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Oligosaccharide polyester and triterpenoid saponins from the roots of *Polygala japonica*

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Abstract

An oligosaccharide polyester, 1-*O*-(*E*)-*p*-coumaroyl-(3-*O*-benzoyl)-β-D-fructofuranosyl-(2 → 1)-[6-*O*-(*E*)-feruloyl-β-D-glucopyranosyl-(1 → 2)]-[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-(4-*O*-acetyl)-β-D-glucopyranosyl-(1 → 3)]-4-*O*-[4-*O*-α-L-rhamnopyranosyl-(*E*)-*p*-coumaroyl]-α-D-glucopyranoside (polygalajaponicose I), and four triterpenoid saponins, 3β, 23, 27-trihydroxy-29-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-olean-12-en-28-oic acid (polygalasaponin XLVII), 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester (polygalasaponin XLVIII), 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-galactopyranosyl-(1 → 5)-β-D-apiofuranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl ester (polygalasaponin XLIX) and 2β, 27-dihydroxy-3-*O*-β-D-glucopyranosyl 11-oxo-olean-12-en-23, 28-dioic acid 28-*O*-β-D-galactopyranosyl-(1 → 5)-β-D-apiofurano-syl-(1 → 4)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester (polygalasaponin L), in addition to five known compounds have been isolated from the roots of *Polygala japonica*.
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1. Introduction

The genus *Polygala* (family Polygalaceae) consists of more than 500 species from all over the world, of which 39 species are distributed throughout China. Some of these species have been used as traditional Chinese medicines for thousands of years for treating amnesia, neurasthenia and inflammation. *Polygala japonica* Houtt., a perennial herbaceous plant widely distributed in southern China, is such a folk herbal medicine and is used as an ataractic, an expectorant and an anti-inflammatory agent for pharyngitis. These activities may be due to the presence of various saponins in *P. japonica* (Zhang et al., 1995a,b, 1996a,b), since pharma-ceutical studies have indicated that the saponins found in *Polygala* have antipsychotic (Chung et al., 2002) and expectorant (Peng and Xu, 1998) effects. Oligosaccharide polyesters are another kind of typical constituents in the genus *Polygala*, and have been reported to have cognition improving and cerebral protective effects (Kouin et al., 2004). As part of research into the bioactive constituents from the genus *Polygala*, the chemical constituents from the roots of *P. japonica* were investigated because previous work was mainly concerned with the aerial parts (Zhang et al., 1995a,b, 1996a). This paper deals with the isolation and structural elucidation of an oligosaccharide polyester and four triterpenoid saponins, respectively, named polygalaja-ponicose I (**1**) and polygalasaponins XLVII-L (**2–5**), along with five known compounds, polygalasaponin XXVIII (**6**), polygalasaponin XXIV (**7**), polygalasaponin XXIX (**8**), sucrose (**9**) and 3-hydroxy-1,2,7-trimethoxyxanthone (**10**).

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2. Results and discussion

The Me₂CO:EtOH (1:1) fraction of a 95% EtOH extract of *P. japonica* Houtt. was subjected to macroporous resin, silica gel column, MPLC and Sephadex LH-20 column chromatographic purification steps, as well as semi-preparative scale HPLC to afford compounds **1**–**9**. Compound **10** was isolated from the EtOAc fraction of the 95% EtOH extract.

Polygalajaponicose I (**1**) was assigned a molecular formula of $C_{75}H_{90}O_{40}$, deduced from the $[M + H]^+$ ion *m/z* at 1631.5085 in the positive high resolution ESI MS (HR–ESI MS), as well as from analysis of the ¹³C NMR spectroscopic data (see Section 4). Its IR spectrum also indicated the presence of hydroxyl (3381 cm⁻¹), carbonyl (1712 cm⁻¹) and phenyl (1631, 1604, 1514, 835, 715 cm⁻¹) groups. After acid hydrolysis of **1**, the EtOAc portion contained benzoic acid, (*E*)-*p*-coumaric and (*E*)-ferulic acid moieties, which were identified by comparing retention times with those of authentic samples on HPLC; the aqueous portion afforded D-glucose, D-fructose and L-rhamnose, identified by HPLC analysis of acyclic diastereoisomeric derivatives of sugars.

The ¹HNMR spectroscopic data showed five anomeric protons at δ 6.45 (*d*, *J* = 2.5 Hz), 6.11 (brs), 5.20 (*d*, *J* = 8.0 Hz), 5.11 (2 H, *d*, *J* = 8.0 Hz), assigned to α-glucose, α-rhamnose, β-glucose, β-glucose and β-glucose, respectively; the 10 configurations were determined from the J_{H1-H2} values and by comparing the ¹³C NMR spectroscopic data for C-3 and C-5 of rhamnose with the literature data for the α-orientation (Kasai et al., 1979). The ¹³C NMR spectrum of **1** indicated the presence of six anomeric (δ 106.2, 105.6, 103.8, 103.4, 99.7, 92.6), two sets of acetyl, a set of benzoyl, a set of feruloyl, and two sets of *p*-coumaroyl carbons. The sugar proton and carbon signals in the NMR spectra were assigned by a combined analysis of NOE, 2D-NOESY, HSQC and HMBC experiments.
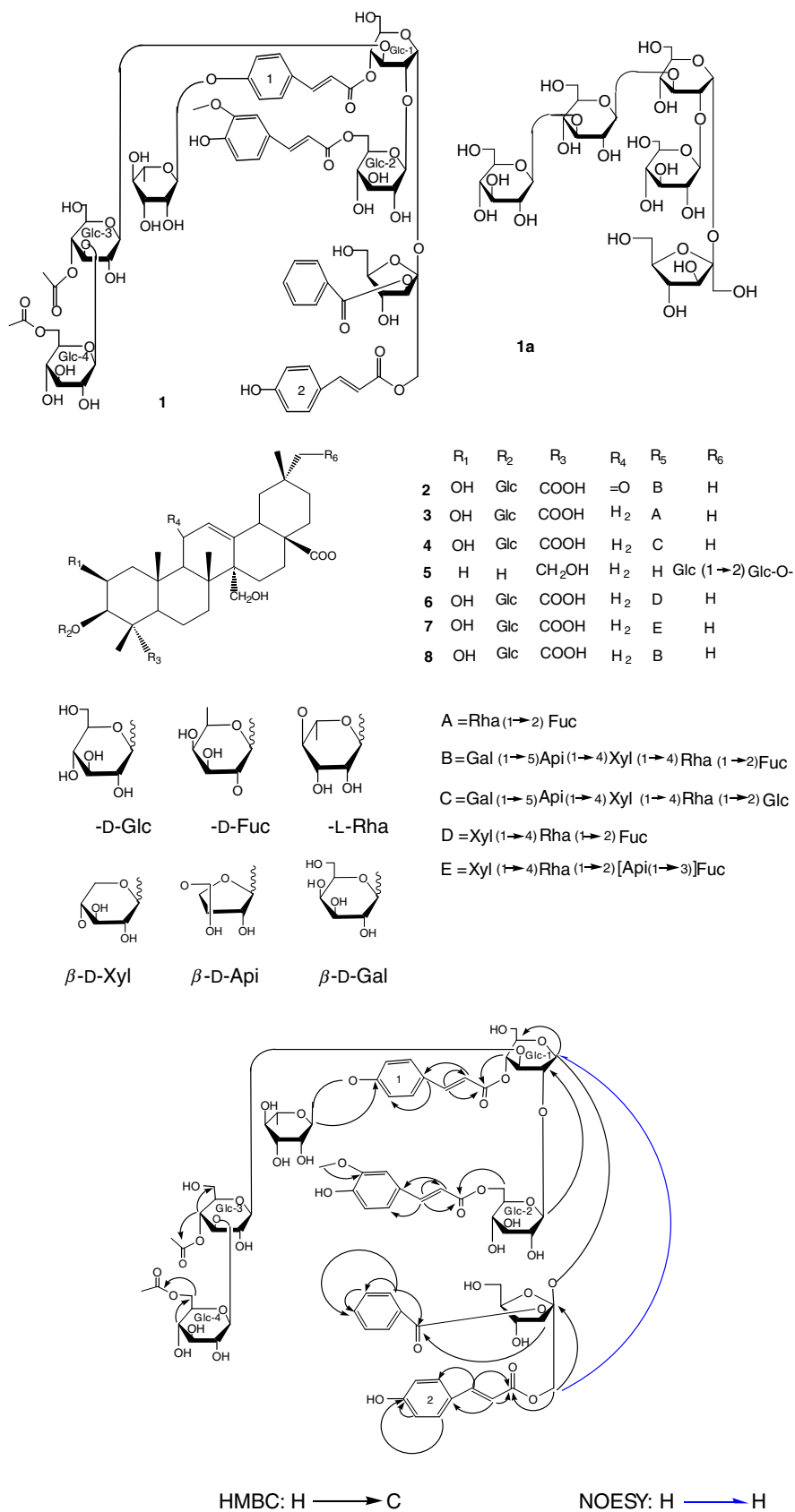
The linkage sites among the monosaccharides were deduced from the following correlations observed in the NOESY and NOE experiments: H-1 of Fru with H-1 of Glc-1, H-1 of Glc-2 with H-2 of Glc-1, H-1 of Glc-3 with H-3 of Glc-1, H-1 of Glc-4 with H-3 of Glc-3, and H-1 of Rha with H-3, H-5 of the set of *p*-coumaroyl protons at δ 7.33. These correlations indicated a pentasaccharide skeleton for **1a** and a rhamnose linked to C-4 of a *p*-coumaroyl group.

The HMBC spectrum further confirmed the above conclusions because long-range correlations were also observed between the following protons and carbons in **1**: H-4 of Glc-3 with a carbonyl carbon of acetyl (δ 170.0), H-6 of Glc-4 with a carbonyl carbon of acetyl (δ 170.6), H-3 of Fru with a carbonyl carbon of benzoyl, H-6 of Glc-2 with a carbonyl carbon of feruloyl, H-4 of Glc-1 with a carbonyl carbon of *p*-coumaroyl (δ 166.33), and H-1 of Fru with a carbonyl carbon of *p*-coumaroyl (δ 166.9). On the basis of these data, the structure of polygalajaponicose I (**1**) was established as 1-*O*-(*E*)-*p*-coumaroyl-(3-*O*-benzoyl)-β-D-fructofuranosyl-

(2 → 1)-[6-*O*-(*E*)-feruloyl-β-D-glucopyranosyl-(1 → 2)]-[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-(4-*O*-acetyl)-β-D-glucopyranosyl-(1 → 3)]-4-*O*-[4-*O*-α-L-rhamnopyranosyl-(*E*)-*p*-coumaroyl]-α-D-glucopyranoside (see Fig. 1).

Polygalasaponin L (**2**) had an $[M + Na]^+$ ion *m/z* at 1435.6013 in the positive HR–ESI MS, 14 mass units higher than that of the known compound **8** (1421$[M + Na]^+$). Combined with analysis of the ¹H and ¹³C NMR spectra of **2**, its molecular formula was deduced as $C_{64}H_{100}O_{34}$. Comparison of the NMR spectroscopic data of **2** and **8** showed that they were almost superimposable in terms of the sugar moieties but differed in the aglycone moiety: **2** had a carbonyl carbon at δ_C 201.4 and the olefinic carbon signals were shifted downfield to δ_C 162.0, 131.8 compared with the alkenyl resonances of **8** at δ_C 138.8, 127.8. The analysis of the HMBC spectrum indicated the carbonyl carbon (δ 201.4) was at C-11 of the aglycone (see Fig. 2). The structure of the sugar chain of **2** was confirmed by analysis of HMBC and NOE spectra. In the HMBC spectrum, long-range correlations were observed between the following protons and carbons: H-1 of Glc with C-3 of aglycone, H-1 of Fuc with C-28 of aglycone, H-1 of Rha with C-2 of Fuc, and H-1 of Xyl with C-4 of Rha. Moreover, correlations were observed between H-1 of Api and H-4 of Xyl and between H-1 of Gal and H-5 of Api in the NOE experiment. Six monosaccharides in the acid hydrolysate of **2** were identified as D-glucose, D-fucose, L-rhamnose, D-xylose, D-apiose and D-galactose by the same methods as described for **1**. Based on the above evidence, the structure of **2** was elucidated as 2β, 3β, 27-trihydroxy-3-*O*-β-D-glucopyranosyl 11-oxo-olean-12-en-23, 28-dioic acid 28-*O*-β-D-galactopyranosyl-(1 → 5)-β-D- apiofuranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 4)-α-L-hamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester.

The following five saponins, polygalasaponin XLVIII (**3**), polygalasaponin XLIX (**4**), polygalasaponin XXVIII (**6**) (Zhang et al., 1996b), polygalasaponin XXIV (**7**) (Zhang et al., 1996a), and polygalasaponin XXIX (**8**) (Zhang et al., 1996b), contained the same aglycone known as presenegenin, which was identified by comparison of the NMR spectroscopic data with the literature values (Toshio et al., 1995). Polygalasaponin XLVIII (**3**) gave a $[M + Na]^+$ ion peak *m/z* at 995.4865 in the positive HR–ESI MS, 132 mass units less than that of **6** (1127 $[M + Na]^+$), implying the absence of a pentose moiety. The NMR spectroscopic data of compounds **3** and **6** were almost identical except for a set of terminal xylose signals, indicating that **3** had a structure similar to **6** except that a proton of **3** was replaced by a xylose moiety in **6**. The NMR spectroscopic data for compounds **4** and **8** were almost identical, except that **4** had one more set of glucose signals than **8** and one less set of fucose resonances, indicating that **4** had a structure similar to **8** except that the fucose in **8** was replaced by glucose in **4**. In addition, the structure of **4** was confirmed by HMBC and NOE spectra. In HMBC, long-range correlations were observed between the following protons and carbons: H-1 (δ_H 5.04) of Glc

	R_1	R_2	R_3	R_4	R_5	R_6
2	OH	Glc	COOH	=O	B	H
3	OH	Glc	COOH	H_2	A	H
4	OH	Glc	COOH	H_2	C	H
5	H	H	CH_2OH	H_2	H	Glc (1→2) Glc-O-
6	OH	Glc	COOH	H_2	D	H
7	OH	Glc	COOH	H_2	E	H
8	OH	Glc	COOH	H_2	B	H

A = Rha(1→2)Fuc

B = Gal(1→5)Api(1→4)Xyl(1→4)Rha(1→2)Fuc

C = Gal(1→5)Api(1→4)Xyl(1→4)Rha(1→2)Glc

D = Xyl(1→4)Rha(1→2)Fuc

E = Xyl(1→4)Rha(1→2)[Api(1→3)]Fuc

-D-Glc   -D-Fuc   -L-Rha

β-D-Xyl β-D-Api β-D-Gal

HMBC: H ——→ C   NOESY: H ——→ H

Fig. 1. The structures of compounds **1**–**8** and key HMBC and NOE correlations of polygalajaponicose I (**1**).

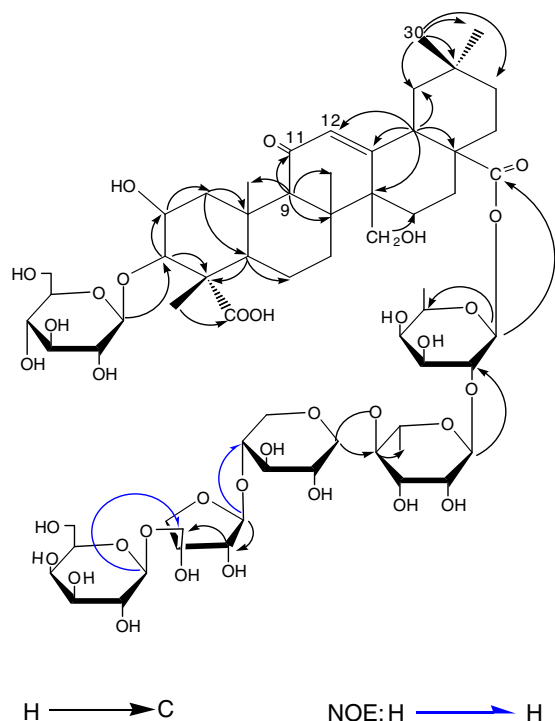Fig. 2. Key HMBC and NOE correlations of polygalasaponin L. (2).

HMBC: H ——→ C   NOE: H ——→ H

3. Conclusions

Compounds **1–5** are new derivatives belonging to the oligosaccharide and triterpenoid classes. Polygalajaponicose I (**1**) is one of the most structurally complex oligosaccharide polyesters found thus far. Polygalasaponin XLVII (**5**) shows an unusual substitution pattern of the Δ-12 oleanene skeleton, since in Δ-12 oleanene structures, the glucosyl moiety is usually linked to C-3 of the aglycone, and very few compounds have the sugar chain attached to C-29 of the aglycone (Hamburger and Hostettmann, 1986). Moreover, from our research and previous reports, it may be concluded that the saponins found in the roots of *Polygala japonica* differ from those in the aerial parts: the saponins found in the aerial parts of *P. japonica* have normal aglycones of Δ-12 oleanene type, while those found in roots all have the hydroxyl group attached to C-27 of the aglycones, which is an uncommon feature in olean-type triterpenoids.

4. Experimental

4.1. General experimental procedures

Optical rotations were determined on a Perkin–Elmer 241 digital polarimeter. Melting points were determined on an XT-4 micro-melting point apparatus and are uncorrected. IR spectra were recorded on an IMPACT 400 spectrometer as KBr pellets. UV spectra were obtained on a Shimadzu UV-260 spectrophotometer. ^{1}H and ^{13}C NMR spectra were acquired on a Varian Unity Inova spectrometer at 500 and 125 MHz, respectively, in pyridine-d_5 at ambient temperature using TMS as internal standard. The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. ^{1}H–^{1}H interactions were determined by NOE and 2D NOESY. One-bond heteronuclear ^{1}H–^{13}C connectivities were determined with 2D HSQC. Two- and three-bond heteronuclear ^{1}H–^{13}C connectivities were determined with 2D HMBC experiments, optimized for $^{2–3}J_{CH}$ of 8 Hz. ESI MS were measured on an Agilent 1100 series LC/MSD Trap SL mass spectrometer and Autospee-Ultima ETOF. Reversed-phase HPLC was performed on a YMC-Pack ODS-A (YMC Co. Ltd.) column. Silica gel (100–200, 200–300 mesh, Qingdao) was used for CC and silica gel GF-254 (Qingdao) for TLC Analysis. Authentic samples were purchased from Sigma and Sinopharm Chemical Reagent Co. Ltd. All chemical solvents used for isolation were of analytical grade or higher.

with C-3 of aglycone, H-1 (δ_H 6.23) of Glc with C-28 of aglycone, H-1 of Rha with C-2 (δ_C 76.6) of Glc, and H-1 of Xyl with C-4 of Rha. The NOE correlations were observed between H-1 of Api and H-4 of Xyl and between H-1 of Gal and H-5 of Api. Thus, polygalasaponin XLVIII (**3**) was determined as 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl ester, and polygalasaponin XLIX (**4**) was established as 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-galactopyranosyl-(1 → 5)-β-D-apiofuranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl ester.

Polygalasaponin XLVII (**5**) displayed a [M + Na]$^+$ ion peak *m/z* at 851.4482 in the positive HR–ESI MS, corresponding to a molecular formula of $C_{42}H_{68}O_{16}$. The NMR spectroscopic data of **5** were characteristic of a triterpenoid glycoside with two sugar units. Detailed analysis of the NMR spectroscopic data established that the aglycone of **5** is 3β, 23, 27, 29-tetrahydroxy olean-12-en-28-oic acid (Hamburger and Hostettmann, 1986). After acid hydrolysis, 5 only afforded D-glucose sugar moieties, and the NMR spectroscopic data indicated the existence of a β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl group (Zhang et al., 1996b). The sugar chain of **5** was also deduced from the HMBC correlations between H-1 of Glc-inn and C-29 of aglycone and between H-1 of Glc-ter and C-2 of Glc-inn. Therefore, the structure of polygalasaponin XLVII was elucidated as 3β, 23, 27-trihydroxy-29-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-olean-12-en-28-oic acid.

4.2. Plant material

The roots of *Polygala japonica* Houtt. were collected in June 2003 in Jiangxi Province, China. The plant was identified by Professor Yongming Luo, Jiangxi College of Traditional Chinese Medicine. A voucher specimen was deposited in the Institute of Materia Medica, Chinese

Academy of Medical Sciences and Peking Union Medical College, Beijing (specimen number: 030621).

4.3. Extraction and isolation

The roots of *P. japonica* Houtt were air-dried after collection at ambient temperature, giving a dry weight of 5.0 kg. The dried roots were pulverized and extracted with 40 l of EtOH–H_2O (95:5, v/v) by heating until reflux began and maintaining this for 2 h. This process was repeated for three times and a residue (0.839 kg) was obtained after removing the solvent under reduced pressure. This residue was subjected to silica gel CC (100–200 mesh), eluted with $CHCl_3$ (8 l), EtOAc (8 l), EtOAc:Me_2CO (1:1, v/v, 8 l), EtOAc:Me_2CO (1:3, v/v, 8 l), Me_2CO (8 l), Me_2CO:EtOH (1:1, v/v, 8 l), EtOH (8 l) and MeOH (8 l), successively. The Me_2CO:EtOH (1:1, v/v) fraction (380 g) was applied to D_{101} macroporous resin (2 kg) CC and eluted gradually in this order: H_2O (9 l), EtOH–H_2O (3:7, v/v, 9 l), EtOH–H_2O (6:4, v/v, 9 l) and EtOH–H_2O (3:7, v/v, 9 l). The EtOH–H_2O (3:7, v/v) fraction (77.2 g) was subjected to a silica gel CC, and eluted with $CHCl_3$:MeOH:H_2O (70:30:5) to afford ten fractions and compound **9**. Fractions 5, 6 and 9 were separated by preparative scale MPLC with a mobile phase of MeOH–H_2O mixture (4:6, 5:5, 6:4, 8:2, v/v), and every 1500 ml were sampled and their homogeneity monitored by UV-detector with the absorbance wavelength at 210 nm, combining the fractions that showing similar UV-spectroscopic profiles. The samples were continuously purified by Sephadex LH-20 using MeOH–H_2O solvents (1:9, 3:7, 6:4, 8:2, v/v, 500 ml) as gradient eluent and semi-preparative scale HPLC (MeOH:H_2O, 50:50/53:47/55:45 + 0.05% TFA, v/v, 7 ml/min) to afford **1** (25 mg), **2** (30 mg), **3** (18 mg), **4** (22 mg), **5** (32 mg), **6** (27 mg), **7** (30 mg) and **8** (300 mg). The EtOAc extract (30.5 g) was subjected to silica gel CC (100–200 mesh), eluting with a gradient of $CHCl_3$–MeOH (100:1 → 100:25, v/v) to yield 61 fractions. Fractions 19–20 were separated by Sephadex LH-20 CC ($CHCl_3$–MeOH, 2:1, v/v) and recrystallized from MeOH to give **10** (4 mg).

4.4. Polygalajaponicose I (**1**)

White powder, $[\alpha]_D^{14}$ −30.25 (MeOH; c0.40). ESI MS m/z: 1631 [M + H]$^+$, HR–ESI MS m/z: 1631.5085 [M + H]$^+$ (calcd. for $C_{75}H_{91}O_{40}$, 1631.5087). IR ν_{max}^{KBr} cm^{-1}: 3381, 1712, 1631, 1604, 1514, 835, 715; UV λ_{max}^{MeOH} nm (log ε): 208.0 (4.60), 228.0 (4.62), 306.6 (4.65). ^{1}H NMR (500 MHz, C_5D_5N): δ 8.40 (2H, d, J = 7.5 Hz; H-2, 6 of benzoyl), 8.02 (1H, d, J = 16.0 Hz; H-7 of feruloyl), 7.92 (1H, d, J = 16.0 Hz; H-7 of p-coumaroyl-1), 7.79 (1H, d, J = 16.0 Hz; H-7 of p-coumaroyl-2), 7.67 (1H, m, H-4 of benzoyl), 7.65 (2H, d, J = 8.0 Hz; H-2, 6 of p-coumaroyl-2), 7.60 (2H, t, J = 7.5 Hz; H-3, 5 of benzoyl), 7.37 (1H, s; H-2 of feruloyl), 7.33 (2H, d, J = 8.0 Hz; H-3, 5 of p-coumaroyl-1), 7.31 (2H, d, J = 8.0 Hz; H-2, 6 of p-coumaroyl-1), 7.30 (1H, d, J = 8.0 Hz; H-6 of feruloyl), 7.15 (1H, d,

J = 8.0 Hz; H-5 of feruloyl), 7.12 (2H, d, J = 8.0 Hz; H-3, 5 of p-coumaroyl-2), 6.76 (1H, d, J = 16.0 Hz; H-8 of feruloyl), 6.70 (1H, d, J = 16.0 Hz; H-8 of p-coumaroyl-1), 6.52 (1H, d, J = 8.0 Hz; H-3 of Fru), 6.45 (1H, d, J = 2.5 Hz; H-1 of Glc-1), 6.38 (1H, d, J = 16.0 Hz; H-8 of p-coumaroyl-2), 6.11 (1H, brs; H-1 of Rha), 5.69 (1H, m; H-4 of Glc-4), 5.46 (1H, t, J = 8.0 Hz; H-4 of Fru), 5.38 (1H, d, J = 9.0 Hz; H-5 of Glc-3), 5.34 (1H, d, J = 11.0 Hz; H-1a of Fru), 5.20 (1H, d, J = 8.0 Hz; H-1 of Glc-3), 5.14 (1H, d, J = 10.5 Hz; H-6a of Glc-2), 5.11 (2H, d, J = 8.0 Hz; H-1 of Glc-2; H-1 of Glc-4), 4.96 (1H, H-6b of Glc-2), 4.94 (1H, H-1b of Fru), 4.74 (1H, H-5 of Fru), 4.67 (1H, H-3 of Glc-1), 3.82 (3H, s; H-OCH$_3$ of feruloyl), 2.20 (3H, s; H-AcO of Glc-3), 1.76 (3H, s; H-AcO of Glc-4), 1.54 (3H, d, J = 6.0 Hz; H-6 of Rha). ^{13}C NMR (125 MHz, C_5D_5N): δ 170.6 [CO-AcO-(Glc-4)], 170.0 [CO-AcO-(Glc-3)], 167.8 (C-9-feruloyl), 166.9 [C-9-(p-coumaroyl-2)], 166.3 [C-9-(p-coumaroyl-1)], 166.3 (C-7-benzoyl), 161.4 [C-4-(p-coumaroyl-2)], 159.1 [C-4-(p-coumaroyl-1)], 151.0 (C-4-feruloyl), 148.9 (C-3-feruloyl), 145.9 (C-7-feruloyl), 145.5 [C-7-(p-coumaroyl-2)], 144.8 [C-7-(p-coumaroyl-1)], 133.7 (C-4-benzoyl), 130.7 [2C; C-2, 6-(p-coumaroyl-2)], 130.4 (2C; C-3, 5-benzoyl), 130.2 [4C; C-2, 6-benzoyl, C-2, 6-(p-coumaroyl-1)], 129.1 (C-1-benzoyl), 128.7 [C-1-(p-coumaroyl-1), 126.7 (C-1-feruloyl), 126.0 [C-1-(p-coumaroyl-2)], 123.9 (C-6-feruloyl), 117.4 [2C, C-3, 5-(p-coumaroyl-1)], 116.7 [2C, C-3, 5-(p-coumaroyl-2)], 116.6 (C-5-feruloyl), 116.5 [C-8-(p-coumaroyl-1)], 115.2 (C-8-feruloyl), 114.7 [C-8-(p-coumaroyl-2)], 111.5 (C-2-feruloyl), 106.2 [C-1-(Gcl-4)], 105.6 [C-1-(Glc-2)], 103.8 [C-1-(Glc-3)], 103.4 (C-2-Fru), 99.7 (C-1-Rha), 92.6 [C-1-(Glc-1)], 84.7 (C-5-Fru), 84.0 [C-3-(Glc-3)], 81.4 [C-2-(Glc-1)], 79.8 (C-3-Fru), 78.5 [C-3-(Glc-1)], 78.4 [C-3-(Glc-2)], 78.0 [2C; C-3, 5-(Glc-4)], 76.0 [C-2-(Glc-4)], 75.7 [C-2-(Glc-2)], 75.0 [C-2-(Glc-3)], 73.6 (C-4-Rha), 73.1 (C-4-Fru), 72.5 [2C; C-3-Rha, C-5-(Glc-1)], 71.8 [C-5-(Glc-3)], 71.5 [C-4-(Glc-4)], 71.0 (C-5-Rha), 70.7 [C-5-(Glc-2)], 70.5 [C-4-(Glc-1)], 69.3 [C-4-(Glc-3)], 63.5 [C-6-(Glc-2)], 62.8 [C-6-(Glc-4)], 62.6 (C-6-Fru), 62.5 [C-6-(Glc-3)], 62.1 [C-6-(Glc-1)], 55.9 (OCH$_3$-feruloyl), 21.0 [CH$_3$-AcO-(Glc-3)], 20.3 [CH$_3$-AcO-(Glc-4)], 18.5 (C-6-Rha).

4.5. Polygalasaponin L (**2**)

White power, $[\alpha]_D^{14}$ −5.56 (MeOH; c0.36). ESI MS m/z: 1435 [M + Na]$^+$; HR–ESI MS m/z: 1435.6013 [M + Na]$^+$ (calcd. for $C_{64}H_{100}O_{34}Na$, 1435.5994). IR ν_{max}^{KBr} cm^{-1}: 3406, 1678; UV λ_{max}^{MeOH} nm (log ε): 204.0 (4.31). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.6. Polygalasaponin XLVIII (**3**)

White power, $[\alpha]_D^{14}$ +11.30 (MeOH; c0.24). ESI MS m/z: 851 [M + Na]$^+$; HR–ESI MS m/z: 851.4482 [M + Na]$^+$ (calcd. for $C_{42}H_{68}O_{16}Na$, 851.4405). IR ν_{max}^{KBr} cm^{-1}: 3413, 1691; UV λ_{max}^{MeOH} nm (log ε): 204.4 (4.01). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

Table 1
^{1}H NMR spectroscopic data of compounds **2–5** (C_5D_5N, 500 MHz, δ ppm)*

	2	**3**	**4**	**5**
Aglycone				
2	4.69(1H, *m*)	4.71(1H, *m*)	4.70(1H, *m*)	1.76
2	–	–	–	1.82
3	4.55(1H, *d*, 3.0)	4.59(1H, *d*, 3.0)	4.58(1H, brs)	4.08
12	6.32(1H, brs)	5.83(1H, *t*-like)	5.81(1H, brs)	5.87(1H, brs)
18	3.27(1H, *dd*, 14, 3)	3.20(1H, *dd*, 14, 4)	3.20(1H, *d*, 12)	3.44(1H, *d*, 14, 4)
24	1.96(3H, *s*)	1.96(3H, *s*)	1.93(3H, *s*)	1.02(3H, *s*)
25	1.85(3H, *s*)	1.56(3H, *s*)	1.51(3H, *s*)	0.92(3H, *s*)
26	1.34(3H, *s*)	1.12(3H, *s*)	1.11(3H, *s*)	1.02(3H, *s*)
27	3.97(1H, *d*, 12)	3.71(1H, *d*, 12)	3.80(1H, *d*, 12)	3.74
27	4.44(1H, *d*, 12)	3.91(1H, *d*, 12)	4.05(1H, *d*, 12)	4.00(1H, *d*, 12)
29	0.71(3H, *s*)	0.79(3H, *s*)	0.75(3H, *s*)	3.35(1H, *d*, 9)
29	–	–	–	3.73(1H, *d*, 9)
30	0.77(3H, *s*)	0.87(3H, *s*)	0.85(3H, *s*)	1.27(3H, *s*)
C-3 sugar				
Glc-1	5.01(1H, *d*, 7.5)	5.07(1H, *d*, 8.0)	5.04(1H, *d*, 6.0)	
2	3.92	3.90(1H, *t*, 6.0)	3.90	
3	4.14	4.14	4.14	
4	4.13	4.14	4.12	
5	3.86	3.92	3.92	
6	4.28	4.29	4.29	
6	4.42	4.46	4.43	
C-28 sugar				
Fuc-1	6.03(1H, *d*, 8.0)	6.04(1H, *d*, 8.0)		
2	4.64(1H, *t*, 8.0)	4.74(1H, *t*, 8.5)		
3	4.13	4.16		
4	3.93	3.95		
5	3.91	3.90		
6	1.45(3H, *d*, 6.0)	1.48(3H, *d*, *J* = 6.0)		
Glc-1				6.23(1H, *d*, 7.5)
2				4.36
3				4.29
4				4.29
5				3.96
6				4.28
6				4.43
Rham-1	6.32(1H, brs)	6.53(1H, brs)	6.41(1H, brs)	
2	4.80(1H, brs)	4.81(1H, brs)	4.80(1H, brs)	
3	4.62	4.71	4.65	
4	4.24	4.33	4.28	
5	4.38	4.51	4.46	
6	1.62(3H, *d*, 6.0)	1.62(3H, *d*, 6.0)	1.71(3H, *d*, 6.0)	
Xyl-1	4.86(1H, *d*, 7.5)		4.89(1H, *d*, 7.0)	
2	4.04		4.02	
3	4.02		4.02	
4	4.13		4.14	
5	3.34(1H, *t*, 11.0)		3.33(1H, *t*, 10.0)	
5	4.34		4.30	
Api-1	6.36(1H, brs)		6.36(1H, brs)	
2	4.38		4.28	
4	4.17		4.15	
4	5.05(1H, *d*, 9.0)		5.05	
5	4.18		4.15	
5	4.66		4.67	
Gal-1	4.83(1H, *d*, 7.5)		4.82(1H, *d*, 6.5)	
2	4.38		4.39	
3	4.40		4.36	
4	4.48		4.48	
5	3.98		4.00	
6	4.36		4.36	
6	4.41		4.43	
C-29 sugar				
Glc-1 inn				4.80(1H, *d*, 7.5)
2				4.15
3				3.95
4				4.15
5				4.21
6				4.06
6				4.35
Glc-1 ter				5.29(1H, *d*, 7.5)
2				4.08
3				3.86
4				4.29
5				4.30
6				4.33
6				4.48

*Assignments were based on COSY, HSQC and HMBC experiments, and due to severe overlapping in the ^{1}H spectrum, only detectable relative *J*(Hz) are reported.

4.7. Polygalasaponin XLIX (4)

White power, $[\alpha]_D^{20}$ −12.16 (MeOH; *c*0.37). ESI MS *m/z*: 1437 [M + Na]$^+$; HR–ESI MS *m/z*: 1437.6144 [M + Na]$^+$ (calcd. for $C_{64}H_{102}O_{34}Na$, 1437.6150). IR v_{max}^{KBr} cm^{-1}: 3413, 1678; UV λ_{max}^{MeOH} nm (log ε): 204.2(4.26). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.8. Polygalasaponin XLVII (5)

Colorless power, $[\alpha]_D^{20}$ +7.50 (MeOH; *c*0.16). ESI MS *m/z*: 995 [M + Na]$^+$; HR–ESI MS *m/z*: 995.48645 [M + Na]$^+$ (calcd. for $C_{48}H_{76}O_{20}Na$, 995.48276). IR v_{max}^{KBr} cm^{-1}: 3411, 1680; UV λ_{max}^{MeOH} nm (log ε): 204.4 (4.46). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.9. Acid hydrolysis of compounds 1–5

To each compound (3 mg) was added 1 ml 10% HCl/H_2O (v/v), with the resulting mixtures heated until reflux began at 75 °C. This was maintained for 3 h, with the mixture cooled and partitioned by EtOAc (1 ml × 3). EtOAc solubles for each experiment was evaporated to dryness

Table 2
^{13}C NMR spectroscopic data of compounds **2–8** (C$_5$D$_5$N, 125 MHz, δ ppm)

	2	**3**	**4**	**5**		**2**	**3**	**4**
Aglycone					*C-3 sugar*			
1	45.1	44.2	44.1	38.5	Glc-1	105.3	105.3	105.4
2	70.5	70.3	70.1	27.6	2	75.1	75.2	75.2
3	85.7	85.9	85.9	73.6	3	78.3	78.3	78.3
4	53.1	52.8	52.8	42.7	4	71.4	71.5	71.5
5	51.9	52.4	52.4	48.7	5	78.3	78.3	78.3
6	20.8	21.3	21.4	18.6	6	62.5	62.6	62.7
7	33.6	33.4	33.5	33.3	*C-28 sugar*			
8	46.4	41.0	41.0	40.4	Fuc-1	94.9	94.9	
9	63.2	49.3	49.3	48.7	2	74.3	73.9	
10	37.5	37.0	36.9	37.3	3	76.5	76.9	
11	201.4	23.4	24.4	24.1	4	73.1	73.3	
12	131.8	127.8	127.8	127.8	5	72.5	72.4	
13	162.0	138.9	138.9	139.5	6	16.8	16.9	
14	49.5	48.0	48.0	47.8	Glc-1			94.7
15	25.1	24.5	23.9	24.3	2			76.6
16	23.1	24.4	23.5	23.8	3			79.3
17	46.6	46.8	46.9	46.8	4			71.2
18	42.4	41.9	41.9	40.8	5			78.8
19	43.3	45.3	45.2	40.3	6			61.9
20	30.5	30.7	30.7	35.6	Rham-1	101.4	101.4	101.3
21	33.5	33.8	33.8	29.1	2	71.7	72.3	71.7
22	31.6	33.2	33.3	32.4	3	72.2	72.5	72.4
23	180.7	180.6	180.7	68.3	4	85.1	73.4	84.8
24	14.0	14.2	14.1	13.0	5	68.3	69.7	68.3
25	18.8	17.5	17.4	16.3	6	18.4	18.6	18.5
26	21.2	18.7	18.5	18.8	Xyl-1	107.3		107.1
27	62.7	64.2	64.3	64.4	2	75.1		75.2
28	176.5	176.6	176.5	180.1	3	76.9		70.0
29	32.7	33.0	33.0	81.1	4	78.2		78.3
30	23.5	23.9	23.9	19.9	5	64.6		64.5
C-29 sugar					Api-1	109.4		109.4
Glc-1 inn				103.4	2	77.8		77.7
2				83.7	3	81.3		81.3
3				78.5	4	76.1		76.1
4				71.3	5	67.6		67.6
5				77.9	Gal-1	103.1		103.2
6				62.8	2	72.5		72.2
Glc-1 ter				106.3	3	73.0		73.2
2				76.9	4	70.1		70.3
3				78.2	5	77.0		76.8
4				71.5	6	62.0		62.1
5				77.9				
6				62.6				

under reduced pressure at room temperature. The dried EtOAc fraction of **1** was next dissolved in MeOH and applied to HPLC to compare retention times with those of authentic samples (YMC-Pack ODS-A, 150 × 4.6 mm, 5 μm, CH$_3$CN–H$_2$O–TFA 450:1550:1, 254 nm, 35 °C, with a flow rate of 1.0 ml/min). As a result, (*E*)-*p*-coumaric acid (t_R 4.25 min), (*E*)-ferulic acid (t_R 4.85 min) and benzoic acid (t_R 7.62 min) were detected. The dried H$_2$O fraction of each compound was redissolved in H$_2$O 0.2 ml, then added to a MeOH solution (0.2 ml) of L-(−)-α-methylbenzylamine (10 mg) and NaBH$_3$CN (2 mg). The mixture was allowed to stand overnight, acidified to pH 3 to 4 by adding glacial AcOH and evaporated to dryness. The resultant oily material was acetylated by reaction with acetic anhydride-dry pyridine (1:1; 1 ml) at 100 °C for 1 h in a sealed tube.

After codistillation of the Ac^{2}O with toluene, H$_2$O (1 ml) was added to the residue and the mixture was extracted with CHCl$_3$. The CHCl$_3$ layer was evaporated to dryness and purified by preparative TLC (CHCl$_3$–MeOH 100:1), then applied to HPLC (ZORBAX SIL-100A, 5 μm, 150 × 4.6 mm, *n*-hexane-EtOH 95:5, 230 nm, 30 °C, with a flow rate of 1.2 ml/min). The authentic samples were treated by the same method and the retention times (t_R) were: D-fructose 26.01 min, D-glucose 26.84 min, D-apiose 26.96 min, L-rhamnose 29.30 min, D-fucose 32.98 min, D-xylose 49.90 min, D-galactose 51.81 min. D-fructose was detected from **1**, D-glucose was detected from **1–5**, D-apiose was detected from **2** and **4**, L-rhamnose was detected from **1**, **2–4**, D-fucose was detected from **2** and **3**, D-xylose was detected from **2–4**, D-galactose was detected from **2** and **4**.

Acknowledgements

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