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Note

# Isolation of 3-*O*-(4'-Hydroxybenzyl)-β-sitosterol and 4-[4'-(4''-Hydroxybenzyloxy)benzyloxy]benzyl methyl Ether from Fresh Tubers of *Gastrodia elata*

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Two new 4-hydroxybenzyl alcohol derivatives (**1** and **2**) were isolated from the methanol extract obtained from fresh tubers of *Gastrodia elata* together with 4-hydroxybenzyl methyl ether, 4-hydroxybenzyl alcohol, bis(4-hydroxyphenyl)methane, 4-hydroxybenzaldehyde, β-sitosterol and palmitic acid. **1** and **2** were identified as 3-*O*-(4'-hydroxybenzyl)-β-sitosterol and 4-[4'-(4''-hydroxybenzyloxy)benzyloxy]benzyl methyl ether, respectively, according to the spectroscopic data.

**Key words :** *Gastrodia elata*, 3-*O*-(4'-Hydroxybenzyl)-β-sitosterol, 4-[4'-(4''-Hydroxybenzyloxy) benzyloxy]benzyl methyl ether

## INTRODUCTION

*Gastrodia elata* Blume (Orchidaceae) is a saprophyte growing in the woods of Korea, China and Japan. The tubers of this plant have been considered as one of the very important herbal medicines in oriental countries and were used for the treatment of headaches, migraine, dizziness, childhood convulsions, epilepsy, rheumatism, neuralgia and other neuralgic and nervous affections (Bensky and Gamble, 1986; Tang and Eisenbrand, 1992). The tubers have also been prescribed for the treatment of symptoms related to various conditions of thrombosis. We have found that a crude extract and several solvent fractions of this plant attenuated the thrombotic symptoms in animal models of thrombosis (Paik *et al.*, 1995; Yun-Choi *et al.*, 1985; Yun-Choi *et al.*, 1986).

Investigations on the chemical components of this plant have revealed the presence of several 4-hydroxybenzyl derivatives including 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol, 4-hydroxybenzyl methyl ether, 4-(4'-hydroxybenzyloxy)benzyl methyl ether, bis(4-hydroxybenzyl)ether, 4-(β-D-glucopyranosyloxy)benzyl alcohol, tris[4-(β-D-glucopyranosyloxy)benzyl]citrate (Yaguchi *et al.*, 1981), 1,2- and 1,3-bis[4-(β-D-glucopyranosyloxy)benzyl]citrate (Lin *et al.*, 1996), 2,4-bis(4-hydroxybenzyl)phenol (Noda *et al.*, 1995) and 4,4'-dihydroxybenzyl sulfoxide (Yun-Choi *et al.*, 1997). Most of the phytochemical studies were carried out with steamed and dried tubers, since these are commercially available in many oriental countries.

From the fresh tubers, the isolation of five 4-hydroxybenzyl derivatives, bis(4-hydroxyphenyl)methane, 4,4'-dihydroxydibenzyl ether, 4-ethoxymethylphenyl-4'-hydroxybenzyl ether, 4-ethoxymethylphenol and 3, 4-dihydroxybenzaldehyde was reported without any spectroscopic data (Zhou *et al.*, 1980). We now describe two new hydroxybenzyl derivatives **1** and **2** from fresh tubers, together with several other components.

## MATERIALS AND METHODS

Melting points were determined on a Mitamura-Riken melting point apparatus and were uncorrected. IR spectra were recorded on a Jasco FT/IR-5300 spectrometer. $^1$H NMR spectra were taken at 300 MHz on a Varian Gemini 2000 spectrometer, and $^{13}$C NMR spectra were taken at 75.5 MHz on a Varian Gemini 2000 spectrometer or at 125 MHz on Bruker AMX-500 spectrometer. Mass spectra were taken with a VG Analytical VG 70-VSEQ. $^{13}$C NMR spectra at 125 MHz and mass spectra were taken at Seoul Branch Analytical Lab., Korea Basic Science Institute. Bis(4-hydroxyphenyl)methane, 4-hydroxybenzaldehyde and 4-hydroxybenzyl alcohol were purchased from Aldrich Chem. Co., Inc., WI, U.S.A..

### Plant materials

Fresh tubers of *Gastrodia elata* were purchased from Korean Agricultural Development Farm in Seoul.

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They were identified by Prof. Hyung Joon Chi and the voucher specimen were deposited at the Herbarium, Natural Products Research Institute, Seoul National University.

### Extraction and isolation

Fresh tubers of *Gastrodia elata* were sliced and percolated in 80% MeOH for several weeks at room temperature. The extract concentrated *in vacuo* was partitioned between $CHCl_3$ and $H_2O$, and the $CHCl_3$ layer upon concentration was further partitioned with hexane and 90% MeOH. The MeOH soluble fraction, after removal of the solvent, was dissolved in hot toluene and filtered on cooling. The precipitate (25 g) was subjected to silica gel chromatography (2 Kg) eluting with $CHCl_3$-MeOH (100:1) affording **1** (10 mg), **3** (30 mg), **4** (6 g) and **5** (50 mg). The mother liquors were concentrated (5 g) and also applied to a silica gel column (500 g) eluting again with $CHCl_3$-MeOH (100:1) to give **3** (40 mg), **2** (10 mg), **4** (800 mg), **6** (50 mg), **7** (80 mg), **8** (10 mg) and **5** (10 mg) in the order of elution.

### 3-*O*-(4'-Hydroxybenzyl)-β-sitosterol (1)

White needles: mp. 189~192°C (from MeOH): $^1$H NMR ($Me_2CO$-$d_6$) δ: 0.72, 1.02 (3H each, s, H-18, 19), 0.83, 0.85, 0.96 (3H each, d, *J*=6.6 Hz, H-21, 26, 27), 0.86 (3H, t, *J*=6.9 Hz, H-29), 3.2 (1H, m, H-3), 4.44 (2H, s, H-α), 5.34 (1H, m, H-6), 6.79, 7.17 (2H each, d, *J*=8.7 Hz, H-2', 3'), 8.17 (1H, s, OH). $^{13}$C NMR ($CDCl_3$) see Table I. IR $\nu_{max}$ (KBr) cm$^{-1}$: 3389 (br, OH), 1616, 1520 (aromatic), 1269 (ether). EIMS *m/z* (relative int., %): 414 (22), 396 (14), 381 (3), 329 (4), 303 (7), 273 (3), 255 (4), 213 (6), 107 (100), 78 (31).

### 4-[4'-(4''-Hydroxybenzyloxy)benzyloxy]benzyl methyl ether (2)

White amorphous solid: mp. 144~145°C (from MeOH): $^1$H NMR ($Me_2CO$-$d_6$) δ: 3.29 (3H, s, $OCH_3$), 4.35 (2H, s, H-α), 5.02, 5.04 (2H each, s, H-α', α''), 6.86, 6.98, 7.02, 7.26, 7.32, 7.40 (2H each, d, *J*=8.7 Hz), 8.39 (1H, s, OH); $^{13}$C NMR ($Me_2CO$-$d_6$) δ: 57.74 ($OCH_3$), 70.24, 70.47 (C-α', C-α''), 74.65 (C-α), 115.51, 115.71, 116.17 (C-3, C-3', C-3''), 129.17, 130.53, 131.90 (C-1, C-1', C-1''), 130.12, 130.27, 130.50 (C-2, C-2', C-2''), 158.35, 159.55, 159.91 (C-4, C-4', C-4''). IR $\nu_{max}$ (KBr) cm$^{-1}$: 3408 (br, OH), 1618, 1518 (aromatic), 1261, 1084 (ether): EIMS *m/z* (relative int., %): 244 (16), 213 (35), 137 (27), 107 (100), 78 (49).

## RESULTS AND DISCUSSION

Fresh tubers of *Gastrodia elata* were extracted with 80% MeOH. The extract was partitioned between $CHCl_3$ and $H_2O$, and the $CHCl_3$-soluble fraction was partitioned again between hexane and 90% MeOH. The 90% MeOH phase was crystallized from toluene. The precipitate and the mother liquor, after removal of the solvent, were separately subjected to column chromatography on silica gel to afford compounds **1, 3, 4** and **5** from the former and compounds **3, 2, 4, 6, 7** and **8** from the latter in the order of elution.

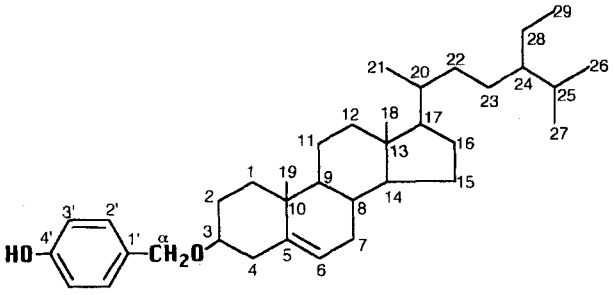
**Table I.** Comparison of the $^{13}$C NMR Data of compound **1** with those of β-sitosterol (**3**) and 4-hydroxybenzyl methyl ether (**5**)

| | 1 | 3 | | 1 | 5 |
|---|---|---|---|---|---|
| 1 | 37.29 | 37.32 | 1' | 129.44 | 129.64 |
| 2 | 26.69 | 31.71 | 2' | 129.34 | 130.07 |
| 3 | 78.39 | 71.94 | 3' | 115.18 | 115.55 |
| 4 | 39.20 | 42.39 | 4' | 154.92 | 155.98 |
| 5 | 141.05 | 141.01 | α | 69.55 | 74.64 |
| 6 | 121.53 | 121.95 | | | |
| 7 | 31.97 | 31.96 | | | |
| 8 | 31.93 | 31.96 | | | |
| 9 | 50.24 | 50.21 | | | |
| 10 | 36.93 | 36.57 | | | |
| 11 | 21.09 | 21.12 | | | |
| 12 | 39.82 | 39.84 | | | |
| 13 | 42.35 | 42.37 | | | |
| 14 | 56.82 | 56.86 | | | |
| 15 | 24.31 | 24.35 | | | |
| 16 | 28.24 | 28.31 | | | |
| 17 | 56.10 | 56.14 | | | |
| 18 | 11.86 | 11.88 | | | |
| 19 | 19.05 | 19.06 | | | |
| 20 | 36.15 | 36.21 | | | |
| 21 | 18.79 | 18.81 | | | |
| 22 | 33.99 | 34.00 | | | |
| 23 | 26.15 | 26.09 | | | |
| 24 | 45.89 | 45.91 | | | |
| 25 | 29.21 | 29.18 | | | |
| 26 | 19.38 | 19.44 | | | |
| 27 | 19.80 | 19.87 | | | |
| 28 | 23.10 | 23.10 | | | |
| 29 | 11.98 | 12.01 | | | |

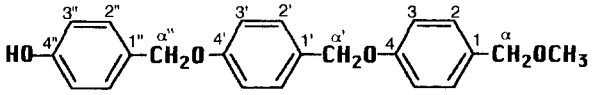
*The spectra were taken in $CDCl_3$.

Compounds **4, 5** and **7** were known components of this plant, 4-hydroxybenzyl methyl ether, 4-hydroxybenzyl alcohol and 4-hydroxybenzaldehyde, respectively (Yaguchi *et al.*, 1981). Compound **6** was identified as bis(4-hydroxyphenyl)methane (Charles and Jacqlynn, 1993), whose isolation from this plant was reported earlier without spectral data (Zhou *et al.*, 1980). Compounds **3** and **8** were β-sitosterol and palmitic acid, two natural products widely distributed in plants (Chang *et al.*, 1981; Charles and Jacqlynn, 1993; Dey and Harborne, 1991).

Compound **1** was obtained as white needles upon recrystallization from MeOH. The IR spectrum exhibited the presence of a hydroxyl (3389 cm$^{-1}$) group. The



1. 3-O-(4'-Hydroxybenzyl)-β-sitosterol



2. 4-[4'-(4''-Hydroxybenzyloxy)benzyloxy]benzyl methyl ether

$^1$H-NMR spectrum of **1** showed two two-proton doublets at δ 7.17 and 6.79 ($J$=8.7 Hz) ascribable to a 1,4-disubstituted aromatic ring, one singlet due to a phenolic hydroxy proton at δ 8.17, and a two-proton singlet at δ 4.44. The above $^1$H-NMR data are indicative of the presence of a 4-hydroxybenzyloxy group. The remaining proton signals are suggestive of a sterol moiety: one unsaturated proton signal at δ 5.34, two methyl singlets at δ 0.72 and 1.02, three methyl doublets at δ 0.83, 0.85 and 0.96 and one methyl triplet at δ 0.86. The $^{13}$C-NMR spectram (with DEPT) confirmed the presence of a 4-hydroxybenzyloxy group with four aromatic signals at δ 115.18, 129.34, 131.46 and 154.92. The carbons at δ 141.05 and 121.53 were assigned to a double bond in the sterol moiety. The remaining carbon signals matched well with those of β-sitosterol (Chang *et al.*, 1981). On the basis of the above spectral data, compound **1** was determined as 3-O-(4'-hydroxybenzyl)-β-sitosterol. The structure of **1** was supported by the mass spectum (Dey and Harborne, 1991), which showed peaks due to fragmentation of the β-sitosterol moiety (*m/z* 414, 396, 381, 329, 303, 273, 255, 213) and a peak due to a hydroxybenzyl fragment (*m/z* 107).

Compound **2** was obtained as a white powder upon recrystallization from MeOH. The IR spectrum exhibited the presence of hydroxyl (3408 cm$^{-1}$), aromatic C=C (1618, 1518 cm$^{-1}$) and ether (1261, 1084 cm$^{-1}$) groups. The $^1$H-NMR spectrum showed a phenolic hydroxyl peak (δ 8.39) and three sets of coupled doublets at δ 6.86, 6.98 and 7.02 and at δ 7.26, 7.32 and 7.40 with $J$=8.7 Hz each. Three singlets ascribable to three pairs of oxygenated methylene protons (δ 4.35, 5.02 and 5.04) and a methoxyl proton singlet (δ 3.29) were also shown. In turn, the $^{13}$C-NMR (with DEPT) spectra exhibited 16 peaks including a methoxyl carbon peak at δ 57.74, three oxygenated methylene carbons at δ 70.24, 70.47 and 74.65 and twelve aromatic carbon signals. The above data suggested that there are three 1,4-disubstituted benzene rings, and the structure of **2** was determined as 4-[4'-(4''-hydroxybenzyloxy)benzyloxy]benzyl methyl ether. The structure of **2** was supported by the mass spectrum, which showed a base peak at *m/z* 107 corresponding to a hydroxybenzyl fragment and a peak at *m/z* 244 corresponding to 4-(4'-hydroxybenzyloxy)benzyl methyl ether. The structure of **2** was further confirmed by comparing the spectral data of **2** with those of 4-(4'-hydroxybenzyloxy)benzyl methyl ether (Yaguchi *et al.*, 1981).

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