

Tenuifoliside Z1, an undescribed glycolipid from *Polygala tenuifolia* Willd. roots with antioxidant activity

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Abstract: A new glycolipid, tenuifoliside Z1 (1), together with four known glycolipid compounds (2–5) has been isolated from the root of *Polygala tenuifolia* Willd. Their structures were determined through spectroscopic analysis, chemical derivatization, and comparison with spectroscopic data reported in the literature. All the compounds were evaluated for antioxidant activity by *in vitro* assays.

Keywords: *Polygala tenuifolia* Willd, polygalaceae, glycolipids, antioxidant activity

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1 Plant Source

Air-dried roots of *Polygala tenuifolia* Willd. (Polygalaceae) were bought from Bozhou City, Anhui Province, China, and authenticated by Jie Li in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (202507/KUN/SCA) was deposited at the herbarium of Kunming Institute of Botany.

2 Previous Studies

Polygala tenuifolia Willd., the botanical source of the traditional Chinese medicine known as Polygalae Radix “Yuanzhi”, has been used for centuries in clinical practice to treat various diseases, including insomnia (Hao et al., 2024), neurasthenia (Shen et al., 2025), amnesia, palpitations with anxiety and restlessness (Zhou et al., 2021). It is also employed to prevent dementia and memory decline (Deng et al., 2020; Liang et al., 2025). With the advancement of research methodologies, more than 140 compounds have been isolated and identified from *P. tenuifolia*. These constituents primarily include triterpenoid saponins (Zeng et al., 2020), xanthenes (Ling et al., 2013), oligosaccharide esters (Yang et al., 2022), and phenylpropanoids.

3 Present Study

Air-dried and powdered roots (15 kg) were infiltrated and extracted five times with 90% EtOH at room temperature (eight hours each). The filtrated solutions were combined and evaporated under reduced pressure at 50°C to a volume of 5.2 L, which was then fractionated successively with CH₂Cl₂ (5.2 L × 3) and *n*-BuOH (5.2 L × 4). After concentration to dryness, the CH₂Cl₂-soluble fraction yielded 169.8 g, and the *n*-BuOH-soluble fraction yielded 705.3 g.

n-BuOH-soluble fraction (705.3 g) was dissolved in MeOH, adsorbed onto macroporous resin (700.2 g), and then concentrated to dryness. The resulting solid was ground, passed through a 40-mesh sieve, and the entire powder was loaded onto a glass column (14.8 cm diameter) that had been pre-packed with 3100 g of macroporous resin in water (bed height: 24.3 cm). The column was eluted sequentially with 25 l each of H₂O, 40% MeOH, 80% MeOH, and pure MeOH. TLC analysis of the eluates guided their combination into four fractions (A–D).

Fraction B (95.2 g) was subjected to silica gel column chromatography (CC) using a stepwise gradient of CH₂Cl₂/MeOH (10:0 to 0:10, v/v), yielding six subfractions (B-1–B-6). Subfraction B-3 (24.4 g) was further separated by ODS gel column with a H₂O/MeOH gradient (4:6 to 0:10, v/v) to give nine fractions (B-3-1–B-3-9). Fraction B-3-8 (2.7 g) was further purified on a Sephadex LH-20 column (MeOH eluent) resulting in five pools (B-3-8-1–B-3-8-5).

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Fraction B-3-8-2 (316.56 mg) was finally purified by semi-preparative HPLC (MeOH–H₂O, 2:8, v/v; 3 mL/min) to yield compound **4** (8.71 mg, $t_R = 15.8$ min). Subfraction B-4 (52.7 g) was further separated on an MCI gel column with a H₂O/MeOH gradient (10:0 to 0:10, v/v), yielding six fractions (B-4-1–B-4-6). Among them, fraction B-4-5 (25.7 g) was subjected to ODS column chromatography with a H₂O/MeOH gradient (4:6 to 0:10, v/v) to obtain ten fractions (B-4-5-1–B-4-5-10). Fraction B-4-5-2 (6.4 g) was subsequently applied to an Sephadex LH-20 column (MeOH eluent) to obtain multiple fractions (B-4-5-2-1–B-4-5-2-5). Fraction B-4-5-2-2 (800.57 mg) was finally purified by semi-preparative HPLC (MeOH–H₂O, 35:65, v/v; 3 mL/min) to yield **3** (10.25 mg, $t_R = 35.7$ min). Subfraction B-4-4 (20.6 g) was chromatographed on an MCI gel column with a H₂O/MeOH gradient (8:2 to 0:10, v/v), yielding five subfractions (B-4-4-1–B-4-4-5). Fraction B-4-4-5 (9.6 g) was further separated on Sephadex LH-20 column and eluted with MeOH to give five fractions (B-4-4-5-1–B-4-4-5-5). Fraction B-4-4-5-1 (269.72 mg) was finally purified by semi-preparative HPLC (MeOH–H₂O, 55:45, v/v; 3 mL/min) to yield **2** (5.25 mg, $t_R = 25.7$ min). Fraction B-4-4-5-2 (238.84 mg) was finally purified by semi-preparative HPLC (MeOH–H₂O, 47:53, v/v; 3 mL/min) to yield **1** (5.52 mg, $t_R = 29.7$ min) and **5** (6.27 mg, $t_R = 34.9$ min).

Compound **1** (Tenuifoliside Z1): White powder (H₂O); $[\alpha]_D^{20} +5.92$ (c 2.50, MeOH); UV (MeOH) λ_{max} nM (log ϵ) 203 (4.82), 235 (4.28), 280 (4.83), HRESIMS m/z 699.2112 [M + Na]⁺ (calcd for C₂₉H₄₀O₁₈, 676.2215, error –0.50 mDa); ¹H (600 MHz) and ¹³C (151 MHz) NMR data in CD₃OD, see Table 1.

Compound **1** was obtained as white powder with the molecular formula C₂₉H₄₀O₁₈ based on its NMR data and a sodiated molecular ion peak at m/z 699.2112 [M + Na]⁺ in HRESIMS spectrum. The ¹H NMR spectra (Table 1) exhibited characteristic signals including: one methyl singlet at δ_H 2.02 (3H, s); two anomeric protons at δ_H 4.49 (1H, d, $J = 7.7$ Hz,

H-1'''), 5.55 (1H, d, $J = 3.6$ Hz, H-1''); a pair of trans-olefinic protons at δ_H 6.56 and 7.72 (1H each, d, $J = 16.1$ Hz, H-8' and 7') and signals corresponding to a monosubstituted phenyl ring at δ_H 7.41 (3H, m, H-3', 4', 5') and 7.62 (2H, d, $J = 7.2$ Hz, H-2', 6'). The ¹³C NMR spectrum (Table 1) revealed a total of 29 carbon signals, which could be classified into one methyl group, four methylene groups, twenty methine groups, and four quaternary carbons by the aid of the HSQC spectrum. Key HMBC correlations established the acyl substituents: correlations from H-2'/6' to the olefinic carbon C-7' (δ_C 117.2), and from H-7' to the acyl carbonyl carbon C-9' (δ_C 166.4) as well as to the aromatic carbons C-2'/6' (δ_C 127.9), identified a cinnamoyl moiety. Meanwhile, a correlation from the methyl singlet (δ_H 2.02) to a carbonyl carbon at δ_C 171.9 (C-2''') indicated an acetyl group. Within the remaining 18 carbon signals, the HSQC spectrum correlated the anomeric carbon resonances at C-1''' (δ_C 104.7) and C-1'' (δ_C 92.6) with their respective anomeric proton signals at H-1''' (δ_H 4.49) and H-1'' (δ_H 5.51). Furthermore, the ¹³C NMR spectrum displayed a characteristic signal for an anomeric, non-protonated carbon at δ_C 102.6 (C-2), suggesting the presence of a fructose unit (Quang et al., 2018). This was confirmed by acid hydrolysis of **1**, which yielded d-glucose and d-fructose. The anomeric configurations of the glucosyl units were determined to be α - and β - based on the characteristic coupling constants of their anomeric protons ($J = 3.6$ and 7.7 Hz, respectively). The HMBC correlations from the methylene protons at H-1 (δ_H 4.09/4.63) to C-9' (δ_C 166.4) and C-2 (δ_C 102.6), indicated the cinnamoyl moiety attached to C-1 of the fructose unit. Furthermore, the HMBC correlations from H-1'' (δ_H 5.51, α -glucose) to fructose C-2 (δ_C 102.6) established an O-glycosidic linkage between the α -glucose and the fructose C-2 (Figure 2). The connectivity of the β -glucose unit was deduced from the HMBC correlation from its anomeric proton H-1''' (δ_H 4.49)

Table 1. ¹H and ¹³C NMR data of compound **1** in CD₃OD

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
1	4.09 (d, 12.3) 4.63 (d, 12.3)	63.0	2''	3.42 (dd, 9.5, 3.6)	80.7
2		102.6	3''	3.90 (t, 9.5)	71.6
3	4.18 (d, 8.7)	76.7	4''	3.44–3.47 (m)	69.4
4	4.03 (t, 8.7)	73.5	5''	3.81–3.83 (m)	72.9
5	3.81–3.83 (m)	82.6	6''	3.79 (dd, 12.5, 6.0) 3.74 (dd, 12.5, 3.0)	60.6
6	3.73 (dd, 12.5, 2.3) 3.81 (dd, 12.5, 6.0)	62.1	1'''	4.48 (d, 7.7)	104.7
1'		134.3	2'''	3.51 (dd, 7.5, 9.0)	73.8
2'/6'	7.62 (d, 7.5)	127.9	3'''	3.36 (d, 9.2)	76.3
3'/5'	7.40 (t, 7.5)	128.7	4'''	3.53 (t, 9.0)	69.6
4'	7.40 (t, 7.5)	130.3	5'''	3.44–3.47 (m)	73.9
7'	7.72 (d, 15.8)	145.3	6'''	4.22 (dd, 11.9, 5.0) 4.50 (dd, 11.9, 2.3)	62.6
8'	6.56 (d, 15.8)	117.2	1''''	2.03 (s)	19.4
9'		166.4	2''''		171.9
1''	5.51 (d, 3.6)	92.6			

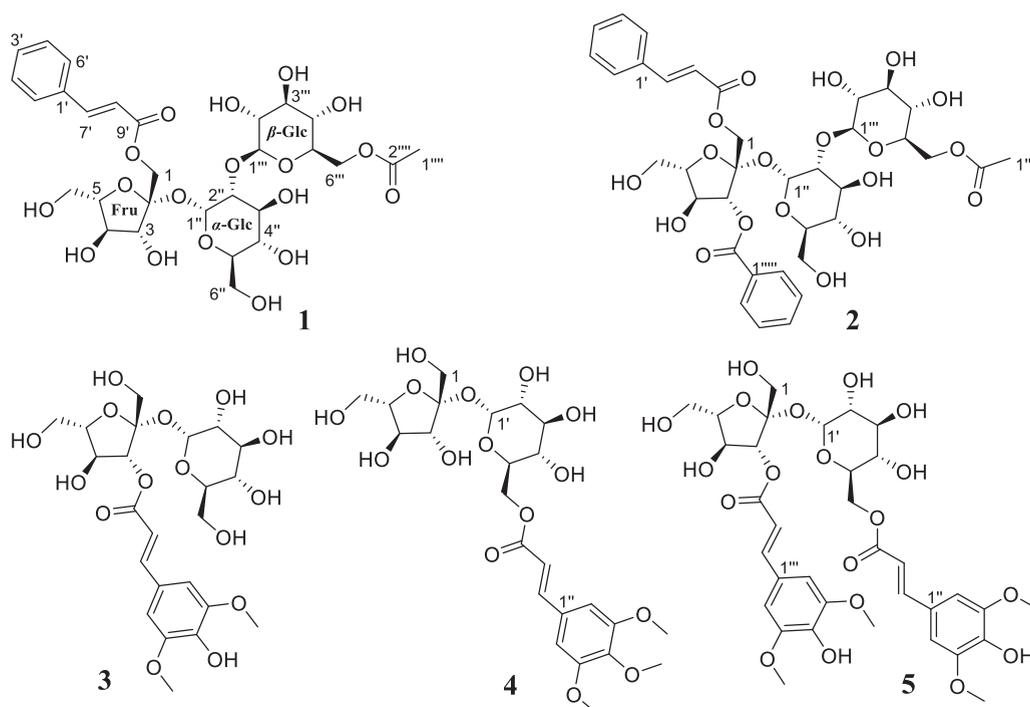


Figure 1. The chemical structures of 1–5

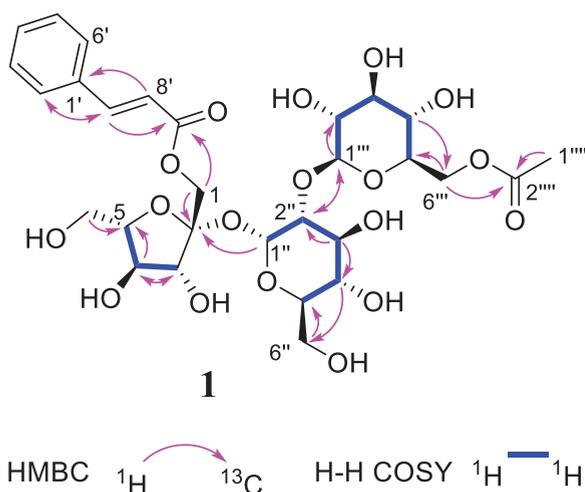


Figure 2. The key ^1H - ^1H COSY, HMBC correlations of 1

to C-2'' (δ_{C} 80.7) of the α -glucose unit, and a COSY correlation between H-1''' and H-2''. Finally, the acetyl group was assigned to C-6''' of the β -glucose unit, as evidenced by HMBC correlations from H-6''' (δ_{H} 4.50/4.22) to the acetyl carbonyl carbon C-2''' (δ_{C} 171.9). Thus, compound 1 was identified as 1-*O*-cinnamoyl- β -D-fructofuranosyl-(2 \rightarrow 1)- α -D-glucopyranosyl-(2 \rightarrow 1)-(6-*O*-acetyl)- β -D-glucopyranoside, and was named tenuifoliside Z1.

The known compounds were identified as (3-*O*-benzoyl)-1-*O*-cinnamoyl- β -D-fructofuranosyl-(2 \rightarrow 1)- α -D-glucopyranosyl-(2 \rightarrow 1)-(6-*O*-acetyl)- β -D-glucopyranoside (2) (Miyase & Ueno, 1993), sibiricose A₆ (3) (Miyase et al., 1999), sibiricose A₂ (4) (Zhou et al., 2014), β -D-(3-*O*-sinapoyl) fructofuranosyl- β -D-(6-*O*-sinapoyl) glucopyranoside (5) (Bashir et al., 1993) by interpretation of their spectroscopic data and comparison of the data with the references (Figure 1).

Table 2. Antioxidant activity of compounds 1–5

Compd	DPPH (IC ₅₀ , μM)	ABTS (IC ₅₀ , μM)	Cellular ROS (IC ₅₀ , μM)
1	>50	>50	>50
2	>50	9.66 \pm 0.23	>50
3	7.88 \pm 0.19	2.71 \pm 0.03	18.17 \pm 0.13
4	23.89 \pm 0.08	2.82 \pm 0.01	42.21 \pm 0.42
5	5.40 \pm 0.27	2.68 \pm 0.02	15.52 \pm 0.25
l-AA	34.07 \pm 1.18	20.66 \pm 0.05	
Curcumin			13.84 \pm 0.13

Note: Values represent mean \pm s.d. ($n = 3$).

ase & Ueno, 1993), sibiricose A₆ (3) (Miyase et al., 1999), sibiricose A₂ (4) (Zhou et al., 2014), β -D-(3-*O*-sinapoyl) fructofuranosyl- β -D-(6-*O*-sinapoyl) glucopyranoside (5) (Bashir et al., 1993) by interpretation of their spectroscopic data and comparison of the data with the references (Figure 1).

The obtained glycolipid compounds were evaluated for antioxidant activity by three in vitro assays. As shown in Table 2, compounds 3–5 showed more potent 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity with IC₅₀ values in the range of 23.89 μM (4) to extract and forsythoside a improved Alzheimer 5.40 μM (5) than l-ascorbic acid (l-AA, 34.07 μM). Compounds 2–5 displayed better ABTS radical cation scavenging activity with IC₅₀ values ranging from 2.68 μM (5) to 9.66 μM (2)

than l-AA (20.66 μM). In cellular reactive oxygen species (ROS) scavenging assay, the IC_{50} values of compounds 3 and 5 ranged from 15.52 μM (5) to 18.17 μM (3), which were equivalent to curcumin (13.8 μM).

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Author Contributions

Yu-chen Zhou: Writing—original draft, Investigation, Data curation, Yong-Jiang Wang: Investigation, Data curation. Da-Le Guo: Data curation. Sheng-Xiong Huang: Supervision, Funding acquisition. Mei-Hong Liu: Validation, Writing—review & editing.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors did not report any potential conflicts of interest.

Supporting Information

Supplementary data to this article can be found online at <http://www.acgpubs.org/journal/records-of-natural-products>.

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