

Isomeric Phenolic Glycosides from *Populus tomentosa*

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Abstract: A new phenolic glycoside named salicyltomenside (**1**), with five similar compounds, salicyltremuloidin (**2**), tremuloidin (**3**), isograndidentatin A (**4**), siebolside B (**5**), and salicin (**6**) were isolated from *Populus tomentosa* Carr. Additionally, salicyltremuloidin (**2**) was obtained from the plant for the first time. Structure of the new compound was determined using spectroscopic methods including ESI-MS and 1D- and 2D-NMR. Others were elucidated through ¹H and ¹³C NMR spectra and comparison with literature data. Notably, salicyltomenside (**1**) and salicyltremuloidin (**2**) were structural isomers.

Keywords: *Populus tomentosa* Carr.; salicyltomenside; salicyltremuloidin; phenolic glycosides; NMR. © 2018 ACG Publications. All rights reserved.

1. Introduction

Populus tomentosa Carr. (Fam. Salicaceae) is a dioecious and economic plant which is endemic to north China [1,2]. The stem bark of *P. tomentosa* has been used as a traditional medicinal herb for the treatment of hepatitis, dysentery, stranguria, cough, and phlegm [3,4]. Doctors in Shandong used its bark with other Chinese herbs to treat chronic bronchitis with the total effective rate of 86% in 1558 cases [5]. Previous pharmacological studies indicated that the leaves and inflorescence of *P. tomentosa* exhibited some biological activities, including analgesic, anti-inflammatory [6], anti-diarrhoeal, and anti-microbial activity [7]. Phytochemical investigations of stem bark of *P. tomentosa* have led to the isolation of several kinds of chemical constituents including lignin [8], flavonoids [9], phenolic acids, phenolic glycosides [10], and fatty acid derivatives [11]. Among which, phenolic glycosides possessed antihyperglycemic [12], antioxidant [13], and antimicrobial effects [14]. Herein, we described the isolation, purification, and structural elucidation of a new phenolic glycoside (**1**) and its isomer (**2**) and four known similar compounds (**3-6**) (Figure 1).

2. Materials and Methods

2.1. General Experimental Procedures

Melting point was measured by an X-6 digital microscopic melting point apparatus without correction (Beijing Focus Instrument Co., Ltd.). The IR spectrum was recorded on an FTIR-8400S spectrophotometer (Shimadzu, Kyoto, Japan). The ESI-MS spectrum was taken on an Agilent 6320 Ion Trap ESI-MS spectrometer (Agilent, California, USA). The HR-ESI-MS spectrum was taken on an LTQ-Orbitrap Velos Pro mass spectrometer (Thermo fisher, USA). The NMR spectra were measured on a Bruker AVANCE II 400 MHz spectrometer in CD₃OD solution. The chemical shifts

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were given as δ values related to tetramethylsilane (TMS) as internal standard. Preparative reversed-phase HPLC was carried out by Varian 50 preparative HPLC (Varian, California, USA). Silica gel (200-300 mesh) for column chromatography, and TLC were obtained from Qingdao Marine Chemical Co. Ltd. (Qingdao, China). Elemental analysis was conducted on an Elementar Vario EL cube elemental analyzer (Hanau, Germany). The filtrate was concentrated in a RE-201D rotary evaporator (Yuhua Instrument Co. Ltd., Gongyi, China) under reduced pressure.

2.2 Plant Material and Chemicals

The stem barks of male adult *P. tomentosa* were collected in March 2017 near the Sanyuan bridge which was located at 39°57'37.31" north latitude, 116°27'28.53" east longitude, and an altitude of 44 m (Beijing, China). The plant materials were identified by Prof. Zhimao Chao (Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences). Voucher specimens were deposited at the 1016 room of Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. Methanol of HPLC grade was provided by Fisher Scientific Worldwide Co. Ltd. (Shanghai, China). Water was Wahaha purified water. Petroleum ether, ethyl acetate, n-butyl alcohol, and chloroform of analytical grade were all produced by Beijing Chemical Works.

2.3 Extraction and Isolation

After scraped off the rough bark, the fresh stem bark (4.8 kg) of *P. tomentosa* was pulverized, extracted with petroleum ether (60-90°C) under reflux (3 × 1 h), and filtered. The residue was extracted with methanol under reflux (3 × 1 h). The MeOH solution was concentrated under reduced pressure to obtain an extract of 432.5 g, which was suspended in H₂O (2.5 L) and partitioned with petroleum ether, ethyl acetate, and n-butyl alcohol in sequence. After evaporation of the solvent under reduced pressure, the ethyl acetate extract (79 g) was obtained with a yield of 18.27% and then subjected to silica-gel column (200-300 mesh, 6 cm i.d.×80 cm, approx. 1.0 kg) chromatography with petroleum ether-ethyl acetate (40:1, 10:1, 5:1, and 2:1) and chloroform-ethyl acetate as the gradient eluent (10:1, 5:1, 2:1, and 1:1). The fraction of chloroform-ethyl acetate (2:1) was subjected to preparative reversed-phase HPLC eluting with MeOH-H₂O (62:38, v/v) to afford compounds **1** (5 mg), **2** (41 mg), and **6** (27 mg). The fractions of chloroform-ethyl acetate (1:1) afforded compounds **3** (157 mg), **4** (562 mg), and **5** (2527 mg).

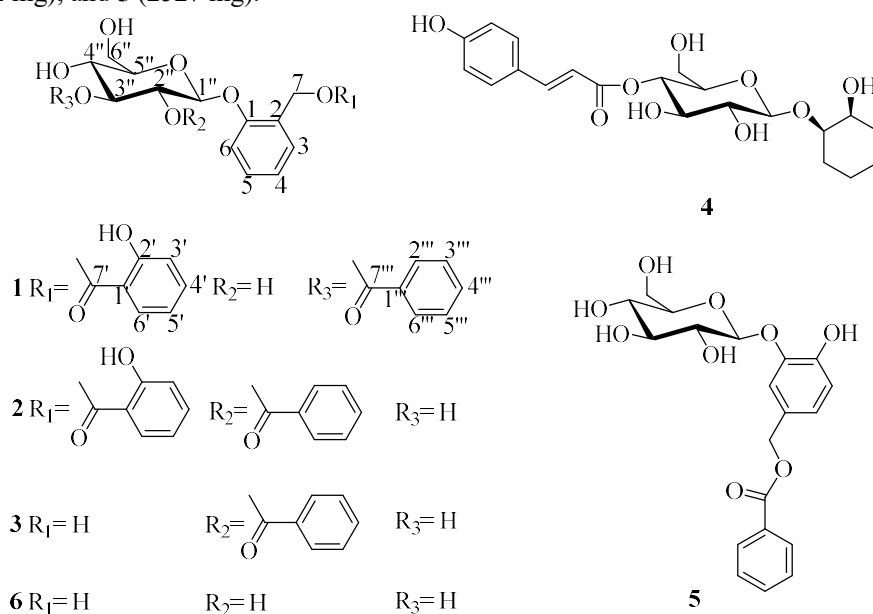


Figure 1. Structures of compounds 1-6

3. Results and Discussion

Compound **1** was obtained as white powder, soluble in chloroform and methanol. The molecular formula was assigned as $C_{27}H_{26}O_{10}$, requiring 15 degrees of unsaturation deduced from 1H and ^{13}C -NMR spectra data, and the high-resolution electrospray ionization mass spectroscopy (HR-ESI-MS) at m/z 533.1400 $[M+Na]^+$ (calcd for $C_{27}H_{26}O_{10}Na$, 533.1418).

1H -NMR data displayed resonances for 13 aromatic protons in downfield region, including two 1,2-bis-substituted benzene rings at δ 7.46 (1H, *d*, $J = 8.0$ Hz, H-3), 7.11 (1H, *t*, $J = 8.0$ Hz, H-4), 7.39 (1H, *t*, $J = 8.0$ Hz, H-5), and 7.33 (1H, *d*, $J = 8.0$ Hz, H-6), and δ 6.96 (1H, *d*, $J = 8.0$ Hz, H-3'), 7.51 (1H, *t*, $J = 8.0$ Hz, H-4'), 6.92 (1H, *t*, $J = 8.0$ Hz, H-5'), and 7.91 (1H, *d*, $J = 8.0$ Hz, H-6'), and a monosubstituted benzene ring at δ 7.51 (2H, *t*, $J = 7.6$ Hz, H-3'', 5''), 7.63 (1H, *t*, $J = 7.6$ Hz, H-4''), and 8.13 (2H, *d*, $J = 7.6$ Hz, H-2'', 6''). 1H -NMR data also showed 9 protons in upfield region (δ 3.62-5.63), assigned to a group of -O-CH₂- at δ 5.49 (1H, *d*, $J = 12.4$ Hz, H-7) and 5.63 (1H, *d*, $J = 12.4$ Hz, H-7), and a glucosyl moiety at δ 5.16 (1H, *d*, $J = 7.6$ Hz, H-1''), 3.83 (1H, *t*, $J = 8.4$ Hz, H-2''), 5.33 (1H, *t*, $J = 9.2$ Hz, H-3''), 3.74 (1H, *m*, H-4''), 3.62 (1H, *m*, H-5''), 3.78 (1H, *m*, H-6''), and 3.93 (1H, *d*, H-6''). ^{13}C NMR data revealed 27 signals of carbons (Table 1), in which there were two carbonyl carbons at δ 171.0 and 166.6, seven carbons bonding to oxygen at δ 60.9, 62.1 (benzyloxy), 68.2, 72.0, 76.7, 78.3, and 101.4 (an anomeric proton of 1''), and eighteen olefinic carbons in the region of δ 113.3-155.7 (in which δ 128.1 and 129.4 were overlapped). By comparing NMR data, compound **1** was shown to be very similar to salicyltremuloidin (**2**), and the assignment of these signals revealed that compound **1** possessed a benzyl group (δ 155.7, 125.2, 130.3, 122.3, 129.9, 115.5, and 62.1), a salicylic acyl group (δ 113.3, 161.4, 117.0, 135.4, 119.0, 129.7, and 171.0), a benzoyl group (δ 129.7, 129.4, 128.1, 132.8, 128.1, 129.4, and 166.6), and a glucosyl moiety (δ 101.4, 72.0, 76.7, 68.2, 78.3, and 60.9). The stereochemistry of the anomeric carbon of glucosyl moiety was determined as β configuration according to the large coupling constant (7.6 Hz) of the anomeric proton at δ 5.16 and the chemical shift of C-1'' at δ 101.4.

In the 1H - 1H COSY spectrum of compound **1**, seven proton signals in upfield (δ 3.62-5.16) had correlation with each other in sequence (δ 5.16-3.83-5.33-3.74-3.62-3.93/3.78), which were assigned to H-1'' to H-6'' of glucosyl moiety. Except for compound **2**, another four compounds **3**, **4**, **5**, and **6** were structurally related to compound **1** by comparison with previous literature data. The NMR data of these compounds were listed in Table 2. C-2'' of compound **1** can't be substituted because its chemical shift (δ 72.0) was in upfield as compounds **4**, **5**, and **6** (δ 73.7, 73.6, and 73.7), while the carbon signal of C-2'' of 1,2-bis-substituted compounds **2** and **3** (δ 74.1 and 74.6) were in downfield. C-4'' of compound **1** can't be substituted because its chemical shift (δ 68.2) was in upfield as compounds **2**, **3**, **5**, and **6** (δ 70.2, 70.2, 70.0, and 70.2), while the carbon signal of C-4'' of 1,4-bis-substituted compound **4** (δ 71.2) was in downfield. C-6'' of compound **1** can't be substituted, since all of six compounds had similar chemical shifts of C-6'' (δ 60.9, 61.1, 61.1, 61.0, 61.2, 61.1) which were in upfield than that of the 1,6-bis-substituted compound salicyloylpopuline (δ 65.1) shown in Table 1. Therefore, compound **1** can only be 1,3-bis-substituted, as the proton signal at δ 5.33 of H-3'' was in further downfield than those of other five compounds (**2-6**) (δ 3.79, 3.84, 3.56, 3.44, and 3.36-3.52). Additionally, compound **1** had the same structure skeleton of 1,3-bis-substituted glucosyl as benzoysalireposide, both of which had the same chemical shift of H-3'' at δ 5.33 shown in Table 1.

The ^{13}C NMR spectroscopic data of glucosyl group of compound **1**, were in the similar pattern and regularity [15] with that of 1,3-bis-substituted benzoysalireposide [16], but different with that of 1,2-bis-substituted compound **2**, 1,4-bis-substituted compound **4**, and 1,6-bis-substituted salicyloylpopuline [17] as shown in Table 1-2.

As C-1'' and C-2'' were substituted in the glucosyl moiety of compound **2**, H-1'' and H-2'' were in downfield chemical shift at δ 5.31 and 5.30 in 1H NMR and correlated with each other in 1H - 1H COSY spectrum. There was also correlation between δ 5.30 (H-2'') and δ 3.79 (H-3'') in 1H - 1H COSY spectrum of compound **2**. Contrarily, two similar protons of downfield chemical shift at δ 5.33 (H-3'') and δ 5.16 (H-1'') were not correlated in 1H - 1H COSY spectrum of compound **1**. Besides, δ 3.83 (H-2'') had correlation with both δ 5.16 (H-1'') and δ 5.33 (H-3'') in 1H - 1H COSY spectrum of compound **1**.

Table 1. NMR spectroscopic data of compound **1**, **2**, salicyloylpopuline, and benzoysalireposide (δ in ppm and J in Hz)

Position	1 (in CD ₃ OD) 1,3-bis-substituted glucosyl			2 (in CD ₃ OD) 1,2-bis-substituted glucosyl			salicyloylpopuline ^[17] (in CD ₃ COCD ₃) 1,6-bis-substituted glucosyl		benzoysalireposide ^[16] (in CDCl ₃) 1,3-bis-substituted glucosyl	
	δ_C	δ_H	¹ H- ¹ H COSY	δ_C	δ_H	¹ H- ¹ H COSY	δ_C	δ_C	δ_H	
1	155.7	-	-	155.4	-	-	156.7	149.7	-	
2	125.2	-	-	124.5	-	-	126.0	134.1	-	
3	130.3	7.46 <i>d</i> (8.0)	4	129.7	7.33 <i>m</i>	4	130.3	116.0	6.78 <i>d</i> (2.9)	
4	122.3	7.11 <i>t</i> (8.0)	3,5	122.4	7.04 <i>br t</i> (7.6)	3,5	123.2	154.3	-	
5	129.9	7.39 <i>t</i> (8.0)	6	129.7	7.33 <i>m</i>	6	130.3	115.4	6.47 <i>dd</i> (3.0, 8.7)	
6	115.5	7.33 <i>d</i> (8.0)	5	115.3	7.32 <i>m</i>	5	116.8	119.6	7.04 <i>d</i> (8.7)	
7	62.1	5.49 <i>d</i> (12.4)	7- ^{5.63}	61.7	5.16 <i>d</i> (12.0)	7- ^{5.22}	63.2	65.1	4.54 <i>d</i> (13.0)	
		5.63 <i>d</i> (12.4)	7- ^{5.49}		5.22 <i>d</i> (12.0)	7- ^{5.16}			4.69 <i>d</i> (13.0)	
1'	113.3	-	-	112.2	-	-	113.6	131.2	-	
2'	161.4	-	-	161.3	-	-	162.5	130.6	8.03 <i>dt</i> (1.4, 6.1)	
3'	117.0	6.96 <i>d</i> (8.0)	4'	116.8	6.89 <i>br d</i> (8.4)	4'	120.2	129.4	7.47 <i>br d</i> (7.5)	
4'	135.4	7.51 <i>d</i> (8.0)	3',5'	135.2	7.41 <i>m</i>	3',5'	136.7	134.2	7.61 <i>m</i>	
5'	119.0	6.92 <i>t</i> (8.0)	4',6'	118.8	6.75 <i>br t</i> (8.4)	4',6'	118.2	129.4	7.47 <i>br d</i> (7.5)	
6'	129.7	7.91 <i>d</i> (8.0)	5'	129.6	7.57 <i>dd</i> (8.4, 1.6)	5'	131.0	130.6	8.03 <i>dt</i> (1.4, 6.1)	
7'	171.0	-	-	169.5	-	-	170.8	167.7	-	
1''	101.4	5.16 <i>d</i> (7.6)	2''	99.4	5.31 <i>m</i>	2''	102.6	104.4	4.88 <i>d</i> (7.8)	
2''	72.0	3.83 <i>t</i> (8.4)	1'',3''	74.1	5.30 <i>m</i>	1'',3''	75.1	73.4	3.86 <i>br t</i> (7.5)	
3''	76.7	5.33 <i>t</i> (9.2)	2'',4''	74.6	3.79 <i>m</i>	2'',4''	77.9	79.3	5.33 <i>t</i> (9.3)	
4''	68.2	3.74 <i>m</i>	3'',5''	70.2	3.57 <i>m</i>	3'',5''	71.5	70.3	3.88 <i>br t</i> (7.5)	
5''	78.3	3.62 <i>m</i>	4'', 6''- ^{3.93} ,	77.1	3.60 <i>m</i>	4'', 6''- ^{3.98} ,	74.6	75.4	3.78 <i>ddd</i> (1.6, 7.7,	
			6''- ^{3.78}			6''- ^{3.76}			9.6)	
6''	60.9	3.93 <i>d</i> (10.4)	5'',6''- ^{3.78}	61.1	3.98 <i>dd</i> (12.0, 2.0)	5'',6''- ^{3.76}	65.1	60.7	4.48 <i>dd</i> (7.2, 11.6)	
		3.78 <i>m</i>	5'',6''- ^{3.93}		3.76 <i>m</i>	5'',6''- ^{3.98}			4.72 <i>dd</i> (7.2, 11.6)	
1'''	129.7	-	-	129.5	-	-	131.2	131.6	-	
2''' ,6'''	129.4	8.13 <i>d</i> (7.6)	3''' ,5'''	129.3	7.99 <i>d</i> (8.4)	3''' ,5'''	130.5	130.7	8.10 <i>dt</i> (1.4, 7.0)	
3''' ,5'''	128.1	7.51 <i>t</i> (7.6)	2''' ,6''' ,4'''	128.0	7.34 <i>m</i>	2''' ,6''' ,4'''	129.4	129.6	7.51 <i>br d</i> (7.7)	
4'''	132.8	7.63 <i>t</i> (7.6)	3''' ,5'''	132.9	7.44 <i>m</i>	3''' ,5'''	133.9	134.4	7.61 <i>m</i>	
7'''	166.6	-	-	165.9	-	-	166.6	167.8	-	

Table 2. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectroscopic data of compound **3**, **4**, **5**, and **6** in CD₃OD (δ in ppm and *J* in Hz)

Position	tremuloidin (3)		isograndidentatin A (4)		siebolside B (5)		salicin (6)	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	154.3	-	78.1	3.90 <i>m</i>	148.6	-	155.7	-
2	130.6	-	69.5	3.86 <i>m</i>	152.6	-	130.8	-
3	127.1	7.33 <i>d</i> (7.6)	26.2	1.32-1.86 <i>m</i>	117.9	7.12 <i>d</i> (8.8)	128.5	7.35 <i>d</i> (7.2)
4	122.3	7.02 <i>t</i> (7.6)	21.0	1.32-1.86 <i>m</i>	115.2	6.73 <i>dd</i> (8.8,3.2)	122.3	7.05 <i>t</i> (7.2)
5	128.2	7.20 <i>t</i> (7.6)	21.7	1.32-1.86 <i>m</i>	127.4	-	128.6	7.26 <i>t</i> (7.2)
6	114.6	7.17 <i>d</i> (7.6)	30.0	1.32-1.86 <i>m</i>	114.9	6.87 <i>d</i> (3.2)	115.6	7.22 <i>d</i> (7.2)
7	58.6	4.32 <i>d</i> (14.0)	-	-	61.8	5.43 <i>d</i> (12.8)	59.6	4.79 <i>d</i> (13.2)
		4.58 <i>d</i> (14.0)				5.52 <i>d</i> (12.8)		4.58 <i>d</i> (13.2)
1'	-	-	-	-	130.1	-	-	-
2'	-	-	-	-	129.2	8.06 <i>dd</i> (7.6,1.6)	-	-
3'	-	-	-	-	128.2	7.48 <i>dd</i> (7.6,1.6)	-	-
4'	-	-	-	-	132.9	7.61 <i>m</i>	-	-
5'	-	-	-	-	128.2	7.48 <i>dd</i> (7.6,1.6)	-	-
6'	-	-	-	-	129.2	8.06 <i>dd</i> (7.6,1.6)	-	-
7'	-	-	-	-	166.7	-	-	-
1''	99.5	5.31 <i>d</i> (8.0)	100.8	4.47 <i>d</i> (7.6)	103.0	4.78 <i>d</i> (7.6)	102.0	4.89 <i>d</i> (7.6)
2''	74.6	5.26 <i>m</i>	73.7	3.38 <i>m</i>	73.6	3.45 <i>m</i>	73.7	3.36-3.52 <i>m</i>
3''	74.2	3.84 <i>m</i>	74.3	3.56 <i>m</i>	76.8	3.44 <i>m</i>	76.6	3.36-3.52 <i>m</i>
4''	70.2	3.58 <i>m</i>	71.2	4.90 <i>m</i>	70.0	3.38 <i>m</i>	70.0	3.36-.52 <i>m</i>
5''	77.0	3.59 <i>m</i>	74.7	3.66 <i>m</i>	76.7	3.37 <i>m</i>	76.9	3.36-3.52 <i>m</i>
6''	61.1	3.98 <i>m</i>	61.0	3.68 <i>m</i>	61.2	3.88 <i>dd</i> (11.2, 1.6)	61.1	3.91 <i>br d</i> (12.0)
		3.78 <i>m</i>		3.55 <i>m</i>		3.69 <i>dd</i> (12.0, 5.2)		3.72 <i>dd</i> (12.0, 5.2)
1'''	129.9	-	125.8	-	-	-	-	-
2''',6'''	129.3	8.10 <i>d</i> (7.2)	129.8	7.49 <i>d</i> (8.4)	-	-	-	-
3''',5'''	128.2	7.49 <i>t</i> (7.2)	115.5	6.83 <i>d</i> (8.4)	-	-	-	-
4'''	133.0	7.60 <i>t</i> (7.2)	160.0	-	-	-	-	-
7'''	166.0	-	145.8	7.69 <i>d</i> (16.0)	-	-	-	-
8'''	-	-	113.4	6.39 <i>d</i> (16.0)	-	-	-	-
9'''	-	-	167.1	-	-	-	-	-

On the basis of above evidence and comparative analysis with the other similar compounds, the structure of the new compound **1** was elucidated as (2-[[[(2-hydroxybenzoyl)oxy]methyl]phenyl- β -D-glucopyranoside 3-benzoate) (Figure 1) and named salicyltomenside. With the same molecular formula of C₂₇H₂₆O₁₀, salicyltomenside (**1**), salicyltremuloidin (**2**), salicyloylpopuline, and benzoylsalireposide were proved to be structural isomers, which enriched the systematic study of phenolic glycosides in *Populus*.

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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