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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-apiofuranosyl-(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*.

Keywords: *Polygala japonica*; Polygalaceae; Oligosaccharide polyester; Triterpenoid saponins; Polygalajaponicose; Polygalasaponin

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 pharmaceutical 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 polygalajaponicose 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 Me_2CO: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 ^{13}C 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 ^{1}HNMR 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 ^{13}C NMR spectroscopic data for C-3 and C-5 of rhamnose with the literature data for the α-orientation (Kasai et al., 1979). The ^{13}C 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 ^{1}H and ^{13}C 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