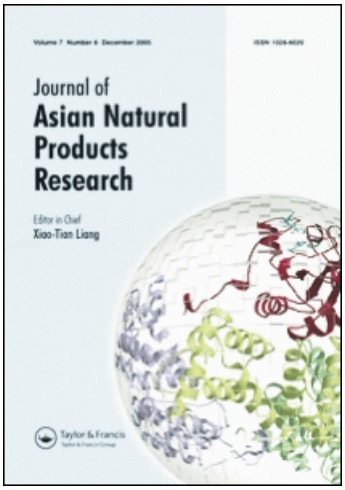

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Three new flavonoid glycosides from *Pinus tabulaeformis* Carr

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Three new flavonoid glycosides from *Pinus tabulaeformis* Carr

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Three new flavonoid glycosides, named 7,8,4′-trihydroxy-3,5-dimethoxy-6-methylflavonol-8-O-β-D-glucopyranoside (**1**), 5,7,4′-trihydroxy-3-methoxy-6-methylflavonol-7-O-β-D-glucopyranoside (**2**), 3,5,7,4′-tetrahydroxy-6-methylflavonol-7-O-β-D-glucopyranoside (**3**), together with two known flavonoid glycosides were isolated from the needles of *Pinus tabulaeformis* Carr. Their structures were established on the basis of various spectroscopic analyses.

Keywords: flavonoid glycosides; needles; *Pinus tabulaeformis* Carr; structure elucidation

1. Introduction

The plants of pine are widely distributed in China, and thirteen of them are used extensively in folk medicine. The modern pharmacology study of them demonstrated that they have various functions such as hypoglycemic [1], antilipemic [2], anti-aging [3], anti-fatigue [4], and antivirus [5] activities. However, few of the phytochemical studies of *Pinus tabulaeformis* Carr are reported by now. This paper deals with the isolation and structural elucidation of three new flavonoid glycosides named 7,8,4′-trihydroxy-3,5-dimethoxy-6-methylflavonol-8-O-β-D-glucopyranoside (**1**), 5,7,4′-trihydroxy-3-methoxy-6-methylflavonol-7-O-β-D-glucopyranoside (**2**), 3,5,7,4′-tetrahydroxy-6-methylflavonol-7-O-β-D-glucopyranoside (**3**), respectively, together with two known compounds, 5,7,8,4′-tetrahydroxy-3-methoxy-6-methyl-flavonol-8-O-β-D-glucopyranoside (**4**), 3,5,7,4′-tetrahydroxy-6-methylflavonol-3-O-β-D-glucopyranoside (**5**).

2. Results and discussion

Compound **1** was isolated as a yellow amorphous powder (MeOH). The molecular formula of **1** was determined as $C_{24}H_{26}O_{12}$ on the basis of HR-ESI-MS at *m/z* 529.1308 $[M + Na]^+$. The IR spectrum indicated the presence of hydroxyl (3399 cm^{-1}), carbonyl (1652 cm^{-1}), and aromatic ring (1577, 1491 cm^{-1}). The ^1H NMR spectrum showed the presence of four aromatic proton signals resonated at δ_H 8.02 (d, 2H, $J = 8.8$ Hz, H-2′, 6′), 6.40 (d, 2H, $J = 8.8$ Hz, H-3′, 5′) as one AA′BB′ system. In addition, the ^1H NMR spectrum showed two methoxyls at δ_H 3.93 (3H, s), 3.75 (3H, s), respectively, and one methyl at δ_H 2.04 (3H, s). Finally, six proton signals were found in the range of δ_H 3.0–5.0, which suggested the existence of a sugar moiety. The signal at δ_H 4.88 (d, 1H, $J = 7.6$ Hz) was assigned as the anomeric proton. Acid hydrolysis of **1** gave glucose, which was identified by comparison with authentic sample. The coupling constant ($J = 7.6$ Hz) of the

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anomeric proton demonstrated that the glucose was in β-orientation. The ^{13}C NMR spectrum clearly indicated that **1** was a flavonoid glycoside including 24 carbons. With the help of the HSQC spectrum, its ^{13}C NMR spectrum showed two methoxyl carbons at δ_C 61.2 and 59.1, one methyl at δ_C 8.3, six carbon signals belonging to the β-D-glucose moiety at δ_C 103.3, 77.5, 76.7, 74.5, 70.6, 61.4, and fifteen carbons of the flavonol aglycone moiety. The locations of all substituted groups can be confirmed by the HMBC spectrum. The HMBC $^1H \rightarrow {}^{13}C$ correlations between the methoxyl at δ_H 3.75 and C-3 at δ_C 135.5; the methoxyl at δ_H 3.93 and C-5 at δ_C 156.3; the methyl at δ_H 2.04 and C-5 at δ_C 156.3, C-6 at δ_C 112.0 and C-7 at δ_C 155.3; H-1″ at δ_H 4.88 (d, 1H, $J = 7.6$ Hz) and C-8 at δ_C 127.9 suggested two methoxyls, a methyl and a glucose moiety located at C-3, C-5, C-6, and C-8, respectively. Thus, the structure of **1** was characterized as 7,8,4′-trihydroxy-3,5-dimethoxy-6-methylflavonol-8-O-β-D-glucopyranoside (Figure 1).

Compound **2** was isolated as a yellow amorphous powder (MeOH). The molecular formula of **2** was determined as $C_{23}H_{24}O_{11}$ on the basis of HR-ESI-MS at *m/z* 499.1224 $[M + Na]^+$. The IR spectrum showed the presence of hydroxyl (3289 cm^{-1}), carbonyl (1650 cm^{-1}), and aromatic ring (1600, 1509 cm^{-1}). The ^1H NMR spectrum showed the presence of five aromatic proton signals. Four aromatic protons resonated at δ_H 7.94 (d, 2H, $J = 8.8$ Hz, H-2′, 6′) and 6.71 (d, 2H, $J = 8.8$ Hz, H-3′, 5′) as one AA′BB′ system, and one aromatic proton resonating at δ_H 6.82 (1H, s) was attributed to H-8. In addition, the ^1H NMR spectrum showed one methoxyl at δ_H 3.75 (3H, s) and one methyl at δ_H 2.13 (3H, s). Furthermore, six proton signals at δ_H 3.0–5.1 suggested the existence of a sugar moiety, and the signal at δ_H 5.09 (1H, d, $J = 7.2$ Hz) was attributed to the anomeric proton. Acid hydrolysis of **2** gave glucose, identified by comparison with authentic sample. The coupling constant ($J = 7.2$ Hz) of the anomeric proton demonstrated that the glucose was in β-orientation. The ^{13}C NMR spectrum clearly indicated that **2** was a flavonoid glycoside with 23 carbons, including fifteen of the flavonol aglycone moiety, six of the β-D-glucose moiety, one of methoxy, and one of methyl. In HMBC spectrum, the methoxy at δ_H 3.75 showed a long-range correlation with C-3 (δ_C 136.4), which revealed that it was attached to C-3; the methyl at δ_H 2.13 showed correlations with C-7 (δ_C 160.3), C-5 (δ_C 157.2), and C-6 (δ_C 108.4), which indicated that it was linked to C-6; the anomeric proton of sugar (δ_H 5.09, d, 1H, $J = 7.2$ Hz) showed the correlation with C-7 (δ_C 160.3), which suggested that the glucose was located at C-7. Through all of the above analysis, the structure of **2** was established as 5,7,4′-trihydroxy-3-methoxy-6-methylflavonol-7-O-β-D-glucopyranoside (Figure 2).

Compound **3** was isolated as a yellow amorphous powder (MeOH). The mole-

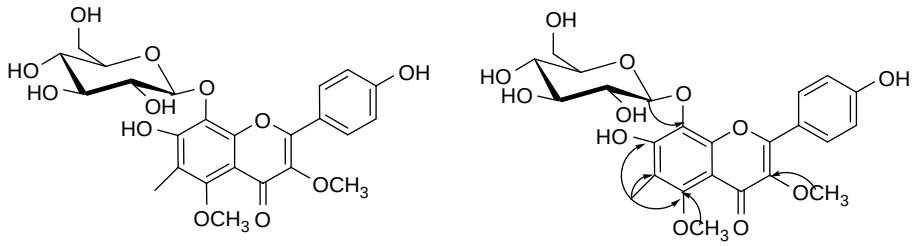

Figure 1. Structure and key HMBC correlations of **1**.

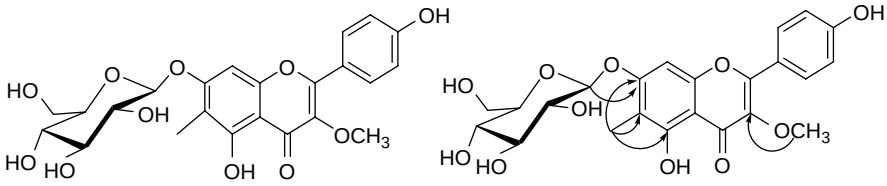

Figure 2. Structure and key HMBC correlations of **2**.

cular formula of **3** was determined as $C_{22}H_{22}O_{11}$ on the basis of HR-ESI-MS at *m/z* 485.1063 $[M + Na]^+$. The IR spectrum exhibited the presence of hydroxyl (3374 cm^{-1}), carbonyl (1646 cm^{-1}), and aromatic ring (1597, 1512 cm^{-1}). The ^1H NMR spectrum showed the presence of five aromatic proton signals. Four aromatic protons resonated at δ_H 8.13 (2H, d, $J = 8.8$ Hz, H-2′, 6′) and 6.93 (2H, d, $J = 8.8$ Hz, H-3′, 5′) as one AA′BB′ system. One aromatic proton resonating at δ_H 6.91 (1H, s) was attributed to H-8. In addition, the ^1H NMR spectrum exhibited the presence of a methyl (δ_H 2.10, 3H, s). Finally, six proton signals were found in the range of δ_H 3.0–5.1, which suggested the existence of a sugar moiety, and the signal at δ_H 5.08 (1H, d, $J = 7.2$ Hz) was assigned as the anomeric proton. Acid hydrolysis of **3** gave glucose, identified by comparison with authentic sample. The coupling constant ($J = 7.2$ Hz) of the anomeric proton demonstrated that the glucose was in β-orientation. The ^{13}C NMR spectrum clearly indicated that **3** was a flavonoid glycoside that possessed 22 carbons, among 15 from the flavonol aglycone moiety, 6 from the β-D-glucose, and 1 from methyl. The locations of all substituted groups were determined by the HMBC experiment. In HMBC spectrum, the correlations of the methyl at δ_H 2.10 with C-7 at δ_C 160.7, C-5 at δ_C 157.2, and C-6 at δ_C 107.7 indicated that the methyl was attached to C-6; the correlation of the anomeric proton at δ_H 5.08 (1H, d, $J = 7.2$ Hz) with C-7 at δ_C 160.7 revealed that the glucose was linked to C-7. Through all of the above analysis, the structure of **3** was established as 3,5,7,4′-tetrahydroxy-6-methylflavonol-7-O-β-D-glucopyranoside (Figure 3).

The known compounds, 5,7,8,4′-tetrahydroxy-3-methoxy-6-methylflavonol-8-O-β-D-glucopyranoside [6] and 3,5,7,4′-tetrahydroxy-6-methylflavonol-3-O-β-D-glucopyranoside [7], were identified by the comparison of their ^1H and ^{13}C NMR, MS spectral data with those reported in the literature.

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained using a Perkin-Elmer 341 polarimeter. UV–vis 2201 spectrophotometer. IR spectra were measured with a Shimadzu FT-IR 8201 PC spectrometer. The ^1H and ^{13}C NMR spectra were obtained on a Bruker DPX-400 spectrometer (400 MHz for ^1H

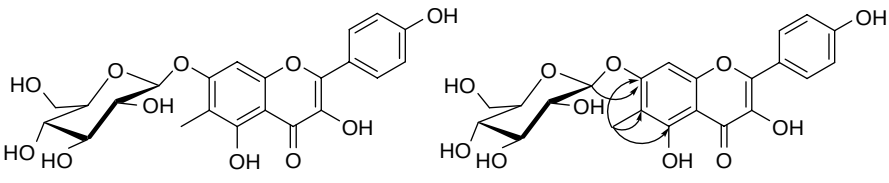

Figure 3. Structure and key HMBC correlations of **3**.

NMR and 100 MHz for ^{13}C NMR) with TMS as internal reference. HR-ESI-MS were recorded on an APEX II spectrometer. Column chromatography was performed on silica gel (160–200 mesh, Qingdao Marine Chemical Industry, Qingdao, China), Toyopearl HW-40 and Sephadex LH-20 (TOSOH Corp., Tokyo, Japan). TLC was conducted on self-made silica gel G (Qingdao Marine Chemical Industry) plates. The chemical reagents were supplied by Beijing Chemical Plant (Beijing, China) and Tianjin NO.3 Reagent Plant (Tianjin, China).

3.2 Plant material

The fresh needles of *P. tabulaeformis* Carr were collected in Xixia County, Henan Province, in July 2009, and authenticated by Prof. Cheng-Ming Dong. A voucher specimen (YS20090705) is deposited in our laboratory.

3.3 Extraction and isolation

The fresh needles of *P. tabulaeformis* Carr (7.8 kg) were extracted with 80% EtOH under reflux for two times. After removal of the solvent under vacuum, the gross extracts (2022 g) were suspended in water and then partitioned with petroleum ether, EtOAc, and *n*-butanol, successively. The EtOAc extract (250 g) was subjected to a silica gel column chromatography with CH_2Cl_2–MeOH (100:1–1:1) as eluent to give fractions A–E. Fraction C (12 g) was rechromatographed on a silica gel column with $CHCl_3$–EtOAc (1:1) to give five fractions. Fraction 2 (2.4 g) was subjected to Toyopearl HW-40 and to Sephadex LH-20 column chromatography eluted with MeOH to yield **1** (15 mg) and **4** (35 mg). Fraction D (18 g) was subjected to a silica gel column with $CHCl_3$–EtOAc (1:1) to give five fractions. Fraction 3 (2.8 g) was subjected to Toyopearl HW-40 and to Sephadex LH-20 column chromatography eluted with MeOH to yield **2** (10 mg), **3** (20 mg), and **5** (18 mg).

3.3.1 7,8,4′-Trihydroxy-3,5-dimethoxy-6-methylflavonol-8-O-β-D-glucopyranoside(*1*)

Yellow amorphous powder. $[\alpha]_D^{20} + 5.0$ ($c = 0.1$, MeOH); UV(MeOH) λ_{max}(nm): 276.6, 333.8; IR(KBr) ν_{max}(cm^{-1}): 3399, 2925, 1652, 1577, 1491, 1435, 1385, 1209, 1174, 1179, 1078, 1026, 842. ^1H and ^{13}C NMR spectral data are shown in Table 1. HR-ESI-MS: *m/z* 529.1308 $[M + Na]^+$ (calcd for $C_{24}H_{26}O_{12}Na$, 529.1322).

3.3.2 5,7,4′-Trihydroxy-3-methoxy-6-methylflavonol-7-O-β-D-glucopyranoside (*2*)

Yellow amorphous powder. $[\alpha]_D^{20} - 53.6$ ($c = 0.12$, MeOH); UV(MeOH) λ_{max}(nm): 271.7, 331.0; IR(KBr)ν_{max}(cm^{-1}): 3289, 2924, 2854, 1650, 1600, 1509, 1457, 1344, 1259, 1071, 1033, 802. ^1H and ^{13}C NMR spectral data are shown in Table 2. HR-ESI-MS: *m/z* 499.1224 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_{11}Na$, 499.1211).

Table 1. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1** in DMSO-d_6.

Position	δ_H (*J*, Hz)	δ_C
2		156.3
3		135.5
4		176.8
5		156.3
6		112.0
7		155.3
8		127.9
9		146.7
10		106.7
1′		128.9
2′, 6′	8.02 (d, $J = 8.8$)	131.2
3′, 5′	6.40 (d, $J = 8.8$)	119.1
4′		158.1
3-OCH_3	3.75 (s)	59.1
5-OCH_3	3.93 (s)	61.2
6-CH_3	2.04 (s)	8.3
Glucose		
1″	4.88 (d, $J = 7.6$)	103.3
2″	3.36 (m)	74.5
3″	3.32 (m)	76.7
4″	3.16 (m)	70.6
5″	3.05 (m)	77.5
6″	3.57, 3.34 (m)	61.4

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of **2** and **3** in CD_3O and DMSO-d_6, respectively.

	2		**3**	
Position	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
2	158.2		147.5	
3	136.4		137.6	
4	177.6		176.9	
5	157.2		157.2	
6	108.4		107.7	
7	160.3		160.7	
8	92.2	6.82 (s)	93.0	6.91 (s)
9	153.8		153.9	
10	105.0		104.5	
1′	124.5		122.1	
2′, 6′	129.6	7.94 (d, $J = 8.8$)	129.5	8.13 (d, $J = 8.8$)
3′, 5′	117.7	6.71 (d, $J = 8.8$)	115.7	6.93 (d, $J = 8.8$)
4′	169.4		159.6	
3-OCH_3	58.2	3.75 (s)		
6-CH_3	5.68	2.13 (s)	7.8	2.10 (s)
Glc				
1″	99.6	5.09 (d, $J = 7.2$)	100.3	5.08 (d, $J = 7.2$)
2″	72.9	3.54 (m)	73.5	3.35 (m)
3″	76.1	3.57 (m)	76.7	3.26 (m)
4″	69.3	3.40 (1 m)	69.8	3.20 (m)
5″	76.3	3.48 (1 m)	77.3	3.48 (m)
6″	60.5	3.91, 3.71 (m)	60.8	3.75, 3.53 (m)

*3.3.3 3,5,7,4′-Tetrahydroxy-6- methyl-flavonol-7-O-β-D-glucopyranoside (**3**)*

Yellow amorphous powder. $[\alpha]_D^{20} - 91.0$ ($c = 0.26$, MeOH); UV(MeOH) λ_{max}(nm): 268.5, 337.7; IR(KBr)ν_{max}(cm^{-1}): 3374, 2930, 2888, 1646, 1597, 1597, 1512, 1481, 1358, 1271, 1072,1025, 802. ^{1}H and ^{13}C NMR spectral data are shown in Table 2. HR-ESI-MS: *m/z* 485.1063 $[M + Na]^+$ (calcd for $C_{22}H_{22}O_{11}Na$, 485.1054).

3.4 Acid hydrolysis of compound 1, 2, and 3

Each compound (3 mg) was heated in 3 ml of HCl–H_2O–EtOH (2:1:2) at 80°C for 4 h. The hydrolysate was partitioned between EtOAc and H_2O, and the aqueous layer was compared with authentic samples of TLC with silica gel [$CHCl_3$–MeOH–H_2O (8:5:1)], which showed that the sugar was glucose.

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