Practical Synthesis of Novel Citryl Glycoside, the Component of the Rhizomes of *Gastrodia elata*

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The rhizome of *Gastrodia elata* Blume (Gastrodiae Rhizoma, Orchidaceae) has been used in traditional medicine as an anticonvulsant and sedatives in Korea, Japan and China.[1] Identification of its constituents has focused mainly on 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, tris[(4-β-D-glucopyranosyloxy)benzyl] citrate (parishin), 1,2-bis[(4-(β-D-glucopyranosyl-oxy)benzyl] citrate (parishin B) and 1,3-bis[(4-(β-D-glucopyranosyl-oxy) benzyl] citrate (parishin C) contain a citrate moiety, further, 1,5-dimethylcitrate[6] was also reported. Recently, we isolated a citrate containing constituent from Gastrodiae Rhizoma, and characterized its structure as trimethylcitryl-β-D-galactopyranoside.[7] This new natural citrate glycoside shows an inhibitory activity on GABA transaminase, suggesting an anticonvulsive effect.

We report the practical synthesis of trimethylcitryl-β-D-galactopyranoside from citric acid.

The synthetic procedure presented in Figure 1 shows that the synthesis proceeded with selective esterification, glycosylation[8] and deacetylation, respectively. In the first step, anhydrous citric acid (**1**) was selectively methylated with methanolic H_2SO_4 to give 1,5-dimethyl citrate (**2**) in 60% yield. This sym-dimethyl citrate was further methylated with methanolic H_2SO_4 by addition of 2,2-dimethoxypropane to produce trimethylcitrate (**3**) in 69% yield. COSY, HMBC, HMQC and NOESY spectra proved this structure (data not shown). The direct esterification of **1** to **3** with methanolic HCl resulted in a poor yield (20%).[9] In the third step, **3** was coupled with galactose pentaacetate by using boron trifluoride diethyl etherate (BF_3-Et_2O) to produce new synthetic trimethylcitryl-β-D-tetraacetylgalatopyranoside (**4**) in 90% yield. The characteristic signal for anomeric proton was observed at δ 4.47 (d, J = 7.7 Hz), which suggested the β-configuration of a sugar unit. The positive specific rotation value (+51.2°) was consistent with the identity of the sugar unit as β-D-galactose. Positive ion-direct chemical ionization mass spectrometry (PI-DCIMS) showed peaks at *m/z* 582 for $[M + NH_4]^+$, at *m/z* 366 for [galactose tetraacetate + $NH_4]^+$, and at *m/z* 252 for [trimethylcitrate + $NH_4]^+$. Its ^{1}H-NMR and ^{13}C-NMR spectral data are given in Table 1 and 2, respectively.

In the final step, compound **4** was deacetylated by sodium methoxide followed by neutralization by passage through an Amberlite IR-120 (H^+) ion exchange column[10] to provide trimethylcitryl-β-D-galactopyranoside (**5**) as colorless crystals. Instrumental and physical analyses of the synthetic compound were identical with the authentic natural compound previously isolated from the roots of *Gastrodia elata*. The

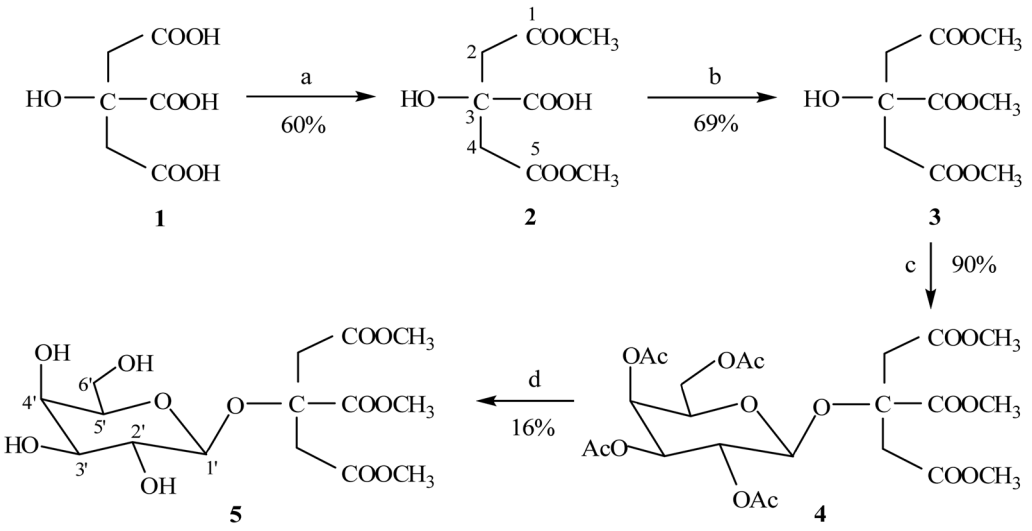

Figure 1. Synthesis of trimethylcitryl-β-D-galactopyranoside (**5**). MeOH, c-H_2SO_4, reflux, 1 h. (b) MeOH, 2,2-dimethoxypropane, c-H_2SO_4, reflux, 7 h. (c) β-D-galactose pentaacetate, BF_3-Et_2O, rt, 3 h. (d) i: CH_3ONa, N_2 stream, rt, 1 h. ii: neutralization by passage through Amberlite IR-120 (H^+) column.

Table 1. ^{1}H-NMR Spectral Data of Compounds **2-5** in CD_3OD

H	2	3	4	5
2 [a]	2.81, 2.95	2.90, 3.11	2.75, 2.98	2.78, 2.95
4 [a]	2.81, 2.95	2.90, 3.11	2.75, 2.98	2.78, 2.95
CH_3	3.66, 3.76	3.73 (2xMe)	3.66 (2xMe)	3.65 (2xMe)
		3.85	3.77	3.76
OAc	–	–	1.98-2.17 (m)	–
1'	–	–	4.47 (d, 7.7)	4.77 (d, 7.8)
2'	–	–	5.43 (dd, 9.4, 7.7)	3.65 (dd, 9.8, 7.6)
3'	–	–	5.33 (dd, 9.4, 7.7)	3.51 (dd, 9.6, 3.2)
4'	–	–	5.25 (dd, 9.4, 7.7)	3.74 (d, 2.4)
5'	–	–	3.35 (m)	3.55 (m)
6'	–	–	4.07 (dd, 12.2, 2.5)	3.71 (dd, 11.6, 4.4)
			4.14 (dd, 12.2, 4.6)	3.82 (dd, 11.6, 7.6)

[a]AB system. J = 15.6 Hz

Table 2. ^{13}C-NMR Spectral Data of Compounds **2-5** in CD_3OD

C	2	3	4	5
1	170.8	172.1	171.5	170.9
2	43.5	43.8	44.2	43.5
3	73.4	74.0	74.6	74.2
4	43.5	43.8	44.2	43.5
5	170.8	172.1	171.5	170.9
CH_3	51.5	52.7 (2xMe)	52.2 (2xMe)	51.4 (2xMe)
	51.5	53.5	53.1	52.3
COO	175.4	175.4	171.8	171.2
Acetyl-CH_3	–	–	20.5	–
Acetyl-CO	–	–	175.0	–
1'	–	–	96.3	97.9
2'	–	–	72.0	70.8
3'	–	–	72.6	73.0
4'	–	–	70.1	70.4
5'	–	–	74.5	75.8
6'	–	–	62.9	61.9

final step of the reaction, however, gave a relatively poor yield (16%). The major product of this deprotection step was, unexpectedly, trimethylcitrate (**3**), which can be produced by nucleophilic attack of sodium methoxide to the glycosidic linkage. Deacetylation of peracetylated glycosides under acidic conditions leads to the cleavage of the glycosidic bond.[11]

In conclusion, we were successful in synthesizing natural trimethylcitryl-β-D-galactopyranoside (**5**), starting from citric acid in a four-step reaction.

Experimental Section

General. Melting point was measured on an Electrothermal IA9100 apparatus (Thermo Scientific, Pittsburgh, PA, USA) and are uncorrected. NMR spectra were recorded on a UNITY-500 or GEMINI-200 spectrophotometer (Varian, Palo Alto, CA, USA) using CD_3OD as a solvent. PI-DCIMS spectra were measured with a Model MAT95 or LCQ mass spectrometer (Thermo Scientific) and fast atom bombardment (FAB) mass spectra were acquired with a JMS 700 mass spectrometer (Jeol, Tokyo, Japan). Specific rotation was measured on a DIP-370 digital polarimeter (Jasco, Easton, MD, USA). Thin layer chromatography utilized a Kieselgel 60F_{254} plate (0.1 mm; Merck, Darmstadt, Germany) using a solvent system of 1,2-dichloroethane:methanol: formic acid (7:3:0.5), a spray consisting of 0.1% alcoholic bromocresol green, then coloration in an iodine chamber for citric acid derivatives. For glycosides, a solvent system consisting of *n*-butanol:acetic acid:diethylether:water (9:6:3:1) and detection by spraying with diphenylamine reagent followed by heat-mediated coloration were used. Citric acid monohydrate, boron trifluoride diethyl etherate, β-D-galactose pentaacetate, 2,2-dimethoxypropane, and Amberlite IR-120 (H^+) ion exchange resin were all purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of 1,5-dimethylcitrate (2). Commercial citric acid monohydrate was dried for 24 h under reduced pressure at 85 °C to give anhydrous citric acid (**1**). Compound **1** (34.6 g, 0.18 mol) in methanol (200 mL) and c-H_2SO_4 (1 mL) was refluxed for 1 h. The reaction mixture was diluted with water and neutralized with 1 N NaOH to generate a clear solution (pH 7.0), which was thoroughly concentrated *in vacuo*. The residue was suspended in acetone and filtered to remove by-products. After the filtrate was concentrated and suspended with water, c-HCl was added slowly in an ice bath, followed by stirring for 10 min. The precipitate was collected and washed with water, then recrystallized with 30% MeOH to yield compound **2** (23.8 g, 60% yield) as colorless amorphous crystals. Rf value: 0.80. mp 109-115 °C (116-121 °C,[12] 122-124 °C[13]). FT-IR (nujol) cm^{-1}: 3476 (OH,), 1742 (ester); PI-DCIMS *m/z*: 221 $[M + H]^+$; ^{1}H- and ^{13}C-NMR data: see Table 1 and Table 2, respectively. The OH signal (δ 4.88 ppm) in COOH group could be assigned after D_2O exchange.

Preparation of trimethylcitrate (3). Compound **2** (15.6 g, 0.07 mol) in MeOH (190 mL), 2,2-dimethoxypropane (10 ml) and c-H_2SO_4 (1.25 mL) was refluxed for 7 h. After the reaction mixture was thoroughly concentrated *in vacuo*, the remaining oily material was crystallized with 30% MeOH to afford compound **3** (11.3 g, 69% yield) as colorless amorphous crystals. Rf value: 0.88. mp 72-76 °C (76 °C[14]). FT-IR (nujol) cm^{-1}: 3486 (OH), 1756 (ester); PI-DCIMS *m/z*: 235 $[M + H]^+$, 252 $[M + NH_4]^+$; ^{1}H- and ^{13}C-NMR data: see Table 1 and Table 2, respectively. The OH proton (δ 1.32 ppm) almost disappeared after D_2O exchange.

Synthesis of trimethylcitryl-β-D-tetraacetylgalatopyranoside (4). BF_3-Et_2O (1.42 g, 0.01 mol) was added dropwise to the mixture of β-D-galactose pentaacetate (3.9 g, 0.01 mol), methylene chloride (60 mL) and trimethylcitrate (2.34 g, 0.01 mol). The mixture was kept in the dark using aluminium foil and stirred for 3 h at room temperature. Excess BF_3-Et_2O was decomposed with a saturated $NaHCO_3$ solution. After dilution with methylene chloride, the mixture was washed with water, dried and concentrated to produce compound **4** (5.08 g, 90%) as a colorless oily material. Rf value: 0.71. $[\alpha]_D$ +51.2° (c = 0.363, CH_3OH); IR (nujol) cm^{-1}: 1753

(ester), 1739 (acetyl); PI-DCIMS *m/z*: 582 $[M + NH_4]^+$, 366 [galactose tetraacetate + $NH_4]^+$, 252 [trimethylcitrate + $NH_4]^+$; ^{1}H- and ^{13}C-NMR data: see Table 1 and Table 2, respectively).

Synthesis of trimethylcitryl-β-D-galactopyranoside (5). Compound **4** (175.4 mg, 0.31 mmol) was dissolved in anhydrous MeOH (5 mL) under a nitrogen atmosphere. Freshly prepared 0.1 N CH_3ONa (0.2 mL) was added dropwise to this solution under a nitrogen stream and stirred for 1 h at room temperature. After the reaction mixture was neutralized by passage through an Amberlite IR-120 (H^+) ion exchange column, the collected solution was concentrated to furnish a colorless oily material. The crystallization of this material with MeOH yielded amorphous crystals, which were identified as trimethylcitrate (**3**). From the mother liquid compound **5** (20 mg, 16%) was obtained as colorless crystals. Rf value: 0.27. mp: 136-139 °C; $[\alpha]_D$ +22.8° (c = 0.309, CH_3OH); IR (nujol) cm^{-1}: 3480 (OH), 1753 (ester); FAB-MS *m/z*: 419 $[M+Na]^+$, 235 [trimethylcitrate + H]$^+$ (base peak); ^{1}H- and ^{13}C-NMR data: see Table 1 and Table 2, respectively).

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