New Megastigmane Glycoside and Aromadendrane Derivative from the Aerial Part of *Piper elongatum*

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A new megastigmane glycoside, called pipeloside A, and a new aromadendrane type sesquiterpenoid, pipelol A, were isolated from the MeOH extract of the aerial part of *Piper elongatum* VAHL. along with a known megastigmane glycoside, byzantionoside B. The structures of these compounds were elucidated on the basis of spectroscopic data and chemical evidence.

Key words *Piper elongatum*; Piperaceae; megastigmane glycoside; pipeloside A; aromadendrane type; pipelol A

Piper elongatum VAHL. (Piperaceae) is a small tree commonly found in the lowlands of the Amazon, and its leaves are used as a folk medicine for the treatment of dermatosis in South America.[1)] In a previous paper, we reported the isolation and structure elucidation of six aromatic compounds in the MeOH extract of the aerial part of *P. elongatum* VAHL. and further that three phenolic compounds among those compounds were found to have stronger antioxidative activity than α-tocopherol.[2)] As part of the continuing study of this folk medicine, this paper describes the isolation and structure elucidation of a new megastigmane glycoside and a new aromadendorane type sesquiterpenoid along with a known megastigmane glycoside, byzantionoside B, from the MeOH extract of the aerial part of *P. elongatum* VAHL.

The MeOH extract of the aerial part of *P. elongatum* VAHL. was successively subjected to Diaion HP20, silica gel, Sephadex LH20, and ODS column chromatography as well as HPLC on silica gel to give three compounds (**1**—**3**). Compound **3** was identified as byzantionoside B based on its physical and spectral data.[3)]

Compound **1**, called pipeloside A, showed an $[M-H]^-$ ion peak at *m*/*z* 695 along with fragment ion peaks at *m*/*z* 533 [M−hexose unit (162)−H]$^-$, 371 [M−162×2−H]$^-$, and 209 [M−162×3−H]$^-$ in the negative FAB-MS. The molecular formula was determined to be $C_{31}H_{52}O_{17}$ by high-resolution (HR) positive FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of **3**, and the chemical shifts of the aglycone (Ag) moiety, in particular, were superimposable, except for the appearance of the signals due to two more monosaccharide units (Tables 1, 2). Acidic hydrolysis of **1** afforded glucose, which was identified as the D-form on the basis of gas chromatographic (GC) analysis according to Hara *et al*.[4)] From these data and the coexistence of **3**, **1** was believed to attach two moles of glucose to **3**, and the coupling constant of anomeric proton signals indicated the mode of glycosidic linkage of these glucose units to be β. To determine the sugar linkages, **1** was converted into the peracetate (**4**), of which the ^{1}H-signals were assigned on the basis of ^{1}H–^{1}H correlation spectroscopy (COSY). In this spectrum, the signals of H-4 of two glucose units showed no acylation shifts, suggesting the sugar linkages of the second glucose unit (Glc′) and terminal glucose unit (Glc″) to be located at OH-4 of first glucose unit (Glc) and OH-4 of Glc′, respectively. Consequently, the structure of pipeloside A was defined to be (6*R*,9*S*)-9-hydroxy-4-megastigmene 9-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (Fig. 1).

Compound **2**, called pipelol A, showed an $[M]^+$ ion peak at *m*/*z* 254 in the electron impact (EI)-MS, which indicated a molecular formula of $C_{15}H_{26}O_3$ in combination with NMR data. The ^{1}H-NMR spectrum (in CD_3OD) showed signals due to three tertiary methyl groups (δ 1.02, 1.04, 1.21), two oxygenated methylene protons [δ 3.61 (d, J=11.5 Hz), 3.70 (d, J=11.5 Hz)], and two cyclopropyl protons [δ 0.43 (dd, J=10.0, 11.0 Hz), 0.62 (ddd, J=6.0, 10.0, 11.0 Hz)]. The ^{13}C-NMR spectrum (in CD_3OD) gave 15 carbon signals including two oxygenated quaternary carbons (δ 76.8, 81.0) and one oxygenated methylene carbon (δ 62.3). These ^{1}H-

Table 1. ^{1}H-NMR Spectral Data for Compounds **1** and **3**

Position	**1**[a)] (500 MHz)	**1**[b)] (400 MHz)	**3**[a)] (500 MHz)	**3**[b)] (500 MHz)
Ag-2a	2.46 d (17.0)	2.43 d (17.0)	2.46 d (17.0)	2.44 d (17.0)
2b	1.97 d (17.0)	2.06 d (17.0)	1.97 d (17.0)	2.03 d (17.0)
4	5.80 s	5.91 s	5.80 s	5.92 s
10	1.19 d (6.0)	1.25 d (6.0)	1.18 d (6.0)	1.27 d (6.0)
11	1.09 s[c)]	0.92 s[c)]	1.09 s[c)]	0.93 s[c)]
12	1.01 s[c)]	0.90 s[c)]	1.01 s[c)]	0.91 s[c)]
13	2.05 s	1.84 s	2.04 d (1.0)	1.86 s
Glc-1	4.45 d (8.0)[d)]	5.18 d (8.0)[d)]	4.32 d (7.5)	4.90 d (8.0)
Glc′-1	4.40 d (7.5)[d)]	5.14 d (7.5)[d)]		
Glc″-1	4.36 d (8.0)[d)]	4.83 d (7.5)[d)]		

δ in ppm from tetramethylsilane (TMS) (coupling constants [J] in Hz are given in parentheses). *a*) In CD_3OD. *b*) In C_5D_5N. *c*, *d*) Assignments may be interchanged in the same column.

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and ^{13}C-NMR signals were assigned with the aid of ^{1}H–^{1}H COSY, heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) spectra as shown in Table 3, and the planar structure of **2**, which was an aromadendrane type sesquiterpene, was characterized as illustrated in Fig. 2. The relative stereochemistry of **2** was defined on the basis of difference nuclear Overhauser effect (NOE) spectra, which were mainly carried out in C_5D_5N, because the ^{1}H-NMR signals were congested in CD_3OD. The NOE correlations were observed between the respective protons, as illustrated in Fig. 3. Moreover, the stable conformation of **2** with minimum steric energy was simulated using CAChe CONFLEX[5—7] and is illustrated in Fig. 3. This conformation supported the NOE correlations and the coupling constant values. Accordingly, pipelol A was concluded to be

Table 2. ^{13}C-NMR Spectral Data for Compounds **1** and **3** (in C_5D_5N, 100 MHz)

Position	**1**	**3**
Ag-1	36.2	36.2
2	47.7	47.7
3	198.4	198.4
4	125.3	125.3
5	165.2	165.2
6	51.0	51.0
7	25.9	25.9
8	36.9	37.0
9	74.7[a)]	75.2
10	19.9	19.9
11	27.1	27.1
12	28.7	28.7
13	24.3	24.3
Glc-1	102.1	102.3
2	74.3[a)]	74.1
3	76.7[b)]	78.6[a)]
4	80.8[c)]	71.9
5	76.4[b)]	78.4[a)]
6	61.7[d)]	63.0
Glc′-1	104.5[e)]	
2	74.5[a)]	
3	76.6[b)]	
4	81.5[c)]	
5	76.3[b)]	
6	62.3[d)]	
Glc″-1	104.9[e)]	
2	74.7[a)]	
3	78.2[f)]	
4	71.5	
5	78.4[f)]	
6	62.4[d)]	

δ in ppm from TMS. *a—f*) Assignments may be interchanged in the same column.

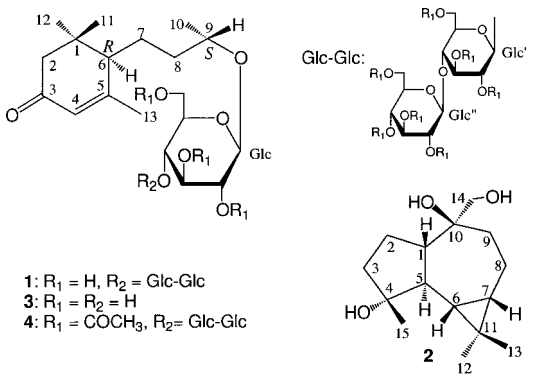

Fig. 1. Structures of **1**—**4**

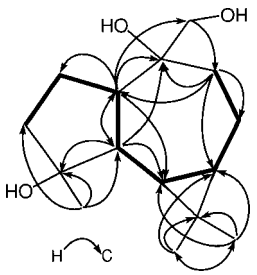

Fig. 2. Partial Structure of **2** Solved by ^{1}H–^{1}H COSY Spectrum (Bold Lines) and ^{1}H–^{13}C Long-Range Correlations Observed for **2** in the HMBC Spectrum (Arrow)

Table 3. ^{1}H- and ^{13}C-NMR Spectral Data for Compound **2** (^{1}H, 500 MHz; ^{13}C, 125 MHz)

Position	^{1}H (CD_3OD)	^{1}H (C_5D_5N)	^{13}C (CD_3OD)	^{13}C (C_5D_5N)
1	1.97 m	*ca.* 2.40	56.8	56.2
2a	*ca.* 1.75	*ca.* 2.36	24.6	24.8
2b	*ca.* 1.62	2.05 m		
3a	*ca.* 1.62	1.99 dd-like (7.0, 12.0)	42.1	42.3
3b	1.48 m	*ca.* 1.69		
4			81.0	79.4
5	1.29 dd (10.5, 11.0)	1.76 dd (10.5, 10.5)	47.9	47.5
6	0.43 dd (10.0, 11.0)	0.54 dd (10.5, 10.5)	30.5	29.9
7	0.62 ddd (6.0, 10.0, 11.0)	0.69 ddd (6.0, 10.5, 11.0)	27.8	27.1
8a	*ca.* 1.75	1.87 ddd-like (6.0, 6.5, 14.5)	20.7	20.5
8b	*ca.* 0.96	1.27 ddd-like (11.0, 13.0, 14.5)		
9a	2.15 dd-like (6.5, 13.5)	2.68 dd-like (6.5, 13.0)	38.4	39.0
9b	*ca.* 1.27	*ca.* 1.70		
10			76.8	75.5
11			20.9	19.9
12	1.04 s	1.17 s	16.7	16.7
13	1.02 s	1.06 s	29.1	28.9
14a	3.70 d (11.5)	4.22 s	62.3	62.0
14b	3.61 d (11.5)	4.22 s		
15	1.21 s	1.46 s	24.3	25.0

δ in ppm from TMS (coupling constants [*J*] in Hz are given in parentheses).

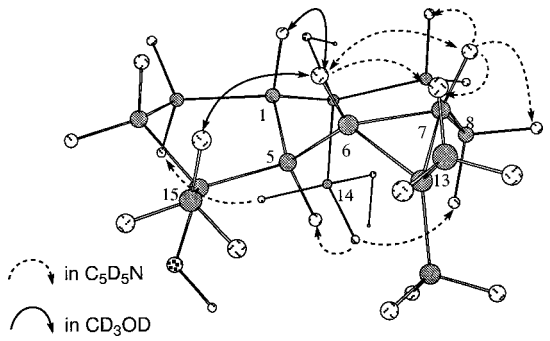

Fig. 3. CAChe Drawings and Selected NOE Correlations Observed in Difference NOE Spectra of **2**

aromadendrane-4α,10β,14-triol (Fig. 1).

As far as we know, **1** and **2** are new compounds, and isolation of **3** from *P. elongatum* VAHL. is described here for the first time.

Experimental

All the instruments and the materials used were the same as cited in the previous reports[2,8] unless otherwise specified.

Extraction and Isolation The air-dried and powdered aerial part of *P. elongatum* VAHL. (5.00 kg) was extracted with MeOH (13 l) under reflux. The MeOH extract (288 g) was defatted by treatment with hexane (300 ml×2) to give a hexane-soluble fraction (fr.) (7.66 g) and the residue (275 g). This residue was subjected to Diaion HP20 (40% MeOH, 60% MeOH, 80% MeOH, MeOH, acetone) to give fr. 1 (62.6 g), fr. 2 (35.7 g), fr. 3 (37.4 g), fr. 4 (93.1 g), and fr. 5 (39.1 g). Chromatography of fr. 2 (29.0 g) over silica gel [$CHCl_3$–MeOH (12 : 1, 10 : 1), $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1), and MeOH] furnished frs. 6—21. Fr. 7 (952 mg) was successively subjected to Sephadex LH20 (MeOH), silica gel [$CHCl_3$–MeOH (15 : 1, 12 : 1, 10 : 1), $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1)], and Chromatorex ODS (50% MeOH, 60% MeOH, 70% MeOH, MeOH) columns to afford **2** (51 mg). Fr. 10 (761 mg) was successively subjected to silica gel [$CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5), MeOH] and HPLC [column, Kusano C.I.G. prepacked Si-5 (100 mm×22 mm i.d., Kusano Kagakukikai Co.); solvent, $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] columns to give **3** (72 mg). Fr. 16 (4054 mg) was chromatographed over Sephadex LH20 (MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1), MeOH] to give **1** (88 mg).

Pipeloside A (**1**): Syrup. $[\alpha]_D^{19}$ +173.7° (c=0.9, MeOH). HR positive FAB-MS *m/z*: 719.3126 $[M+Na]^+$ (Calcd. for $C_{31}H_{52}O_{17}Na$: 719.3102). Negative FAB-MS *m/z*: 695 $[M-H]^+$, 533 $[M-162-H]^-$, 371 $[M-162\times2-H]^-$, 209 $[M-162\times3-H]^-$. ^{1}H- and ^{13}C-NMR spectral data: see Tables 1, 2.

Pipelol A (**2**): Syrup. $[\alpha]_D^{19}$ −14.3° (c=1.2, MeOH). EI-MS *m/z*: 254 $[M]^+$. ^{1}H- and ^{13}C-NMR spectral data: see Table 3.

Byzantionoside B (**3**): Syrup. $[\alpha]_D^{19}$ +21.1° (c=0.7, MeOH). Positive FAB-MS *m/z*: 373 $[M+H]^+$, 211 $[M-162+H]^-$. ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data (in CD_3OD, 125 MHz) δ: 202.5 (C-3-Ag), 170.1 (C-5-Ag), 125.5 (C-4-Ag), 102.2 (C-1-Glc), 78.2 (C-3-Glc), 77.9 (C-5-Glc), 75.6 (C-2-Glc), 75.2 (C-9-Ag), 71.9 (C-4-Glc), 63.0 (C-6-Glc), 52.5 (C-6-Ag), 48.2 (C-2-Ag), 37.9 (C-8-Ag), 37.4 (C-1-Ag), 29.1 (C-12-Ag), 27.6 (C-11-Ag), 26.9 (C-7-Ag), 25.0 (C-13-Ag), 19.9 (C-10-Ag), ^{13}C-NMR (in C_5D_5N): see Table 2.

Acidic Hydrolysis of 1 Compound **1** (3 mg) in 2 N HCl (2 ml) was heated at 95 °C for 120 min and the reaction mixture was neutralized with 2 N NaOH. After removal of the solvent under reduced pressure, the residue was extracted with MeOH (1 ml). The MeOH extract was subjected to GC analysis [detector, FID; column, silicone OV-1 (30 m×0.32 mm i.d., Ohio Valley Specialtly Chem); column temperature, 230 °C; injector temperature, 270 °C; detector temperature, 280 °C; carrier gas, He; flow rate, 0.8 ml/min] as trimethylsilyl ether of the methyl thiazolidine 4(*R*)-carboxylate derivatives according to Hara *et al.*[4] The retention time of this product was identical to that of an authentic sample of D-glucose derivative.

Acetylation of 1 A solution of **1** (10 mg) in Ac_2O–pyridine (1 : 1, 1 ml) was allowed to stand at room temperature overnight. After removal of the reagent under a stream of N_2, the residue was suspended in H_2O (1 ml) and then extracted with ether (1 ml). The ether layer was concentrated to afford **4** (4 mg). Syrup. ^{1}H-NMR spectral data (in C_5D_5N, 500 MHz) δ: 5.95 (1H, s, H-4-Ag), 5.71—5.64 (3H, H-3-Glc, H-3-Glc′, H-3-Glc″), 5.47 (1H, dd, J=9.5, 9.5 Hz, H-4-Glc″), 5.38—5.31 (3H, H-2-Glc, H-2-Glc′, H-2-Glc″), 5.06 (1H, d, J=8.0 Hz, H-1-Glc or H-1-Glc′ or H-1-Glc″), 5.01 (1H, d, J=7.9 Hz, H-1-Glc or H-1-Glc′ or H-1-Glc″), 4.85 (1H, dd, J=2.0, 11.5 Hz, Ha-6-Glc or Ha-6-Glc′ or Ha-6-Glc″), 4.84 (1H, d, J=7.9 Hz, H-1-Glc or H-1-Glc′ or H-1-Glc″), 4.73 (1H, dd, J=2.0, 11.5 Hz, Ha-6-Glc or Ha-6-Glc′ or Ha-6-Glc″), 4.70 (1H, dd, J=4.0, 11.5 Hz, Ha-6-Glc or Ha-6-Glc′ or Ha-6-Glc″), 4.47 (1H, dd, J=6.0, 11.5 Hz, Hb-6-Glc or Hb-6-Glc′ or Hb-6-Glc″), 4.45 (1H, dd, J=6.5, 11.5 Hz, Hb-6-Glc or Hb-6-Glc′ or Hb-6-Glc″), 4.35 (1H, dd, J=2.0, 11.5 Hz, Hb-6-Glc or Hb-6-Glc′ or Hb-6-Glc″), 4.21 (1H, dd, J=9.5, 9.5 Hz, H-4-Glc or H-4-Glc′), *ca.* 4.19 (1H, H-5-Glc or H-5-Glc′ or H-5-Glc″), 4.17 (1H, dd, J=9.5, 9.5 Hz, H-4-Glc or H-4-Glc′), 4.05 (1H, m, H-5-Glc or H-5-Glc′ or H-5-Glc″), 3.93 (1H, m, H-5-Glc or H-5-Glc′ or H-5-Glc″), 3.84 (1H, m, H-9-Ag), 2.50 (1H, d, J=17.0 Hz, Ha-2-Ag), 2.21 (3H, s, $COCH_3$), 2.20 (3H, s, $COCH_3$), 2.19 (3H, s, $COCH_3$), 2.17 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 2.07 (6H, s, $COCH_3\times2$), 2.04 (3H, s, $COCH_3$), 2.00 (3H, s, $COCH_3$), 1.99 (3H, s, $COCH_3$), 1.88 (3H, s, H_3-13-Ag), 1.19 (3H, d, J=6.0 Hz, H_3-10-Ag), 0.97 (3H, s, H_3-11-Ag or H_3-12-Ag), 0.94 (3H, s, H_3-11-Ag or H_3-12-Ag).

Computational Method Calculations were performed using CAChe (Version 4.1.1) with extended MM2 parameters[9] (Fujitsu Co.), which was run on a Macintosh Powerbook G-3/400 personal computer. Energies were minimized with the conjugate gradient. Convergence was obtained when the difference in the energies between two successive interactions was less than 0.00000001 kcal/mol. Drawing was performed using the Chem3D program (Cambridge Scientific Computing Inc., Cambridge, MA, U.S.A.).

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