Pergamon

PII: S0031-9422(97)00065-4

Phytochemistry, Vol. 45, No. 4, pp. 733–741, 1997

0031-9422/97 $17.00+0.00

OLIGOSACCHARIDE POLYESTERS FROM ROOTS OF *POLYGALA FALLAX*

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(*Received in revised form* 25 *November* 1996)

Key Word Index—*Polygala fallax*; Polygalaceae; roots; oligosaccharide polyesters; fallaxoses A–E.

Abstract—Five new oligosaccharide polyesters, fallaxoses A–E, along with four known ones, reiniose D, senegose G, tenuifolioses C and P, were isolated from the roots of *Polygala fallax*. Fallaxoses A–E were elucidated as 3-*O*-{4-*O*-[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]-feruloyl}-β-D-fructofuranosyl-(2 → 1)-(4,6-di-*O*-benzoyl)-α-D-glucopyranoside, 3-*O*-{4-*O*-[β-D-gluocopyranosyl-(1 → 3)-(2-*O*-acetyl)-α-L-rhamnopyranosyl]-feruloyl}-β-D-fructofuranosyl-(2 → 1)-(4,6-di-*O*-benzoyl)-α-D-glucopyranoside, 1-*O*-*p*-coumaroyl-(3-*O*-benzoyl)-β-D-fructofuranosyl-(2 → 1)-[β-D-glucopyranosyl-(1 → 2)]-[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)]-(4-*O*-*p*-coumaroyl)-α-D-glucopyranoside, 1-*O*-*p*-coumaroyl-(3-*O*-benzoyl)-β-D-fructofuranosyl-(2 → 1)-[β-D-glucopyranosyl-(1 → 2)]-[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)]-(4-*O*-feruloyl)-α-D-glucopyranoside, 1-*O*-feruloyl-(3-*O*-benzoyl)-β-D-fructofuranosyl-(2 → 1)-[β-D-glucopyranosyl-(1 → 2)]-[β-D-glucopyranosyl-(1 → 3)-(6-*O*-acetyl)-β-D-glucopyranosyl-(1 → 3)]-(6-*O*-feruloyl)-α-D-glucopyranoside, respectively, by spectroscopic and chemical means.

INTRODUCTION

We previously reported the isolation and structural elucidation of triterpene saponins, called polygalasaponins XXXIII–XLI, and seven known saponins from the roots of *Polygala fallax* [1]. We continued our investigation of the constituents of the roots of this species and isolated five new oligosaccharide polyesters, called fallaxoses A–E (**2–6**), together with four known oligosaccharides, reiniose D (**1**) [2], senegose G (**7**) [3], tenuifolioses C (**8**) [4] and P (**9**) [5]. This paper deals with the isolation and structural elucidation of the new oligosaccharide polyesters.

RESULTS AND DISCUSSION

A 70% aqueous methanol extract of roots was concentrated and the residue suspended in water and passed through a porous polymer gel, Mitsubishi Diaion HP-20, column. Adsorbed materials were eluted successively with 30 and 60% aqueous methanol, and methanol. The methanol eluate was chromatographed on silica gel and octadecyl silica (ODS) columns, followed by repeated semi-preparative HPLC on a reverse-phase (ODS, PhA) column to give compounds **1–9**. Compounds **7–9** were identified as senegose G (**7**), tenuifolioses C (**8**) and P (**9**), respectively, by comparison of their ^{1}H and ^{13}C NMR data with reported data.

Compound **1** showed a $[M+Na]^+$ at *m/z* 895 in the FAB-mass spectrum. The ^{1}H and ^{13}C NMR data of **1** are the same as those of reiniose D [2]. Previously, we reported that the positions of two benzoyl residues were at C-6 of glucose and C-3 of fructose and the position of the feruloyl residue was at the C-4 of glucose in reiniose D. In the heteronuclear multiple bond coherence (HMBC) spectrum of **1**, long-range correlations ($^3J_{HCOC}$) were observed between the carbonyl carbon signals at δ 167.2, 167.8 (C-7 of each benzoyl) and the proton signals at δ 5.19, 4.37 due to the H-4 and H-6 of glucose, respectively, between the carbonyl carbon signal at δ 167.6 (C-9 of feruloyl) and the proton signal at δ 5.42 due to the H-3 of fructose. Based on the above data, the structure of reiniose D was corrected as **1**.

Fallaxose A (**2**), $C_{48}H_{58}O_{25}$, showed a $[M+Na]^+$ at *m/z* 1057 in the FAB-mass spectrum. On acid hydrolysis, it afforded D-glucose, L-rhamnose and D-fructose, while on alkaline hydrolysis, it gave sucrose, benzoic acid and ferulic acid. The ^{1}H NMR spectrum suggested the presence of three anomeric protons [δ 4.63

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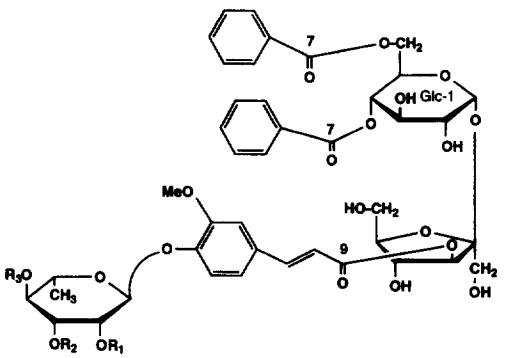
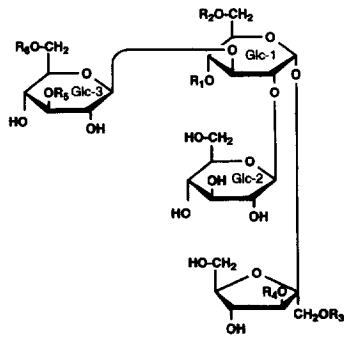


	R_1	R_2	R_3
1	H	H	H
2	H	H	Glc
3	Ac	Glc	H

(Glc-2: Glc at R_2 or R_3)



	R_1	R_2	R_3	R_4	R_5	R_6
4	*p*-coumaroyl	H	*p*-coumaroyl	benzoyl	H	Ac
4a	H	H	H	H	H	H
5	feruloyl	H	*p*-coumaroyl	benzoyl	H	Ac
6	H	feruloyl	feruloyl	benzoyl	Glc	Ac
6a	H	H	H	H	Glc	H
7	feruloyl	H	feruloyl	benzoyl	H	Ac
8	feruloyl	H	*p*-coumaroyl	benzoyl	Glc	Ac
9	feruloyl	H	feruloyl	benzoyl	Glc	Ac

(Glc-4: Glc at R_5)

(1H, *d*, J = 8 Hz), 5.38 (1H, *d*, J = 1.5 Hz), 5.61 (1H, *d*, J = 3.5 Hz)], two sets of benzoyl protons [δ 7.38 (2H, *t*, J = 8 Hz), 7.55 (1H, *tt*, J = 8, 2 Hz), 7.97 (2H, *dd*, J = 8, 2 Hz); 7.19 (2H, *t*, J = 8 Hz), 7.51 (1H, *tt*, J = 8, 2 Hz), 7.81 (2H, *dd*, J = 8, 2 Hz)] and one set of feruloyl protons [δ 3.69 (3H, *s*), 6.55 (1H, *d*, J = 16 Hz), 7.01 (1H, *d*, J = 8 Hz), 7.13 (1H, *dd*, J = 8, 2 Hz), 7.24 (1H, *d*, J = 2 Hz), 7.78 (1H, *d*, J = 16 Hz)]. The ^{13}C NMR spectrum indicated the presence of four anomeric carbons (δ 93.1, 100.8, 105.6, 105.7), two sets of benzoyl carbons [δ 129.5 (2C), 130.7 (3C), 134.3, 167.9; 129.5 (2C), 130.6 (2C), 130.8, 134.3, 167.3 and one set of feruloyl carbons [δ 56.5, 112.7, 116.9, 119.0, 123.4, 130.9, 147.1, 149.3, 152.2, 167.6]. The sugar proton and carbon signals in the NMR spectra (Tables 1 and 2) were assigned by ^{1}H-^{1}H correlation spectroscopy (COSY), homonuclear Hartmann–Hahn (HOHAHA), HMBC and heteronuclear single quantum coherence (HSQC). The sugar and acyl residue linkages were assigned from a nuclear Overhauser effect (NOE) difference spectrum and HMBC (see Fig. 1). In the NOE difference spectra of **2**, when the proton signals at δ 4.63 (H-1 of Glc-2), 5.38 (H-1 of Rha) were irradiated, NOEs were observed at δ 3.71 (H-4 of Rha) and 7.01 (H-5 of feruloyl), respectively. In the HMBC spectrum, long-range correlations ($^3J_{HCOC}$) were observed between the anomeric proton signal at δ 5.61 (H-1 of Glc-1) and the anomeric carbon signal at δ 105.6 (C-2 of Fru); between the anomeric proton signal at δ 4.63 (H-1 of Glc-2) and the carbon signal at δ 83.1 due to the C-4 of rhamnose; between the anomeric proton signal at δ 5.38 (H-1 of Rha) and the carbon signal at δ 149.3 due to the C-4 of feruloyl; between the carbonyl carbon signal at δ 167.6 (C-9 of feruloyl) and the proton signal at δ 5.42 due to the H-3 of fructose; between the carbonyl carbon signals at δ 167.3, 167.9 (C-7 of each benzoyl) and the proton signals at δ 5.18, 4.37 due to the H-4 and H-6 of Glc-1, respectively. These data led us to assign the structure of fallaxose A as 3-*O*-{4-*O*-[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]-feruloyl}-β-D-fructofuranosyl-(2 → 1)-(4,6-di-*O*-benzoyl)-α-D-glucopyranoside.

Fallaxose B (**3**) was obtained as an amorphous powder and it exhibited a $[M+Na]^+$ at m/z 1099 in the FAB-mass spectrum. The NMR spectra were similar to those of fallaxose A except for the presence of one set of acetyl signal (see Tables 1 and 2). It was composed of one fructose, two glucoses, one rhamnose, one acetic acid, two benzoic acids and one ferulic acid. Sugar proton and carbon signals in the NMR spectra were assigned by ^{1}H-^{1}H COSY, HOHAHA, HMBC and HSQC. The sugar and acyl residue linkages were determined by NOE and HMBC spectra. In the NOE difference spectra of **3**, when the anomeric proton signals at δ 4.59 (H-1 of Glc-2) and 5.37 (H-1 of Rha) were irradiated, NOEs were observed at the signals of H-3 [δ 4.14 (1H, *dd*, J = 9.5, 3.5 Hz)] of rhamnose and the H-5 [δ 6.99 (1H, *d*, J = 8 Hz)] of ferulic acid, respectively. In the HMBC spectrum, long-range correlations ($^3J_{HCOC}$) were observed between the anomeric proton signal at δ 4.59 (H-1 of Glc-2) and the carbon signal at δ 80.2 due to the C-3 of rhamnose; between the anomeric proton signal at δ 5.37 (H-1 of Rha) and carbon signal at δ 148.9 due to the C-4 of ferulic acid; between the proton signal at δ 5.17 (H-4 of Glc-1) and the carbon signal at δ 167.3 due to the C-7 of benzoic acid; between the proton signal at δ 4.37 (H-6 of Glc-1) and the carbon signal at δ 167.9 due to the C-7 of another benzoic acid. Comparing the ^{1}H NMR spectrum of **3** with that of **2**, the H-2 signal of rhamnose moiety in **3** was shifted downfield at δ 5.53 (Δ +1.44 ppm). The position of the acetyl group was thus assigned to C-2 of the rhamnose moiety. From these data, the structure

Table 1. ^{1}H NMR spectral data (400 MHz, CD_3OD) for compounds **1–6**

	1	**2**	**3**
Glc-1	5.61 *d* (3.5)	5.61 *d* (3.5)	5.61 *d* (3.5)
1	3.63 *dd* (9.5, 3.5)	3.63 *dd* (9.5, 3.5)	3.63 *dd* (9.5, 3.5)
3	3.94 *t* (9.5)	3.94 *t* (9.5)	3.93 *t* (9.5)
4	5.19 *t* (9.5)	5.18 *t* (9.5)	5.17 *t* (9.5)
5	4.55 *m*	4.55 *m*	4.54 *m*
6	4.37*	4.37*	4.37 *dd* (12, 5)
6	4.58 *dd* (12, 3)	4.58 *dd* (12, 3)	4.59 *dd* (12, 3)
Glc-2			
1		4.63 *d* (8)	4.59 *d* (8)
2		3.24 *dd* (8.5, 8)	3.26 *dd* (8.5, 8)
3		3.39*	3.40 *t* (9)
4		3.30*	3.33*
5		3.33*	3.32*
6		3.70*	3.67*
6		3.85*	3.85*
Glc-3			
1			
2			
3			
4			
5			
6			
6			
Ac			
Glc-4			
1			
2			
3			
4			
5			
6			
6			
Fru			
1	3.67 *d* (12)	3.67 *d* (12)	3.67 *d* (12)
1	3.74 *d* (12)	3.73 *d* (12)	3.73 *d* (12)
3	5.42 *d* (8)	5.42 *d* (8)	5.42 *d* (8)
4	4.36 *t* (8)	4.36 *t* (8)	4.35 *t* (8)
5	3.97 *m*	3.96 *m*	3.96 *m*
6	3.72*	3.69*	3.71*
6	3.78*	3.77*	3.77*
Rha			
1	5.39 *d* (1.5)	5.38 *d* (1.5)	5.37 *d* (1.5)
2	4.07 *dd* (3.5, 1.5)	4.09 *dd* (3.5, 1.5)	5.53 *dd* (3.5, 1.5)
3	3.88 *dd* (9.5, 3.5)	4.11 *dd* (9.5, 3.5)	4.14 *dd* (9.5, 3.5)
4	3.48 *t* (9.5)	3.71*	3.64 *t* (9.5)
5	3.73*	3.78*	3.86*
6	1.22 *d* (6)	1.29 *d* (6)	1.25 *d* (6)
Ac			2.15 *s*
Benzoyl	in C-4 of Glc-1	in C-4 of Glc-1	in C-4 of Glc-1
2	7.81 *dd* (8, 2)	7.81 *dd* (8, 2)	7.79 *dd* (8, 2)
3	7.19 *t* (8)	7.19 *t* (8)	7.17 *t* (8)
4	7.51 *tt* (8, 2)	7.51 *tt* (8, 2)	7.50 *tt* (8, 2)
5	7.19 *t* (8)	7.19 *t* (8)	7.17 *t* (8)
6	7.81 *dd* (8, 2)	7.81 *dd* (8, 2)	7.79 *dd* (8, 2)
Benzoyl	in C-6 of Glc-1	in C-6 of Glc-1	in C-6 of Glc-1
2	7.97 *dd* (8, 2)	7.97 *dd* (8, 2)	7.97 *dd* (8, 2)
3	7.38 *t* (8)	7.38 *t* (8)	7.38 *t* (8)
4	7.55 *tt* (8, 2)	7.55 *tt* (8, 2)	7.55 *tt* (8, 2)
5	7.38 *t* (8)	7.38 *t* (8)	7.38 *t* (8)
6	7.97 *dd* (8, 2)	7.97 *dd* (8, 2)	7.97 *dd* (8, 2)

Table 1. *Continued.*

	1	**2**	**3**
Cinn			
2			
3			
5			
6			
7			
8			
MeO			
Cinn	in C-3 of Fru	in C-3 of Fru	in C-3 of Fru
2	7.24 *d* (2)	7.24 *d* (2)	7.25 *d* (2)
3			
5	7.02 *d* (8)	7.01 *d* (8)	6.99 *d* (8)
6	7.13 *dd* (8, 2)	7.13 *dd* (8, 2)	7.13 *dd* (8, 2)
7	7.78 *d* (16)	7.78 *d* (16)	7.78 *d* (16)
8	6.55 *d* (16)	6.55 *d* (16)	6.57 *d* (16)
MeO	3.69 *s*	3.69 *s*	3.70 *s*
	4	**5**	**6**
Glc-1			
1	5.82 *d* (3.5)	5.84 *d* (3.5)	5.83 *d* (3)
2	3.79 *dd* (9.5, 3.5)	3.78 *dd* (9.5, 3.5)	3.73*
3	3.97 *t* (9.5)	3.98 *t* (9.5)	3.74*
4	5.00 *dd* (10, 9.5)	5.02 *dd* (10, 9.5)	3.47*
5	4.22 *m*	4.21 *m*	4.32*
6	3.56 *dd* (12, 5)	3.56 *dd* (12, 5)	4.32*
6	3.69 *dd* (12, 2)	3.68 *dd* (12, 2)	4.60*
Glc-2			
1	4.59 *d* (8)	4.59 *d* (8)	4.51 *d* (8)
2	3.31*	3.31*	3.30*
3	3.32*	3.32*	3.29*
4	3.33*	3.33*	3.29*
5	3.32*	3.32*	3.30*
6	3.71*	3.72*	3.69*
6	3.93*	3.93*	3.92*
Glc-3			
1	4.47 *d* (8)	4.47 *d* (8)	4.31 *d* (8)
2	3.01 *t* (8.5)	3.00 *t* (8.5)	3.37*
3	3.19*	3.16 *t* (9.5)	3.37*
4	3.20*	3.22 *t* (9.5)	3.36*
5	3.08 *m*	3.05 *m*	3.36*
6	3.97*	3.99 *dd* (12, 2)	4.13*
6	4.05*	4.06 *dd* (12, 4)	4.36*
Ac	1.63 *s*	1.59 *s*	2.06 *s*
Glc-4			
1			4.46 *d* (8)
2			3.24 *t* (8.5)
3			3.36*
4			3.64*
5			3.34*
6			3.62 *dd* (12, 5)
6			3.88*
Fru			
1	4.21 *d* (12)	4.21 *d* (12)	4.20 *d* (12)
1	4.71 *d* (12)	4.72 *d* (12)	4.63 *d* (12)
3	5.71 *d* (8)	5.72 *d* (8)	5.76 *d* (8)
4	4.51 *t* (8)	4.50 *t* (8)	4.55 *t* (8.5)
5	4.04 *m*	4.04 *m*	4.08 *m*
6	3.85*	3.84 *dd* (12, 3)	3.83 *dd* (12, 3)
6	3.90*	3.89*	3.94 *dd* (12, 8)
Rha			
1			
2			
3			
4			
5			
6			

Table 1. *Continued.*

	4	5	6
Benzoyl			
2			
3			
4			
5			
6			
Benzoyl	in C-3 of Fru	in C-3 of Fru	in C-3 of Fru
2	8.19 *dd* (8, 1)	8.20 *dd* (8, 1.5)	8.18 *dd* (8, 1.5)
3	7.58 *t* (8)	7.60 *t* (8)	7.57 *t* (8)
4	7.69 *tt* (8, 1)	7.68 *tt* (8, 1.5)	7.70 *tt* (8, 1.5)
5	7.58 *t* (8)	7.60 *t* (8)	7.57 *t* (8)
6	8.19 *dd* (8, 1)	8.20 *dd* (8, 1.5)	8.18 *dd* (8, 1.5)
Cinn	in C-4 of Glc-1	in C-4 of Glc-1	in C-6 of Glc-1
2	7.45 *d* (8.5)	7.21 *d* (2)	7.22 *d* (2)
3	6.85 *d* (8.5)		
5	6.85 *d* (8.5)	6.84 *d* (8)	6.80 *d* (8)
6	7.45 *d* (8.5)	7.05 *dd* (8, 2)	7.10 *dd* (8, 2)
7	7.57 *d* (16)	7.57 *d* (16)	7.64 *d* (16)
8	6.23 *d* (16)	6.27 *d* (16)	6.45 *d* (16)
MeO		3.95 *s*	3.88 *s*
Cinn	in C-1 of Fru	in C-1 of Fru	in C-1 of Fru
2	7.42 *d* (8.5)	7.43 *d* (8.5)	7.18 *d* (2)
3	6.81 *d* (8.5)	6.80 *d* (8.5)	
5	6.81 *d* (8.5)	6.80 *d* (8.5)	6.79 *d* (8)
6	7.42 *d* (8.5)	7.43 *d* (8.5)	6.90 *dd* (8, 2)
7	7.68 *d* (16)	7.68 *d* (16)	7.61 *d* (16)
8	6.36 *d* (16)	6.37 *d* (16)	6.35 *d* (16)
MeO			3.90 *s*

Assignments were based on ^{1}H-^{1}H COSY, HOHAHA, NOE difference and HMBC spectra.
* Overlapping with other signals.

Table 2. ^{13}C NMR spectral data (100 MHz, CD_3OD) for compounds **1**–**6**

	1	2	3	4	5	6
Glc-1						
1	93.1	93.1	93.0	93.2	93.2	92.8
2	73.1	73.2	73.2	81.5	81.6	80.4
3	72.7	72.8	72.8	79.2	79.2	85.1
4	73.5	73.7	73.7	70.4	70.3	70.7
5	70.0	70.1	70.1	72.3	72.3	71.9
6	65.1	65.2	65.2	62.1	62.1	65.2
Glc-2						
1		105.7	105.9	105.4	105.4	105.9
2		76.1	75.5	75.3	75.3	74.8
3		78.1	77.8	78.5	78.5	78.3
4		71.6	71.4	71.7	71.7	71.6
5		78.3	78.0	78.5	78.5	78.5
6		62.8	62.9	63.1	63.1	63.1
Glc-3						
1				104.5	104.4	104.8
2				75.5	75.5	74.5
3				77.9	77.9	87.1
4				71.1	71.0	70.2
5				74.7	74.7	75.2
6				64.4	64.3	64.6
Ac				20.5	20.5	20.8
				172.7	172.6	172.6

Table 2. *Continued*

	1	2	3	4	5	6
Glc-4						
1						105.1
2						75.4
3						77.8
4						71.6
5						78.1
6						62.6
Fru						
1	65.9	66.0	66.0	66.0	65.9	65.9
2	105.5	105.6	105.6	103.8	103.8	103.6
3	80.1	80.3	80.3	80.2	80.1	80.0
4	74.5	74.6	74.7	73.6	73.6	74.0
5	84.9	84.9	84.9	84.5	84.5	84.5
6	63.3	63.4	63.4	63.2	63.2	64.3
Rha						
1	100.9	100.8	98.3			
2	72.0	71.9	73.2			
3	72.2	72.2	80.2			
4	73.7	83.1	72.8			
5	71.0	69.7	70.7			
6	18.1	18.2	18.1			
Ac			21.0			
			172.7			
Benzoyl	in C-4 of Glc-1	in C-4 of Glc-1	in C-4 of Glc-1			
1	130.8	130.8	130.9			
2,6	130.6	130.6	130.6			
3,5	129.5	129.5	129.4			
4	134.2	134.3	134.4			
7	167.2	167.3	167.3			
Benzoyl	in C-6 of Glc-1	in C-6 of Glc-1	in C-6 of Glc-1	in C-3 of Fru	in C-3 of Fru	in C-3 of Fru
1	130.7	130.7	130.7	131.0	131.1	130.2
2, 6	130.7	130.7	130.7	131.1	131.1	131.1
3, 5	129.5	129.5	129.5	129.9	129.9	129.9
4	134.2	134.3	134.3	134.8	134.8	134.8
7	167.8	167.9	167.9	167.3	167.3	167.3
Cinn.	in C-3 of Fru	in C-3 of Fru	in C-3 of Fru	in C-1 of Fru	in C-1 of Fru	in C-1 of Fru
1	130.9	130.9	131.0	127.2	127.1	127.7
2	112.5	112.7	112.7	131.2	131.3	111.6
3	152.1	152.2	152.1	117.0	116.9	149.4
4	149.3	149.3	148.9	161.4	161.4	150.8
5	118.8	119.0	119.2	117.0	116.9	116.4
6	123.4	123.4	123.4	131.2	131.3	124.4
7	147.1	147.1	146.9	147.0	147.0	147.2
8	116.8	116.9	117.2	114.9	114.9	115.2
9	167.6	167.6	167.6	168.5	168.5	168.4
MeO	56.4	56.5	56.6			56.5
Cinn.				in C-4 of Glc-1	in C-4 of Glc-1	in C-6 of Glc-1
1				127.1	127.6	127.7
2				131.2	111.6	111.6
3				116.9	149.5	149.4
4				161.3	150.9	150.8
5				116.9	116.5	116.5
6				131.2	124.5	124.4
7				146.6	146.9	147.2
8				115.2	115.4	115.3
9				168.1	168.1	169.0
MeO					56.5	56.5

Assigned by HSQC and HMBC spectra.

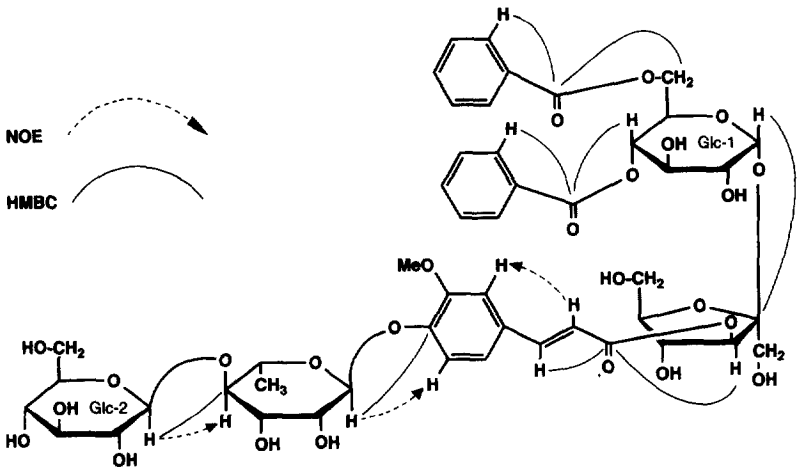


Fig. 1. HMBC and NOE correlations of compound **2**.

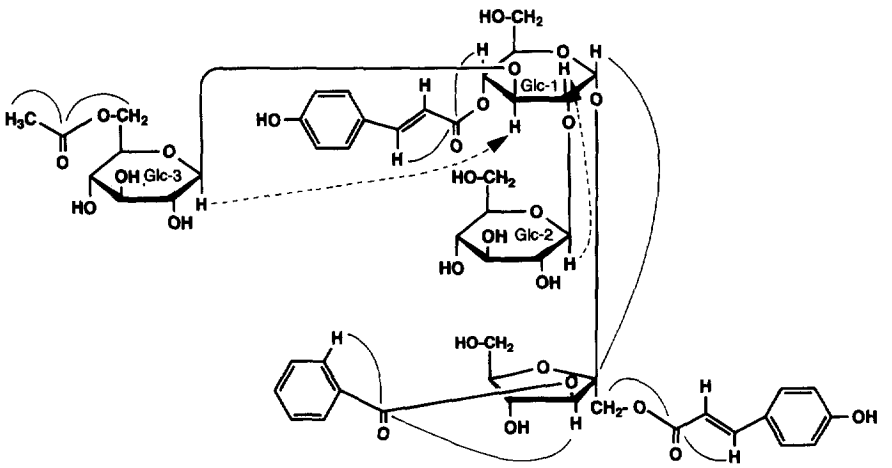


Fig. 2. HMBC and NOE correlations of compound **4**.

of fallaxose **B** was elucidated as 3-*O*-{4-*O*-[β-D-glucopyranosyl-(1 → 3)-(2-*O*-acetyl)-α-L-rhamnopyranosyl]-feruloyl}-β-D-fructofuranosyl-(2 → 1)-(4,6-di-*O*-benzoyl)-α-D-glucopyranoside.

Fallaxose C (**4**) was obtained as an amorphous powder and showed a $[M+Na]^+$ at *m/z* 1127 in the FAB-mass spectrum. It gave D-glucose and D-fructose on acid hydrolysis, while on alkaline hydrolysis, it gave a tetrasaccharide **4a** [3] (see Experimental) and a mixture of benzoic and *p*-coumaric acids. The position of each acyl residue was deduced from NOEs in the NOE difference and long-range correlations ($^3J_{HCOC}$) in the HMBC spectra (see Fig. 2). Therefore, the structure of fallaxose C was determined to be 1-*O*-*p*-coumaroyl-(3-*O*-benzoyl)-β-D-fructofuranosyl-(2 → 1)-[β-D-glucopyranosyl-(1 → 2)]-[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)]-(4-*O*-*p*-coumaroyl)-α-D-glucopyranoside.

The ^1H and ^{13}C NMR spectra of fallaxose D (**5**) showed that this compound was composed of a tetrasaccharide **4a**, one benzoic, one *p*-coumaric, one ferulic and one acetic acid moieties. The NMR spectra were similar to those of fallaxose C, except for the presence of one feruloyl residue instead of one *p*-coumaroyl residue. The feruloyl and *p*-coumaroyl proton and carbon signals in the NMR spectra were assigned by NOE difference, HMBC and HSQC spectra. In the HMBC spectrum, long-range correlations ($^3J_{HCOC}$) were observed between the feruloyl carbonyl carbon signal at δ 168.1 and the proton signal at δ 5.02, due to H-4 of Glc-1 and between the *p*-coumaroyl carbonyl carbon signal at δ 168.5 and the proton signals at δ 4.21, 4.72, due to H_2-1 of fructose. These data led us to assign the structure of fallaxose D as 1-*O*-*p*-coumaroyl-(3-*O*-benzoyl)-β-D-fructofuranosyl-(2 → 1)-[β-D-glucopyranosyl-(1 → 2)]-[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)]-(4-*O*-feruloyl)-α-D-glucopyranoside.

Fallaxose E (**6**) showed a $[M+Na]^+$ at *m/z* 1349 in the FAB-mass spectrum. The ^1H NMR spectrum suggested the presence of four anomeric proton signals at δ 4.31 (1H, *d*, *J* = 8 Hz), 4.46 (1H, *d*, *J* = 8 Hz), 4.51 (1H, *d*, *J* = 8 Hz) and 5.83 (1H, *d*, *J* = 3 Hz). The ^{13}C NMR spectrum indicated the presence of five anomeric carbons (δ 92.8, 103.6, 104.8, 105.1, 105.9), one set of acetyl, one set of benzoyl and two

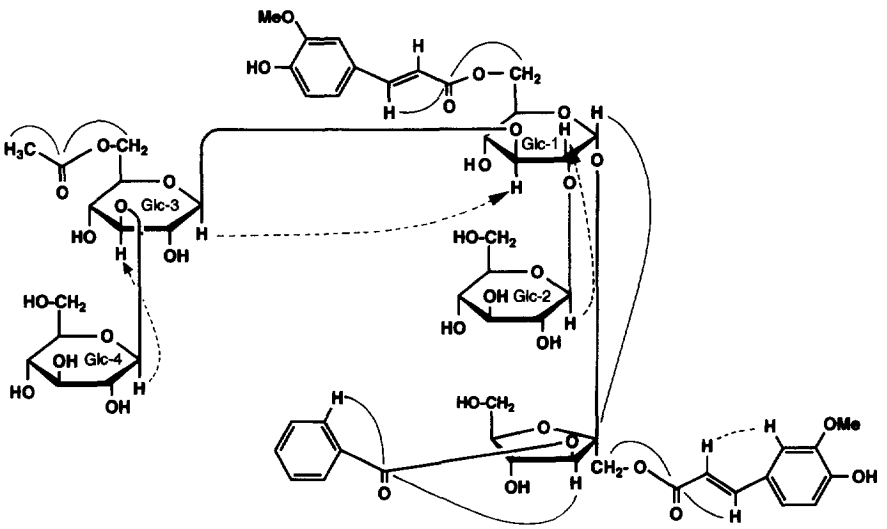


Fig. 3. HMBC and NOE correlations of compound **6**.

sets of feruloyl signals. On acid hydrolysis, **6** afforded D-glucose and D-fructose, while on alkaline hydrolysis, it gave a pentasaccharide **6a** [4] and benzoic and ferulic acids. Sugar proton and carbon signals in the NMR spectra were assigned by ^{1}H-^{1}H COSY, HOHAHA, NOE, HMBC and HSQC spectra. The sugar and acyl linkages were determined by NOE difference and HMBC spectra (see Fig. 3). In the NOE difference spectra of **6**, when the signals at δ 4.31 (1H, *d*, $J = 8$ Hz, H-1 of Glc-3), 4.46 (1H, *d*, $J = 8$ Hz, H-1 of Glc-4), 4.51 (1H, *d*, $J = 8$ Hz, H-1 of Glc-2) were irradiated, NOEs were observed at the signals at H-3 (δ 3.74) of Glc-1, the H-3 (δ 3.37) of Glc-3 and the H-2 (δ 3.73) of Glc-1. In the HMBC spectrum, long-range correlations were observed between the following carbons and protons in **6**: carbonyl carbon of acetyl group and H-6 of Glc-3, carbonyl carbon of feruloyl group and H_2-6 of Glc-1, carbonyl carbon of another feruloyl group and H_2-1 of Fru, C-2 of Fru and H-1 of Glc-1, C-1 of Glc-2 and H-2 of Glc-1, C-1 of Glc-4 and H-3 of Glc-3. Based on this evidence, the structure of fallaxose E was elucidated as 1-*O*-feruloyl-(3-*O*-benzoyl)-β-D-fructofuranosyl-(2 → 1)-[β-D-glucopyranosyl-(1 → 2)]-[β-D-glucopyranosyl-(1 → 3)-(6-*O*-acetyl)-β-D-glucopyransoyl-(1 → 3)]-(6-*O*-feruloyl)-α-D-glucopyranoside. The anomeric configurations of Glc-1, Glc-2, Glc-3 and Glc-4 were determined, to be α, β, β and β, respectively, from these $J_{H1\text{-}H2}$ values and that of rhamnose was determined to be α, by comparison with the ^{13}C NMR data of C-3 and C-5 of rhamnose [6].

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR were with a 400 MHz instrument; chemical shifts are given in δ with TMS as int. standard. Prep. and semi-prep. HPLC were carried out on a column of Develosil Lop-ODS (5 × 50 cm) and YMC ODS-7 (2 × 25 cm) or Develosil PhA-7 (2 × 25 cm), respectively.

Extraction and isolation. Polygala fallax Hemsl. was collected in Guangxi, China in August 1994 and identified by Prof. Deng Xi Qin, Guangxi Zhuangzu Zizhigu Medicinal Botanical Garden, Nan Ning, China; a voucher specimen is deposited in the Herbarium of his institute. Dried roots (960 g) were extracted × 2 with 70% aq. MeOH. The extract was passed through a porous polymer gel (Mitsubishi Diaion HP-20) column after evapn of MeOH. After the contents of the column were washed with H_2O, adsorbed materials were eluted successively with 30 and 60% aq. MeOH, and MeOH. The MeOH eluate (25 g) was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (85:14:1), increasing the proportion of MeOH to give 18 frs (A–R). From frs F, H–L, compounds **1**–**9** were isolated by prep. and semi-prep. HPLC (MeCN–H_2O); yields, **1** (91 mg), **2** (21 mg), **3** (3 mg), **4** (17 mg), **5** (6 mg), **6** (3 mg), **7** (19 mg), **8** (4 mg) and **9** (5 mg).

Fallaxose A (**2**). Amorphous powder. $[\alpha]_D^{25}$ −6.2° (MeOH; *c* 0.49). UV λ_{max}^{MeOH} nm (log ε): 229 (4.50), 293 (4.11), 320 (4.11). FAB-MS *m/z*: 1057 $[M+Na]^+$, ^{1}H and ^{13}C NMR: Tables 1 and 2.

Fallaxose B (**3**). Amorphous powder. $[\alpha]_D^{28}$ −27.4° (MeOH; *c* 0.31). UV λ_{max}^{MeOH} nm (log ε): 230 (4.50), 292 (4.15), 319 (4.13). FAB-MS *m/z*: 1099 $[M+Na]^+$. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Fallaxose C (**4**). Amorphous powder. $[\alpha]_D^{25}$ −6.8° (MeOH; *c* 1.22). UV λ_{max}^{MeOH} nm (log ε): 229 (4.52), 300 (sh 4.49), 316 (4.56). FAB-MS *m/z*: 1127 $[M+Na]^+$. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Fallaxose D (**5**). Amorphous powder. $[\alpha]_D^{28}$ −6.5° (MeOH; *c* 0.57). UV λ_{max}^{MeOH} nm (log ε): 230 (4.53), 300 (sh 4.49), 318 (4.58). FAB-MS *m/z*: 1157 $[M+Na]^+$. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Fallaxose E (**6**). Amorphous powder. $[\alpha]_D^{28}$ +16.1°

(MeOH; *c* 0.27). UV λ_{max}^{MeOH} nm (log ε): 220 (4.50), 231 (4.49), 297 (sh 4.35), 327 (4.52). FAB-MS *m/z*: 1349 $[M+Na]^+$. ^1H and ^{13}C NMR: Tables 1 and 2.

Alkaline hydrolysis of **2–6**. Each compound (2 mg) was treated with 5% aq. NaOH (0.1 ml) for 3 hr at room temp. and the reaction mixt. passed through a column of Amberlite IR-120B. From the aq. eluate of the reaction mixt., sugars were detected by HPLC {Asahipak NH2P-50, 4.6 mm × 25 cm, MeCN–H_2O (13:7), 1 ml min^{-1}, UV 195 nm [7]} as follows: sucrose (R_t 5.2 min) from **2** and **3**; tetrasaccharide **4a** (R_t 6.8 min) [3] from **4** and **5**; pentasaccharide **6a** (R_t 9.1 min) [4] from **6**. The MeOH eluate was concd and subjected to HPLC [YMC R-ODS-7, 4.6 mm × 25 cm, MeCN–H_2O–TFA (22.5:77.5:0.05), 1 ml min^{-1}, UV 270 nm] to reveal a peak due to benzoic acid (R_t 12.5 min) from **2–6**, *p*-coumaric acid (R_t 7.7 min) from **4** and **5** and ferulic acid (R_t 8.4 min) from **2**, **3**, **5** and **6**.

Acid hydrolysis of **2–6**. Each compound (1 mg) was heated at 100° with dioxane (0.05 ml) and 5% H_2SO_4 (0.05 ml) for 1 hr. After diln with H_2O, the reaction mixt. was extracted ×2 with EtOAc and the H_2O layer passed through an Amberlite IRA-60E column. The H_2O eluate was concd and the residue treated with D-cysteine [8] (0.05 mg) in H_2O (0.03 ml) and pyridine (0.015 ml) at 60° for 1 hr with stirring. After the soln was concd and the reaction mixt. dried, pyridine (0.015 ml), hexamethyldisilazine (0.015 ml) and trimethylsilylchloride (0.015 ml) were added to the residue. The reaction mixt. was heated at 60° for 30 min. The supernatant was analysed by GC. GC conditions: column, Supelco SPB™-1, 0.25 mm × 27 m; column temp., 230°; carrier gas, N_2; R_t (min): L-rhamnose 12.4, D-rhamnose 12.2, D-fructose 13.9, L-fructose 14.3, D-glucose 18.1 and L-glucose 17.1. The R_t for D-rhamnose and L-fructose were obtained from their enantiomers (L-rhamnose + L-cysteine and D-fructose + L-cysteine, respectively). D-Glucose and D-fructose were detected from **2–6**. L-Rhamnose was detected from **2** and **3**.

Acknowledgements—We thank the staff of the Central Analytical Laboratory of this university for the measurement of MS.

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