TENUIFOLIOSES A-F, OLIGOSACCHARIDE MULTI-ESTERS FROM THE ROOTS OF *POLYGALA TENUIFOLIA* WILLD.

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From the roots of *Polygala tenuifolia* WILLD. six new oligosaccharides, named tenuifolioses A-F, were isolated and their structures were elucidated by spectroscopic data and chemical evidence. These oligosaccharides were esterified with acetic, benzoic, *p*-coumaric and ferulic acid.

KEYWORDS *Polygala tenuifolia*; Polygalaceae; tenuifoliose; oligosaccharide; acetate; benzoate; *p*-coumarate; ferulate

The root of *Polygala tenuifolia* WILLD. is a famous crude drug used as an expectorant and a tonic in China and Japan and is studied on saponins¹⁾ and xanthones.²⁾ But there are few studies on the other type constituents of this crude drug, so we investigated with a view to isolating the biologically active substances other than saponin and xanthone. Now we wish to report the isolation and the structural elucidation of six new multi-acylated oligosaccharides, named tenuifolioses A-F (1-6).

The water extract of the roots of *P. tenuifolia* WILLD. was chromatographed on MITSUBISHI Diaion HP-20 and the adsorbed material was eluted with 50% MeOH aq., 70% MeOH aq. and 100% MeOH, successively. The 70% MeOH aq. eluate was rechromatographed on silica gel using CHCl₃-MeOH system to give four fractions (Fr. 1-4). Six oligosaccharides were isolated by HPLC using an ODS column from Fr. 2 and 3.

Tenuifoliose A (1), [α]_D²⁵ -32.8°, C₆₂H₇₆O₃₅,³⁾ was obtained as an amorphous powder, and it showed [M + Na]⁺ and [M + H]⁺ ions at *m/z* 1403 and 1381, respectively, in the FAB-MS. 1 gave glucose and fructose in the ratio 4:1 on acid hydrolysis, while it gave pentasaccharide 1a⁴⁾ and an acid mixture composed of benzoic, *p*-coumaric and ferulic acid on alkaline hydrolysis.⁵⁾ The ¹H-NMR spectrum showed three acetyl methyl signals (δ 1.53, 1.99, 2.02), one α -linked glucosyl anomeric proton signal [δ 5.90 (1H, d, *J*=3.5 Hz)], three β -linked ones [δ 4.46 (1H, d, *J*=8 Hz), 4.53 (1H, d, *J*=8 Hz), 4.59 (1H, d, *J*=8 Hz)] and the signals due to a benzoyl, a *p*-coumaroyl and a feruloyl residue.⁶⁾ So, 1 was assumed to be a hexa-acylated pentasaccharide. Detailed proton spin decoupling experiments, difference NOE experiments on irradiation at each anomeric proton signal and ¹H-¹³C COSY spectrum led us to presume the sugar linkages and the acylated sites of tenuifoliose A (1) as shown. The position of each acyl residue was decided by observation of ³*J*_(COCH) using a long-range selective proton decoupling method. On acetylation, 1 gave peracetate 1b⁷⁾, which had two aromatic and fourteen aliphatic acetoxyl signals in the ¹H-NMR spectrum. The glycosylation and acylation shifts in the ¹³C-NMR spectrum of tenuifoliose A supported structure 1.

The ¹H-NMR spectra of tenuifolioses B (2), C (3), D (4) and E (5)^{3,8)} showed that these four compounds were composed of a pentasaccharide 1a, a benzoic acid, a *p*-coumaric acid, a ferulic acid and one or two acetic acids. On acetylation 2-5 gave the same peracetate 1b and pentasaccharide 1a on alkaline hydrolysis as in the case of 1. Therefore the positions of benzoyl, *p*-coumaroyl, feruloyl and one or two acetyl residues were the same as 1.

In the ¹H-NMR spectrum of 2, two acetyl methyl signals were observed at δ 1.54 and 1.99, while H₂-6 of Glc ① shifted up-field at δ 3.58 and 3.67 compared with those of 1. Therefore, two acetyl groups were located at C-4 and C-6 of Glc ①.

The ¹H-NMR spectrum of 3 showed one acetyl methyl signal at δ 1.59 and up-field shifted carbonyl at δ 3.32 and hydroxymethyl proton signals at δ 3.57, 3.68 which were assigned to H-4 of Glc ① and H₂-6 of Glc ②, respectively. So, one acetyl group located at C-6 of Glc ②.

	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	R ₉
1 :	H	C	B	D	A	A	A	H	H
1a :	H	H	H	H	H	H	H	H	H
1b :	A	C	B	D	A	A	A	A	A
2 :	H	C	B	D	H	A	A	H	H
3 :	H	C	B	D	H	H	A	H	H
4 :	H	C	B	D	A	H	A	H	H
5 :	H	C	B	D	A	H	H	H	H
6 :	H	C	B	D	A	A	A	H	Rham
6b :	A	C	B	D	A	A	A	A	Rham(OAc) ₃

A : -CO-CH₃

B : -CO-

C : -CO-CH=CH-

D : -CO-CH=CH-

Table II. ¹³C-NMR Data of 1 - 6 in CD₃OD

	1	2	3	4	5	6
Glc ①						
1	92.7	92.9	93.2	92.8	92.3	92.7
2	81.3	81.4	81.5	81.3	80.9	81.3
3	79.3	79.5	79.3	79.0	79.7	79.2
4	70.3	70.2	70.3	70.3	71.0	70.4
5	69.6	72.2	72.3	69.6	69.7	69.6
6	64.4	62.1	62.1	64.3	64.4	64.3
Glc ②						
1	105.4	105.3	105.4	105.3	105.4	105.3
2	75.2	75.3	75.4	75.2	75.3	75.3
3	78.5	78.4	78.5	78.4	78.5	78.4
4	71.5	71.5	71.6	71.5	71.1	71.5
5	77.7	77.7	77.7	77.6	77.7	77.7
6	63.0	63.0	63.1	63.0	63.0	63.0
Glc ③						
1	104.1	104.1	104.2	104.1	104.5	104.1
2	75.4	75.3	75.4	75.2	74.9	75.2
3	83.4	83.4	87.2	87.2	87.1	83.4
4	69.6	69.6	69.7	69.5	70.0	69.6
5	72.3	72.3	74.4	74.3	77.1	72.4
6	63.0	63.0	64.3	64.1	62.7	63.0
Glc ④						
1	105.4	105.3	105.1	105.0	105.0	105.3
2	75.7	75.7	74.8	74.6	75.3	75.7
3	78.4	78.4	78.5	78.3	78.3	78.4
4	71.5	71.5	71.6	71.5	71.5	71.5
5	77.9	77.8	78.0	77.9	78.0	77.8
6	62.8	62.8	62.6	62.6	62.6	62.8
Fru						
1	66.3	66.3	66.0	65.9	65.8	66.3
2	103.7	103.6	103.8	103.8	103.9	103.7
3	80.4	80.4	80.2	80.1	79.9	80.4
4	74.0	73.6	73.6	73.9	73.9	74.0
5	84.7	84.5	84.5	84.6	84.6	84.6
6	63.9	63.2	63.2	63.8	63.8	63.8
Rham						
1						100.7
2						71.9
3						72.1
4						73.7
5						71.0
6						18.0
Ac (R ₅)						
1	172.5			172.5	172.5	172.5
2	20.8			20.8	20.8	20.8
Ac (R ₆)						
1	171.9	171.9				171.9
2	21.1	21.1				21.1
Ac (R ₇)						
1	172.3	172.3	172.5	172.4		172.2
2	20.3	20.3	20.5	20.4		20.3

Table I. ¹H-NMR Data of 1 - 6 in CD₃OD and Yields

	1	2	3	4	5	6
Yield	0.14 %	0.062 %	0.020 %	0.072 %	0.011 %	0.018 %
Glc ①						
1	5.90 (d, 3.5)	5.88 (d, 3.5)	5.85 (d, 3.5)	5.89 (d, 3.5)	5.87 (d, 3.5)	5.90 (d, 3.5)
2	3.83 (dd, 9, 3.5)	3.79 (dd, 9.5, 3.5)	3.79 (dd, 9.5, 3.5)	3.85 a)	3.83 (dd, 9, 3.5)	3.85 a)
3	3.98 (t, 9)	3.98 (t, 9.5)	3.98 (t, 9.5)	3.98 (t, 9.5)	4.04 (t, 9.5)	3.98 (t, 9)
4	5.03 (t, 9.5)	5.03 (t, 9.5)	5.02 (t, 9.5)	5.04 (t, 9.5)	5.02 (t, 9.5)	5.03 (t, 9)
5	4.43 (m)	4.25 (ddd, 9.5, 4, 1.5)	4.22 (m)	4.41 (m)	4.39 (ddd, 9.5, 5, 3)	4.43 (m)
6	4.15 (dd, 12.5, 6)	3.58 (dd, 12, 5)	3.57 (dd, 12.5, 5.5)	4.15 (dd, 12, 5.5)	4.15 (dd, 12, 5.5)	4.16 (dd, 12.5, 5.5)
	4.20 (dd, 12.5, 2)	3.67 (dd, 12, 2)	3.68 (dd, 12.5, 1.5)	4.19 (dd, 12, 3)	4.19 (dd, 12, 2)	4.21 (dd, 12.5, 2)
Glc ②						
1	4.59 (d, 8)	4.59 (d, 8)	4.58 (d, 7.5)	4.60 (d, 8)	4.58 (d, 8)	4.60 (d, 8)
Glc ③						
1	4.53 (d, 8)	4.50 (d, 8)	4.49 (d, 8)	4.54 (d, 8)	4.48 (d, 8)	4.53 (d, 8)
2	3.30 (dd, 9, 8)	3.30 (dd, 9, 8)	3.23 (t, 9)	3.25 a)	3.23 a)	3.31 a)
3	3.61 (t, 9)	3.60 (t, 9.5)	3.37 (t, 9)	3.37 (t, 9)	3.38 (t, 9)	3.61 (t, 9)
4	4.73 (t, 9.5)	4.74 (t, 9.5)	3.32 a)	3.35 a)	3.28 a)	4.73 (t, 9)
5	3.20 (td, 3, 9.5)	3.18 (td, 3, 10)	3.10 (ddd, 9, 3, 1.5)	3.13 (td, 3, 9.5)	3.05 (ddd, 9.5, 4.5, 2)	3.20 (td, 2, 10)
6	3.68 (dd, 12, 2)	3.68 (dd, 12, 2)	4.01 (dd, 12, 2)	4.01 (dd, 12, 2)	3.45 (dd, 12, 5)	3.66 (dd, 12, 2)
	3.98 (dd, 12, 3.5)	3.98 (dd, 12, 3.5)	4.06 (dd, 12, 3.5)	4.07 (dd, 12, 4)	3.62 (dd, 12, 2)	3.98 (dd, 12, 3.5)
Glc ④						
1	4.46 (d, 8)	4.46 (d, 8)	4.47 (d, 8)	4.48 (d, 8)	4.47 (d, 8)	4.47 (d, 8)
Fru						
1	4.29 (d, 12)	4.30 (d, 12)	4.23 (d, 12)	4.23 (d, 12)	4.19 (d, 12)	4.30 (d, 12)
2	4.60 (d, 12)	4.63 (d, 12)	4.70 (d, 12)	4.69 (d, 12)	4.70 (d, 12)	4.61 (d, 12)
3	5.75 (d, 8)	5.73 (d, 8)	5.72 (d, 8)	5.75 (d, 8)	5.75 (d, 8)	5.75 (d, 8)
4	4.43 (t, 9)	4.51 (t, 8)	4.50 (t, 8)	4.44 (t, 8)	4.43 (t, 8)	4.43 (t, 8)
5	4.09 (ddd, 8, 7, 3)	4.06 (ddd, 8, 6, 3)	4.03 (m)	4.09 (m)	4.07 (ddd, 8, 7, 3)	4.09 (m)
6	3.84 (dd, 12, 3)	3.86 (dd, 12.5, 3)	3.84 (dd, 12.5, 3)	3.86 a)	3.84 (dd, 12.5, 3)	3.83 a)
	3.89 (dd, 12, 6)	3.90 (dd, 12.5, 6)	3.88 (dd, 12.5, 6)	3.88 (dd, 12.5, 6)	3.89 (dd, 12.5, 6)	3.87 (dd, 12.5, 6)
Rham						
1						5.49 (d, 1.5)
2						4.11 (dd, 3.5, 1.5)
3						3.93 a)
4						3.49 (t, 9.5)
5						3.75 (m)
6						1.24 (d, 6.5)
Ac (R ₅)	2.02 (s)			2.07 (s)	2.07 (s)	2.08 (s)
Ac (R ₆)	1.99 (s)	1.99 (s)				1.99 (s)
Ac (R ₇)	1.53 (s)	1.54 (s)	1.59 (s)	1.59 (s)		1.50 (s)

a) Overlapped with other signals.

The ^1H -NMR spectrum of **4** showed the presence of two acetyl groups at C-6 of Glc ① and C-6 of Glc ②, and that of **5** showed only one acetyl group at C-6 of Glc ① by down-field shifts.

Tenuifoliose F (**6**), $[\alpha]_D^{25} -52.3^\circ$, $\text{C}_{68}\text{H}_{86}\text{O}_{39}$,³⁾ was obtained as an amorphous powder, and it showed an $[\text{M} + \text{Na}]^+$ ion at m/z 1549 in the FAB-MS. **6** gave glucose, fructose and rhamnose in the ratio 4:1:1 on acid hydrolysis, while **6** gave pentasaccharide **1a** and the same acid mixture as in the case of **1** on alkaline hydrolysis. The ^{13}C -NMR spectrum revealed the presence of rhamnose other than **1**. On acetylation **6** gave peracetate **6b**⁹⁾, and its ^1H -NMR spectrum showed the presence of one aromatic and seventeen aliphatic acetoxyl signals. An NOE was observed at H-5 of feruloyl residue by irradiation at the anomeric proton signal of rhamnose, suggesting **6** to be a phenolic rhamnoside. The rhamnosyl anomeric configuration was α from the $J_{\text{C1-H1}}$ (171.3 Hz). These data led us to conclude the structure of **6**.

From *P. amarella*, a tetrasaccharide hexa-ester amarelliside,¹⁰⁾ and from *P. chamaebuxus*, three sucrose di-esters¹¹⁾ were isolated, but tenuifolioses A-F (**1-6**) were the first examples of pentasaccharide multi-esters from *Polygala* species.

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- 3) The molecular formula was determined by elemental analysis.
- 4) Amorphous powder, $[\alpha]_D^{27} +4.8^\circ$ (H_2O), FAB-MS m/z : 851 $[\text{M} + \text{Na}]^+$. ^1H -NMR (D_2O): δ 4.01 (1H, t, $J=8.5$ Hz, H-3 of Glc ①), 4.13 (1H, t, $J=9.5$ Hz, H-3 of Glc ②), 4.20 (1H, d, $J=9$ Hz, H-1 of Glc ①), 4.69 (1H, d, $J=8$ Hz, H-1 of Glc ②), 4.82 (1H, d, $J=8$ Hz, H-1 of Glc ③), 5.52 (1H, d, $J=3$ Hz, H-1 Glc ④). ^{13}C -NMR (D_2O): δ 60.8, 61.4 (2C), 61.6, 61.7, 63.1, 68.5, 69.0, 70.3, 70.5, 72.8, 74.0, 74.2 (2C), 74.7, 76.3 (2C), 76.5, 76.7 (3C), 79.5, 80.2, 82.3, 85.4, 92.7, 102.6, 103.6, 103.9, 104.7. Dioxane (δ 3.73, 67.3) was used as an internal standard.
- 5) These acids were identified by HPLC. Conditions: Column, YMC R-ODS-7 4.6 x 250 mm; Solvent, $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ (22.5 : 77.5) + 0.05% TFA; Flow rate, 1.0 ml/min; Detector, UV 270 nm; t_R , 8.4 min (*p*-coumaric acid), 10.0 min (ferulic acid), 17.2 min (benzoic acid).
- 6) NMR data of ester moieties other than acetates. ^1H -NMR (CD_3OD): δ (benzoate) 7.61 (2H, t, $J=8$ Hz, H-3, H-5), 7.69 (1H, br t, $J=8$ Hz, H-4), 8.21 (2H, br d, $J=8$ Hz, H-2, H-6), (*p*-coumarate) 6.31 (1H, d, $J=16$ Hz, H- β), 6.80 (2H, d, $J=8.5$ Hz, H-3, H-5), 7.35 (2H, d, $J=8.5$ Hz, H-2, H-6), 7.64 (1H, d, $J=16$ Hz, H- γ), (ferulate) 3.93 (3H, s, OMe), 6.26 (1H, d, $J=16$ Hz, H- β), 6.85 (1H, s, $J=8$ Hz, H-5), 7.05 (1H, dd, $J=8, 2$ Hz, H-6), 7.17 (1H, d, $J=2$ Hz, H-2), 7.61 (1H, d, $J=16$ Hz, H- γ). ^{13}C -NMR (CD_3OD): δ (benzoate) 130.0 (C-3, C-5), 131.1 (C-1, C-2, C-6), 134.8 (C-4), 167.1 (C- α), (*p*-coumarate) 114.9 (C- β), 116.9 (C-3, C-5), 127.0 (C-1), 131.2 (C-2, C-6), 146.8 (C- γ), 161.3 (C-4), 168.3 (C- α), (ferulate) 56.4 (OMe), 111.5 (C-2), 115.1 (C- β), 116.6 (C-5), 124.4 (C-6), 127.5 (C-1), 147.1 (C- γ), 149.4 (C-3), 150.9 (C-4), 167.8 (C- α). These carbon signals were assigned by a ^1H - ^{13}C COSY and a long-range ^1H - ^{13}C COSY spectra.
- 7) Amorphous powder, FAB-MS m/z : 1950 $[\text{M} + \text{Na}]^+$, 1928 $[\text{M} + \text{H}]^+$. ^1H -NMR (CDCl_3): δ 1.75, 1.96, 1.97, 2.08, 2.10, 2.15 (each 3H, s, aliphatic OAc), 2.32, 2.33 (each 3H, s, aromatic OAc), 2.00, 2.01, 2.05, 2.08 (each 6H, s, aliphatic OAc).
- 8) **2**: Amorphous powder, $[\alpha]_D^{25} -35.9^\circ$, $\text{C}_{60}\text{H}_{74}\text{O}_{34}$, FAB-MS m/z : 1361 $[\text{M} + \text{Na}]^+$, 1339 $[\text{M} + \text{H}]^+$. **3**: Amorphous powder, $[\alpha]_D^{25} -8.3^\circ$, $\text{C}_{58}\text{H}_{72}\text{O}_{33}$, FAB-MS m/z : 1319 $[\text{M} + \text{Na}]^+$, 1297 $[\text{M} + \text{H}]^+$. **4**: Amorphous powder, $[\alpha]_D^{25} -10.5^\circ$, $\text{C}_{60}\text{H}_{74}\text{O}_{34}$, FAB-MS m/z : 1361 $[\text{M} + \text{Na}]^+$, 1339 $[\text{M} + \text{H}]^+$. **5**: Amorphous powder, $[\alpha]_D^{25} -2.2^\circ$, $\text{C}_{58}\text{H}_{72}\text{O}_{33}$, FAB-MS m/z : 1319 $[\text{M} + \text{Na}]^+$, 1297 $[\text{M} + \text{H}]^+$.
- 9) Amorphous powder, FAB-MS m/z : 2180 $[\text{M} + \text{Na}]^+$, 2158 $[\text{M} + \text{H}]^+$. ^1H -NMR (CDCl_3): δ 1.75, 1.96, 1.97, 2.03, 2.08, 2.10, 2.20 (each 3H, s, aliphatic OAc), 2.00, 2.01, 2.06 (each 6H, s, aliphatic OAc), 2.08 (12H, s, aliphatic OAc), 2.32 (3H, s, aromatic OAc).
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(Received September 5, 1991)