Two New Phenolic Compounds from the Rhizomes of *Gastrodia elata* BLUME

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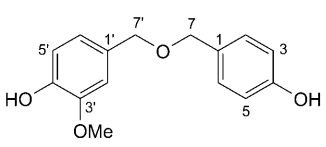
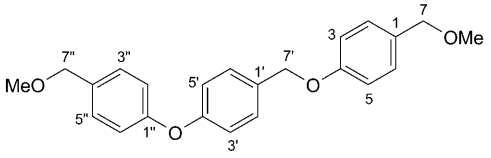
Phytochemical investigation of the rhizomes of *Gastrodia elata* BLUME (Orchidaceae) led to the isolation and identification of twelve compounds, including two new phenolic compounds, 4-hydroxybenzyl vanillyl ether (=4-{[(4-hydroxyphenyl)methoxy]methyl}-2-methoxyphenol; **1**) and 4-{{4-[4-(methoxymethyl)phenoxy]benzyl}oxy}benzyl methyl ether (=1-[4-(methoxymethyl)phenoxy]-4-{[4-(methoxymethyl)phenoxy]methyl}benzene; **2**). The structures of **1** and **2** were elucidated by spectroscopic data analysis including 1D- and 2D-NMR experiments.

Introduction. – The Rhizomes of *Gastrodia elata* BLUME (Orchidaceae) have been used as a traditional Chinese herbal medicine for treating headaches, dizziness, rheumatism, convulsion, and epilepsy [1]. Previous phytochemical work on this rhizome has resulted in the isolation of various types of phenolic compounds such as 4-hydroxybenzyl alcohol (gastrodigenin), 4-hydroxybenzaldehyde, gastrodin, gastrol, parishin, and bis(4-hydroxybenzyl) sulfoxide [2–4]. The extracts and some isolates of this rhizome were reported to have diverse biological activities such as neuroprotective [5–8], GABAergic-neuromodulatory [9–11], anti-inflammatory [12], antidepressant [13], anti-asthmatic [14], and anti-osteoporotic [15] effects.

As part of our ongoing search for bioactive compounds from traditional herbal medicines, the rhizomes of *G. elata* were investigated, and two new phenolic compounds, 4-hydroxybenzyl vanillyl ether[1]) (**1**) and 4-{{4-[4-(methoxymethyl)phenoxy]benzyl}oxy}benzyl methyl ether[1]) (**2**), were isolated, together with ten known compounds. The structure elucidation of **1** and **2** is described herein.



1[1])



2[1])

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

Results and Discussion. – Compound **1** gave a molecular-ion peak at *m/z* 260.1041 (M^+) in the HR-ESI-MS, corresponding to an elemental formula $C_{15}H_{16}O_4$. The IR spectrum showed absorption bands at 3361 cm^{-1} for one or more OH groups and at 1612 and 1516 cm^{-1} for aromatic groups [16]. The UV spectrum of **1** exhibited absorption maxima at 228 and 278 nm, indicating the presence of a phenolic structure [16]. The ^{1}H- and ^{13}C-NMR spectra of **1** showed signals for a 1,4-disubstituted benzene moiety at δ(H) 7.16 (*d*, *J* = 8.8 Hz, H–C(2,6))/δ(C) 130.9 (C(2,6)) and 6.76 (*d*, *J* = 8.8 Hz, H–C(3,5))/116.2 (C(3,5)) and for a 1,2,4-trisubstituted benzene moiety at δ(H) 6.89 (br. *s*, H–C(2′))/δ(C) 113.0 (C(2′)), 6.77 (*dd*, *J* = 8.0, 1.2 Hz, H–C(6′))/122.3 (C(6′)), and 6.75 (*d*, *J* = 8.0 Hz, H–C(5′))/115.9 (C(5′)). In addition, two *s*s integrating for two H-atoms at δ(H) 4.40 (CH_2(7′)) and 4.39 (CH_2(7)) in the ^{1}H-NMR spectrum were attributed to two O-bearing CH_2 groups of different Bn groups, as evidenced by the HMBC cross-peaks CH_2(7′)/C(1′), C(2′), and C(6′) and CH_2(7)/C(1) and C(2,6). A MeO group was assigned to position C(3′) by the HMBC δ(H) 3.83 (*s*, MeO)/δ(C) 149.0 (C(3′)) and the ^{1}H,^{1}H-NOESY correlations MeO/H–C(2′) and H–C(2′)/CH_2(7′). Two O-bearing C-atoms at δ(C) 158.3 and 147.4 were assigned at C(4) and C(4′), which were substituted by an OH group, by the three-bond HMBCs H–C(2,6)/C(4) and H–C(2′)/C(4′), respectively. The above data suggested that there were two sets of Bn groups, a 3-methoxy-4-hydroxybenzyl (=vanillyl) group and a 4-hydroxybenzyl group, and they were connected by an ether link, as indicated by the HMBC CH_2(7)/C(7′). Compound **1** had a structure similar to that of the known benzyl vanillyl ether [17], except for the presence of a 4-hydroxyphenyl group in **1** instead of a Ph group. Further detailed analysis of the ^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC data (*Fig.*) allowed unambiguous assignments for all of the ^{1}H- and ^{13}C-NMR signals of **1**. Accordingly, the structure of **1** was elucidated as the new compound 4-hydroxybenzyl vanillyl ether[1]).

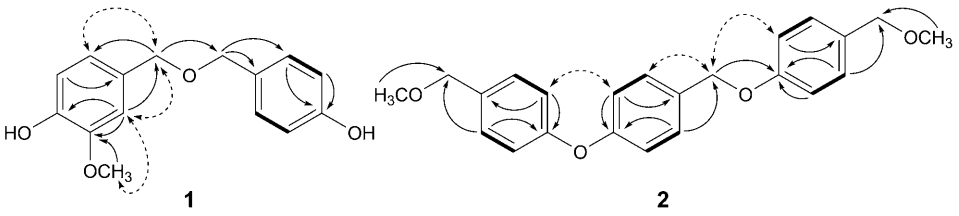


Figure. *Important ^{1}H,^{1}H-COSY* (—), *NOESY* (H ↔ H), *and HMBC* (H → C) *features of* **1** *and* **2**

Compound **2** showed a molecular-ion peak at *m/z* 364.1695 (M^+) in the HR-ESI-MS, consistent with an elemental formula $C_{23}H_{24}O_4$. The IR absorption bands at 1615, 1516, and 1448 cm^{-1} and the UV absorption maxima at 226 and 276 nm indicated the presence of a phenolic structure [16]. The ^{1}H- and ^{13}C-NMR spectra of **1** showed signals for three sets of a 1,4-disubstituted benzene moiety at δ(H) 7.24 (*d*, *J* = 8.8 Hz, H–C(2′,6′))/δ(C) 130.6 (C(2′,6′)), 7.23 (*d*, *J* = 8.8 Hz, H–C(2,6))/130.5 (C(2,6)), 7.15 (*d*, *J* = 8.8 Hz, H–C(3″,5″))/130.8 (C(3″,5″)), 6.95 (*d*, *J* = 8.8 Hz, H–C(3,5))/115.9 (C(3,5)), 6.77 (*d*, *J* = 8.8 Hz, H–C(3′,5′))/116.2 (C(3′,5′)), and 6.75 (*d*, *J* = 8.8 Hz, H–C(2″,6″))/116.1 (C(2″,6″)). In addition, the ^{1}H-NMR spectrum of **2** displayed signals for three pairs of O-bearing CH_2 groups at δ(H) 4.94 (*s*, CH_2(7′)), 4.37 (*s*, CH_2(7)), and 4.33 (*s*, CH_2(7″)), and two MeO groups at δ(H) 3.33 (*s*, 2 MeO). In the

^{13}C-NMR spectrum of **2**, signals of three O-bearing C-atoms were present at δ(C) 160.2 (C(4)), 158.5 (C(4′)), and 158.4 (C(1″)), and those of three quaternary C-atoms at δ(C) 131.5 (C(1)), 130.1 (C(4″)), and 129.4 (C(1′)). These observations suggested the presence of two (methoxymethyl)phenoxy groups and a Bn group in the molecule, supported by the HMBCs MeO/C(7) and C(7″), H–C(2,6)/C(7) and C(4), H–C(3,5)/C(1), C(2,6) and C(4), H–C(3″,5″)/C(7″) and C(1″), H–C(2″,6″)/C(4″) and C(1″), H–C(2′,6′)/C(7′) and C(4′), and H–C(3′,5′)/C(4′) and C(1′) (*Fig.*). The HMBC CH_2(7′)/C(4) and the ^{1}H,^{1}H-NOESY correlations CH_2(7′)/H–C(3) and H–C(2′,6′), and H–C(3′,5′)/H–C(2″,6″) provided evidence that the Bn group was between two (methoxymethyl)phenoxy groups. Thus, compound **2** was identified as a new compound, namely, 4-{{4-[4-(methoxymethyl)phenoxy]benzyl}oxy}benzyl methyl ether[1]).

Other known compounds previously isolated from this plant were also identified in the present investigation, *i.e.*, as 4-hydroxybenzyl alcohol (= gastrodigenin) [3], 4-hydroxybenzyl methyl ether [3], 4-hydroxybenzaldehyde [3], 3-methoxy-4-hydroxybenzaldehyde (= vanillin) [18], 3-methoxy-4-hydroxybenzyl alcohol (= vanillyl alcohol) [19], 4-[(4-hydroxybenzyl)oxy]benzyl methyl ether [3], bis(4-hydroxybenzyl) ether [3], 4-(4-hydroxybenzyl)phenol [20], 2,4-bis(4-hydroxybenzyl)phenol [4], and 5-(hydroxymethyl)furan-2-carboxaldehyde [21], respectively, by comparison of their physical and spectroscopic data with those reported previously.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 230–400 mesh; *Merck*, Germany) and *YMC*Gel ODS-A* (S-150 μm; *YMC Co., Ltd.*, Japan). TLC: *silica gel 60* F_{254} and *RP-18* F_{254s} SiO_2 plates (*Merck*, Germany). HPLC: *Waters* instrument composed of a *1525* binary HPLC pump and a *2487* dual-wavelength absorbance detector, with an *YMC-Pack-Pro-C18* column (250 mm × 20 mm i.d.; *YMC Co., Ltd.*, Japan); t_R in min. UV Spectra: *Hitachi-U-3000* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad-FTS 135* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian-Unity-INOVA-400* FT-NMR instrument; chemical shift δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *Waters Acquity-UPLC* system coupled to a *Micromass-Q-Tof-Micro* spectrometer and *Agilent-6220-Accurate-Mass* TOF LC/MS system; in *m*/*z*.

Plant Material. The rhizomes of *Gastrodia elata* BLUME (Orchidaceae) were collected in Sangju, Gyeongsangbuk-do, Korea, in November 2009, and identified by one of the authors, *J.-H. L.* (Dongguk University, Geongju 780-714, Korea). A voucher specimen (No. EAD271) has been deposited with the College of Pharmacy, Ewha Womans University.

Extraction and Isolation. The dried rhizomes of *G. elata* (20 kg) were extracted with MeOH at r.t. (3 × 10 l, overnight). The extracts were concentrated *in vacuo* at 40° to afford a MeOH-soluble residue (790 g), which was then suspended in H_2O (5 l), and partitioned with hexane (3 × 5 l), AcOEt (3 × 5 l), and BuOH (3 × 5 l), sequentially. The AcOEt extracts (70 g) were separated by vacuum liquid CC (SiO_2 (700 g), 0.5–50% MeOH/CH_2Cl_2); *Fr. I*–*Fr. XXI*). From *Fr. V* and *VII*, 4-[(4-hydroxybenzyl)oxy]benzyl methyl ether (59.1 mg) and 4-hydroxybenzyl alcohol (= gastrodigenin, 3.57 g), resp., were isolated by

precipitation in $CHCl_3$. The residual portion of *Fr. VII* (12 g) was subjected to CC (*ODS-A* (200 g), MeOH/H_2O 1:1 → 4:1): *Fr. VII.1 – VII.12*). *Fr. VII.2* (776.8 mg) was subjected again to CC (*ODS-A* (100 g), MeCN/H_2O 3:2): 4-hydroxybenzyl methyl ether (215.2 mg). *Fr. FVII.3* (1.7 g) was subjected to CC (*ODS-A* (100 g), MeCN/H_2O 3:2): bis(4-hydroxybenzyl) ether (811.8 mg). *Fr. VII.5* (160.1 mg) was subjected to CC (*ODS-A* (100 g), MeCN/H_2O 3:2) and then purified by prep. HPLC (*RP-C_{18}*, MeOH/H_2O 3:2, 3 ml/min): 2,4-bis(4-hydroxybenzyl)phenol (t_R 86.4; 12.7 mg). *Fr. II* (16 mg) was subjected to prep. HPLC (*RP-C_{18}*, MeOH/H_2O 3:2, 4 ml/min): 3-methoxy-4-hydroxybenzaldehyde (=vanillin; t_R 17.3; 1.0 mg). *Fr. III* (9 g) was subjected to flash CC (SiO_2 (150 g), hexane/AcOEt 8:1 → 1:1): 4-hydroxybenzaldehyde (119.4 mg) and *Fr. III.1 – III.11*). *Fr. III.2* was purified by prep. HPLC (*RP-C_{18}*, MeOH/H_2O 9:1, 4 ml/min): **2** (t_R 33.1; 2.1 mg). *Fr. IV* (695 mg) was subjected to flash CC (SiO_2 (150 g), hexane/AcOEt 4:1): **1** (24.6 mg), 4-(4-hydroxybenzyl)phenol (155.4 mg), and *Fr. IV.1 – IV.14*). *Frs. IV.10 – IV.12* (37.9 mg) were subjected to prep. HPLC (*RP-C_{18}*, MeOH/H_2O 2:3, 3 ml/min): 5-(hydroxymethyl)furan-2-carboxaldehyde (t_R 24.4; 2.0 mg) and 3-methoxy-4-hydroxybenzyl alcohol (=vanillyl alcohol) (t_R 29.9; 5.0 mg).

4-Hydroxybenzyl Vanillyl Ether (=*4-{[(4-Hydroxyphenyl)methoxy]methyl}-2-methoxyphenol*; **1**): White amorphous powder. UV (MeOH): 228 (4.51), 278 (3.94). IR (film): 3361, 3246, 2986, 2931, 1612, 1516, 1359, 1273. ^{1}H-NMR (CD_3OD, 400 MHz): 7.16 (*d*, *J* = 8.8, H–C(2,6)); 6.89 (br. *s*, H–C(2′)); 6.77 (*dd*, *J* = 8.0, 1.2, H–C(6′)); 6.76 (*d*, *J* = 8.8, H–C(3,5)); 6.75 (*d*, *J* = 8.0, H–C(5′)); 4.40 (*s*, CH_2(7′)); 4.39 (*s*, CH_2(7)); 3.83 (*s*, MeO). ^{13}C-NMR (CD_3OD, 100 MHz): 158.3 (C(4)); 149.0 (C(3′)); 147.4 (C(4′)); 131.0 (C(1)); 130.9 (C(2,6)); 130.3 (C(1′)); 122.3 (C(6′)); 116.2 (C(3,5)); 115.9 (C(5′)); 113.0 (C(2′)); 72.9 (C(7′)); 72.7 (C(7)); 56.4 (MeO). HR-ESI-MS: 260.1041 (M^+, $C_{15}H_{16}O_4^+$; calc. 260.1049).

4-{{4-[4-(Methoxymethyl)phenoxy]benzyl}oxy}benzyl Methyl Ether (=*1-[4-(Methoxymethyl)phenoxy]-4-{[4-(methoxymethyl)phenoxy]methyl}benzene*; **2**): White amorphous powder. UV (MeOH): 226 (4.23), 276 (3.47). IR (film): 2928, 1615, 1516, 1448, 1378, 1233. ^{1}H-NMR (CD_3OD, 400 MHz): 7.24 (*d*, *J* = 8.8, H–C(2′,6′)); 7.23 (*d*, *J* = 8.8, H–C(2,6)); 7.15 (*d*, *J* = 8.8, H–C(3″,5″)); 6.95 (*d*, *J* = 8.8, H–C(3,5)); 6.77 (*d*, *J* = 8.8, H–C(3′,5′)); 6.75 (*d*, *J* = 8.8, H–C(2″,6″)); 4.94 (*s*, CH_2(7′)); 4.37 (*s*, CH_2(7)); 4.33 (*s*, CH_2(7″)); 3.33 (*s*, 2 MeO). ^{13}C-NMR (CD_3OD, 100 MHz): 160.2 (C(4)); 158.5 (C(4′)); 158.4 (C(1″)); 131.5 (C(1)); 130.8 (C(3″,5″)); 130.6 (C(2′,6′)); 130.5 (C(2,6)); 130.1 (C(4″)); 129.4 (C(1′)); 116.2 (C(3′,5′)); 116.1 (C(2″,6″)); 115.9 (C(3,5)); 75.6 (C(7″)); 75.4 (C(7)); 71.1 (C(7′)); 57.9 (MeO); 57.8 (MeO). HR-ESI-MS (pos.): 364.1695 (M^+, $C_{23}H_{24}O_4^+$; calc. 364.1675).

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