Supporting Information

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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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Experimental

General experimental procedures

Silica gel (100–200 mesh, Qingdao PuKe separation material Co., Ltd), D-101 macroporous adsorption resin (Sinopharm chemical reagent Co., Ltd.), MCI GEL CHP20/P120 (Mitsubishi Chemical Group Co., Ltd.), ODS (50 μm, YMC Co., Ltd., Kyoto, Japan) and Sephadex LH-20 were used for column chromatography (CC). TLC was conducted on pre-coated silica gel $HSGF_{254}$ plates (Jiangyou Silica Gel Dev elopment Co., Yantai, China) and visualized under UV light (λ = 254 nm) and by heating after sprayed 10% sulfuric acid in EtOH (v/v). EtOH, $CHCl_2$, *n*-BuOH, and MeOH for extraction and CC were of analytical grade (Yunnan juke Trading Co., Ltd, China). MeOH and CH_3CN for LC were of HPLC grade (Guangdong Guanghua Technology Co., Ltd, China). D-glucose, D-fructose, *N*-(trimethylsilyl)imidazole, L-Cysteine methyl ester hydrochloride and *O*-tolylisothiocyanate (Macklin, Shanghai, China) were used. DPPH, 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), *t*-BHP and DCFH-DA were purchased from Sigma-Aldrich (St. Louis, MO). L-Ascorbic acid and curcumin were obtained from Shanghai Boao Biotech Co. (Shanghai, China). The murine macrophage RAW 264.7 cell line was obtained from Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China).

HPLC was run on a Chromaster 5430 liquid chromatograph (Hitachi, Kyoto, Japan), and the columns used were 250 mm × 4.6 mm i.d. and 250 mm × 10 mm i.d., Cosmosil 5C18-MS-II, 5 μm (Nacalai Tesque, Inc., Kyoto, Japan) for analytic and preparative purposes. 1D and 2D NMR spectra were recorded on a Bruker AV 600 MHz NMR spectrometer at 25°C in CD_3OD (Qingdao Tenglong Weibo Technology Co., Shandong, China) using solvent residual peaks as reference. HRESIMS spectra were measured on an Agilent 6540 Ultra High Definition Accurate-Mass Q-TOF LC/MS system equipped with an Agilent 1290 UPLC system and Aglient 6550 QTOF & Thermo Fisher Q Exactive Orbitrap instrument. UV spectra, Optical rotation (α_D) was acquired on an Autopol VI polarimeter (Rudolph, New Jersey, USA). The supernatants of cell cultures and absorbance (OD) were measured by ELX808 microplate reader (Charles River Laboratories Inc., USA).

Acid hydrolysis of compound 1

compound **1** (2 mg) was dissolved in a mixture of MeOH (1.5 mL) and 3.3 mol/L aqueous

HCl (1.5 mL) and refluxed for 2 h. The reaction mixture was concentrated under reduced pressure and then partitioned with CH_2Cl_2 and H_2O. The aqueous layer was concentrated and then diluted with H_2O. These operations were repeated until the pH solution became near-neutral, as determined by using litmus paper. L-cysteine ethyl ester hydrochloride (4 mg) was added after the residue was diluted in pyridine (2 mL). The mixture was stirred at 60 °C in a N_2 atmosphere for 2 h. After evaporation in a N_2 stream, the residue was dried in vacuum and then added to *N*-trimethylsilylimidazole (0.4 mL). The resulting solution was stirred at 60 °C for 2 h. After the addition of n-hexane and H_2O (each 2.0 mL), the mixture was stirred vigorously (for 1.0 min). The *n*-hexane layer was analyzed through GC-MS with a capillary column HP-5MS (30 m × 250 μm × 0.25 μm, He flow [1 mL/min], 100 °C for 2.0 min, then ramped at 10 °C/min to 280 °C, detected with FID) by using an Agilent 7890 B gas chromatograph.

DPPH radical scavenging assay

DPPH was freshly prepared in methanol at a concentration of 0.1 mM. Test compounds and the positive control (L-ascorbic acid) were separately dissolved in methanol. Each compound solution was then serially diluted to six different concentrations (31.25, 62.5, 125.0, 25.0 and 500 μM). In a 96-well plate, 20 μL of a compound solution (or L-ascorbic acid solution for the positive control) was mixed with 180 μL of the DPPH solution. The control contained methanol instead of the compound solution, and the blank contained methanol in place of the DPPH solution. All assays were performed in quadruplicate. The plates were incubated at 37 °C for 30min in the dark. The absorbance (OD) in each well was read at 515 nm on a microplate reader. The inhibitory rates of DPPH radicals were calculated according to the formula.

$$\text{inhibition (\%)} = [1 - (OD_{treated} - OD_{blank}) / OD_{control}] \times 100$$

SC_{50} values (the concentrations required to scavenge 50% DPPH radicals present in the test solution) were calculated and expressed as means ± SD in micromolar.

ABTS radical cation decolorization assay

ABTS was dissolved in deionized water to aconcentration of 7 mM. $ABTS^{\bullet+}$ was produced by reacting the ABTS solution with 2.45 mM (final concentration) potassium persulfate, and the mixture was kept at room temperature for 12−16 h in the dark before being used. The $ABTS^{\bullet+}$ solution was diluted with phosphate-buffered saline (PBS, pH7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and

diluted to 500, 250, 125, 62.5, 31.25, and 12.5 μM. In a 96-well plate, 20 μL of a compound solution (or L-ascorbic acid solution for the positive control) was mixed with 180 μL of the diluted $ABTS^{\bullet+}$ solution. The control contained DMSO instead of the compound solution. Each treatment was conducted in triplicate. After a mixing time of 10 s and an incubation period of 6 min at 37 °C in the dark, the absorbance (OD) in each well was read at 415 nm on a microplate reader. The inhibitory rates of $ABTS^{\bullet+}$ were calculated according to the formula.

inhibition (%) = $[1 - (OD_{treated} - OD_{blank}) / OD_{control}] \times 100$

SC_{50} values were calculated and expressed as means ± SD in micromolar.

Cellular antioxidant activity assay

In the MTT cell viability assay, 50 μM of *t*-BHP treatment for 3 h provoked about 60% of cell death and this condition was selected for the subsequent experiments as reported. Briefly, RAW264.7 cells (4×10^4 cells/well) were seeded into a black 96-well plate and allowed to growth for 24 h, then treated with either compounds or the positive control curcumin (100, 50, 25, 12.5, and 6.25 μM) for 24 h. After stained with 20 μM DCFH-DA for 1 h in darkness, the cells were exposed to 50 μM *t*-BHP for 1 h to induce ROS generation. The supernatants of cell cultures were measured for intracellular ROS levels using the fluorescence intensity at excitation wavelength of 485 nm and the emission wavelength of 530 nm by micro plate reader.

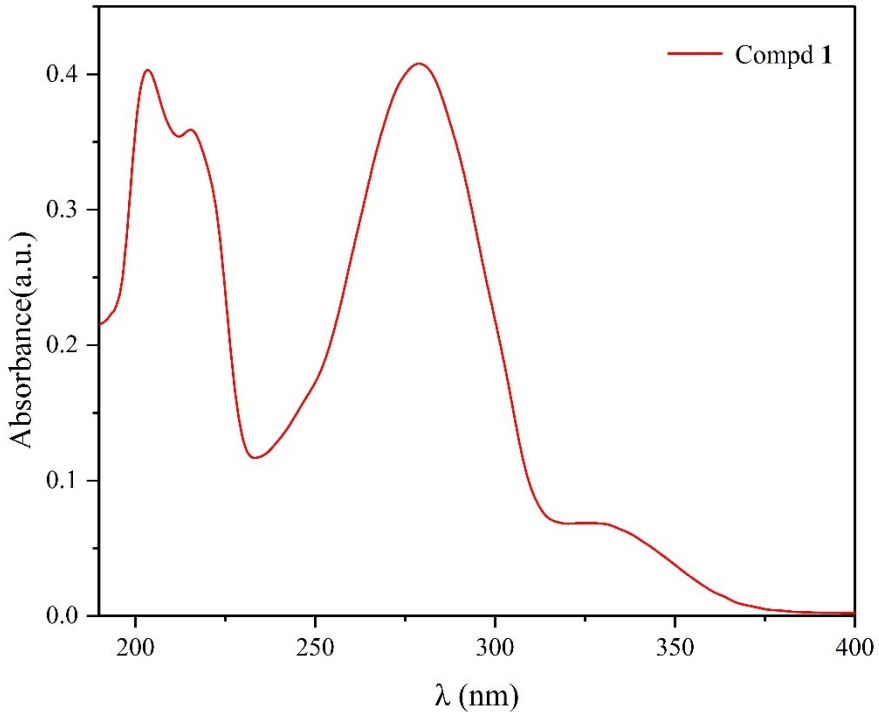
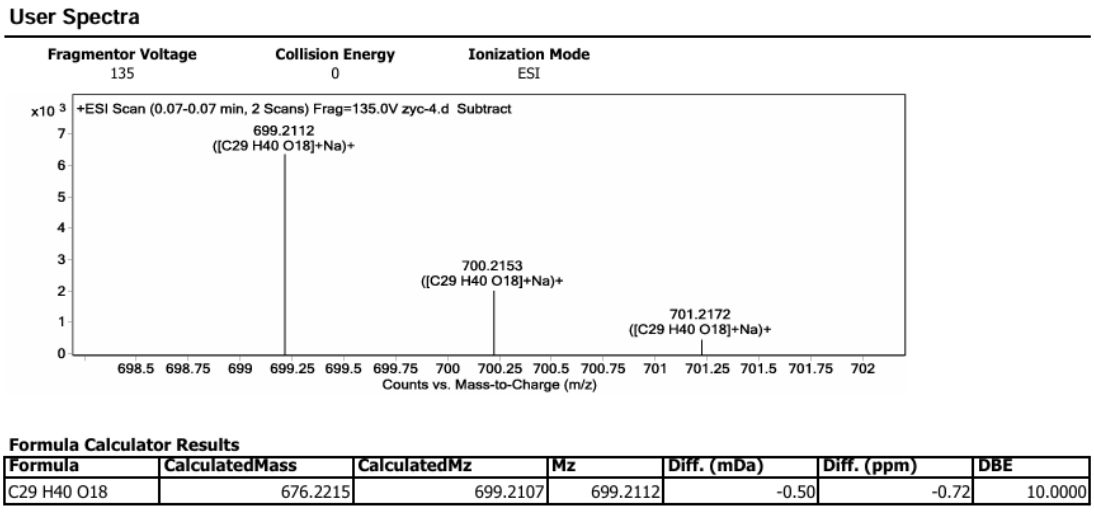


Fig. S1. The UV spectrum of **1**



Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C29 H40 O18	676.2215	699.2107	699.2112	-0.50	-0.72	10.0000

Fig. S2. The HRESIMS spectrum of **1**

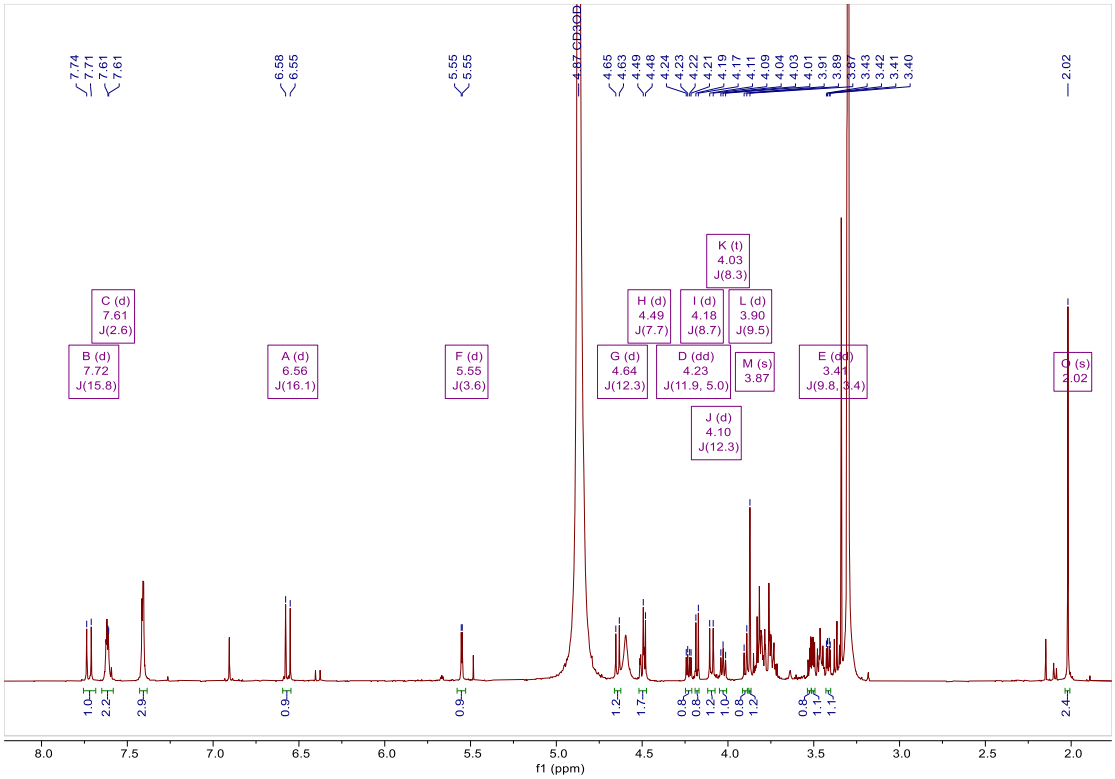


Fig. S3. The ^{1}H NMR spectrum of **1** (in CD3OD)

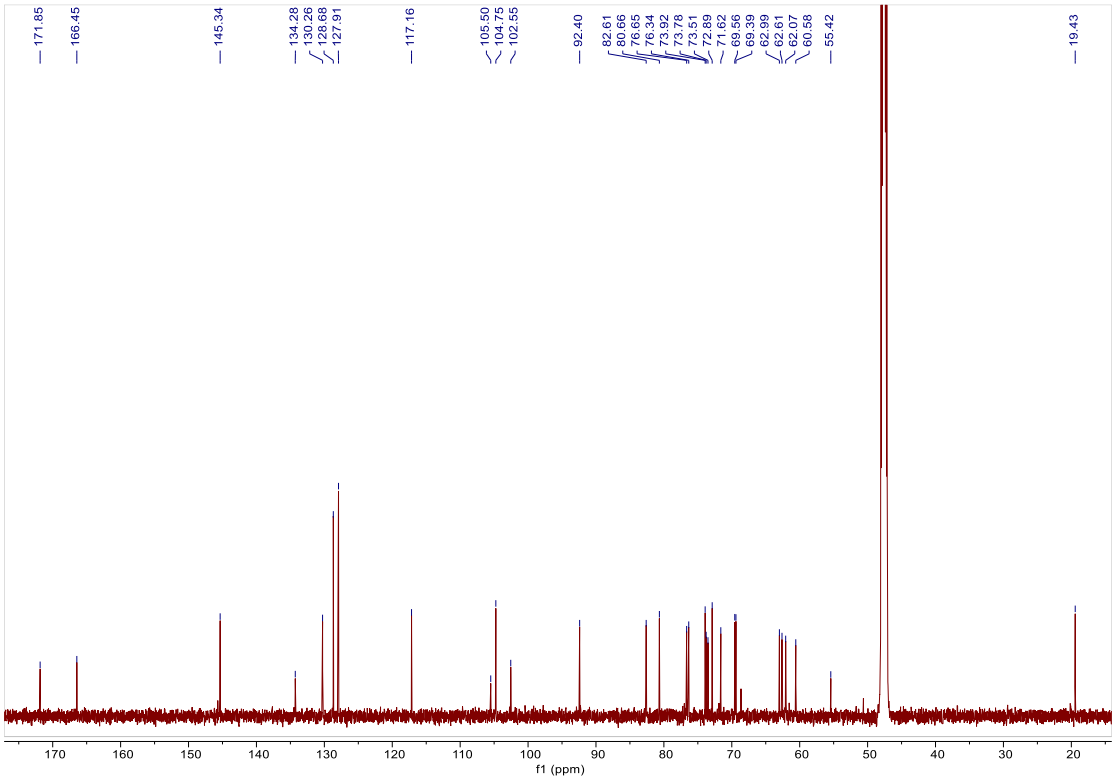


Fig. S4. The ^{13}C NMR spectrum of **1** (in CD3OD)

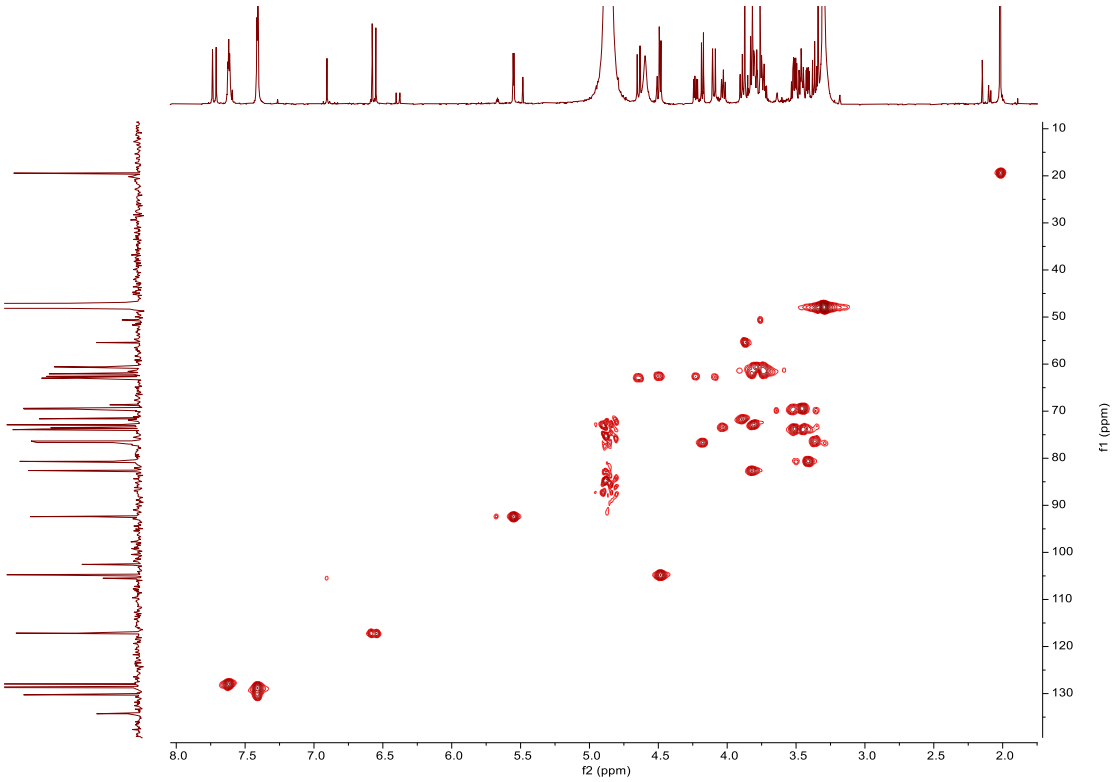


Fig. S5. The HSQC spectrum of **1** (in CD3OD)

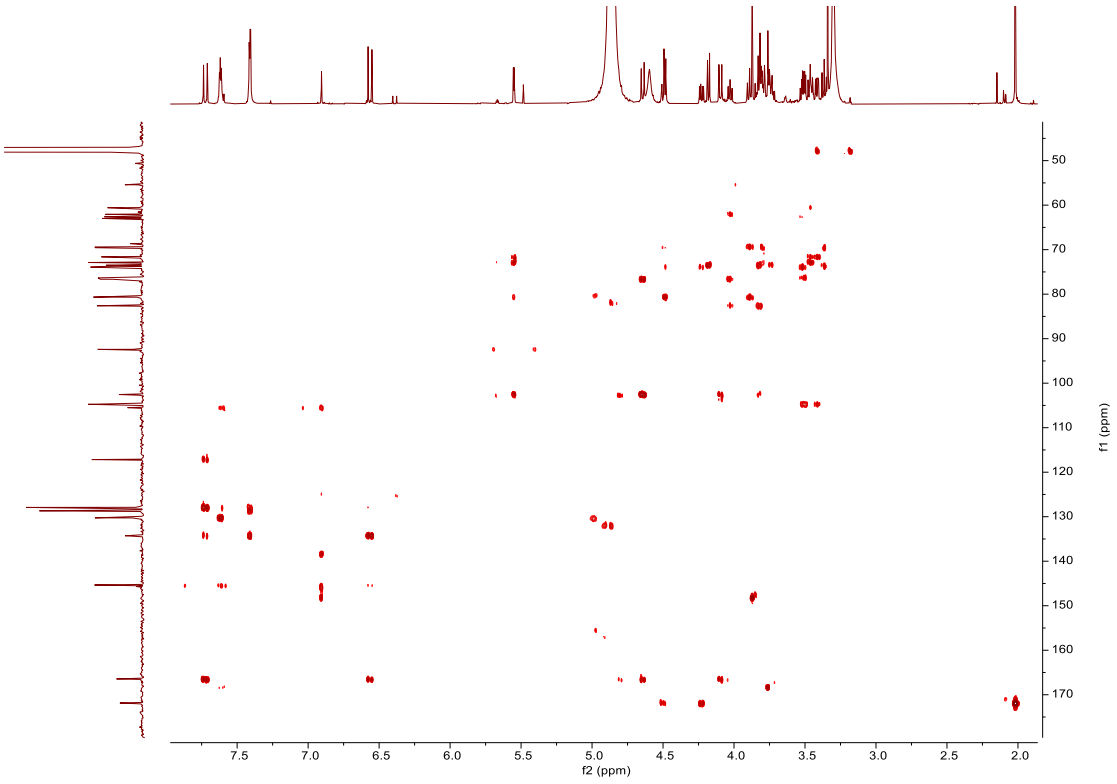


Fig. S6. The HMBC spectrum of **1** (in CD3OD)

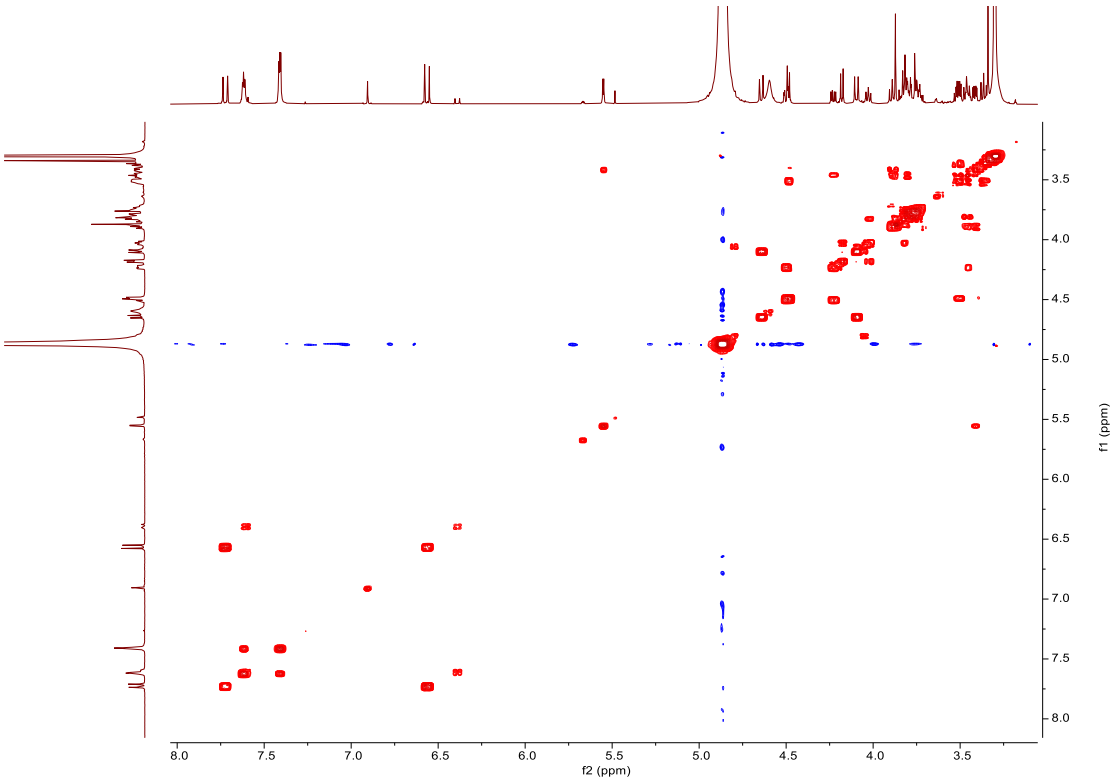


Fig. S7. The COSY spectrum of **1** (in CD3OD)

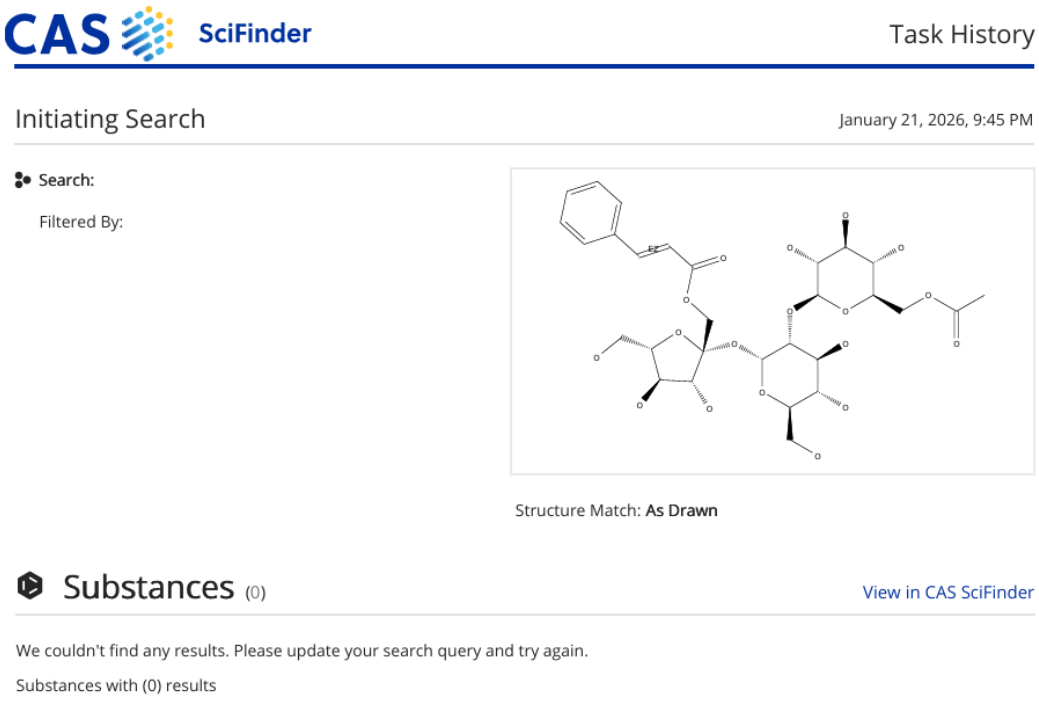


Figure S8: The SciFinder search result for compound **1**

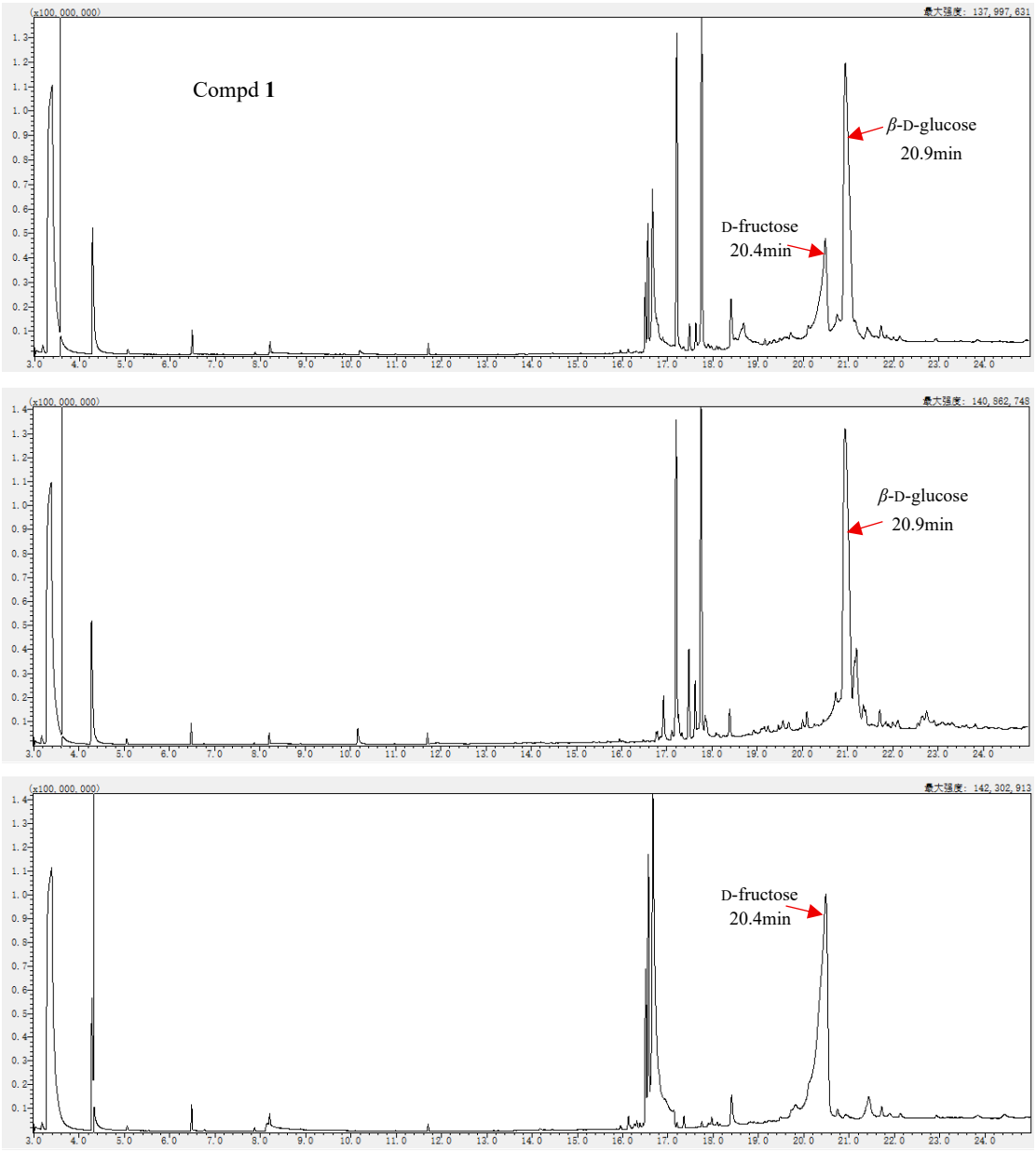
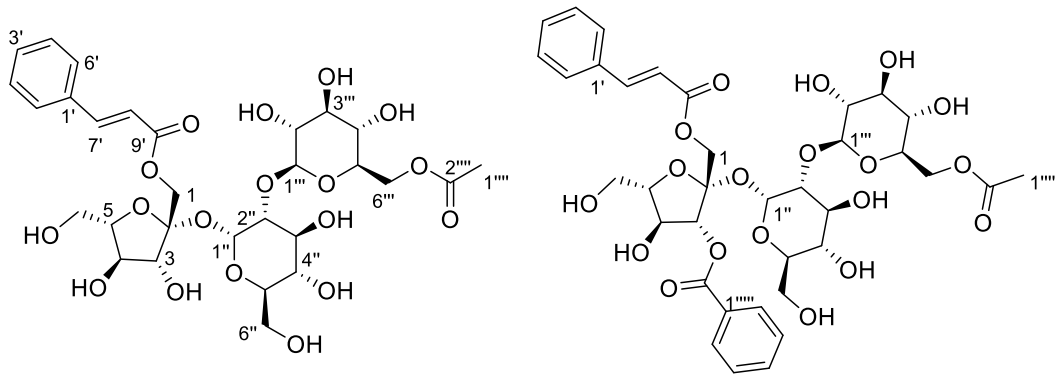


Fig. S9. The Gas chromatograms of acid hydrolysates of **1** and authentic sugars



1 **2**

Figure S10: Structure of compounds **1** and **2**

Table S1: Comparison of NMR data between compounds **1** and **2** in CD_3OD

pos	**1**		**2**	
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	63.0, CH_2	4.09 (d, 12.3)	66.1, CH_2	4.21 (d, 12)
		4.63 (d, 12.3)		4.60 (d, 12)
2	102.6, C		103.2, C	
3	76.7, CH	4.18 (d, 8.7)	79.4, CH	5.78 (d, 8)
4	73.5, CH	4.03 (t, 8.7)	73.1, CH	4.54 (t, 8)
5	82.6, CH	3.81-3.83 (m)	84.0, CH	4.08 (ddd, 8, 6, 3)
6	62.1, CH_2	3.73 (dd, 12.5, 2.3)	63.0, CH_2	3.87 (dd, 12.5, 2)
		3.81 (dd, 12.5, 6.0)		3.93 (dd, 12.5, 5.5)
1'	134.3, C		135.4, C	
2' / 6'	127.9, CH	7.62 (d, 7.5)	129.2, CH	7.51 (*)
3' / 5'	128.7, CH	7.40 (t, 7.5)	129.9, CH	7.39 (*)
4'	130.3, CH	7.40 (t, 7.5)	131.5, CH	7.39 (*)
7'	145.3, CH	7.72 (d, 15.8)	146.6, CH	7.71 (d, 16)
8'	117.2, CH	6.56 (d, 15.8)	118.1, CH	6.51 (d, 16)
9'	166.4, C		167.6, C	
1"	92.6, CH	5.51 (d, 3.6)	93.6, CH	5.64 (d, 3.5)
2"	80.7, CH	3.42 (dd, 9.5, 3.6)	82.1, CH	3.42 (dd, 9.5, 3.5)
3"	71.6, CH	3.90 (t, 9.5)	73.2, CH	3.79 (t, 9.5)
4"	69.4, CH	3.44-3.47 (m)	70.6, CH	3.52 (t, 9.5)
5"	72.9, CH	3.81-3.83 (m)	74.4, CH	3.98 (ddd, 9.5, 4, 2)
6"	60.6, CH_2	3.79(dd, 12.5, 6.0)	62.1, CH_2	3.82(dd, 12.5, 5)
		3.74 (dd, 12.5, 3.0)		3.91 (dd, 12.5, 2)
1"'	104.7, CH	4.48 (d, 7.7)	106.0, CH	4.44 (d, 8)
2"'	73.8, CH	3.51 (dd, 7.5, 9.0)	75.2, CH	3.40 (t, 8)
3"'	76.3, CH	3.36 (d, 9.2)	77.5, CH	3.37 (t, 8.5)
4"'	69.6, CH	3.53 (t, 9.0)	70.8, CH	3.51 (t, 8.5)
5"'	73.9, CH	3.44-3.47 (m)	75.2, CH	3.46 (m)
6"'	62.6, CH_2	4.22 (dd, 11.9, 5.0)	63.9, CH_2	4.27 (dd, 12, 4)
		4.50 (dd, 11.9, 2.3)		4.51 (dd, 12, 2)
1""	19.4, CH_3	2.03 (s)	19.4, CH_3	2.06 (s)
2""	171.9, C		171.9, C	
1""'			130.7, C	
2""' / 6""'			130.9, CH	8.13 (d, 16)
3""' / 5""'			129.7, CH	7.51 (t, 8)
4""'			134.5, CH	7.63 (tt, 8, 1)
7""'			167.2, C	