A New Citryl Glycoside from *Gastrodia elata* and Its Inhibitory Activity on GABA Transaminase

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Received May 15, 2006; accepted September 19, 2006

A new citryl glycoside, trimethylcitryl-β-D-galactopyranoside (1) along with a known phenolic compound, gastrodigenin (2) have been isolated from the active fraction of the rhizomes of *Gastrodia elata* (Orchidaceae). Their structures were elucidated on the basis of spectroscopic data and chemical reaction. 1 inhibited GABA transaminase activity by 56.8% at the final concentration of 10 μg/ml.

Key words *Gastrodia elata*; Orchidaceae; trimethylcitryl-β-D-galactopyranoside; gastrodigenin; GABA transaminase

The rhizomes of *Gastrodia elata* Blume (Gastrodiae Rhizoma, Orchidaceae) have been used in traditional medicine in Korea, Japan and China as an anticonvulsant, an analgesic and a sedative against general paralysis, epilepsy, vertigo and tetanus.[1] Its constituents have mainly been examined for phenolic compounds: besides a major phenolic glucoside—gastrodin [4-(β-D-glucopyranosyloxy)benzyl alcohol], more than 15 phenolics have been isolated.[2—5] Among them, three compounds—tris[(4-β-D-glucopyranosyloxy)benzyl] citrate (parishin), 1,2- and 1,3-bis[(4-(β-D-glucopyranosyloxy)benzyl] citrate (parishin B and C, respectively) contain citrate moiety, further, 1,5-dimethylcitrate[6] was recently reported. Gastrodiae Rhizoma showed a variety of pharmacological effects including GABAergic neuromodulatory,[7] anticonvulsant,[8] anxiolytic,[9] anti-aggregating[10] and antioxidative activities.[11] Besides that, the extracts of this medicinal plant reduced focal ischemic brain injury,[12] protected erythrocyte membrane damage,[13] and prevented rat pheochromocytoma cells from serum-deprived apoptosis.[14] Even though this herbal medicine has been prescribed for a long time for the purpose of sedation, its constituents have not thoroughly been investigated. As a part of our continuing search[7,8,15—19] for natural or synthetic sedatives, we have investigated the inhibitory activity of the extracts of Gastrodiae Rhizoma on GABA transaminase (GABA-T) which degrades GABA (γ-aminobutyric acid), a major CNS inhibitory neurotransmitter. Activity-guided fractionation of the crude extracts resulted in the isolation of a new glycoside together with a known phenolic compound. In this paper, the isolation, structure elucidation and enzyme assay of the isolated components from the rhizomes of *G. elata* are described.

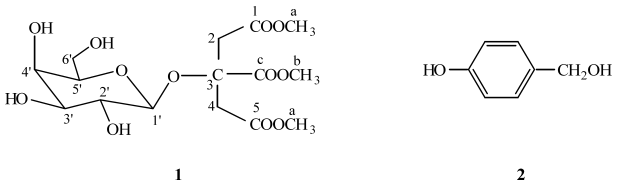
Table 1. Inhibitory Effects of the Tested Compounds on the Activity of GABA Transaminase *in Vitro*

	% Inhibition
Control	0
Methanol extract	8.99±3.71
Methylene chloride fraction	20.64±9.53
Ethylacetate fraction	39.63±1.70**
Butanol fraction	46.48±5.24*
1	56.80±0.21**
2	30.87±3.06*
Oily material	30.47±3.42
Valproic acid	65.38±1.45**

Final concentration: 10 μg/ml. Data represent mean±S.E. (n=3) with significant differences from control at ** $p<0.01$ and * $p<0.05$.

The crude methanolic extracts of Gastrodiae Rhizoma inhibited the GABA-T activity. Activity-guided fractionation of the extracts indicated that butanol fraction is the most effective (46.5% inhibition, Table 1), so we tried to isolate the active components from this fraction. Chromatographic separation of the butanol fraction gave three compounds, among them, the structures of two pure crystals (**1**, **2,** Fig. 1) were determined but an oily material could not be identified.

Compound **1** appeared as colorless powder. Its sugar test (Molish test) was positive, indicating **1** to be a glycoside. Its FAB-MS showed a peak at m/z 419 for $[M+Na]^+$ and at m/z 235 as a base peak. Its ^{1}H-NMR spectrum (Table 2) exhibited three methoxy signals at δ 3.65 and 3.76 and a single AB quartet spin system for germinal protons ($2\times CH_2$) at δ 2.78 and 2.95 (J=15.6 Hz). The characteristic signal for anomeric



1 **2**

Fig. 1. Structures of the Isolated Components **1** and **2**

Table 2. ^{1}H- and ^{13}C-NMR Data of Compounds **1** and Trimethylcitrate

H/C	**1**		Trimethylcitrate
	δ ^{1}H (m, J Hz)	δ ^{13}C	δ ^{13}C
1	—	170.9	170.8
2	2.78, 2.95 (AB, 15.6)	43.5	43.2
3	—	74.2	73.8
4	2.78, 2.95 (AB, 15.6)	43.5	43.2
5	—	170.9	170.8
CH_3 [a)]	3.65	51.4	51.5
CH_3 [b)]	3.76	52.3	52.4
COO [c)]	—	171.2	174.2
1′	4.77 (d, 7.8)	97.9	
2′	3.65 (dd, 9.8, 7.6)	70.8	
3′	3.51 (dd, 9.6, 3.2)	73.0	
4′	3.74 (d, 2.4)	70.4	
5′	3.55 (m)	75.8	
6′	3.71 (dd, 11.6, 4.4) 3.82 (dd, 11.6, 7.6)	61.9	

a—*c*) Protons and/or carbons of ester groups (see Fig. 1).

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proton was observed at δ 4.77 (d, J=7.8 Hz), which suggested the β-configuration of a sugar unit. In the ^{13}C-NMR spectrum of **1**, signals at δ 170.9 (2×CO) and 171.2 (CO), three methoxy carbons, two methylene carbons proposed the presence of trimethylcitrate moiety of which NMR data were identical with those of the authentic compound (Table 2). The anomeric carbon signal at δ 97.9, C-4′ signal at δ 70.4, further H-4′ proton at δ 3.74 (d, J=2.4 Hz) and the positive specific rotation value (+22.8°) proposed the sugar unit should be β-D-galactose. To confirm the structure, acid hydrolysis of **1** was performed. The sugar unit in the aqueous phase was identified as galactose and an aglycone moiety in the organic phase was characterized as citric acid derived from further hydrolysis of trimethylcitrate by comparison of their TLC patterns with those of authentic compounds. From above results, the structure of **1** was concluded to be trimethylcitryl-β-D-galactopyranoside. The base peak at m/z 235 in FAB-MS can now be confirmed as a peak of protonated trimethylcitrate.

To the best of our knowledge, this is the first report of its occurrence as a natural product and also a synthetic material. Compound **2** could easily be identified by ^{1}H- and ^{13}C-NMR as gastrodigenin (4-hydroxybenzyl alcohol), which is an aglycone of gastrodin,[2] a major phenolic glucoside of *G. elata*.

Compounds **1** and **2** and an oily material were examined their inhibitory activities on GABA-transaminase *in vitro*, exerting 56.8%, 30.9% and 30.5% inhibition against control, respectively. Valproic acid, a known anticonvulsant, revealed 65.4% inhibition.

Experimental

General Melting points were measured on an Electrothermal IA9100 apparatus and are uncorrected. NMR spectra were recorded on a Varian UNITY-500 or Varian GEMINI-200 spectrophotometer using CD_3OD as a solvent. FAB-MS spectra were acquired with a Jeol JMS 700 mass spectrometer. Specific rotation was measured on a Jasco DIP-370 digital polarimeter. Optical density was determined with Ultraspec 2000 spectrophotometer. HPLC analysis was carried out with a Shimadzu LC-10AD system equipped with an Eclipse XDB-C18 column (4.6×250 mm) using a solvent system of 0.05 M Na_2HPO_4 : acetonitrile 80 : 20 (flow rate: 1 ml/min) at 260 nm at room temp. TLC was done on Kieselgel 60F_{254} plate (Merck, 0.1 mm).

Plant Material The dried rhizomes of *Gastrodia elata* were purchased from one of the oriental drugstores in Youngchon, Korea, and identified by Prof. Byung-Soo Kang, College of Oriental Medicine, Dongguk University, Gyeongju, Korea. A voucher specimen of this plant material is deposited under the Herbarium (No. SKK-F001) at Sungkyunkwan University, Seoul, Korea.

Extraction and Isolation The dried and pulverized rhizomes of the plant (600 g) were extracted with 80% methanol (2×2 l) and then concentrated to give a brown oily extract (25 g), which was successively fractionated with 200 ml of each of CH_2Cl_2 (2.5 g), EtOAc (4.5 g) and *n*-BuOH (3.2 g) in sequence to yield the corresponding dried extracts. The most active *n*-BuOH fraction was subjected to column chromatography on silica gel (5—40 μm) using $CHCl_3$–MeOH (gradient, 19 : 1 to 9 : 1) to furnish five fractions. Successive column chromatography of subfraction 4 (solvent, $CHCl_3$–MeOH 1 : 1) afforded compounds **1**, **2** and an oily material. Compound **1** was further purified by YMC gel (12 nm, S-75 μm) column chromatography (solvent, EtOAc–MeOH, 100 : 1) yielding a pure crystal. Its purity was analyzed by reverse-phase HPLC to be 98.8% (t_R=2.458 min).

Trimethylcitryl-β-D-galactopyranoside (**1**): Coloress powder. mp 136—139 °C, $[\alpha]_D^{25}$ +22.8° (c=0.309, MeOH), FAB-MS m/z: 419 [(M+Na]$^+$, 235 (trimethylcitrate+H$^+$, base peak), ^{1}H- and ^{13}C-NMR data: see Table 2.

4-Hydroxybenzyl Alcohol (**2**, Gastrodigenin): Pink powder. mp 119—121 °C (116—117 °C),[2] ^{1}H-NMR δ: 7.19 and 7.16 (2H, AA′ part of Ar protons), 6.78 and 6.74 (2H, BB′ part of Ar protons), 4.92 (br, OH after D_2O exchange), 4.48 (2H, s, CH_2). ^{13}C-NMR δ: 64.26 (CH_2), 115.18 (C-2, C-6), 128.93 (C-3, C-5), 132.54 (C-1), 153.58 (C-4).

Acid Hydrolysis of 1 Compound **1** (1.0 mg) was refluxed with 2 N HCl in MeOH for 5 h. The reaction mixture was diluted with water and extracted with EtOAc. The aqueous layer was neutralized with saturated sodium carbonate. The sugar unit in the filtrate was identified as galactose and the EtOAc residue was confirmed to be citric acid by comparison of their TLC patterns with those of authentic samples. Galactose: *Rf* 0.34, *n*-butanol–acetic acid–diethylether–H_2O 9 : 6 : 3 : 1. Citric acid: *Rf* 0.52, 1,2-dichloroethane–MeOH–formic acid 7 : 3 : 0.5 (colorization using iodine chamber after spraying with 0.1% alcoholic bromocresol green).

Enzyme Assay One tenth milliliter of GABA (873 mM), 0.05 ml of α-ketoglutaric acid (174 mM), 2.1 ml of potassium phosphate buffer (pH 8.0, 0.15 M), 0.05 ml of GABAse (2.5 mg/ml) and 0.1 ml of compounds (0.1 mg/ml) were incubated in 37 °C for 30 min, and followed by addition of $NADP^+$ according to the method of Bergmeyer.[20] Amount of NADPH generated for 20 min was measured by spectrophotometer at 340 nm as an activity of GABA transaminase.

Acknowledgments This work was supported by grant No. R05-2002-000-00682-0 from the Basic Research Program of the Korea Science & Engineering Foundation.

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