Stilbenoids in Lianas of *Gnetum parvifolium*

Toshiyuki Tanaka,*a Ibrahim Iliya,a Tetsuro Ito,a Miyuki Furusawa,a Ken-ichi Nakaya,a Munekazu Inuma,a Yoshiaki Shirataki,a Nobuyasu Matsuura,a Makoto Uubukata,a Jin Murata,d Fumio Simozono,c,d and Kazunori Hirad

Gifu Prefectural Institute of Health and Environmental Sciences,*a 1–1 Naka-fudagaoa, Kakamigahara, Gifu 504–0838, Japan, Faculty of Pharmaceutical Sciences, Josai Universityb,c, 1–1 Keyakidai, Saitama, Saitama 350–0295, Japan, Biotechnology Research Center, Toyama Prefectural University, 5180 Kosugi-machi, Izumi-gun, Toyama 939–0398, Japan, and Botanical Gardens, Koishikawa, Graduate School of Science, The University of Tokyo, d 3–7–1 Hakusan, Bunkyo-ku, Tokyo 112–0001, Japan. Received February 7, 2001; accepted April 10, 2001

Five new stilbene dimers were isolated from the lianas of *Gnetum parvifolium* in addition to known stilbenoids. The structures of the compounds were established on the basis of spectroscopic evidence, including long-range coupling and nuclear Overhauser effect experiments, in NMR spectrum. Among the isolates, 2b-hydroxyamelpolosin F showed potent inhibitory activity in the Maillard reaction.

Key words *Gnetum parvifolium*; Gnetaceae; parvifolol A—D; 2b-hydroxyamelpolosin F; gnetulin; inhibitory activity in the Maillard reaction.

The genus *Gnetum* (Gnetaceae) comprises about 40 species distributed in South America (Amazon), southwest Africa, and in tropical and subtropical zones of Asia.1,2) The fruit and seed of some species like *Gnetum gnemon*, called “melinjo” in Indonesia, are used as food, while others have been used as folk medicines.3) In the present study, an acetone extract of lianas of *Gnetum parvifolium* was found to exhibit a strong inhibitory activity (79%) in the Maillard reaction at 1 g/ml. In this paper, we describe the isolation and structural determination of stilbenoids in the lianas of *G. parvifolium*.

The lianas of *Gnetum parvifolium* (Warb) C. Y. Cheng ex Chun was extracted with acetone and MeOH, successively. The acetone and the methanol extracts were fractionated to give several fractions by open column chromatography on silica gel. The resulting fractions were further purified with silica gel, Sephadex LH 20 columns and preparative TLC to give 11 stilbenoids. Six compounds among them were known stilbenoids (resveratrol, isorhapontigenin, isorhapontigenin-4’-O-β-glucopyranoside, isorhapontigenin-3-O-β-glucopyranoside, gnetulin, (−)-ε-viniferin).3) The other five compounds are new compounds.

Compound 1 (parvifolol A), an amorphous powder, showed the [M–H]– at *m/z* 469 in negative ion FAB-MS, indicating the molecular formula 

C_{28}H_{22}O_{7}.

The 1H-NMR showed the presence of four benzylic protons coupled in the order [δ 5.36 (1H, br, d, J=10 Hz, H-7a), 3.35 (1H, br dd, J=10, 11 Hz, H-8a), 3.51 (1H, br dd, J=11, 11 Hz, H-7b), 4.07 (1H, br, d, J=11 Hz, H-8b)], a p-substituted phenyl group [δ 7.43 (2H, br, d, J=9 Hz, H-2a, 6a), 6.95 (2H, d, J=9 Hz, H-3a, 5a)], and a 3,5-disubstituted phenyl group in an A_2B spin system [δ 6.56 (2H, d, J=2 Hz, H-10b, 14b); δ 6.33 (1H, t, J=2 Hz, H-12b)]. The spectrum also exhibited the presence of a 1,2,4-trisubstituted benzene ring [δ 6.26 (1H, d, J=2 Hz, H-3b), 6.23 (1H, dd, J=8, 2 Hz, H-5b), 6.99 (1H, br dd, J=8, 1 Hz, H-6b)] and a 1,2,3,5-tetrasubstituted benzene ring [δ 5.54 (1H, dd, J=2, 1 Hz, H-14a), 6.07 (1H, d, J=2 Hz, H-12a)] in addition to six phenolic hydroxyl groups [δ 6.40, 8.03, 8.07, 8.21 (× 2), 8.53]. The number of hydroxyl groups was confirmed by acetylation and methylation, which gave a hexacetate (1a) and a hexamethyl ether (1b), respectively. All protonated carbon signals in the 13C-NMR spectrum were assigned by 1H detected single quantum coherence (HMQC) spectrum (Table 2). In the 1H–1H long-range correlation spectroscopy (COSY) (HH long-range COSY) spectrum, significant correlations were observed between H-2a(6a)/H-7a, H-14a/H-8a, H-6b/H-7b and H-10b(14b)/H-8b. In addition, 13C long range couplings were observed between H-2a(6a)/C-7a, H-14a/C-8a, H-6b/C-7b and H-10b(14b)/C-8b in the 1H detected multiple quantum coherence (HMBC) spectrum, which showed that rings A_1, A_2, B_1 and B_2 were connected at C-7a, C-8a, C-7b, C-8b, respectively. The long-range correlations observed between H-14a/C-10a and H-8b/C-10a in the HMBC spectrum indicated that a resveratrol unit (ring A_1–C7a–C8a–ring A_2) formed a five-membered ring with C-7b and C-8b. Although no correlation was observed between H-7a/C-2b, a linkage through an oxygen exist in 1. The planar structure was therefore concluded to be as shown in Fig. 2. Significant nuclear Overhauser effect (NOE) interactions in the NOE spectroscopy (NOESY) spectrum supported the relative stereostructure of 1 (Fig. 3).

Compound 2 (parvifolol B), an amorphous powder, showed [M–H]– at *m/z* 469 in negative ion FAB-MS, indicating that the molecular formula is C_{28}H_{22}O_{8}. As the 1H- and the 13C-NMR spectral data were closely similar to those of 1, it was considered that 2 will be one of the stereoisomers of 1. As NOE correlations in the NOESY spectrum were observed between H-7a/H-7b, H-7a/H-14a, H-8a/H-2a(6a), H-7b/H-10b(14b) and H-6b/H-8b, the configuration at C-8b would be β as compared with 1, where C-8b is α (Fig. 1). Compounds 1 and 2 are dimers consisting of a resveratrol and an oxyresveratrol. Although gnetuhainins D and E isolated as a mixture from *G. hainanense*3) have structures similar to 1 and 2, the structures are completely different, that is, a bridge is formed between C-7a–O–C-2b in 1 and 2, while both C-7a and C-2b are substituted with hydroxyl groups in gnetuhainins D and E.

Compound 3 (parvifolol C), an amorphous powder, showed [M–H]– at *m/z* 485 in negative ion FAB-MS, indicating the molecular formula to be C_{28}H_{22}O_{9}. The 1H-NMR spectrum was very similar to those of 1 and 2 except that a p-
The hydroxyl phenyl group was substituted with a 2,4-dihydroxy phenyl group. In the HMBC spectrum, long range correlations were observed between H-7a/C-2a and H-7a/C-6a, which indicated that the 2,4-dihydroxy phenyl group is connected at C-7a. In the NOESY spectrum, correlations were observed between H-7a/H-14a, H-6a/H-8a, H-7b/H-10b(14b), H-6b/H-8b and H-7a/H-8b, which permitted the conclusion that the relative stereochemistry could be drawn as 3 in Fig. 4. Compound 3 is a dimer consisting of two oxyresveratrol units.

Compound 4 (parvifolol D), an amorphous powder, showed [M−H]− at m/z 513 in negative ion FAB-MS corresponding to the molecular formula C30H26O8. The 1H-NMR showed the presence of two benzylic protons coupled with each other [δ 4.54 (1H, br d, J=9 Hz, H-8a), 5.45 (1H, d, J=9 Hz, H-7a)], two trans olefinic protons [δ 6.92 (1H, d, J=16 Hz, H-8b), 7.02 (1H, d, J=16 Hz, H-7b)] and two sets of 3,5-disubstituted phenyl groups in an A2B spin system [δ 6.20 (2H, d, J=2 Hz, H-10a, 14a), 6.28 (1H, t, J=2 Hz, H-12a); δ 6.53 (2H, d, J=2 Hz, H-10b, 14b), 6.25 (1H, t, J=2 Hz, H-12b)]. The spectrum also exhibited the presence of a 1,2,4-trisubstituted benzene ring [δ 7.05 (1H, d, J=2 Hz, H-2a), 6.28 (2H, m, H-5a, 6a)] and a 1,2,3,5-tetrasubstituted benzene ring [δ 7.17 (1H, d, J=1 Hz, H-2b), 6.28 (1H, d, J=1 Hz, H-6b)]. In addition, two methoxyls (δ 3.84, 3.94) and five hydroxyl groups [δ 7.70, δ 8.21 (×2), 8.23 (×2)] were observed. Correlations were observed between H-2a/C-
7a, H-6a/C-7a, H-10a(14a)/C-8a, H-2b/C-7b, H-6b/C-7b and H-10b(14b)/C-8b in the HMBC spectrum (Fig. 5), indicating that rings A1, A2, B1 and B2 were attached to C-7a, 8a, 7b and 8b, respectively. NOE correlations in the NOESY spectrum (Fig. 5) observed between OMe (δ 3.84)/H-2a and OMe (δ 3.94)/H-2b also indicated that the two methoxyl groups were substituted at C-3a and C-3b. Although no long-range correlation was observed between H-7a/C-4b in the HMBC spectrum, it could be concluded that a furan ring is present in 4 after consideration of the number of hydroxyl groups and the molecular formula. The planar structure of 4 was then concluded to be as shown in Fig. 5. The configuration of the dihydrofuran ring was confirmed to be trans on the basis of NOESY correlations between H-2a/H-8a, H-6a/H-8a and H-10a(14a)/H-7a. Consequently, the relative stereostructure of 4 could be drawn as in Fig. 5.

Compound 5 (2b-hydroxyampelopsin F), an amorphous powder, showed [M−H]− at m/z 469 in negative ion FAB-MS indicating the molecular formula C28H22O7. The 1H-NMR spectrum, including 1H–1H COSY showed, the presence of a sequence of four benzylic protons that were successively coupled in the order [δ 4.28 (1H, br s, H-7a), 3.40 (1H, br s, H-7a), 3.92 (1H, br s, H-7b), 4.14 (1H, br s, H-8b)]. The presence of a p-substituted phenyl group [δ 7.10 (2H, br d, J=9 Hz, H-2a, 6a); δ 6.17 (2H, d, J=9 Hz, H-3a, 5a)], a 2,4-disubstituted phenyl group in an ABM spin system [δ 6.58 (1H, br d, J=8 Hz, H-6b); δ 6.28 (1H, d, J=2 Hz, H-3b), 6.03 (1H, dd, J=8, 2 Hz, H-5b)] and two sets due to a 1,2,3,5-tetrasubstituted benzene ring [δ 6.04 (1H, d, J=2 Hz, H-12a), 5.68 (1H, br d, J=2 Hz, H-14a); δ 6.14 (2H, d, J=2 Hz H-12b), 6.24 (1H, br d, J=2 Hz, H-14b)] were exhibited in the spectrum in addition to seven phenolic hydroxyl groups (δ 7.17, 7.70, 7.81, 7.84, 7.87, 7.90, 7.95). The correlations [C-2a(6a)/H-7a, C-2b/H-7b] in the COSY involving long-range coupling (COLOC) spectrum and [H-2a(6a)/H-7a, H-6b/H-7b] in the 1H–1H long range COSY spectrum indicated that rings A1 and B1 were connected at C-7a and C-7b, respectively. Furthermore, the results of HMBC spectrum (Fig. 6) and 1H–1H long range correlations between H-14a/H-8a and H-14b/H-8b showed that rings A1 and B1 were connected as shown in Fig. 6. The NOEs observed in the difference NOE (DIFNOE) experiment are depicted in Fig. 6. The spectral data indicated that 5 consists of a dibenzobicyclo[3,2,1]octadiene ring system like ampelopsis F.9 The small coupling constant values of the four benzylic methine protons indicated that all dihedral angles of the methine protons are approximately 90 °C, which is equal to ampelopsis F. Therefore, the structure of 5 was determined to be 2b-hydroxyampelopsin F. Although gnetuhiainin C isolated from G. hainanense has the same planar structure as 5, the coupling constants of H-7a and H-8a are different (gnetuhiainin C: 5.4 Hz) from those of 5, which showed that 5 and gnetuhiainin C are diastereomers.

Compound 6 (gnetulin), an amorphous solid, showed the [M−H]− at m/z 513 in the negative ion FAB-MS, corresponding to the molecular formula C30H26O8. The 1H-NMR showed the presence of two benzylic protons [δ 4.19 (1H, br s, H-8b), 4.27 (1H, br s, H-7b)], an olefinic proton [δ 7.05 (1H, br s, H-7a)] and two sets of 3,4-disubstituted phenyl groups in an ABX type [δ 6.89 (1H, d, J=2 Hz, H-2a), 6.69 (1H, d, J=8 Hz, H-5a), 6.83 (1H, dd, J=8, 2 Hz, H-6a); δ 6.74 (1H, d, J=2 Hz, H-2b), 6.64 (1H, d, J=8 Hz, H-5b), 6.51 (1H, dd, J=8, 2 Hz, H-6b)]. The presence of a 3,5-disubstituted phenyl group in an A2B type [δ 6.31 (1H, d, J=2 Hz, H-12a), 6.78 (1H, d, J=2 Hz, H-14a)] was also exhibited in the spectrum. All protonated carbons in the 13C-NMR spectrum were assigned by the HMQC spectrum (Table 2). Considering the HMBC correlations observed between H-7a/C-
2a, H-7a/C-6a, H-7b/C-2b and H-10b(14b)/C-8b demonstrated that rings A1, B1 and B2 were connected at C-7a, C-7b and C-8b, respectively. In addition, the HMBC correlations depicted in Fig. 7 indicated that C-8a–C-8b–C-7b forms a double bond is E. The NOEs between H-7b/H-10b(14b) and H-8b/H-6b also suggested that ring B1 and B2 are trans. Although gnetulin5) was isolated as an acetate derivative from G. ula, this is the first instance of isolation of the compound as an intact form. A compound, gnetifolin C (1), was isolated from G. parvifolium, but direct comparison of the two compounds has not yet been carried out. As the compounds are identical, re-examination of gnetifolin C called for.

It is reported that protein glycation (Maillard reaction) is one of the causes of diabetic complications and aging of the skin. Among the isoforms, 2b-hydroxyamelpespin F (5) showed a potent inhibitory activity in the Maillard reaction6) (70%: 10 μg/ml).

**Experimental**

1H and 13C-NMR spectra were recorded on α-500 and LA-300 (JEOL) spectrometers. Chemical shifts are shown as δ values with tetramethylsilane (TMS) as an internal reference. Peak multiplicities are quoted in Hz. Negative ion FAB-MS were measured on a JMS-DX-300 spectrometer equipped with a JMA 3500 data analysis system (JEOL). UV spectra were recorded on a UV-2200 spectrometer (Shimadzu), and optical rotations were recorded on a P-1020 (JASCO) polarimeter. Silica gel 60 (70—230 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used for column chromatography, and Kiesel-gel 60F (Merck) was used for analytical and preparative TLC.

### Extraction and Isolation

The dried bark of *Gnetum parvifolium* (1.6 kg), collected in the Botanical Garden of the University of Tokyo in April, 1999, was powdered and extracted successively with acetone (3×3 l), and MeOH (3 l) at room temperature. An acetone extract (80 g) and a methanol extract (45 g) were chromatographed on silica gel and eluted with CHCl3–MeOH mixtures. The CHCl3–MeOH (10:1) fraction was further chromatographed on Sephadex LH 20 and eluted with MeOH. Final purification was made by preparative TLC (benzene: EtOAc: acetone: EtOH=10:2:2:1) to give resveratrol (14 mg) and isoraphontigine (22 mg).
CHCl3–MeOH (5:1) fraction was chromatographed on Sephadex LH 20 and eluted with acetone. The resulting fractions were purified by preparative TLC (benzene–acetone–EtOAc–MeOH=4:1:1:1) to give isohapontinigenin-4’-O-β-glucopyranoside (12 mg), isohapontinigenin-3-O-β-glucopyranoside (7 mg), 1 (22 mg), 3 (5 mg) and 5 (18 mg), respectively. The CHCl3–MeOH (5:1) fraction of the methanol extract was repeatedly chromatographed on Sephadex LH 20 (MeOH). The resulting fractions were further purified by preparative TLC (CHCl3:MeOH:H2O=45:15:2 and EtOAc:CHCl3:MeOH:H2O=15:8:4:1) to give 1 (16 mg), 2 (15 mg), 3 (11 mg), 4 (14 mg), 5 (25 mg), 6 (17 mg) and (−)-e-viniferin (7 mg), respectively.

**Compound 1 (Parvifolol A):** A pale brownish amorphous solid. Negative ion FAB-MS m/z [M−H]− 469. HR-FABMS m/z 469.1292 (Calcd 469.1287 for C32H31O11). UV (MeOH) λmax (log ε) 215 (4.3), 239sh, 283 (3.7), 325sh. 

**Acetylation of Compound 1** To a solution of 1 (3 mg) in pyridine (1 ml), acetic anhydride (1 ml) was added and the mixture was kept at room temperature for 24 h. The reaction mixture was poured into cold water and then extracted with EtOAc. After removing the solvent in vacuo, the crude product was purified with PTLC (n-hexane : EtOAc=3:2) to afford 1a as an amorphous solid (2 mg). 1H-NMR (400 MHz, CDCl3) δ: 1.80, 2.20, 2.22 (3H each, s, OAc), 2.29 (6H, s, 2×OAc), 2.35 (3H, s, OAc), 3.60 (2H, t, H-8a, b), 4.17 (1H, br, J=11 Hz, H-8b), 5.49 (1H, br, J=10 Hz, H-7a), 5.90 (1H, brs, H-1a), 6.55 (1H, dd, J=8, 2 Hz, H-5a), 6.55 (1H, d, J=2 Hz, H-12a), 6.58 (1H, d, J=2 Hz, H-3b), 6.93 (1H, t, J=2 Hz, H-12b), 7.03 (2H, d, J=2 Hz, H-10b, 14b), 7.03 (2H, d, J=9 Hz, H-3a, 5a), 7.19 (1H, brd, J=8 Hz, H-6b), 7.52 (2H, brd, J=9 Hz, H-2a, 6a).

**Methylation of 1** Compound 1 (5 mg) was allowed to react with MeI (0.5 g) and K2CO3 (2 g) in dry acetone under reflux for 3 h. The reaction mixture was treated in the usual manner and the resulting crude product was purified with PTLC (n-hexane : acetone=3:1) to afford 1b as an amorphous colorless solid (3 mg). 1H-NMR (400 MHz, CDCl3) δ: 3.40, 3.51, 3.72 (3H each, s, OMe), 3.80 (6H, s, 3×OAc), 3.87 (3H, s, OMe), 4.18 (1H, brd, J=11 Hz, H-8b), 5.43 (1H, brd, J=10 Hz, H-7a), 5.55 (1H, brs, H-1a), 6.20 (1H, d, J=2 Hz, H-12a), 6.37 (1H, dd, J=8, 2 Hz, H-5b), 6.37 (1H, t, J=2 Hz, H-12b), 6.59 (2H, d, J=2 Hz, H-10b, 14b), 7.03 (2H, d, J=9 Hz, H-3a, 5a), 7.19 (1H, brd, J=8 Hz, H-6b), 7.52 (2H, brd, J=9 Hz, H-2a, 6a), H-8a and 7b were overlapped with methoxyl signals.

**Compound 2 (Parvifolol B):** A pale brownish amorphous solid. Negative ion in FAB-MS ([M−H]−) m/z 469. HR-FAB-MS m/z 469.1291 (Calcd 469.1287 for C32H31O11). UV (MeOH) λmax (log ε) 213 (4.3), 230 sh, 283 (3.7), 326 sh. [δH0 +4° (c=0.13, MeOH). The 1H- and 13C-NMR spectral data are listed in Tables 1 and 2.

**Compound 3 (Parvifolol C):** A pale brownish amorphous solid. Negative ion FAB-MS ([M−H]−) m/z 485. HR-FAB-MS m/z 485.1228 (Calcd 485.1236 for C32H31O11). UV (MeOH) λmax (log ε) 215 (4.3), 230 sh, 282 (3.7), [δH0 −10° (c=0.16, MeOH). The 1H- and 13C-NMR spectral data are listed in Tables 1 and 2.

**Compound 4 (Parvifolol D):** A pale brownish amorphous solid. Negative ion FAB-MS ([M−H]−) m/z 513. HR-FAB-MS m/z 513.1573 (Calcd 513.1549 for C32H31O11). UV (MeOH) λmax (log ε) 217 (4.3), 287 (3.8), 316 sh, 327 (3.8). [δH0 +7° (c=0.11, MeOH). The 1H- and 13C-NMR spectral data are listed in Tables 1 and 2.

**Compound 5 (2b-Hydroxyampelopsin F):** A pale brownish amorphous solid. Negative ion FAB-MS ([M−H]−) m/z 469. HR-FAB-MS m/z 469.1292 (Calcd 469.1287 for C32H31O11). UV (nm, MeOH) 215 (4.1), 283 (3.5), 325 sh. [δH0 +12° (c=0.1, MeOH). The 1H- and 13C-NMR spectral data are listed in Tables 1 and 2.

Inhibitory activity of the Maillard reaction was measured as described in ref. 6.

**References**


