets of *meta*-coupled H-atoms in AB systems on tetra-substituted benzene rings [δ 6.55 (1H, *d*, *J* = 2.0 Hz, H-14a)/6.07 (1H, *d*, *J* = 2.0 Hz, H-12a); δ 6.44 (1H, *d*, *J* = 2.4 Hz, H-14b)/6.16 (1H, *d*, *J* = 2.4 Hz, H-12b). Four benzylic methine singlets [δ 4.21 (H-7a); 3.45 (H-8a); 4.14 (H-8b); 3.70 (H-7b)], two signals for methoxyl groups [δ 3.80 (OMe-3a); 3.65 (OMe-3b)], and six phenolic OH H-atoms (7.95, 7.88, 7.80, 7.33, 7.23 and 7.16) were also observed in the spectrum. The correlations between H-2a/H-7a, H-2b/H-7b, and H-8b/H-14b in the long range ^{1}H–^{1}H COSY spectrum (Fig. 5) revealed the linkages of C-1a/C-7a, C-1b/C-7b, and C-9b/C-8b, respectively. These results indicated that **3** is a stilbene dimer composed of two isorhapontigenin units. The ^{1}H–^{1}H COSY spectrum (Fig. 5) showed that the four benzylic methines were coupled successively in the order of H-7a/H-8a/H-7b/H-8b and their appearance as singlets revealed that all dihedral angles are almost 90 degrees. The relative stereochemistry of the chiral centers were assigned with the aid of NOEs observed in a DIFNOE experiment (Fig. 5) and found to be similar to those of 2b-hydroxyampelopsin F (Tanaka et al., 2001).

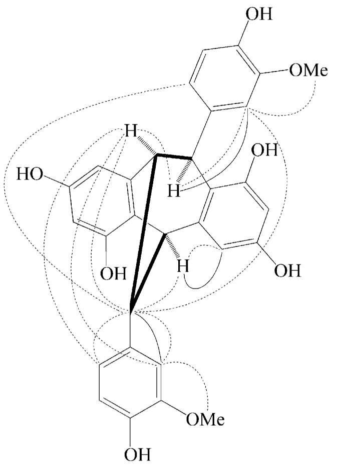


Fig. 5. —, ^{1}H–^{1}H long range correlations; - - - -, DIFNOE interactions;—COSY correlations for **3**.

Gnemonoside K (**4**), a colorless amorphous powder, gave a blue color to Gibbs reagent. The [M–H]$^{-}$ ion peak at *m*/*z* 1165 in the negative FAB-MS and 1165.3558 in the negative HRFAB-MS corresponded to the molecular formula $C_{60}H_{62}O_{24}$. The ^{1}H NMR spectrum exhibited the signals for three sets of *ortho*-coupled H-atoms in A_2B_2 systems on *p*-substituted phenyl moieties [δ 7.20 (2H, *d*, *J* = 8.8 Hz, H-2a,6a)/7.00 (2H, *d*, *J* = 8.8 Hz, H-3a,5a); δ 7.23 (2H, *d*, *J* = 8.8 Hz, H-2b,6b)/7.02 (2H, *d*, *J* = 8.8 Hz, H-3b,5b)]; δ 7.23 (2H, *d*, $J = 8.8$ Hz, H-2c,6c)/6.92 (2H, *d*, $J = 8.8$ Hz, H-3c,5c)], two sets of *meta*-coupled H-atoms in AB systems on tetra-substituted benzene rings [δ 6.20 (1H, *br.s*, H-10b)/6.17 (1H, *br.s*, H-14b); δ 6.37 (1H, *br.s*, H-14c)/6.28 (1H, *br.s*, H-10c)] and a set of *meta*-coupled H-atoms in an A_2B system on a 1,3,5-trisubstituted benzene ring [δ 6.05 (1H, *t*, $J = 2.0$ Hz, H-12a)/5.97 (2H, *d*, $J = 2.0$ Hz, H-10a,14a)]. Furthermore, a set of *cis*-coupled olefinic H-atoms [δ 6.48 (1H, *d*, $J = 12.2$ Hz, H-7c)/6.42 (1H, *d*, $J = 12.2$ Hz, H-8c)], and two sets of mutually coupled methines [δ 5.32 (1H, *d*, $J = 5.6$ Hz, H-7a)/4.21 (1H, *d*, $J = 5.6$ Hz, H-8a); δ 5.43 (1H, *d*, $J = 3.9$ Hz, H-7b)/4.31 (1H, *d*, $J = 3.9$ Hz, H-8b)], together with three anomeric H-atoms [δ 4.86 (3H, *d*, $J = 8.0$ Hz, Glc(a,b,c)-H-1′,1″,1‴)] were observed in the spectrum. The presence of three anomeric signals in the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) suggested that **4** has three sugar moieties. The position of three sugar functions was deduced by the results of a NOESY experiment (Fig. 6). The correlations between H-7a/H-2a(6a), H-8a/H-10a(14a), H-7b/H-2b(6b), H-8b/H-10b(14b), H-7c/H-2c(6c) and H-8c/H-10c(14c) in the long-range ^{1}H–^{1}H COSY spectrum (Fig. 6) revealed the respective linkages between C-7a/C-1a, C-8a/C-9a, C-7b/C-1b, C-8b/C-9b, C-7c/C-1c and C-8c/C-9c. The C–H COSY, ^{1}H–^{1}H COSY and long range ^{1}H–^{1}H COSY spectra allowed the assignment of all methines and protonated C-atoms in **4**. The quaternary C-atoms were assigned comparatively with gnemonoside F (**5**) (Iliya et al., 2002c). The ^{1}H and ^{13}C NMR spectral data of **4** (Tables 1 and 2) showed a close resemblance to those of gnemonoside F except for the appearance of *cis* olefinic H-atoms instead of *trans* and thus **4** was characterized as a (*Z*)-geometric isomer of gnemonoside F (**5**). The *trans* configuration at the two dihydrofuran rings was deduced from a NOESY experiment (Fig. 6). Compounds **4** and **5** were found stable under cold and dark storage conditions. The antioxidant activity of some other stilbenoids such as gnemanol B (Iliya et al., 2002b), gnemanol I (Iliya et al., 2002e) and latifolol (Iliya et al., 2002d) and vitamin E as a positive control is shown in Table 3.

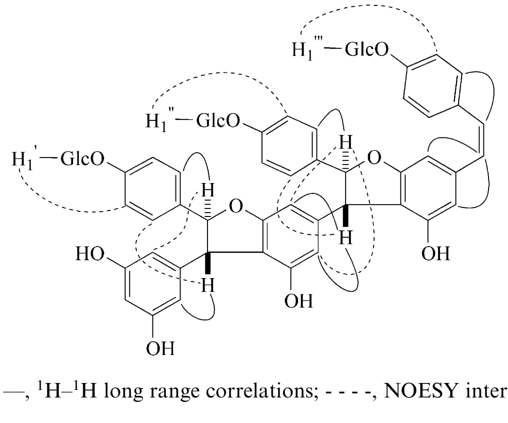


Fig. 6. —, ^{1}H–^{1}H long range correlations; - - - -, NOESY interactions for **4**.

Table 3

Scavenging activity for super oxide and lipid peroxide inhibition activity of some stilbenoids

	IC_{50} (μM)	
	Super oxide scavenging activity	Lipid peroxide inhibition activity
Gnemonol K (**1**)	69	19
Gnemonol L (**2**)	59	7
(−)-ε-Viniferin	20	33
Gnetol	66	61
Isorhapontigenin	29	45
Resveratrol	15	75
Latifolol	68	32
Gnemonol B	79	50
Gnemonol I	57	25
Vitamin E	2100	1000

3. Experimental

3.1. General

NMR spectra were recorded on JEOL EX-400 spectrometer. Chemical shift values are presented as δ values with tetramethylsilane (TMS) as an internal reference. Peak multiplicities were quoted in Hz. Optical rotations were recorded on a JASCO P-1020 polarimeter and UV spectra were recorded on a Shimadzu UV 2200 spectrometer. Negative ion FAB-MS were measured on Jeol JMS-DX 300 spectrometer equipped with a JMA 3500 data analysis system. Silica gel 60 (70–230 mesh, Merck), Sephadex LH-20 (Pharmacia), ODS (100–200 mesh, Fuji Silysia Chemical) and ODS Sep-Pak C_{18} Cartridge (Merck) were used for CC. Kieselgel 60 F_{254} (Merck) was used as analytical and preparative TLC.

3.2. Plant material

Gnetum gnemon is cultivated at Bogor Botanical Garden, Indonesia, from where its roots were collected in April 2001, and a voucher specimen is deposited there in the herbarium.

3.3. Extraction and isolation

The dried root of *G. gnemon* (2.0 kg) was powdered and extracted successively with acetone, MeOH and 70% MeOH. The acetone extract (60 g) was subjected to silica gel column chromatography eluted with a mixture of $CHCl_3$/MeOH of increasing polarity to give 11 fractions (A–K). ε-Viniferin (78 mg), gnetol (15 mg), isorhapontigenin (17 mg) and resveratrol (200 mg) were purified from Fr. A after CC over Sephadex LH-20 (MeOH). Compounds **1** (270 mg) and **2** (193 mg) were obtained from Fr. C after CC over Sephadex LH-20 (MeOH) and ODS (MeOH/H_2O 4:6), respectively. Fr. E was chromatographed over Sephadex LH-20 (MeOH) to give latifolol (98 mg). Gnetifolin E (37 mg) and isorhapontigenin-3-*O*-β-D-glucopyranoside (17 mg) were purified from Fr. F by CC over Sephadex LH-20 (MeOH) followed by prep. TLC with EtOAc/$CHCl_3$/MeOH/H_2O 15:15:9:2. The MeOH extract (12 g) was applied to a silica gel column which was eluted with a mixture of EtOAc/$CHCl_3$/MeOH/H_2O 20:10:12:5 to give eight fractions (MA–MH). Compound **3** (8 mg) and (+)-lirioresinol (11 mg, $[\alpha]_D + 49°$) were obtained from Fr. MA after CC over Sephadex LH-20 (MeOH) followed by prep. TLC with benzene/EtOAc/MeOH/H_2O 20:14:6:1. Gnemonosides A (17 mg) and B (13 mg) were obtained from Fr. MF by CC over Sephadex LH-20 (MeOH) followed by prep. TLC with EtOAc/$CHCl_3$/MeOH/H_2O 20:10:12:5. The 70% MeOH extract (35 g) was treated similarly as MeOH extract to give 8 fractions (WA–WH). Fraction WH was applied to a Sephadex LH-20 column (MeOH) to give 24 subfractions (WH_1–WH_{24}, 30 ml each). Compound **4** (36 mg), gnemonoside F (76 mg) and gnetifolin K (29 mg) were purified from sub-Frs. WH_1–WH_{10} by CC over silica gel eluted with EtOAc/$CHCl_3$/MeOH/H_2O 20:10:12:5.

3.4. Gnemonol K (1)

Colorless amorphous powder; $[\alpha]_D + 6°$ ($c = 0.72$, MeOH); UV (MeOH) λ_{max} (log ε): 227 (4.9), 286 (4.3), 323 (4.4) nm; negative ion FAB-MS *m*/*z*: 679 $[M–H]^-$; negative ion HRFAB-MS *m*/*z*: 679.1962 (calc. 679.1968 for $C_{42}H_{31}O_9$); for ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively.

3.5. Gnemonol L (2)

Colorless amorphous powder; $[\alpha]_D + 23°$ ($c = 0.49$, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.9), 225 *sh*, 285 (4.3), 325 *sh* nm; negative ion FAB-MS *m*/*z*: 679 $[M–H]^-$; negative ion HRFAB-MS *m*/*z*: 679.1964 (calc. 679.1968 for $C_{42}H_{31}O_9$); for ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively.

3.6. Gnemonol M (3)

Colorless amorphous powder; $[\alpha]_D - 28°$ ($c = 0.12$, MeOH); UV (MeOH) λ_{max} (log ε): 207 (4.9), 230 *sh*, 282 (4.4) nm; negative ion FAB-MS *m*/*z*: 513 $[M–H]^-$; EI-MS *m*/*z*: 514 $[M]^+$; HREI-MS *m*/*z*: 514.1638 (calc. 514.1628 for $C_{30}H_{26}O_8$); for ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively.

3.7. Gnemonoside K (4)

Colorless amorphous powder; $[\alpha]_D - 36°$ ($c = 0.52$, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.9), 225 *sh*, 280 (3.4), 325 *sh* nm; negative ion FAB-MS *m*/*z*: 1165 $[M–H]^-$; negative ion HRFAB-MS *m*/*z*: 1165.3558 (calc. 1165.3553 for $C_{60}H_{61}O_{24}$); for ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively.

References

Briggs, L.H., Cambie, R.C., Couch, R.A.F., 1968. Lirioresinol-C dimethyl ether, a diaxially substituted 3,7-dioxabicyclo[3,3,0]octane lignan from *Macropiper excelsum* (Forst. f.) Miq. J. Chem. Soc. C, 3042–3045.

Huang, K.S., Li, R.L., Wang, Y.H., Lin, M., 2001. Three new stilbene trimers from the lianas of *Gnetum hainanense*. Planta Medica 67, 61–64.

Huang, K.S., Wang, Y.H., Li, R.L., Lin, M., 2000a. Stilbene dimers from the lianas of *Gnetum hainanense*. Phytochemistry 54, 875–881.

Huang, K.S., Wang, Y.H., Li, R.L., Lin, M., 2000b. Five new stilbene dimers from the lianas of *Gnetum hainanense*. J. Nat. Prod 63, 86–89.

Iliya, I., Tanaka, T., Furusawa, M., Ali, Z., Nakaya, K., Iinuma, M., Shirataki, Y., Murata, J., Darnaedi, D., 2001. Four new glucosides of stilbene oligomers from the stem of *Gnetum gnemonoides*. Heterocycles 55, 2123–2130.

Iliya, I., Tanaka, T., Furusawa, M., Ali, Z., Nakaya, K., Iinuma, M., Shirataki, Y., Murata, J., Darnaedi, D., 2002a. Dimeric stilbenes from stem lianas of *Gnetum africanum*. Heterocycles 57, 1057–1062.

Iliya, I., Tanaka, T., Furusawa, M., Ali, Z., Nakaya, K., Iinuma, M., Shirataki, Y., Murata, J., Darnaedi, D., 2002b. Stilbene derivatives from two species of Gnetaceae. Chem. Pharm. Bull 50, 796–801.

Iliya, I., Tanaka, T., Furusawa, M., Iinuma, M., Ali, Z., Nakaya, K., Murata, J., Darnaedi, D., 2002c. Five stilbene glucosides from *Gnetum gnemonoides* and *Gnetum africanum*. Helv. Chim. Acta 85, 2394–2402.

Iliya, I., Ali, Z., Tanaka, T., Iinuma, M., Furusawa, M., Nakaya, K., Murata, J., Darnaedi, D., 2002d. Stilbenoids from the stem of *Gnetum latifolium* (Gnetaceae). Phytochemistry 61, 959–961.

Iliya, I., Ali, Z., Tanaka, T., Iinuma, M., Furusawa, M., Nakaya, K., Murata, J., Darnaedi, D., 2002e. Four new stilbene oligomers in root of *Gnetum gnemon*. Helv. Chim. Acta 85, 2538–2546.

Ingham, J.L., 1976. Trihydroxy stilbene as phytoalexin from groundnuts (*Arachis hypogaea*). Phytochemistry 15, 1791–1793.

Ito, T., Akao, Y., Tanaka, T., Iinuma, M., Nozawa, Y., 2002. Vaticanol C, a novel resveratrol tetramer, inhibits cell growth through induction of apoptosis in colon cancer cell lines. Biol. Pharm. Bull 25, 147–148.

Li, X.M., Wang, Y.H., Lin, M., 2001. Stilbenoids from the lianas of *Gnetum pendulum*. Phytochemistry 58, 591–594.

Lin, M., Li, J.B., Li, S.Z., Yu, D.Q., Liang, X.T., 1992. A dimeric stilbene from *Gnetum parvifolium*. Phytochemistry 31, 633–638.

Tanaka, T., Iliya, I., Ito, T., Furusawa, M., Nakaya, K., Iinuma, M., Shirataki, Y., Matsuura, M., Ubukata, M., Murata, J., Simozono, F., Hirai, K., 2001. Stilbenoids in lianas of *Gnetum parvifolium*. Chem. Pharm. Bull 49, 858–862.

Xu, Q., Lin, M., 1997. Gnetfolin K, a new stilbene diglucoside from *Gnetum parvifolium*. Chin. Chem. Lett. 8, 509–510.