Reinioses A—J, Oligosaccharide Multi-Esters from the Roots of *Polygala reinii* Fr. *et* Sav.

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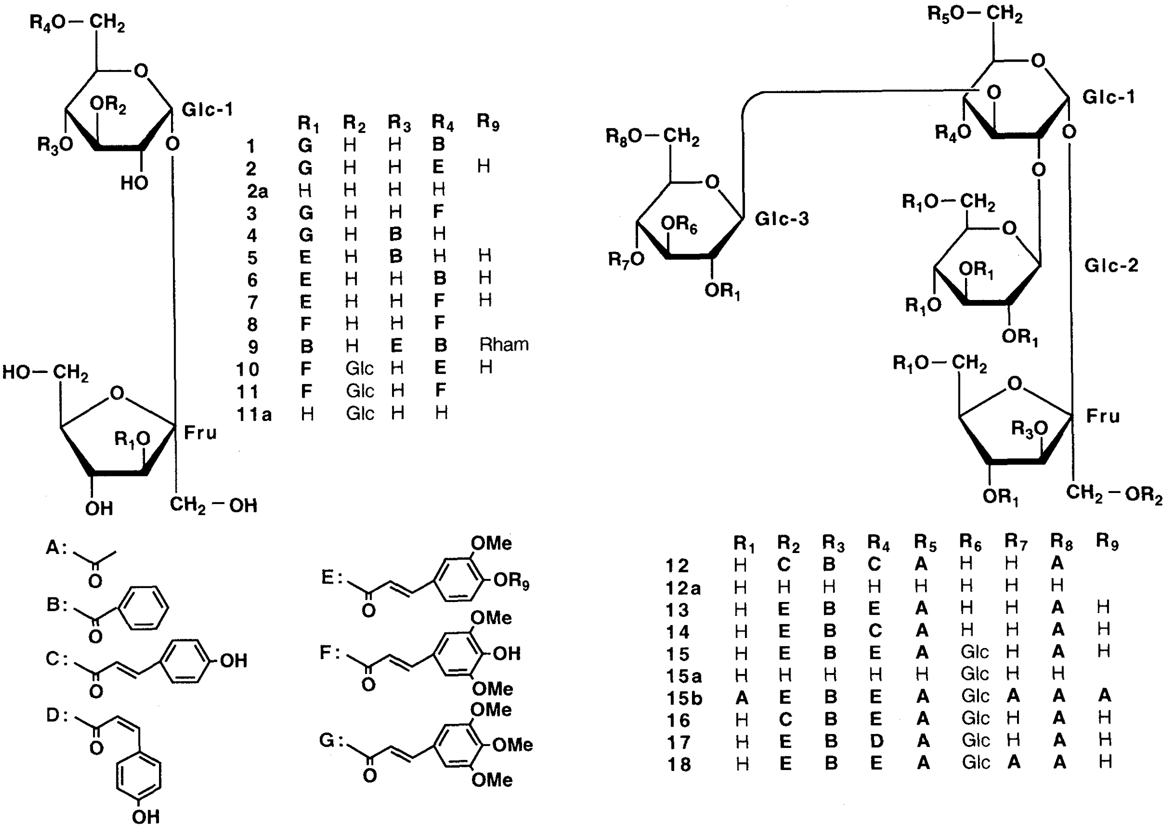
Ten new acylated oligosaccharides, called reinioses A—J, and seven known oligosaccharides were isolated from the roots of *Polygala reinii* Fr. *et* Sav. and their structures were elucidated by spectroscopic and chemical means. These oligosaccharides were found to be di-, tri-, tetra- and pentasaccharides having two or more acyl residues.

Keywords *Polygala reinii*; reiniose; acylated oligosaccharide; Polygalaceae; acetate; benzoate

In previous papers, we have reported the isolation and structural elucidation of fifteen new oligosaccharide multi-esters called senegoses A—I from the roots of *Polygala senega* var. *latifolia* Torr. *et* Gray[1,2] and senegoses J—O from the roots of *Polygala senega* L.[3] So we have continued our investigations with a view to isolating similar constituents from *Polygala reinii* Fr. *et* Sav. belonging to the genus *Polygala* and have been successful in isolating other new oligosaccharide multi-esters. This paper reports the isolation and structural elucidation of ten new di-, tri-, tetra- and pentasaccharides, having two or more acyl residues, called reinioses A—J. Seven known saccharides were also isolated from this material.

The structures of seven known oligosaccharides **1**, **3**, **4**, **7**, **8**, **13** and **16** were elucidated by comparison of the ^{1}H- and ^{13}C-NMR spectral data with reported data as the [3-*O*-(3,4,5-trimethoxycinnamoyl)]-*β*-D-fructofuranosyl-(6-*O*-benzoyl)-*α*-D-glucopyranoside,[4] tenuifoliside C,[5] [3-*O*-(3,4,5-trimethoxycinnamoyl)]-*β*-D-fructofuranosyl-(4-*O*-benzoyl)-*α*-D-glucopyranoside,[4] (3-*O*-feruloyl)-*β*-D-fructofuranosyl-(6-*O*-sinapoyl)-*α*-D-glucopyranoside,[4] (3-*O*-sinapoyl)-*β*-D-fructofuranosyl-(6-*O*-sinapoyl)-*α*-D-glucopyranoside,[5,6] senegose F[2] and tenuifoliose D,[7] respectively.

Reiniose A (**2**), $[\alpha]_D$ −51.8°, $C_{34}H_{42}O_{18}$, was obtained as an amorphous powder and exhibited $[M+Na]^+$ and $[M+H]^+$ ions at *m*/*z* 761 and 739, respectively, under FAB-MS. On alkaline hydrolysis, compound **2** gave sucrose (**2a**) and an acid mixture composed of ferulic and 3,4,5-trimethoxycinnamic acids (see Experimental). In the NMR spectra of **2**, one feruloyl and one 3,4,5-trimethoxycinnamoyl signals were observed (see Tables I and III). Detailed proton spin decoupling experiments,



	R1	R2	R3	R4	R9
1	G	H	H	B	
2	G	H	H	E	H
2a	H	H	H	H	
3	G	H	H	F	
4	G	H	B	H	
5	E	H	B	H	H
6	E	H	H	B	H
7	E	H	H	F	H
8	F	H	H	F	
9	B	H	E	B	Rham
10	F	Glc	H	E	H
11	F	Glc	H	F	
11a	H	Glc	H	H	

	R1	R2	R3	R4	R5	R6	R7	R8	R9
12	H	C	B	C	A	H	H	A	
12a	H	H	H	H	H	H	H	H	
13	H	E	B	E	A	H	H	A	H
14	H	E	B	C	A	H	H	A	H
15	H	E	B	E	A	Glc	H	A	H
15a	H	H	H	H	H	Glc	H	H	
15b	A	E	B	E	A	Glc	A	A	A
16	H	C	B	E	A	Glc	H	A	H
17	H	E	B	D	A	Glc	H	A	H
18	H	E	B	E	A	Glc	A	A	H

Chart 1

which started from irradiation of each anomeric proton signal, enabled us to assign all the proton signals (see Table I). The position of each acyl residue was determined by observation of ${}^3J_{(COCH)}$ using the ${}^1H$ detected heteronuclear multiple bond connectivity (HMBC) method and differential nuclear Overhauser effect (NOE) experiments (see Chart 2).[8] The carbon signals of the ester moiety were assigned from the HMBC (see Chart 2 and Table III). The details are as follows. NOEs were observed at the signals δ 3.88, 6.43 and 7.60 on irradiation at the signal δ 7.19, suggesting that these signals were assigned to the feruloyl residue (see Chart 2). There was a ${}^2J_{(CCH)}$ and ${}^3J_{(CCCH)}$ observed between an ester carbonyl carbon signal (δ 169.2) and two *trans*-olefinic proton signals (δ 6.43, 7.60), and ${}^3J_{(COCH)}$ between the carbonyl carbon signal and the H_2-6 (δ 4.23, 4.66) of α-glucose. So, the feruloyl residue

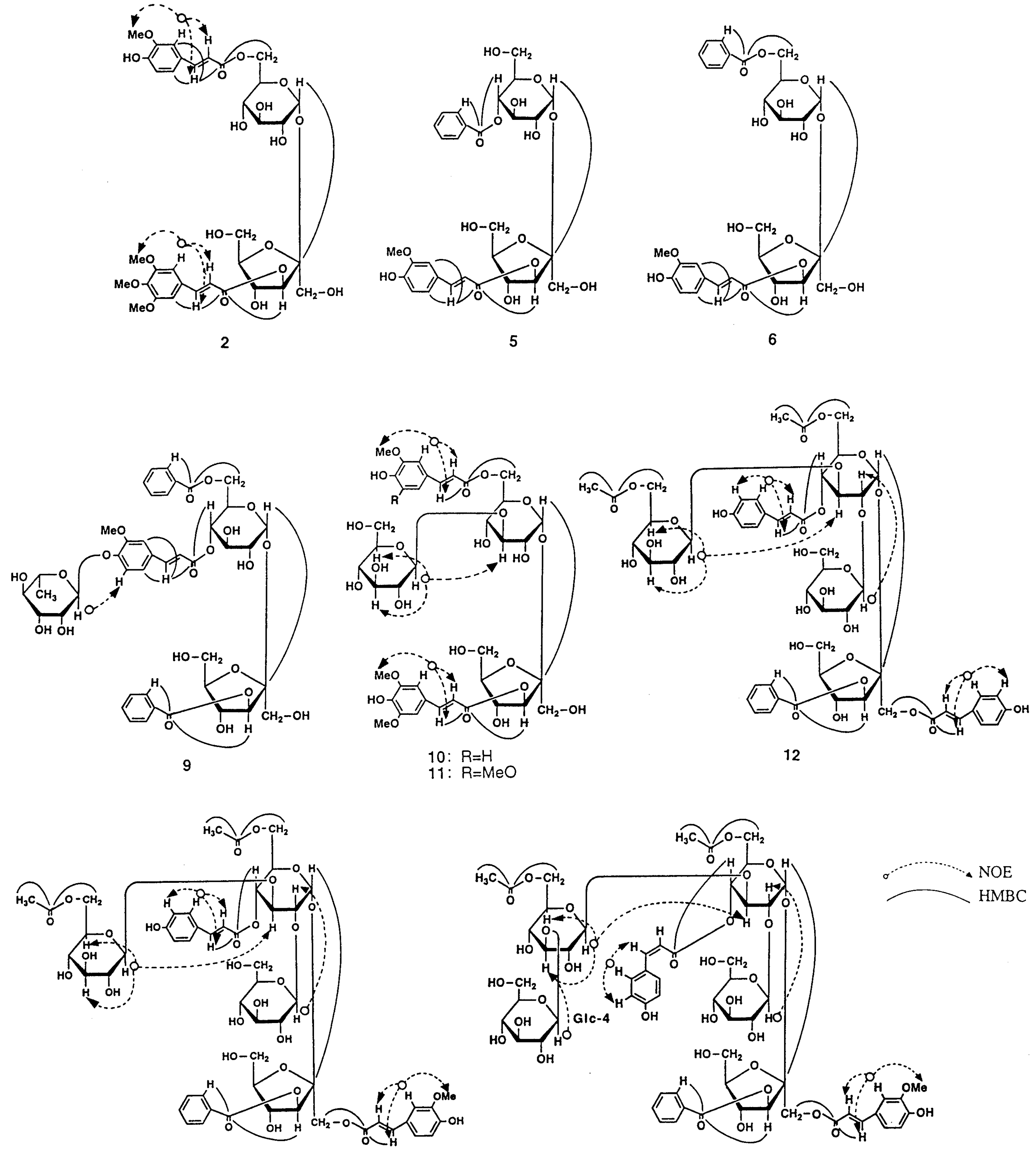


Chart 2

TABLE I. ^{1}H-NMR Data for Oligosaccharides from the Roots of *Polygala reinii* in MeOH-d_4

	2	**5**	**6**	**9**	**10**	**11**
Glc-1						
1	5.51 (1H, d, 3.5)	5.56 (1H, d, 3.5)	5.50 (1H, d, 3.5)	5.61 (1H, d, 3.5)	5.56 (1H, d, 3.5)	5.58 (1H, d, 3.5)
2	3.48 (1H, dd, 9.5, 3.5)	3.59 (1H, dd, 9.5, 3.5)	3.49 a)	3.63 (1H, dd, 9.5, 3.5)	3.67 a)	3.96 a)
3	3.66 (1H, t, 9.5)	3.91 (1H, t, 9.5)	3.71 (1H, t, 9.5)	3.94 (1H, t, 9.5)	3.68 a)	3.70 (1H, t, 9.5)
4	3.32 (1H, t, 9.5)	5.04 (1H, t, 9.5)	3.49 (1H, t, 9.5)	5.19 (1H, t, 9.5)	3.43 (1H, dd, 10, 9.5)	3.42 (1H, t, 9.5)
5	4.25 (1H, m)	4.24 (1H, m)	4.25 (1H, m)	4.55 (1H, m)	4.30 (1H, m)	4.32 (1H, m)
6	4.23 a)	3.60 a)	4.50 (1H, dd, 12, 6)	4.37 a)	4.23 (1H, dd, 12, 7)	4.21 (1H, dd, 12, 7)
	4.66 (1H, br d, 11)	3.71 (1H, dd, 12, 3)	4.71 (1H, dd, 12, 2)	4.57 (1H, dd, 12, 3)	4.70 (1H, dd, 12, 2)	4.73 (1H, dd, 12, 2)
Glc-2						
1					4.35 (1H, d, 8)	4.36 (1H, d, 8)
2					3.24 a)	3.24 a)
3					3.26 a)	3.27 a)
4					3.27 a)	3.28 a)
5					3.05 (1H, m)	3.07 (1H, m)
6					3.55 (1H, dd, 12, 6)	3.55 (1H, dd, 12, 5.5)
					3.66 a)	3.67 a)
Fru						
1	3.59 (1H, d, 12)	3.66 (1H, d, 12)	3.61 (1H, d, 12)	3.68 (1H, d, 12)	3.62 (1H, d, 12)	3.62 (1H, d, 12)
	3.64 (1H, d, 12)	3.73 (1H, d, 12)	3.67 (1H, d, 12)	3.73 (1H, d, 12)	3.66 (1H, d, 12)	3.66 (1H, d, 12)
3	5.50 (1H, d, 8)	5.42 (1H, d, 8)	5.48 (1H, d, 8)	5.42 (1H, d, 8)	5.49 (1H, d, 8)	5.51 (1H, d, 8)
4	4.48 (1H, t, 8)	4.41 (1H, t, 8)	4.38 (1H, t, 8)	4.36 (1H, t, 8)	4.49 (1H, t, 8)	4.57 (1H, t, 8)
5	3.98 (1H, m)	3.98 (1H, m)	3.93 (1H, m)	3.96 (1H, m)	3.99 (1H, m)	4.00 (1H, m)
6	3.83 (1H, dd, 12, 3)	3.81 (1H, dd, 12, 4)	3.70 (1H, dd, 12, 3)	3.74 (1H, dd, 12, 4)	3.82 a)	3.83 a)
	3.89 (1H, dd, 12, 6)	3.86 (1H, dd, 12, 6)	3.80 (1H, dd, 12, 6)	3.79 a)	3.88 a)	3.88 a)
Rham						
1				5.39 (1H, d, 1.5)		
2				4.07 (1H, dd, 3.5, 1.5)		
3				3.88 (1H, dd, 9.5, 3.5)		
4				3.48 (1H, t, 9.5)		
5				3.73 a)		
6				1.23 (3H, d, 6)		
Bz		(R_3)	(R_4)	(R_1)		
2, 6		7.81 (2H, dd, 8, 1)	8.05 (2H, dd, 8, 1)	7.81 (2H, dd, 8, 1)		
3, 5		7.24 (2H, t, 8)	7.46 (2H, t, 8)	7.19 (2H, t, 8)		
4		7.51 (1H, tt, 8, 1)	7.59 (1H, tt, 8, 1)	7.51 (1H, tt, 8, 1)		
Bz (R_2)				(R_4)		
2, 6				7.97 (2H, dd, 8, 1)		
3, 5				7.38 (2H, t, 8)		
4				7.55 (1H, tt, 8, 1)		
Cinn	(R_1)	(R_1)	(R_1)	(R_3)	(R_1)	(R_1)
2	6.95 (1H, s)	7.17 (1H, d, 2)	7.20 (1H, d, 2)	7.24 (1H, d, 2)	6.93 (1H, s)	6.91 (1H, s)
3						
5		6.73 (1H, d, 8)	6.79 (1H, d, 8)	7.02 (1H, d, 8)		
6	6.95 (1H, s)	7.10 (1H, dd, 8, 2)	7.10 (1H, dd, 8, 2)	7.13 (1H, dd, 8, 2)	6.93 (1H, s)	6.91 (1H, s)
β	6.54 (1H, d, 16)	6.47 (1H, d, 16)	6.43 (1H, d, 16)	6.55 (1H, d, 16)	6.47 (1H, d, 16)	6.46 (1H, d, 16)
γ	7.71 (1H, d, 16)	7.77 (1H, d, 16)	7.71 (1H, d, 16)	7.78 (1H, d, 16)	7.68 (1H, d, 16)	7.67 (1H, d, 16)
OMe	3.79 (3H, s)	3.75 (3H, s)	3.88 (3H, s)	3.69 (3H, s)	3.88 (6H, s)	3.87 (6H, s)
	3.88 (6H, s)					
Cinn	(R_4)				(R_4)	(R_4)
2	7.19 (1H, d, 2)				7.18 (1H, d, 2)	6.86 (1H, s)
3						
5	6.78 (1H, d, 8)				6.78 (1H, d, 8)	
6	7.03 (1H, dd, 8, 2)				7.03 (1H, dd, 8, 2)	6.86 (1H, s)
β	6.43 (1H, d, 16)				6.44 (1H, d, 16)	6.46 (1H, d, 16)
γ	7.60 (1H, d, 16)				7.60 (1H, d, 16)	7.58 (1H, d, 16)
OMe	3.88 (3H, s)				3.84 (3H, s)	3.83 (6H, s)

a) Overlapping with other signals. Figures in parentheses are coupling constants (*J*) in Hz.

was located at the C-6 of α-glucose. In a similar way the 3,4,5-trimethoxycinnamoyl residue was located at the C-3 of fructose. These data led us to assign the structure of **2** to reiniose A. The glycosylation and acylation shifts in the ^{13}C-NMR spectrum of reiniose A supported this conclusion.

The ^{1}H-NMR spectrum of reiniose B (**5**), $[\alpha]_D$ −4.8°, $C_{29}H_{34}O_{15}$ and reiniose C (**6**), $[\alpha]_D$ −29.3°, $C_{29}H_{34}O_{15}$, showed that these compounds were composed of single sucrose, benzoic and ferulic acid moieties. On alkaline hydrolysis, these compounds gave sucrose and an acid mixture composed of benzoic and ferulic acids (see Experimental). The position of each acyl residue was determined by observation of ^2J and ^3J using the HMBC (see Chart 2). These data led us to assign the structure of **5** and **6** to reinioses B and C, respectively.

Table II. ^{1}H-NMR Data for Oligosaccharides from *Polygala reinii* in MeOH-d_4

	12	14	15	17
Glc-1				
1	5.86 (1H, d, 3.5)	5.88 (1H, d, 3.5)	5.88 (1H, d, 3.5)	5.85 (1H, d, 3.5)
2	3.81 (1H, dd, 9.5, 3.5)	3.81 (1H, dd, 9.5, 3.5)	3.82 (1H, dd, 9.5, 3.5)	3.81 (1H, dd, 9.5, 3.5)
3	3.95 a)	3.95 (1H, t, 9.5)	3.98 (1H, t, 9.5)	3.93 a)
4	5.01 (1H, dd, 10, 9.5)	5.00 (1H, t, 9.5)	5.03 (1H, t, 9.5)	5.00 (1H, dd, 10, 9.5)
5	4.39 (1H, m)	4.39 (1H, m)	4.40 (1H, m)	4.37 (1H, m)
6	4.13 (1H, dd, 12, 5.5)	4.13 (1H, dd, 12, 5.5)	4.14 (1H, dd, 12, 5.5)	4.16 a)
	4.19 (1H, dd, 12, 2)	4.19 (1H, dd, 12, 2)	4.19 (1H, dd, 12, 2)	4.18 a)
Glc-2				
1	4.59 (1H, d, 8)	4.60 (1H, d, 8)	4.59 (1H, d, 8)	4.59 (1H, d, 8)
Glc-3				
1	4.49 (1H, d, 8)	4.49 (1H, d, 8)	4.53 (1H, d, 8)	4.52 (1H, d, 8)
2	3.01 (1H, dd, 8.5, 8)	3.01 (1H, dd, 8.5, 8)	3.21 a)	3.24 a)
3	3.18 a)	3.17 (1H, t, 8.5)	3.38 (1H, t, 9.5)	3.39 (1H, t, 9.5)
4	3.19 a)	3.18 (1H, t, 8.5)	3.35 a)	3.33 a)
5	3.09 (1H, m)	3.09 (1H, m)	3.14 (1H, m)	3.16 (1H, m)
6	3.97 a)	3.96 a)	4.01 (1H, dd, 12, 2)	4.01 (1H, dd, 12, 3.5)
	4.05 (1H, dd, 12, 3)	4.05 (1H, dd, 12, 3.5)	4.06 (1H, dd, 12, 3.5)	4.05 a)
Glc-4				
1			4.47 (1H, d, 8)	4.48 (1H, d, 8)
Fru				
1	4.21 (1H, d, 12)	4.21 (1H, d, 12)	4.23 (1H, d, 12)	4.20 (1H, d, 12)
	4.69 (1H, d, 12)	4.71 (1H, d, 12)	4.69 (1H, d, 12)	4.69 (1H, d, 12)
3	5.73 (1H, d, 8)	5.73 (1H, d, 8)	5.74 (1H, d, 8)	5.74 (1H, d, 8)
4	4.43 (1H, t, 8)	4.43 (1H, t, 8)	4.43 (1H, t, 8)	4.42 (1H, t, 8)
5	4.06 (1H, m)	4.08 (1H, m)	4.07 (1H, m)	4.06 (1H, m)
6	3.83 (1H, dd, 12, 3)	3.83 (1H, dd, 12, 3)	3.84 (1H, dd, 12, 3)	3.83 (1H, dd, 12, 2)
	3.88 (1H, dd, 12, 7)		3.88 (1H, dd, 12, 6)	3.88 a)
Ac (R_5)				
2	2.06 (3H, s)	2.06 (3H, s)	2.06 (3H, s)	2.04 (3H, s)
Ac (R_7)				
2				
Ac (R_8)				
2	1.62 (3H, s)	1.62 (3H, s)	1.61 (3H, s)	1.80 (3H, s)
Bz (R_3)				
2, 4	8.18 (2H, dd, 8, 1)	8.18 (2H, dd, 8, 1)	8.18 (2H, dd, 8, 1)	8.14 (2H, dd, 8, 1)
3, 5	7.59 (2H, t, 8)	7.59 (2H, t, 8)	7.60 (2H, t, 8)	7.56 (2H, t, 8)
6	7.70 (1H, tt, 8, 1)	7.70 (1H, tt, 8, 1)	7.69 (1H, tt, 8, 1)	7.70 (1H, tt, 8, 1)
Cinn (R_2)				
2	7.42 (1H, d, 8.5)	7.20 (1H, d, 2)	7.20 (1H, d, 2)	7.19 (1H, d, 2)
3	6.81 (1H, d, 8.5)			
5	6.81 (1H, d, 8.5)	6.81 (1H, d, 8)	6.81 (1H, d, 8)	6.81 (1H, d, 8)
6	7.42 (1H, d, 8.5)	7.02 (1H, dd, 8, 2)	7.00 (1H, dd, 8, 2)	7.00 (1H, dd, 8, 2)
β	6.36 (1H, d, 16)	6.40 (1H, d, 16)	6.39 (1H, d, 16)	6.39 (1H, d, 16)
γ	7.68 (1H, d, 16)	7.68 (1H, d, 16)	7.67 (1H, d, 16)	7.67 (1H, d, 16)
OMe		3.91 (3H, s)	3.90 (3H, s)	3.90 (3H, s)
Cinn (R_4)				
2	7.45 (1H, d, 8.5)	7.44 (1H, d, 8.5)	7.20 (1H, d, 2)	7.60 (1H, d, 8.5)
3	6.85 (1H, d, 8.5)	6.85 (1H, d, 8.5)		6.75 (1H, d, 8.5)
5	6.85 (1H, d, 8.5)	6.85 (1H, d, 8.5)	6.85 (1H, d, 8)	6.75 (1H, d, 8.5)
6	7.45 (1H, d, 8.5)	7.44 (1H, d, 8.5)	7.05 (1H, dd, 8, 2)	7.60 (1H, d, 8.5)
β	6.22 (1H, d, 16)	6.21 (1H, d, 16)	6.25 (1H, d, 16)	5.69 (1H, d, 13)
γ	7.56 (1H, d, 16)	7.56 (1H, d, 16)	7.57 (1H, d, 16)	6.84 (1H, d, 13)
OMe			3.95 (3H, s)	

a) Overlapping with other signals. Figures in parentheses are coupling constants (*J*) in Hz.

The ^{1}H-NMR spectrum of reiniose D (**9**), $[\alpha]_D$ −51.1°, $C_{42}H_{48}O_{20}$, showed that this compound was composed of single sucrose, rhamnose, and ferulic acid moieties and two benzoic acid moieties. On alkaline hydrolysis this compound gave a sugar mixture composed of sucrose and rhamnose and an acid mixture composed of benzoic and ferulic acids (see Experimental). An NOE was observed at δ 7.02 due to the H-5 of the feruloyl residue, on irradiation at the anomeric proton signal at δ 5.39 of rhamnose, suggesting **9** to be a phenolic rhamnoside. The position of each acyl residue was determined by observation of ^2J and ^3J using the HMBC (see Chart 2). These data led us to assign the structure of **9** to reiniose D.

Reiniose E (**10**), $[\alpha]_D$ −52.2°, $C_{39}H_{50}O_{23}$, was obtained as an amorphous powder and it exhibited $[M+Na]^+$ and $[M+H]^+$ ions at *m/z* 909 and 887, respectively, under FAB-MS. Compound **10** gave glucose and fructose in the ratio 2 : 1 on acid hydrolysis, while alkaline hydrolysis gave an acid mixture composed of ferulic and sinapic acids. In the ^{1}H-NMR spectrum of **10**, one feruloyl and one

Table III. ^{13}C-NMR Data for Oligosaccharides from the Roots of *Polygala reinii* in MeOH-d_4

	2	**5**	**6**	**9**	**10**	**11**
Glc-1						
1	92.8	93.2	93.1	93.0	92.4	92.3
2	73.1	72.9	73.1	73.2	72.0[e]	72.0[h]
3	75.1	72.6	74.8	72.8	86.4	86.4
4	71.9	73.4	71.5	73.7	70.5	70.5
5	72.5	73.2	72.3	70.0	72.2[e]	72.2[h]
6	65.5	62.4	65.3	65.9[b]	65.7[f]	65.7[i]
Glc-2						
1					105.4	105.3
2					75.5	75.5
3					77.8[g]	77.8[j]
4					71.1	71.1
5					77.6[g]	77.6[j]
6					62.1	62.1
Fru						
1	65.8	65.8	65.5	65.2[b]	65.4[f]	65.5[i]
2	104.9	105.7	104.8	105.6	105.2	105.1
3	79.8	80.2	79.6	80.3	79.4	79.3
4	74.3	74.6	74.0	74.6	74.3	74.2
5	84.4	85.1	84.0	84.9	84.6	84.5
6	63.8	63.0	63.3	63.4	63.7	63.7
Rham						
1				101.0		
2				72.0		
3				72.2		
4				73.8		
5				71.0		
6				18.0		
Bz		(R_3)	(R_4)	(R_1)		
1		131.0	131.2	130.9[c]		
2, 6		130.5	130.6	130.6[d]		
3, 5		129.4	129.6	129.5		
4		134.2	134.3	133.3		
α		167.5	168.0	167.7		
Bz				(R_4)		
1				130.7[c]		
2, 6				130.7[d]		
3, 5				129.5		
4				133.3		
α				167.8		
Cinn	(R_1)	(R_1)	(R_1)	(R_3)	(R_1)	(R_1)
1	131.5	127.7	127.6	130.9[c]	126.5	126.5
2	107.1	111.9	112.0	112.7	107.1	107.1[k]
3	154.8	149.5	149.3	152.2	149.4	149.3
4	141.5	150.9	150.6	149.4	139.7	139.7[l]
5	154.8	116.7	116.5	118.9	149.4	149.3
6	107.1	124.3	124.2	123.4	107.1	107.1[m]
α	167.8	168.0	168.3	167.3	168.2	169.0
β	117.8	115.1	114.9	116.9	115.4	115.8[n]
γ	147.2[a]	147.7	147.7	147.0	148.0	148.0[o]
OMe	56.8	56.4	56.5	56.5	56.9	56.9[p]
	61.2					
Cinn	(R_4)				(R_4)	(R_4)
1	127.8				127.7	126.5
2	111.8				111.6	106.9[k]
3	149.4				149.3	149.3
4	150.7				150.5	139.4[l]
5	116.4				116.3	149.3
6	124.3				124.3	106.9[m]
α	169.2				169.1	168.2
β	115.4				115.3	115.4[n]
γ	147.1[a]				147.1	147.3[o]
OMe	56.5				56.5	56.8[p]

a—p) Assignments may be interchanged in each column.

sinapoyl signal were observed (see Table I). Detailed proton spin decoupling experiments, which started from the irradiation at each anomeric proton signal and NOE experiments involving irradiation at each anomeric proton signal, enabled us to assign all proton signals. An NOE was observed at the H-3 (δ 3.68) of α-glucose, on irradiation at the anomeric proton signal at δ 4.35 of β-glucose. The above mentioned ^{1}H-NMR data led us to assume that the sugar linkage and acylated sites of reiniose E (**10**) were as shown. The position of each acyl residue was determined by observation of ^{2}J and ^{3}J using the HMBC and NOE experiments (see Chart 2). These data led us to assign the structure of **10** to reiniose E.

Reiniose F (**11**), $[\alpha]_D$ −59.0°, $C_{40}H_{52}O_{24}$, was obtained as an amorphous powder and it showed $[M+Na]^+$ and $[M+H]^+$ ions at *m*/*z* 939 and 917, respectively, under FAB-MS. The ^{1}H-NMR spectrum was similar to that of reiniose E (**10**), but it showed the presence of two sinapoyl residues (see Table I). Compound **11** gave glucose and fructose in the ratio 2 : 1 on acid hydrolysis, while alkaline hydrolysis gave sinapic acid (see Experimental). Detailed proton spin decoupling and NOE experiments led us to assume that the sugar linkage and the acylated sites of reiniose F (**11**) were as shown. The position of each acyl residue was determined by observation of ^{2}J and ^{3}J using the HMBC and NOE experiments (see Chart 2). These data led us to assign the structure of **11** to reiniose F.

Reiniose G (**12**), $[\alpha]_D$ −30.6°, $C_{53}H_{62}O_{28}$, was obtained as an amorphous powder and it showed an $[M+Na]^+$ ion at *m*/*z* 1169 under FAB-MS. The ^{1}H-NMR spectrum showed the presence of one benzoyl, two acetyl and two *p*-coumaroyl groups (see Table II). Compound **12** gave a tetrasaccharide **12a** which was identical to the deacyl compound of senegoses F—H and an acid mixture composed of benzoic and *p*-coumaric acids (see Experimental). Detailed proton spin decoupling and NOE experiments enabled us to assign all proton signals of the Glc-1 and Glc-3 moieties (see Chart 2 and Table II) and led us to assume that the sugar linkage and the acylated sites of reiniose G (**12**) were as shown. The position of each acyl residue was determined by observation of ^{2}J and ^{3}J using HMBC and NOE experiments (see Chart 2). These data led us to assign the structure of **12** to reiniose G.

The ^{1}H-NMR spectrum of reiniose H (**14**), $[\alpha]_D$ −15.7°, $C_{54}H_{64}O_{29}$, showed that this compound was composed of a tetrasaccharide **12a**, single benzoic, *p*-coumaric and ferulic acid moieties and two acetic acid moieties. The position of each acyl residue was determined by observation of ^{2}J and ^{3}J using HMBC and NOE experiments (see Chart 2). These data led us to assign the structure of **14** to reiniose H.

Reiniose I (**15**), $[\alpha]_D$ −21.0°, $C_{61}H_{76}O_{35}$, was obtained as an amorphous powder and it showed $[M+Na]^+$ and $[M+H]^+$ ions at *m*/*z* 1391 and 1369, respectively, under FAB-MS. Compound **15** gave a pentasaccharide **15a** which was identical to the deacyl compound of tenuifolioses A—P on alkaline hydrolysis.[7,9] The ^{1}H-NMR spectrum was very similar to that of tenuifoliose N (**18**).[9] Results of the proton spin decoupling experiments showed that one acetyl methyl signal and a downfield-shifted oximethine proton signal assigned to the H-4 of Glc-3 which were

TABLE IV. ^{13}C-NMR Data for Oligosaccharides from the Roots of *Polygala reinii* in MeOH-d_4

	12	14	15	17
Glc-1				
1	93.0	93.0	92.9	93.0
2	81.4	81.4	81.3	81.3
3	79.1	79.0	79.1	79.5
4	70.5	70.5	70.5	70.0
5	69.7	69.7	69.7	69.6
6	64.4	64.4	64.3	64.4
Glc-2				
1	105.4	105.4	105.4	105.4
2	75.3	75.3	75.4	75.4
3	78.5	78.5	78.5	78.6
4	71.7	71.7	71.6	71.6
5	77.9	77.9	77.8	77.8
6	63.1	63.1	63.1	63.1
Glc-3				
1	104.5	104.5	104.2	104.1
2	75.6	75.6	75.4	75.4
3	78.5	78.5	87.3	87.4
4	71.1	71.1	69.7	69.8
5	74.8	74.8	74.5	74.4
6	64.4	64.4	64.3	64.4
Glc-4				
1			105.1	105.2
2			74.8	74.9
3			78.5	78.6
4			71.6	71.6
5			78.1	78.1
6			62.7	62.6
Fru				
1	66.0	65.9	66.0	66.0
2	103.9	104.0	104.0	103.9
3	80.2	80.2	80.2	80.1
4	74.0	74.0	73.9	74.0
5	84.7	84.7	84.6	84.7
6	63.8	63.8	63.8	63.8
Ac (R_5)				
1	172.7	172.7	172.5	172.5
2	20.8	20.8	20.8	20.8[m]
Ac (R_7)				
1				
2				
Ac (R_8)				
1	172.5	172.5	172.5	172.7
2	20.5	20.5	20.5	20.7[m]
Bz (R_3)				
1	131.1	131.0	131.1	130.9
2, 6	131.2	131.0	131.1	131.0
3, 5	130.0	129.9	130.0	130.0
4	134.9	134.9	134.9	134.9
α	167.3	167.3	167.3	167.3
Cinn (R_2)				
1	127.2	127.7	127.7[f]	127.7
2	131.3	111.8	111.8	111.8
3	117.0	149.5	149.5	149.5
4	161.5[a]	150.8	150.8[g]	150.8
5	117.0	116.6	116.5[h]	116.5[n]
6	131.3	124.4	124.5[i]	124.4
α	168.4	168.4	168.4	168.4
β	114.9[b]	115.1[d]	115.2[j]	115.2
γ	147.0[c]	147.3[e]	147.3[k]	147.3
OMe		56.6	56.6[l]	56.6
Cinn (R_4)				
1	127.1	127.0	127.6[f]	127.5
2	131.3	131.3	111.8	133.7
3	117.0	117.0	149.5	116.6
4	161.4[a]	161.7	151.0[g]	160.2
5	117.0	117.0	116.6[h]	116.6[n]
6	131.3	131.3	124.4[i]	133.7
α	167.9	167.9	167.9	166.9
β	115.1[b]	115.0[d]	115.3[j]	115.9
γ	146.9[c]	146.9[e]	147.2[k]	145.4
OMe			56.5[l]	

a—n) Assignments may be interchanged in each column.

observed in the ^{1}H-NMR spectrum of **18**, were absent in **15**. In the ^{13}C-NMR spectrum, the C-3 and C-5 of Glc-3 were shifted downfield by 3.8 ppm and 2.1 ppm, respectively, compared with those of tenuifoliose N (**18**) (see Table IV). On acetylation, **15** gave a peracetate **15b** which was identical to the peracetate of tenuifoliose N (**18**).[9] Therefore, the structure of reiniose I (**15**) was concluded to be as shown in Chart 1.

Reiniose J (**17**), $[\alpha]_D$ +2.6°, $C_{60}H_{74}O_{34}$, was obtained as an amorphous powder and it showed an $[M+Na]^+$ ion at *m/z* 1361 under FAB-MS. The ^{1}H-NMR spectrum showed the presence of single benzoyl, *cis-p*-coumaroyl and feruloyl groups and two acetyl groups (see Table II). Compound **17** gave a pentasaccharide **15a** and an acid mixture composed of benzoic, *cis-p*-coumaric and ferulic acids (see Experimental). The position of each acyl group was determined by observation of ^2J and ^3J using HMBC and NOE experiments, except for the *cis-p*-coumaroyl group. The ^2J and ^3J between an ester carbonyl carbon signal and two *cis*-olefinic proton signals (δ 5.69, 6.84) of the *cis-p*-coumaroyl group were not observed in the HMBC spectrum (see Chart 2). But the H-4 of Glc-1 was shifted downfield to δ 5.00 and the only residual acyl residue was that of the *cis-p*-coumaroyl group. This group was therefore determined to be at the C-4 of Glc-1. These data led us to assign the structure of **17** to reiniose J.

The anomeric configurations of Glc-1, Glc-2, Glc-3 and Glc-4 were determined to be α, β, β and β, respectively, from each $^3J_{H_1-H_2}$ value and that of the Rham moiety was determined as α from the chemical shifts of the C-3 and C-5 of Rham.[10] The absolute configuration of each monosaccharide was not determined.

Experimental

General Procedure Instrumental analyses were carried out as described previously.[9]

Extraction and Isolation *Polygala reinii* FR. *et* SAV. was collected in Shizuoka, Japan, in June, 1993 and the dried roots (350 g) were extracted three times with boiling water (5 l). The extract was passed through a Mitsubishi Diaion HP-20 column (9 cm × 28 cm) and the adsorbed material eluted successively with 50% MeOH aq., 70% MeOH aq. and MeOH to give a pale yellow powder (50% MeOH aq. eluate 7.57 g, 70% MeOH aq. eluate 6.58 g and MeOH eluate 6.09 g). From the 70% MeOH aq. eluate (6.58 g), oligosaccharides were isolated by preparative HPLC [Develosil Lop-ODS, 5 cm × 50 cm × 2, CH_3CN-H_2O (20 : 80)—(28 : 72) linear gradient]: **1** (24 mg), **2** (18 mg), **3** (315 mg), **4** (12 mg), **5** (9 mg), **6** (161 mg), **7** (36 mg), **8** (261 mg), **9** (24 mg), **10** (124 mg), **11** (196 mg), **12** (9 mg), **13** (15 mg), **14** (12 mg), **15** (15 mg), **16** (12 mg), **17** (9 mg).

Reiniose A (2) Amorphous powder. $[\alpha]_D^{20}$ −51.8° (c=1.37, MeOH). UV λ_{max}^{MeOH} nm (log ε): 219 (4.63), 233 (4.64), 302 (sh 4.58), 321 (4.63). *Anal.* Calcd for $C_{34}H_{42}O_{18} \cdot H_2O$: C, 53.97; H, 5.86. Found: C, 53.97; H, 5.91. FAB-MS *m/z*: 761 $[M+Na]^+$, 739 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables I and III.

Reiniose B (5) Amorphous powder. $[\alpha]_D^{20}$ −4.8° (c=0.52, MeOH). UV λ_{max}^{MeOH} nm (log ε): 221 (4.31), 231 (4.34), 301 (sh 4.05), 328 (4.22). *Anal.* Calcd for $C_{29}H_{34}O_{15} \cdot 2H_2O$: C, 52.89; H, 5.82. Found: C, 53.09; H, 5.89. FAB-MS *m/z*: 645 $[M+Na]^+$, 623 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables I and III.

Reiniose C (6) Amorphous powder. $[\alpha]_D^{20}$ −29.3° (c=0.87, MeOH). UV λ_{max}^{MeOH} nm (log ε): 219 (4.36), 230 (4.36), 301 (sh 4.11), 327 (4.23). *Anal.* Calcd for $C_{29}H_{34}O_{15} \cdot 2H_2O$: C, 52.89; H, 5.82. Found: C, 53.14; H, 5.88. FAB-MS *m/z*: 645 $[M+Na]^+$, 623 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables I and III.

Reiniose D (9) Amorphous powder. $[\alpha]_D^{20}$ −51.1° (c=0.94, MeOH). UV λ_{max}^{MeOH} nm (log ε): 230 (4.56), 298 (4.21), 322 (4.25). *Anal.* Calcd for $C_{42}H_{48}O_{20} \cdot 3H_2O$: C, 54.43; H, 5.87. Found: C, 54.51; H, 5.86.

FAB-MS *m*/*z*: 895 $[M+Na]^+$, 873 $[M+H]^+$. ^1H- and ^{13}C-NMR: Tables I and III.

Reiniose E (10) Amorphous powder. $[\alpha]_D^{20}$ $-52.2°$ ($c=0.91$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 236 (4.54), 328 (4.45). *Anal.* Calcd for $C_{39}H_{50}O_{23}\cdot 1/4H_2O$: C, 52.55; H, 5.71. Found: C, 52.58; H, 6.01. FAB-MS *m*/*z*: 909 $[M+Na]^+$, 887 $[M+H]^+$. ^1H- and ^{13}C-NMR: Tables I and III.

Reiniose F (11) Amorphous powder. $[\alpha]_D^{20}$ $-59.0°$ ($c=0.94$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 238 (4.58), 330 (4.57). *Anal.* Calcd for $C_{40}H_{52}O_{24}\cdot H_2O$: C, 51.39; H, 5.82. Found: C, 51.53; H, 6.05. FAB-MS *m*/*z*: 939 $[M+Na]^+$, 917 $[M+H]^+$. ^1H- and ^{13}C-NMR: Tables I and III.

Reiniose G (12) Amorphous powder. $[\alpha]_D^{20}$ $-30.6°$ ($c=0.36$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 229 (4.54), 302 (sh 4.55), 315 (4.61). *Anal.* Calcd for $C_{53}H_{62}O_{28}\cdot 7H_2O$: C, 50.00; H, 6.02. Found: C, 50.23; H, 6.06. FAB-MS *m*/*z*: 1169 $[M+Na]^+$. ^1H- and ^{13}C-NMR: Tables II and IV.

Reiniose H (14) Amorphous powder. $[\alpha]_D^{20}$ $-15.7°$ ($c=1.08$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 230 (4.57), 301 (sh 4.53), 319 (4.62). *Anal.* Calcd for $C_{54}H_{64}O_{29}\cdot 3H_2O$: C, 52.68; H, 5.73. Found: C, 52.86; H, 5.80. FAB-MS *m*/*z*: 1197 $[M+Na]^+$. ^1H- and ^{13}C-NMR: Tables II and IV.

Reiniose I (15) Amorphous powder. $[\alpha]_D^{20}$ $-21.0°$ ($c=0.62$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 219 (4.61), 233 (4.60), 300 (sh 4.49), 327 (4.65). *Anal.* Calcd for $C_{61}H_{76}O_{35}\cdot 4H_2O$: C, 50.83; H, 5.87. Found: C, 51.11; H, 5.93. FAB-MS *m*/*z*: 1391 $[M+Na]^+$, 1369 $[M+H]^+$. ^1H- and ^{13}C-NMR: Tables II and IV.

Reiniose J (17) Amorphous powder. $[\alpha]_D^{20}$ $+2.6°$ ($c=0.38$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 210 (4.73), 227 (4.71), 301 (sh 4.58), 320 (4.63). *Anal.* Calcd for $C_{60}H_{74}O_{34}\cdot 5H_2O$: C, 50.42; H, 5.92. Found: C, 50.58; H, 6.04. FAB-MS *m*/*z*: 1361 $[M+Na]^+$. ^1H- and ^{13}C-NMR: Tables II and IV.

Acetylation of 15 Compound **15** (3 mg) was treated with acetic anhydride–pyridine (1 : 1) (3 drops) overnight at 35 °C and the reagents were then evaporated to give an amorphous powder **15b**. ^1H-NMR and FAB-MS were identical to those of tenuifoliose N peracetate.[9]

Alkaline Hydrolysis of 2, 5, 6, 9—12, 14, 15, 17 Each compound (1 mg) was treated with 5% NaOH aq. (3 drops) for 3 h at room temperature and the reaction mixture was passed through a column filled with Amberlite IR-120B. From the aqueous eluates of the reaction mixtures sugars were detected by HPLC [Asahipak NH2P-50, 4.6 mm × 25 cm, CH_3CN–H_2O (65 : 35), 1.0 ml/min, UV 195 nm,[11]] as follows: from **2**, **5** and **6**, sucrose (**2a**); from **9**, sucrose (**2a**) and rhamnose; from **12** and **14**, a tetrasaccharide **12a**; from **15** and **17**, a pentasaccharide **15a**. The t_R values were 3.8 min (rhamnose); 4.9 min (sucrose **2a**); 6.4 min (tetrasaccharide **12a**); 7.2 min (pentasaccharide **15a**) and the retention times of **12a** and **15a** were identical to that of the deacyl product of senegose F[2] and tenuifoliose A,[7] respectively. From the methanol eluate of the reaction mixture of each compound benzoic, *trans*-*p*-coumaric, *cis*-*p*-coumaric, ferulic, sinapic and 3,4,5-trimethoxycinnamic acids were identified by HPLC [YMC R-ODS-7, 4.6 mm × 25 cm, CH_3CN–H_2O–trifluoroacetic acid (22.5 : 77.5 : 0.05), 1.0 ml/min, UV 270 nm, t_R 8.2 min (*trans*-*p*-coumaric acid); 8.6 min (sinapic acid); 9.0 min (*cis*-*p*-coumaric acid); 9.3 min (ferulic acid); 14.1 min (benzoic acid); 26.2 min (3,4,5-trimethoxycinnamic acid)].

Acid Hydrolysis of 10 and 11 A solution of each compound (2 mg) in 5% H_2SO_4 (3 drops) was heated in a boiling water bath for 30 min. The solution was passed through a column filled with Amberlite IRA-60E and the aqueous eluate concentrated. From **10** and **11**, glucose and fructose were detected in the ratio 2 : 1 using HPLC [Asahipak NH2P-50 4.6 mm × 25 cm, CH_3CN–H_2O (80 : 20), 1.0 ml/min, UV 195 nm,[11] t_R 8.4 min (fructose); 10.6 min (glucose)].

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