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Stilbenoids from the stem of *Gnetum latifolium* (Gnetaceae)

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Abstract

An acetone extract of the stem of *Gnetum latifolium* Blume afforded the stilbene trimer (latifolol) together with five known stilbenoids (gnetin E, gnetin D, gnetin C, (−)ε-viniferin and resveratrol). Their structures were elucidated on the basis of spectral evidence, in particular by using 2D NMR methods.

Keywords: *Gnetum latifolium*; Gnetaceae; Stilbenoid; Latifolol

1. Introduction

In continuation of the study of *Gnetum parvifolium, G. gnemonoides*, *G. gnemon*, *G. africanum* (Tanaka et al., 2001; Iliya et al., 2001, 2002a,b), the isolation and structure elucidation of a new stilbene trimer, latifolol (**1**) is reported along with five known stilbenoids; gnetin E (**2**, Lins et al., 1982; Boralle et al., 1993), gnetin D (Lins et al., 1982), gnetin C (Lins et al., 1982), (−)ε-viniferin (Lins et al., 1982) and resveratrol (Ingham, 1976) from the acetone extract of the stem of *Gnetum latifolium*. The compounds were purified by chromatography on silica gel, Sephadex LH-20 and ODS, and by PTLC. The structures were characterized on the basis of analyses of spectroscopic data.

2. Results and discussion

Latifolol (**1**) gave a positive reaction towards Gibbs reagent. The UV spectrum (λ_{max} 326 nm) revealed the presence of a highly conjugated system in the molecule. The negative FAB–MS exhibited an $[M–H]^-$ ion peak at *m*/*z* 695, indicating the molecular weight to be 696. The molecular formula $C_{42}H_{32}O_{10}$ was deduced from HRFAB-MS *m*/*z* 695.1920 $[M–H]^-$ (requires 695.1917).

The ^1H NMR spectrum exhibited the signals of two sets of *ortho*-coupled protons in the A_2B_2 system on the *p*-substituted phenyl moieties (rings B_1 and C_1) at δ 7.25 (2H, *d*, *J* = 8.8 Hz, H-2b, 6b)/6.87 (2H, *d*, *J* = 8.8 Hz, H-3b, 5b), δ 7.45 (2H, *d*, *J* = 8.8 Hz, H-2c, 6c)/6.85 (2H, *d*, *J* = 8.8 Hz, H-3c, 5c); two sets of *meta*-coupled protons in the AB system on 1,3,4,5-tetrasubstituted benzene rings (rings B_2 and C_2) at δ 6.35 (1H, *br s*, H-10b)/6.21 (1H, *br s*, H-14b), δ 6.73 (1H, *br s*, H-10c)/6.63 (1H, *br s*, H-14c) and a set of protons in the A_2X system on the 1,3,5-trisubstituted benzene ring (ring A_2) at δ 6.28 (2H, *d*, *J* = 2.0 Hz, H-10a, 14a)/6.22 (1H, *t*, *J* = 2.0 Hz, H-12a) and a set of protons in ABX system on 1,2,4-trisubstituted benzene ring (ring A_1) at δ 7.03 (1H, *d*, *J* = 8.4 Hz, H-6a)/6.46 (1H, *d*, *J* = 2.4 Hz, H-3a)/6.30 (1H, *dd*, *J* = 2.4, 8.4 Hz, H-5a). A set of *trans*-coupled olefinic protons at δ 7.09 (1H, *d*, *J* = 16.0 Hz, H-7c) /6.96 (1H, *d*, *J* = 16.0 Hz, H-8c), two sets of mutually coupled methines at δ 5.76 (1H, *d*, *J* = 4.0 Hz, H-7a)/4.46 (1H, *d*, *J* = 4.0 Hz, H-8a), δ 5.47 (1H, *d*, *J* = 4.4 Hz, H-7b)/4.49 (1H, *d*, *J* = 4.4 Hz, H-8b) and eight phenolic hydroxyl protons at δ 8.53 (OH-2a), 8.28 (OH-4a), 8.10 (2H, OH-11a, 13a), 8.52, 8.53 (1H each, OH-4b, 4c), 8.03 (OH-13b), 8.25 (OH-13c) were also observed in the spectrum.

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The molecular formula ($C_{42}H_{32}O_{10}$) and NMR (^1H and ^{13}C) spectral data revealed that **1** is a resveratrol trimer. The correlations of all protons to the respective carbons were clarified with the help of HMQC spectrum. The HMBC correlations between H-6a/C-7a; H-8a/C-10a(14a); H-7b/C-2b(6b); H-10b(14b)/C-8b; H-7c/C-2c(6c); H-8c/C-10c(14c) (Fig. 2) and the correlations between H-6a/H-7a; H-10a(14a)/H-8a; H-2b(6b)/H-7b; H-10b(14b)/H-8b; H-2c(6c)/H-7c; H-10c(14c)/H-8c in the long range ^1H–^1H COSY spectrum (Fig. 3) revealed the connectivities between C-1a/C-7a, C-8a/C-9a, C-1b/C-7b, C-8b/C-9b, C-1c/C-7c and C-8c/C-9c. The correlations between H-8a/C-12b and H-8b/C-12c showed that the resveratrol unit A is connected to the resveratrol unit B which in turn is connected to the other resveratrol unit C through the bonds C-8a/C-12b and C-8b/C-12c, respectively.

The upfield chemical shift of C-1a (δ 120.3) as compared to that of gnetin E (**2**) (Lins et al., 1982) and the appearance of a set of protons in the ABX system based on the 1,2,4-trisubstituted benzene ring (ring A_1) revealed the substitution of the hydroxyl group at C-2 on ring A_1. The chemical shifts of all protons and carbons were assigned by the HMBC correlations (Fig. 2). Although the presence of two dihydrofuran rings (7a-8a-12b-11b-O) and (7b-8b-12c-11c-O) were not supported

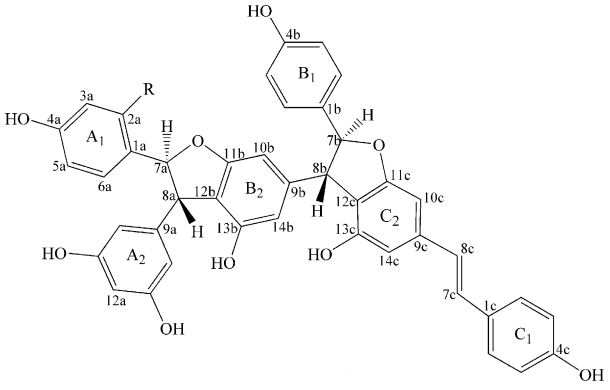

Fig. 1. 1: R = OH; 2: R = H.

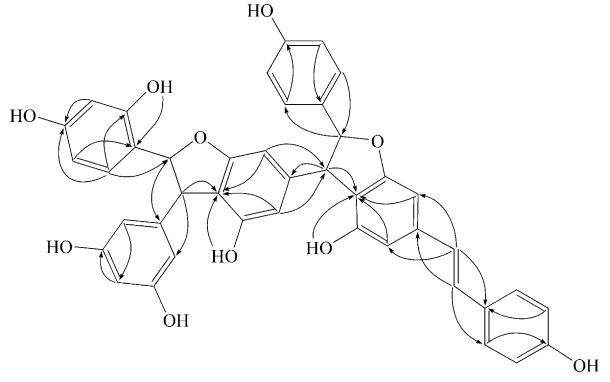

Fig. 2. Selected HMBC correlations.

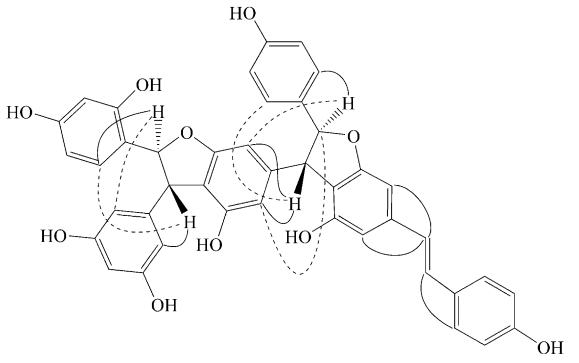

Fig. 3. — ^1H–^1H Long range correlations; - - - - NOESY interactions.

Table 1
^1H and ^{13}C NMR spectroscopic data for latifonol (**1**)[a]

Position	^1H (J in Hz)	^{13}C[b]
1a		120.3
2a		156.1
3a	6.46 *d* (2.4)	103.4
4a		158.8
5a	6.30 *dd* (8.4, 2.4)	108
6a	7.03 *d* (8.4)	128.1
7a	5.76 *d* (4.0)	89.1
8a	4.46 *d* (4.0)	54.1
9a		146
10a(14a)	6.28 *d* (2.0)	107
11a(13a)		159
14a	6.22 *t* (2.0)	101.6
1b		133.9
2b(6b)	7.25 *d* (8.8)	127.6
3b(5b)	6.87 *d* (8.8)	116.1
4b		158.0*
7b	5.47 *d* (4.4)	93.5
8b	4.49 *d* (4.4)	55.8
9b		146.7
10b	6.35 *br s*	100.9
11b		162.9$^+$
12b		114.8*
13b		155.3*
14b	6.21 *br s*	107.9
1c		129.8
2c(6c)	7.45 *d* (8.8)	128.6
3c(5c)	6.85 *d* (8.8)	116.3
4c		158.0*
7c	7.09 *d* (16.0)	129.1
8c	6.96 *d* (16.0)	126.6
9c		141.1
10c	6.73 *br s*	99.1
11c		163.0$^+$
12c		114.8*
13c		155.3*
14c	6.63 *br s*	107.2
OH-2a	8.53* *br s*	
OH-4a	8.28 *br s*	
OH-11a(13a)	8.10 *br s*	
OH-4b	8.53^{+*} *br s*	
OH-13b	8.03 *br s*	
OH-4c	8.52$^+$ *br s*	
OH-13c	8.25 br s	

[a] Measured in CD_3COCD_3.
[b] *Overlapped, $^+$Interchangeable.

by the HMBC spectrum due to the absence of correlations between H-7a/C-11b and H-7b/C-11c, out of the 27 degrees of unsaturation, 25 were satisfied by six benzene rings and one olefinic moiety. Therefore, the remaining must be attributed to two dihydrofuran rings.

The orientation of two dihydrofuran rings was found to be *trans* as in gnetin E (**2**) (Lins et al., 1982; Boralle et al., 1993) by the NOESY experiment (Fig. 3) and thus the structure of latifolol was drawn as **1** in Fig. 1.

3. Experimental

3.1. General

NMR spectra were recorded on Jeol LA 400, Jeol α-400 spectrometers. 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 a 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. Kiesel-gel 60 F_{254} (Merck) was used as analytical and preparative TLC.

3.2. Plant material

The stem of *Gnetum latifolium* was collected at Bogor Botanical Garden, Indonesia in April 2001.

3.3. Extraction and isolation

The dried stem of *G. latifolium* (800 g) was powdered and extracted successively with acetone, MeOH and MeOH–H_2O (70:30). The acetone extract (12 g) was subjected to CC over silica gel eluted with a mixture of EtOAc–$CHCl_3$–MeOH–H_2O (75:75:35:7) to provide eight fractions (each 75 ml). Fraction 4 was further subjected to chromatography over Sephadex LH-20 (MeOH) and ODS Sep-Pak C_{18} Cartridge (MeOH–H_2O, 1:1) to yield a mixture of two compounds. The mixture was purified by PTLC ($CHCl_3$—EtOAc–MeOH–H_2O 20:14:6:1) to give latifolol (**1**, 55 mg) and gnetin D (**3**, 60 mg). Gnetin E (**2**, 46 mg) was purified from fraction 3 by VLC on ODS eluted with MeOH–H_2O (1:1). Gnetin C (**4**, 35 mg) and resveratrol (**5**, 80 mg) were obtained from fraction 2 by chromatography over Sephadex LH-20 (MeOH).

3.4. Latifolol (*1*)

A brown amorphous powder; $[\alpha]_D$ -42° ($c = 0.15$; MeOH); UV λ_{max} (MeOH): 326, 286, 225 nm; negative ion FAB–MS m/z: 695 $[M-H]^-$; negative ion HR—FAB–MS m/z: 695.1920, (calc. 695.1917 for $C_{42}H_{31}O_{10}$); NMR spectral data: see Table 1.

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