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Chinese Chemical Letters 20 (2009) 1215–1217
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

# Minor antifungal aromatic glycosides from the roots of *Gentiana rigescens* (Gentianaceae)

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Received 9 January 2009

**Abstract**

Two new phenolic glycosides, 2,3-dihydroxybenzoic acid methyl ester 3-*O*-*β*-D-glucopyranosyl-(1-6)-*β*-D-glucopyranoside (**1**) and 2,5-dihydroxylbenzofuran 5-*O*-*β*-D-xylopyranosyl-(1-6)-*O*-*β*-D-glucopyranoside (**2**), were isolated as the minor chemical constituents from the roots of *Gentiana rigescens*, along with 15 known compounds. Their structures were elucidated by detailed spectroscopic analysis, including 1D, 2D NMR and chemical method. All of these compounds were isolated for the first time from the title plant. Moreover, compounds **1** and **2** were tested for the antifungal activities on three plant pathogens *Peronophythora litchi*, *Glomerella cingulata*, and *Glorosprium musarum*.


*Keywords:* *Gentiana rigescen*; Phenolic glycosides; Antifungal activity; Plant pathogen

''Long-Dan'' is a well-known traditional Chinese medicinal (TCM) herbs commonly used for the treatment of inflammation, hepatitis, and cholecystitis. In the Chinese Pharmacopoeia, the roots of four species from the genus *Gentiana* (Gentianaceae), e.g. *G. scabra* Bge, *G. manshurica* Kitag, *G. triflora* Pall, and *G. rigescens* Franch, are used as the raw materials of ''Long-Dan''. Of them, the former three species distribute mainly in northeast of China, called as ''Guan-Long-Dan'', while the latter one grows in southwest part of China, particularly in the mountainous areas of Yunnan province, called as ''Jian-Long-Dan''. As a part of our ongoing phytochemical studies on Gentianaceous medicinal plants, we have previously reported nine iridoidal glycosides as major constituents from the roots of this herb [1]. In addition, six new minor dammarane-type triterpenoids were identified [2]. Further investigation on the minor components in the roots of *G. rigescens* led to the isolation of two new phenolic glycosides (**1**, **2**), together with 15 known glycosides, (−)-7*R*,8*S*-dehydrodiconiferyl alcohol-4,9′-di-*O*-*β*-D-glucopyranoside, (−)-7*R*,8*S*-dehydro-diconiferyl alcohol-4-*O*-*β*-D-glucopyranoside, (−)-pinoresinol-*O*-*β*-D-glucopyranoside, (−)-syringaresinol-*O*-*β*-D-glucopyranoside, liriodendrin, lariciresinol-4′-*O*-*β*-D-glucopyranoside, tortoside B, 2,3-dihydroxybenzoic acid methyl ester 3-*O*-*β*-D-glucopyranoside, 2-hydroxy-3-methoxy benzoic acid glucose ester, vanillyl alcohol-*O*-*β*-D-glucopyranoside, 3,5-dimethoxy-4-hydroxybenzyl alcohol 4-*O*-*β*-D-glucopyranoside, 3-*O*-*β*-D-glycosylcaffeate, 4-hydroxy-3-methoxyphenyl-*O*-*β*-D-xylopyranosyl-(1-6)-*O*-*β*-D-glucopyranoside, isoorientine-3′-*O*-*β*-D-glucopyranoside and oct-1-en-3-yl

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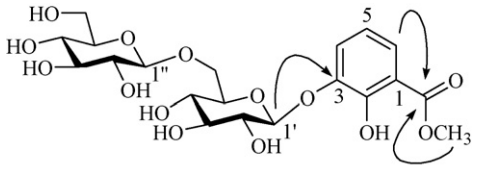
1001-8417/$ – see front matter 
doi:10.1016/j.cclet.2009.05.006




**1**

Fig. 1. Structure and key HMBC correlations of compound **1**.

arabinopyranosyl-(1-6)-*O*-*β*-D-glucopyranoside. This paper presents the isolation and structural elucidation of the new compounds.

Compound **1** showed a quasi-molecular ion peak at *m/z* 491.1419 $[M-H]^-$ in the HR-FAB-MS, suggesting the molecular formula of $C_{20}H_{28}O_{14}$ for **1**. The IR spectrum of **1** exhibited strong absorptions at 3418, 1676, 1616, 1467, 1441, 1252, 1072 $cm^{-1}$, indicating the existence of hydroxyl, carbonyl and aromatic ring. The $^1H$ and $^{13}C$ NMR spectra of **1** showed the presence of a methoxyl [$\delta_H$ 3.53 (3H, s)], one 1,2,3-trisubstituted benzene ring [$\delta_H$ 7.52, 7.46 (dd, each 1H, $J$ = 8.1, 1.2 Hz) and 6.88 (t, 1H, $J$ = 8.1 Hz)], two β-glucopyranosyl moieties [anomeric H at $\delta_H$ 4.89 (d, 1H, $J$ = 8.2 Hz) and 4.36 (d, 1H, $J$ = 7.7 Hz)], and a carbonyl group ($\delta_C$ 171.1). Acidic hydrolysis of **1** with 1 M HCl afforded D-glucose as sugar components, which were determined by GC analysis of their corresponding trimethylsilylated L-cysteine derivatives [3]. The spectroscopic features of **1** were similar to those of 2,3-dihydroxybenzoic acid methyl ester 3-*O*-*β*-D-glucopyranoside [4], except for the appearance of one more *β*-D-glucopyranosyl unit in **1**. The obvious downfield shift of glucosyl C-6′ by +7.5 ppm suggested that the second glucosyl unit was linked at C-6′ of the inner glucosyl unit in **1**, which was confirmed by the HMBC correlation of the anomeric proton ($\delta_H$ 4.36) of the terminal glucosyl unit with C-6′ ($\delta_C$ 69.7) of the inner glucosyl unit. Moreover, HMBC correlations of the anomeric proton ($\delta_H$ 4.89, H-1′) of the inner glucosyl unit with C-3 ($\delta_C$ 123.6), and both methoxyl ($\delta_H$ 3.53) and aromatic ($\delta_H$ 7.52, H-6) protons with the carbonyl group ($\delta_C$ 171.7) were also observed (Fig. 1). Accordingly, compound **1** was elucidated as 2,3-dihydroxy benzoic acid methyl ester 3-*O*-*β*-D-glucopyranosyl-(1-6)-*β*-D-glucopyranoside.

The molecular formula of compound **2** was established as $C_{19}H_{24}O_{12}$ on the basis of its HR-FAB-MS (*m/z* 443.1179 $[M-H]^-$) and DEPT data. The IR spectrum exhibited strong absorptions at 3425, 2922, 2887, 1616, 1463, 1198, 1072, 1043 $cm^{-1}$, indicating the existence of hydroxyl group and aromatic ring. The $^1H$ NMR spectrum showed the presence of a trisubstituted double bond [olefinic proton at $\delta_H$ 7.06 (s, 1H, H-3)] and one 1,2,4-trisubstituted aromatic ring [$\delta_H$ 7.37 (d, 1H, $J$ = 2.4 Hz, H-4), 7.17 (dd, 1H, $J$ = 2.4, 8.9 Hz, H-6) and [$\delta_H$ 7.36 (d, 1H, $J$ = 8.9 Hz, H-7)]. In addition, the $^1H$ and $^{13}C$ NMR spectra of **2** displayed signals assignable to a *β*-glucopyranosyl [$\delta_H$ 4.87 (d, 1H, $J$ = 7.8 Hz, anomeric H); $\delta_C$ 103.4, 74.9, 77.8, 71.4, 77.6, and 69.8 (C-1 to C-6)] and a *β*-xylopyranosyl [$\delta_H$ 4.35 (d, 1H, $J$ = 8.9 Hz, anomeric H); $\delta_C$ 105.4, 74.9, 77.3, 71.2, and 66.8 (C-1 to C-5)] units (Table 1).

Acetylation of **2** afforded **2a**, whose FAB-MS and NMR spectral data indicated the presence of 2,5-dihydroxylbenzofuran skeleton and one pentosyl and one hexosyl units in **2**. Acid hydrolysis of **2** with 1 mol/L HCl afforded D-glucose and D-xylose as sugar residues which were determined by GC analysis of their corresponding trimethylsilylated L-cysteine adducts [3].

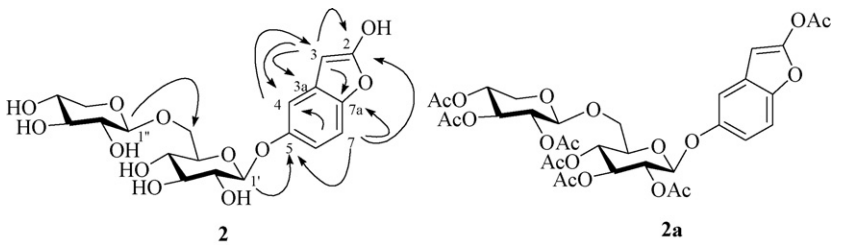
The sugar sequence and linkage site to the aglycone of **2** were determined by 2D NMR experiments. In the HMBC spectrum of **2**, correlations of $\delta_H$ 4.35 (xylosyl H-1) with $\delta_C$ 69.8 (glucosyl C-6), and $\delta_H$ 4.87 (glucosyl H-1) with $\delta_C$ 155.8 (C-5) were observed. Other key HMBC correlations are shown in Fig. 2. Moreover, the anomeric proton of glucosyl unit at $\delta_C$ 4.86 was correlated with both aromatic protons of H-4 ($\delta_H$ 7.37) and H-6 ($\delta_H$ 7.17) in the ROESY spectrum. Therefore, the structure of compound **2** was unambiguously established to be 2,5-dihydroxyl benzofuran 5-*O*-*β*-D-xylopyranosyl-(1-6)-*O*-*β*-D-glucopyranoside.

Compounds **1** and **2** were tested for their antifungal activities against plant pathogens, *Peronophythora litchi*, *Glomerella cingulata*, and *Glorosprium musarum*, using the disc diffusion method [2], with carbendazim as positive control. With a concentration of 0.98 mg/mL, the new phenolic glycoside **1** showed inhibitory activities against the growth of *P. litchi* and *G. musarum*, whose diameters of the inhibitory zone were 2.0 and 0.8 cm, respectively. The compound **2** was not active with a concentration of 1.02 mg/mL.

Table 1
$^{1}H$ and $^{13}C$ NMR data of **1**, **2** in $CD_3OD$ ($\delta$ ppm).

| Position | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| | $\delta_H$[a] | *J* (Hz) | $\delta_C$ | $\delta_H$[a] | *J* (Hz) | $\delta_C$ |
| 1 | | | 114.6 | | | – |
| 2 | | | 154.8 | | | 152.0 |
| 3 | | | 123.6 | 7.06 (s) | | 113.7 |
| 3a | | | – | | | 129.3 |
| 4 | 7.46 (dd) | 8.1, 1.2 | 120.3 | 7.37 (d) | 2.4 | 109.2 |
| 5 | 6.88 (d) | 8.1 | 123.4 | | | 155.8 |
| 6 | 7.52 (dd) | 8.1, 1.2 | 147.0 | 7.17 (dd) | 8.9, 2.4 | 117.9 |
| 7 | | | – | 7.36 (d) | 8.9 | 112.4 |
| 7a | | | – | | | 140.1 |
| *CO*OCH$_3$ | | | 171.7 | | | - |
| CO*OCH$_3$* | 3.53 (s) | | 53.0 | | | - |
| glc-1′ | 4.89 (d) | 8.2 | 102.7 | 4.87 (d) | 7.8 | 103.4 |
| 2′ | 3.19 (dd) | 8.2, 3.9 | 74.7 | 3.47 (m) | | 74.9 |
| 3′ | 3.20 (m) | 3.9 | 77.9 | 3.48 (m) | | 77.8 |
| 4′ | 3.25 (m) | | 71.4 | 3.34 (m) | | 71.4 |
| 5′ | 3.67 (m) | | 78.5 | 3.65 (m) | | 77.6 |
| 6′ | 3.83 (dd) 4.13 (dd) | 10.5, 1.2 10.5, 5.4 | 69.7 | 3.76 (dd) 4.11 (dd) | 11.7, 4.8 11.7, 2.3 | 69.8 |
| 6′-glc-1″ | 4.36 (d) | 7.7 | 104.7 | | | |
| 2″ | 3.46 (m) | | 74.4 | | | |
| 3″ | 3.27 (m) | | 77.5 | | | |
| 4″ | 3.52 (m) | | 71.3 | | | |
| 5″ | 3.52 (m) | | 77.8 | | | |
| 6″ | 3.65 (dd) 3.80 (dd) | 11.0, 2.1 11.0, 5.3 | 62.5 | | | |
| xyl-1″ | | | | 4.35 (d) | 8.9 | 105.4 |
| 2″ | | | | 3.20 (t) | 7.9 | 74.9 |
| 3″ | | | | 3.27 (m) | | 77.3 |
| 4″ | | | | 3.41 (m) | | 71.2 |
| 5″ | | | | 3.12 (dd) 3.85 (dd) | 11.0, 2.1 11.0, 5.3 | 66.8 |

[a] Multiplicity: s, singlet; d, doublet; dd, double doublet; m, multiplet.




**2** **2a**

Fig. 2. Structure and key HMBC correlations of compounds **2** and **2a**.

## Acknowledgments

The authors are grateful to the members of the Analytical Group in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for measurements of all spectra.

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