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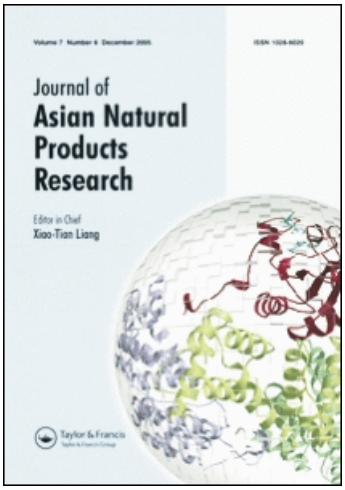

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A novel oligosaccharide ester from *Syringa pubescens*

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Pubescenside A (**1**), a novel oligosaccharide ester, has been isolated from the flowers of *Syringa pubescens*, together with five known compounds D-mannitol (**2**), meso-inositol (**3**), hydrostytosol (**4**), glucose (**5**), and sucrose (**6**). The structure of **1** was elucidated as 1-*O*-[β-D-glucopyranosyl-(1–6)-β-D-galactopyranosyl-(1–1)-β-D-galactopyranosyl-6]-4,4-dimethylpelargonicate by chemical and spectroscopic means. The water extract of the flowers and leaves of *S. pubescens* showed cytotoxicity against L2215 cell line ($IC_{50} = 78\ \mu g/ml$).

Keywords: *Syringa pubescens* Turcz; Oleaceae; Oligosaccharide ester; Pubescenside A

1. Introduction

Syringa pubescens Turcz (Oleaceae) is a shrub widely distributed in China [1–3]. Its flowers and leaves have long been used to treat chronic hepatitis, enteritis, cirrhosis, and liver and oesophagus cancers among the traditional communities [4–5]. During an *in vitro* assay of hepatitis virus B, the water extract of its flowers and leaves inhibited L2215 cell lines with an IC_{50} value of 78 μg/ml. No phytochemical investigation on this plant has been reported to date. In this paper, we report the isolation and structural elucidation of a novel oligosaccharide ester, named pubescenside A (**1**), and five known compounds D-mannitol (**2**) [6], meso-inositol (**3**) [7], 3,4-dihydroxyphenethyl alcohol (**4**) [8], glucose (**5**) [9], and sucrose (**6**) [10].

2. Results and discussion

An EtOAc-soluble fraction of the EtOH extract of the flowers and leaves of *Syringa pubescens* Turcz was repeatedly chromatographed on active charcoal column to give a novel

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oligosaccharide ester, pubescenside A (**1**), together with five known compounds, D-mannitol (**2**) [6], meso-inositol (**3**) [7], 3,4-dihydroxyphenethyl alcohol (**4**) [8], glucose [9], and sucrose [10].

Compound **1** (figure 1) was obtained as pale yellow solid. Its molecular weight (672) was determined by FAB-MS [$m/z = 764$ $[M + 92]^+$ (M + glycerin)]. Its IR spectrum showed absorption bands for hydroxyl groups at 3540 and 1070 cm^{-1}, the ester carbonyl group at 1720 cm^{-1}. The carbon signal at δ 174.1 in the ^{13}C NMR spectrum further revealed the presence of an ester carbonyl group. The EI-MS showed ion peaks at m/z 503 $[R'O]^+$ and 185 $[OOC{-}R]^+$, which indicated the presence of α-fragment and β-fragment of the ester. On alkaline hydrolysis [11], the Et_2O soluble fraction of the hydrolysate gave a long-chain saturated fatty acid (**7**) (figure 2) which was identified on the basis of spectral analysis. The ^1H NMR spectrum of **7** showing proton signals at δ 2.40 (2H, t, CH_2), 1.59 (10H, br, $CH_2 \times 5$), 1.26 (6H, s, $CH_3 \times 2$), and 0.88 (3H, t, CH_3) and the IR spectrum of **7** exhibiting absorption bands at 3471, 2923, 2855, 1734, 1459, and 1375 cm^{-1} suggested that **7** was a long-chain saturated fatty acid. The molecular formula of **7** was determined as $C_{11}H_{22}O_2$ by HRESI-MS at m/z 186.2921 (calcd for $C_{11}H_{22}O_2$, 186.2936). On acid hydrolysis [12], its hydrolysate gave glucose and galactose which were identified by comparison of the R_f values on HPTLC with those of the authentic sample. The chemical composition of ion peak at m/z 503 was determined as $C_{18}H_{31}O_{16}$ by HRESI-MS at m/z 503.4329 $[R'O]^+$ (calcd for $C_{18}H_{31}O_{16}$, m/z 503.4333). The molecular formula of **1** was deduced to be $C_{29}H_{52}O_{17}$ ($672 = 503 + 185 - 16$), which was supported by the total 29 carbons in the ^{13}C NMR spectrum. The ^1HNMR spectrum of **1** revealed three hexosyl moieties by the presence of three anomeric protons at δ 4.33 (2H, br) and 4.65 (1H, d, $J = 7.6$ Hz) and the residual 18 protons at δ 3.08–4.04, which was consistent with the result of acid hydrolysis.

In the FAB-MS of **1**, the fragment ions at m/z 526 $[503 + Na]^+$ and 554 $[531 + Na]^+$ supported the presence of three hexosyl groups; the fragment ion at m/z 582 $[559 + Na]^+$ suggesting the location of the branch moiety of a long-chain saturated fatty acid. Meanwhile, in the FAB-MS of **7**, the fragment ions at m/z 43 (100%), 57, 71 supported the location of the branch moiety of a long-chain saturated fatty acid. By HSQC and HMBC experiments, all the proton and carbon signals of **1** were well assigned as shown in table 1, thus suggesting **1** to be a triglycoside of 4,4-dimethylpelargonicacid ester.

In the ^{13}C NMR spectrum of **1**, 15 carbon signals within δ 62.4–100.7 represented 18 carbons, in which the signal at δ 100.6 represented two anomeric carbons, and the signals at δ 75.3 and 66.8 represented two carbons, respectively. Thus, **1** had three sugar moieties and the anomeric carbons of the three sugars were all occupied. The signals at δ 66.8 and 62.4 indicated two 6-position carbons of two sugars were occupied. The ^{13}C NMR spectral data

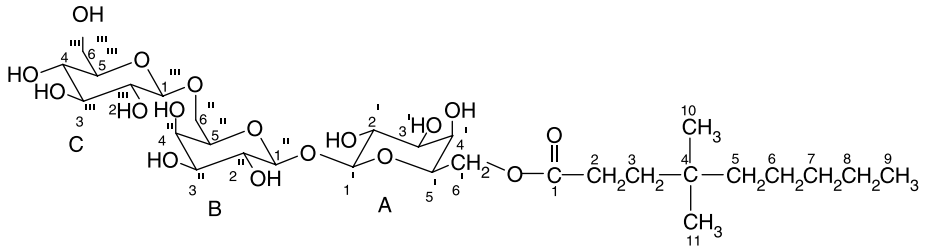

Figure 1. Structure of **1**.

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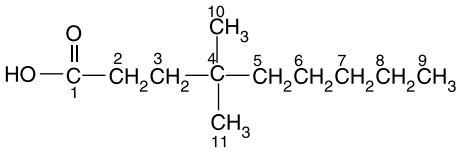

Figure 2. Structure of **7**.

mentioned above and the carbon signals at δ 174.1 and 16.5–38.4 suggested that **1** was an oligoglycoside ester, and the aglycone was a fatty acid which connected with three sugars to form a glycoside ester. If an anomeric carbon is connected with the aglycone, its chemical shift value should be at a higher field ($\delta \sim 95.0$) owing to the esterification effect [13,14]. For **1**, the chemical shifts of three anomeric carbons were all within δ 100.6–100.7. Thus, the above-mentioned linkage was excluded. The aglycone should be linked at 6-position of the sugar, implying the presence of the $1 \rightarrow 1$ bond joint of the two sugars of **1** [15]. Meanwhile, the HSQC and HMBC experiments coinstantaneously showed the correlation between the proton at δ 4.33 and the carbon at δ 100.6, suggesting that two sugars of **1** were $1 \rightarrow 1$ linkage. Moreover, the HSQC correlation between the proton at δ 4.33 and the carbon at δ

Table 1. ^{1}H NMR (400 MHz, D_2O) and ^{13}CNMR (100 MHz, D_2O) spectral data of **1**.

	No.	δ_C	*DEPT*	δ_H	*HMQC* δ_C/δ_H	*HMBC* δ_C/δ_H
	1	174.7	C			174.7/3.50 (C-1/H-6′)
	2	38.4	CH_2	2.64 brs	38.4/2.64 (C-2/H-2)	38.4/2.43 (C-2/H-3)
	3	29.2	CH_2	2.43 brs	29.2/2.43 (C-3/H-3)	29.2/2.43 (C-3/H-5)
	4	36.6	C			
	5	29.2	CH_2	2.43 brs	29.2/2.43 (C-5/H-5)	29.2/2.43 (C-5/H-3, 6, 7)
	6	29.2	CH_2	2.43 brs	29.2/2.43 (C-6/H-6)	29.2/2.43 (C-6/H-5, 7)
	7	29.2	CH_2	2.43 brs	29.2/2.43 (C-7/H-7)	29.2/2.43 (C-7/H-5, 6)
	8	29.2	CH_2	2.20 m	29.2/2.20 (C-8/H-8)	29.2/2.43 (C-8/H-6, 7)
	9	13.1	CH_3	1.05 t	13.1/1.05 (C-9/H-9)	
	10	16.5	CH_3	1.08 s	16.5/1.08 (C-10/H-10)	
	11	16.5	CH_3	1.08 s	16.5/1.08 (C-11/H-11)	
Gal A	1′	100.6	CH	4.33 br	100.6/4.33 (C-1′/H-1′)	100.6/4.33 (C-1′/H-1′)
	2′	69.8[a]	CH	3.51–3.60 m	69.8/3.51–3.60 (C-2′/H-2′)	
	3′	71.9	CH	3.15–3.22 m	71.9/3.15–3.22 (C-3′/H-3′)	
	4′	68.6[a]	CH	3.42–3.51 m	68.6/3.42–3.51 (C-4′/H-4′)	
	5′	74.7	CH	3.35–3.42 m	74.7/3.35–3.42 (C-5′/H-5′)	
	6′	66.8	CH_2	3.42–3.51 m	66.8/3.42–3.51 (C-6′/H-6′)	
				3.75–3.85 m	66.8/3.75–3.85 (C-6′/H-6′)	
Gal B	1″	100.6	CH	4.33 br	100.6/4.33 (C-1″/H-1″)	100.6/4.33 (C-1″/H-1′)
	2″	69.9[a]	CH	3.75–3.85 m	69.9/3.75–3.85(C-2″/H-2″)	
	3″	72.6	CH	3. 08 m	72.6/3.08 (C-3″/H-3″)	
	4″	69.5[a]	CH	3.15–3.22 m	69.5/3.15–3.22(C-4″/H-4″)	
	5″	75.3	CH	3.15–3.22 m[b]	75.3/3.15–3.22(C-5″/H-5″)	
	6″	66.8	CH_2	3.42–3.51 m	66.8/3.42–3.51(C-6″/H-6″)	66.8/4.65 (C-6″/1‴)
				3.75–3.85 m	66.8/3.75–3.85(C-6″/H-6″)	
Glc C	1‴	100.7	CH	4.65 d (7.6)	100.7/4.65 (C-1‴/H-1‴)	
	2‴	73.2	CH	3.42–3.51 m	73.2/3.42–3.51 (C-2‴/H-2‴)	
	3‴	75.3	CH	3.22–3.30 m[b]	75.3/3.22–3.30 (C-3‴/H-3‴)	
	4‴	70.2	CH	3.90–4.04 m	70.2/3.90–4.04 (C-4‴/H-4‴)	
	5‴	76.0	CH	3.22–3.30 m	76.0/3.22–3.30 (C-5‴/H-5‴)	
	6‴	62.4	CH_2	3.90–4.04 m	62.4/3.90–4.04 (C-6‴/H-6‴)	

[a,b]The assignments of these signals may be interchangeable.

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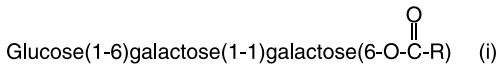
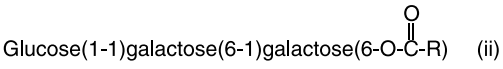
100.6 indicated that two carbons at δ 100.6 were assigned to the anomeric carbons of the two $1 \rightarrow 1$ bond sugars, and the carbon at δ 100.7 was assigned to that of the remaining sugar.

The free movement, shorter correlation time and longer relaxation time caused the outer sugar to have lower peak height of carbon signals [16,17]. In the three sugars, the lower peak height of one sugar group (δ 62.4, 70.2, 73.2, 75.3, 76.0) suggested this sugar should be a glucose located at the outer side of the whole molecular structure. The other two sugars should be two galactoses and be located at the middle of the whole molecular structure, based on the chemical shift values of their carbon signals.

Thus, the possible structures of **1** are listed as follows:





Based on the ^{13}CNMR spectrum of **1**, we know the relative peak height ratio between the outer glucose and the inner two galactoses was 1:1.5. Therefore, for structure (i), the peak height ratio between δ 100.7 and 100.6 should be $1:(1.5 + 1.5) = 1:3$; for structure (ii), the peak height ratio between δ 100.7 and 100.6 should be $1.5:(1.5 + 1) = 1.5:2.5$. The relative peak height ratio between δ 100.7 and 100.6 was 1:3, closely similar to that of structure (i).

The anomeric proton of the three sugar signals at δ 4.33, 4.65 indicated all β-configuration [15]. Moreover, the bigger coupling constant ($J = 7.6$ Hz) of the outer glucose and the ^{13}C NMR spectral data of **1** in table 1 further confirmed its β-configuration [15]. Consequently, the structure of **1** was established as 1-*O*-[β-D-glucopyranosyl-(1–6)-β-D-galactopyranosyl-(1–1)-β-D-galactopyranosyl-6]-4,4-dimethylpelargonicate.

3. Experimental

3.1 General experimental procedures

Melting points were measured on an XT_4 instrument and are uncorrected. IR spectra were obtained on a PE-580B infrared spectrometer. Optical rotations were taken on a PE-341 polarimeter. The FAB-MS spectra were recorded on a JEOL JMS-HX-110 instrument, and EI-MS on a VG ZAB-2f and MM7070 spectrometer. NMR spectra were recorded on a Bruker DPX400 spectrometer. Silica gel for TLC and column chromatography was obtained from Qingdao Marine Chemical Inc., China. HPTLC silica gel plates (20×10 cm) were purchased from Taizhou Siqing Biochemical Material Inc., Taizhou, China.

3.2 Plant material

Flowers and leaves of *Syringa pubescens* Turcz were collected in Songxian county, Henan province, China, in May 2000, and were identified by Professor C.S. Zhu and S.Y. Wang, Henan Agricultural University. A voucher specimen (4378) is deposited at the College of Agronomy, Henan Agricultural University, China.

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3.3 Extraction and isolation

Dried flowers and leaves (3.5 kg) were extracted (6 L × 2) with 75% ethanol at room temperature and filtered. The filtrate (31.2 *g*) was concentrated and extracted with ethyl acetate/hexane (8:2) and EtOAc, respectively. The EtOAc extract (10 *g*) was submitted to an active charcoal column (150 × 6 cm) eluting with a gradient system of H_2O/EtOH (H_2O, 10%, 20%, 30%, 40%, 50%, 60%, 70% EtOH). Compound **1** (42 mg) was isolated from the 10% EtOH fraction as a pale yellow amorphous powder. Compounds **2** (200 mg) and **3** (59 mg) were isolated from the 20% EtOH fraction and purified by crystallisation. Compound **4** (60 mg) was obtained from 30% and 40% EtOH fraction and purified by crystallisation. The water fraction afforded glucose (70 mg) and sucrose (40 mg) after being chromatographed over silica gel column eluting with EtOAc/MeOH (4:6–2:8) and MeOH/H_2O (8:2).

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3.3.1 Pubescenside A (1). $C_{29}H_{52}O_{17}$. Pale yellow solid, mp 139–141°C (MeOH). $[\alpha]_D^{20}$ −5.63 (MeOH, *c* 0.50). IR (KBr) ν_{max} (cm^{-1}): 3540, 1720, 1590, 1440, 1385, 1205, 1075, 1050. ^1H NMR and ^{13}C NMR spectral data: see table 1. EI-MS *m/z*: 503, 341, 326, 207, 185 (100%), 163, 115; HRESI-MS *m/z*: 503.4329 (calcd for $C_{18}H_{31}O_{16}$, 503.4333); FAB-MS *m/z*: 764 $[M + 92]^+$ (M + glycerin), 661 $[638 + Na]^+$, 607 $[584 + Na]^+$, 582 $[559 + Na]^+$, 554 $[531 + Na]^+$, 526 $[503 + Na]^+$, 316 $[293 + Na]^+$, 288 $[265 + Na]^+$.

3.4 Alkaline hydrolysis of 1

Compound **1** (10 mg) was dissolved in 1.5 ml H_2O and heated at 90°C for 150 min under a liquid NH_3 treatment until the hydrolysis reaction was completed [11]. The reaction mixture was extracted with Et_2O. The resulting oil layer was evaporated to give a long chain saturated fatty acid (**7**).

Compound **7**: $C_{11}H_{22}O_2$, IR (film) ν_{max} (cm^{-1}): 3471, 2923, 2855, 1734, 1459, 1375, 726; ^1H NMR (400 MHz, $CDCl_3$): δ 2.40 (2H, t, H-2), 1.59 (10H, br, H-3, 5, 6, 7, 8), 1.26 (6H, s, H-10, 11), 0.88 (3H, t, H-9). EI-MS *m/z*: 186, 141, 111, 97, 83, 71, 57, 43 (100%); HRESI-MS *m/z* 186.2921 (calcd for $C_{11}H_{22}O_2$, 186.2936).

3.5 Acid hydrolysis of 1

Compound **1** (0.5 mg) and lactose (0.5 mg) were dissolved in 1.0 ml EtOH, respectively, and both the solutions were added to an HPTLC silica gel plate. A strong HCl of 10.0 ml was poured into a 1000-ml beaker with a support at the bottom. The sample plate was placed on the support, and the top of the beaker was airproofed by a two-double filter paper and a plastic film. The beaker was heated at 50–60°C for 20 min until the hydrolysis reaction was completed [12]. After glucose was added as a control, the plate was developed using $CHCl_3$/MeOH/H_2O (6:3:0.5). The hydrolysate of **1** gave both glucose (R_f = 3.5) and galactose (R_f = 4.3), comparing with glucose and the hydrolysate of lactose (glucose and galactose).

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