

A NEW CHLOROPHENYL GLYCOSIDE FROM THE BULBS OF *Lilium brownii* var. *viridulum*

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Thirteen compounds were isolated from the bulbs of *Lilium brownii* var. *viridulum* Baker, including a new chlorophenyl glycoside identified as 5-chloro-6-methoxy-4-methylphenyl 1-O- β -D-glucopyranosyl-(1 \rightarrow 6)- α -L-rhamnopyranoside. The structure of this new compound was elucidated using comprehensive spectroscopic analyses, acid hydrolysis, and derivatization.

Keywords: Liliaceae, *Lilium brownii* var. *viridulum*, chlorophenyl glycoside.

Lilium, an important medicinal herb with a long history, has been widely used in traditional Chinese medicine and the food industry. It can be found in various regions across Asia, Europe, and North America and it is native to China [1]. *Lilium* possesses multiple medicinal and nutritional values, which include nourishing Yin, moisturizing the lungs, and calming the mind. Previous studies have indicated that *Lilium* contains at least 82 steroidal saponins, 12 phenylpropanoid glycosides, 5 chlorophenyl glycosides, and some phenyl glycosides [2–4]. To further explore the chemical composition and new bioactive compounds from *Lilium*, we reported a new chlorophenyl glycoside, along with 12 known phenylpropanoid compounds.

The methanol extract of *Lilium* bulbs was subjected to both normal and reverse-phase silica column chromatography, as well as preparative HPLC to isolate a new chlorophenyl glycoside (**1**), 1-*O*-feruloylglycerol (**2**) [5], 1-*O*-*p*-coumaroylglycerol (**3**) [6], caffeic acid methyl ester (**4**) [7], 1-*O*-*p*-coumaroyl-3-*O*-feruloylglycerol (**5**) [8], 1,3-*O*-diferuloylglycerol (**6**) [9], 1-*O*-feruloyl-3-*O*-caffeoylglycerol (**7**) [8], 1-*O*-caffeoyl-3-*O*-*p*-coumaroylglycerol (**8**) [8], regaloside A (**9**) [10], regaloside B (**10**) [10], syringaresinol (**11**) [11], pinosresinol (**12**) [11], and epipinosresinol (**13**) [11]. Compounds **11**–**13** were isolated from the *Lilium* genus to our knowledge for the first time.

Compound **1** was obtained as a white amorphous powder. Its molecular formula was deduced as C₂₀H₂₉O₁₁Cl based on the HR-ESI-MS (*m/z* 479.1337 [M – H][–]; calcd 479.1318). Additionally, the isotopic molecular ion peak was observed at *m/z* 481.1311 with an abundance ratio of 3:1, indicating the presence of one chlorine atom. The ¹H NMR spectrum of **1** showed two aromatic protons at δ 6.66 (1H, d, *J* = 8.0 Hz, H-2) and 6.65 (1H, d, *J* = 8.0 Hz, H-3), a methoxy proton at δ 3.84 (3H, s, 6-OCH₃), a methyl proton at δ 2.33 (3H, s, CH₃-4), and two anomeric proton signals at δ 4.86 (1H, d, *J* = 7.5 Hz, H-1') and δ 4.70 (1H, d, *J* = 2.0 Hz, H-1''). The ¹³C NMR spectrum of **1** displayed 20 carbon signals, combining with the HSQC and HMBC spectrum, which were assigned as follows: δ 158.0 (C-1), 157.1 (C-6), 139.0 (C-5), 117.1 (C-4), 111.6 (C-3), and 100.9 (C-2) for the six aromatic carbons, 10 oxymethine carbons at δ 102.5 (C-1'), 74.9 (C-2'), 77.9 (C-3'), 71.5 (C-4'), 76.9 (C-5'), 102.1 (C-1''), 72.1 (C-2''), 72.4 (C-3''), 74.1 (C-4''), and 69.8 (C-5''), one oxymethylene carbon at δ 67.8 (C-6'), one methoxy carbon at δ 56.7 (6-OCH₃), and two methyl carbon at δ 20.7 (CH₃-4) and 17.9 (C-6''). The acid hydrolysis and derivatization of **1**, as determined by HPLC analysis, indicated the absolute configuration of the monosaccharide units to be one D-glucose and one L-rhamnose [12]. The coupling constant of the anomeric protons at δ 4.86 (1H, d, *J* = 7.5 Hz, H-1') and δ 4.70 (1H, d, *J* = 2.0 Hz, H-1'') indicated the presence of a β -configured glucosyl and an α -rhamnosyl [4].

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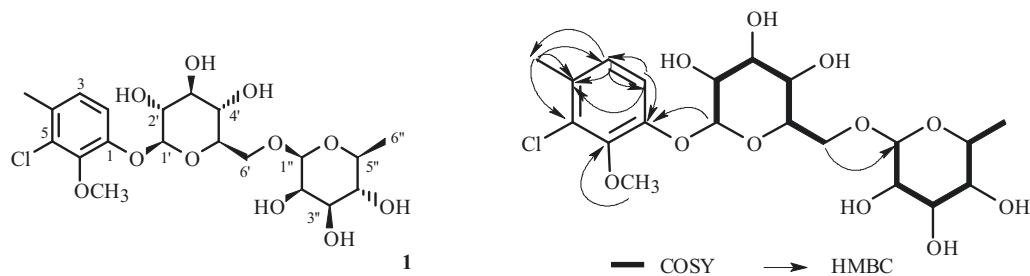


Fig. 1. Key HMBC and ^1H - ^1H COSY correlations of compound **1**.

Correlations from H-2 to C-1, C-3, and C-4, from H-3 to C-2, C-4, and CH₃-4, from CH₃-4 to C-3, C-4, and C-5, and from 6-OCH₃ to C-6, as well as correlations from H-1' to C-1 and from H-6' to C-1'' in the HMBC spectrum confirmed the structure of **1** as 5-chloro-6-methoxy-4-methylphenyl 1-*O*- β -D-glucopyranosyl-(1 \rightarrow 6)- α -L-rhamnopyranoside.

EXPERIMENTAL

Plant Material. The *Lilium* were purchased from Jingshiqiao Town, Longhui County, Shaoyang City, Hunan Province in 2020. They were identified as the bulbs of *Lilium brownii* var. *viridulum* Baker by Prof. Guo Tao from the School of Pharmacy, Henan University of Chinese Medicine. The voucher specimens are stored at the Henan Engineering Research Center of Medicinal and Edible Chinese Medicine Technology.

Extraction and Isolation. Forty kilograms of fresh *Lilium* bulbs were extracted twice with 70% ethanol (25 L) for 14 days each time at room temperature. The combined extract was concentrated under reduced pressure and partitioned with petroleum ether, ethyl acetate, and *n*-butanol. The ethyl acetate fraction (34 g) was obtained. The ethyl acetate fraction was subjected to column chromatography on a silica gel column (700 g, 8 \times 77 cm, 200 mesh) using a eluent mixture of dichloromethane–methanol (20:1 \rightarrow 2:1). This resulted in the separation of 18 fractions (A1–A18), with each fraction collected under the guidance of a UV detector (210 nm and 254 nm). Fraction A5 was further subjected to reverse-phase chromatography (YMC-pack ODS-A, 10 \times 250 mm, S-5 μm) using a mobile phase of MeOH–H₂O (60:40, 3 mL/min) to isolate caffeic acid methyl ester (**4**, 14.3 mg). Fraction A6 was eluted using a mobile phase of MeOH–H₂O (46:54, 3 mL/min) to isolate pinoresinol (**12**, 29.6 mg) and epipinoresinol (**13**, 3.0 mg). Fraction A7 was eluted using a mobile phase of MeCN–H₂O (32:68, 3 mL/min) to isolate 1,3-*O*-diferuloylglycerol (**6**, 5.0 mg); using a mobile phase of MeOH–H₂O (50:50, 3 mL/min) to isolate syringaresinol (**11**, 29.6 mg). Fraction A8 was eluted using a mobile phase of MeOH–H₂O (40:60, 3 mL/min) to isolate 1-*O*-feruloylglycerol (**2**, 127.7 mg). Fraction A9 was eluted using a mobile phase of MeCN–H₂O (26:74, 3 mL/min) to isolate 1-*O*-*p*-coumaroyl-3-*O*-feruloylglycerol (**5**, 5 mg), 1-*O*-feruloyl-3-*O*-caffeoylglycerol (**7**, 10.1 mg), 1-*O*-caffeoyl-3-*O*-*p*-coumaroylglycerol (**8**, 5.3 mg). Fraction A10 was eluted using a mobile phase of MeOH–H₂O (25:75, 3 mL/min) to isolate 1-*O*-*p*-coumaroylglycerol (**3**, 21.6 mg). Fraction A13 was eluted using a mobile phase of MeOH–H₂O (34:66, 3 mL/min) to isolate regaloside B (**10**, 173.0 mg). Fraction A16 was eluted using a mobile phase of MeCN–H₂O (18:82, 3 mL/min) to isolate **1** (5.0 mg) and regaloside A (**9**, 5.3 mg).

Acid Hydrolysis and Derivatization. Compound **1** (1.0 mg) was placed in a solution of 0.5 M HCl (0.2 mL) and heated at 90°C for 2 h. After drying, the reaction mixture was dissolved in anhydrous pyridine (0.2 mL). Then, L-cysteine methyl ester hydrochloride (1 mg) was added. The mixture was heated at 60°C for 1 h. Then, 1 mg of *o*-tolyl isothiocyanate was added, and the reaction mixture was heated at 60°C for 1 h. After drying the solution, the residue was partitioned between H₂O and cyclohexane. The H₂O layer was concentrated, dissolved in methanol, and analyzed using semi-preparative HPLC with a mobile phase of 52% methanol. The wavelength was set at 254 nm for detection. The peaks at 15.85 and 20.35 min coincided with derivatives of D-glucose and L-rhamnose in compound **1**.

1-*O*- β -D-Glucopyranosyl-(1 \rightarrow 6)- α -L-rhamnopyranoside (1**),** white amorphous powder, $[\alpha]_{\text{D}}^{20}$ –63.0° (*c* 0.10, MeOH). UV (MeOH, λ_{max} , nm) (log ϵ): 210 (2.92). HR-ESI-MS *m/z* 479.1337 [M – H][–] (calcd for C₂₀H₂₈O₁₁Cl, 479.1318). ¹H NMR (500 MHz, CD₃OD, δ , ppm, J/Hz): 1.21 (3H, d, J = 6.5, H-6''), 2.33 (3H, s, CH₃-4), 3.35–3.37 (1H, m, H-4'), 3.35–3.37 (1H, m, H-4''), 3.42–3.46 (1H, m, H-3'), 3.49–3.53 (1H, m, H-2'), 3.55–3.60 (1H, m, H-5'), 3.56–3.59 (1H, m, H-6'b), 3.61–3.65 (1H, m, H-5''), 3.64–3.67 (1H, m, H-3''), 3.81–3.82 (1H, m, H-2''), 3.84 (3H, s, OCH₃), 4.05 (1H, dd, J = 13.5, 5.0, H-6'a), 4.70 (1H, d, J = 2.0, H-1''), 4.86 (1H, d, J = 7.5, H-1'), 6.65 (1H, d, J = 8.0, H-3), 6.66 (1H, d, J = 8.0, H-2).

¹³C NMR (125 MHz, CD₃OD, δ, ppm): 17.9 (C-6''), 20.7 (CH₃), 56.7 (OCH₃), 67.8 (C-6'), 69.8 (C-5''), 71.5 (C-4'), 72.1 (C-2''), 72.4 (C-3''), 74.1 (C-4'), 74.9 (C-2'), 76.9 (C-5'), 77.9 (C-3'), 102.1 (C-1''), 102.5 (C-1'), 100.9 (C-2), 111.6 (C-3), 117.1 (C-4), 139.0 (C-5), 157.1 (C-6), 158.0 (C-1).

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