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Glycosidic constituents from the roots and rhizomes of *Melicope pteleifolia*

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ABSTRACT

Seven new diglycosidic constituents, named pteleifosides A–G (**1**–**7**), along with ten known glycosides, were isolated from the roots and rhizomes of *Melicope pteleifolia* (Champ. ex Benth.) T. Hartley. The structures of the isolated compounds were established on the basis of chemical and spectroscopic methods, mainly 1D and 2D NMR data and mass spectrometry.

1. Introduction

Melicope pteleifolia (Champ. ex Benth.) T. Hartley (= *Melicope ptelefolia*, Rutaceae) is a deciduous shrub or arbor distributed in southern China and Southeast Asia. Its fresh leaves are a traditional vegetable among the Malaysian community,[1] and are also a main material of *Guangdong Herbal Tea*—a popular healthy drink in China, whereas its roots and barks, known as '*San-ya-ku*' in traditional Chinese medicines, are acted as an antipyretic, anti-inflammatory, and analgesic agent to treat trauma, abscess, eczema, dermatitis, and hemorrhoids.[2] *M. pteleifolia* had been taxonomically assigned as *Euodia* genus with Latin name of *Euodia lepta* (Spreng.) Merr. (= *Evodia lepta* = *E. roxburghiana* Pierre) before 2009.[3] Previous studies on the title plant disclosed numbers of ester-soluble constituents, such as benzopyrans, alkaloids, acetophenones, sesquiterpenes, and flavonoids.[4,5] However, little is known about the water-soluble chemical profiles despite its water decoction is a main ingredient of '*999 weitai*'—a famous traditional Chinese medicine formula for the treatment of various gastritis. Our investigation focused on the water-soluble constituents of the roots and rhizomes, which led to the isolation of seven new diglycosidic compounds, named pteleifosides A–G (**1**–**7**), structurally as (3*S*,5*R*,6*R*,9*S*)-megastigman-3,6,9-triol 9-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside (**1**), (3*R*,9*S*)-megastigman-5-ene-3,9-diol 9-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside (**2**), citroside A 6′-*O*-β-D-apiofuranoside (**3**), 2-pinen-5,10-diol 5-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (**4**), homovanillyl alcohol 4-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (**5**), (2*R*,3*R*)-dihydrokaempferol 3-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (**6**), and (+)-lyoniresinol 9′-*O*-β-D-glucopyranosyl (1→3)-β-D-glucopyranoside (**7**), together with ten known glycosides, tachioside (**8**),[6] isotachioside (**9**),[6] (+)-lyoniresinol 9′-*O*-β-D-glucopyranoside (**10**),[7] cuneataside D (**11**),[8] canthoside D (**12**),[9] 1-*O*-(β-D-apiosyl(1→6)-β-D-glucopyranosyl)-3-*O*-methylphloroglucinol (**13**),[10] vanillyl alcohol 4-*O*-β-D-glucopyranoside (**14**),[11] melia-ionoside B (**15**),[12] syrigoylglycerol 9-*O*-β-D-glucopyranoside (**16**),[13] and dihydrophaseic acid 3-*O*-β-D-glucopyranoside (**17**).[14] The above compounds were reported from the title plant for the first time.

2. Results and discussion

Pteleifoside A (**1**) was isolated as an amorphous powder and its molecular formula was determined to be $C_{25}H_{46}O_{12}$ by HR-ESI-MS. Acid hydrolysis and GC analysis of **1** gave an L-rhamnose and a D-glucose as sugar moiety. The ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) showed signals for a β-glucopyranosyl at δ_H 4.41 (1H, *d*, *J* = 7.7 Hz) and δ_C 100.6, 79.5, 78.4, 77.8, 71.8, and 62.8, an α-rhamnopyranosyl at δ_H 5.24 (1H, *d*, *J* = 1.4 Hz) and 1.22 (3H, *d*, *J* = 6.2 Hz) as well as δ_C 101.9, 74.1, 72.1, 72.0, 69.5, and 17.9, and a C_{13}-aglycone unit which comprised four methyls at δ_H 1.22 (d, *J* = 6.2 Hz), 1.02 (s), 0.99 (s), and 0.96 (d, *J* = 6.6 Hz), four methylenes at δ_C 47.6, 40.7, 31.9, 32.1, three methines at δ_C 76.0, 67.3,

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Table 1
^{1}H NMR spectroscopic data for compounds **1**–**4**[a]

No.	**1** (500 MHz)	**2** (500 MHz)	**3** (400 MHz)	**4** (400 MHz)
1	—	—	—	2.05 dd (6.6 2.1)
2	1.63 m, 1.29 m	1.67 br d (12.1) 1.39 t (12.0)	1.91 ddd (12.5, 3.7, 1.7) 1.37 dd (12.5, 11.0)	—
3	3.76 tt (11.4, 4.6)	3.85 m	4.29 tt (11.2, 3.8)	5.62 br s
4	1.60 m, 1.39 m	2.19 dd (16.3, 5.2) 1.93 dd (16.3, 11.1)	2.46 ddd (13.4, 3.7, 1.7) 1.40 dd (13.4, 11.4)	2.39 dd (17.0, 2.0) 2.52 dd (17.0, 2.0)
5	1.92 dqd (10.4, 6.6, 4.4)	—	—	—
7	1.64 m, 1.61 m	2.06 td (13.3, 5.0) 1.95 td (13.3, 5.0)	—	2.71 dd (8.4, 6.6) 1.53 d (8.4)
8	1.74 m, 1.51 m	1.69 m, 1.58 m	5.95 s	1.32 s
9	3.88 m	3.94 m	—	0.93 s
10	1.22 d (6.2)	1.25 d (5.6)	2.20 s	3.91 br d (1.2)
11	0.99 s	1.05 s	1.16 s	
12	1.02 s	1.073/1.068 s[b]	1.37 s	
13	0.96 d (6.6)	1.65 s	1.47 s	
1′	4.41 d (7.7)	4.440/4.438 d (7.7)[b]	4.50 d (7.7)	4.31 d (7.8)
2′	3.37 dd (8.9, 7.6)	3.35 dd (9.3, 7.8)	3.13 dd (9.3, 7.8)	3.14 dd (8.6, 7.8)
3′	3.46 dd (8.9, 8.5)	3.45 t (9.0)	3.34 m	3.35 m
4′	3.26 dd (9.7, 8.5)	3.27 dd (9.8, 8.8)	3.22 dd (9.5, 9.2)	3.23 dd (9.4, 9.0)
5′	3.21 ddd (9.7, 5.8, 1.6)	3.22 ddd (9.8, 5.6, 2.1)	3.32 m	3.32 m
6′	3.85 dd (11.9, 1.6) 3.65 dd (11.9, 5.8)	3.85 dd (11.8, 2.1)3.65 dd (11.8, 5.6)	3.94 dd (10.9, 1.2)3.50 dd (10.9, 6.0)	3.95 dd (11.0, 1.5)3.55 dd (11.0, 6.5)
1″	5.24 d (1.4)	5.18 br s	4.93 d (2.4)	4.99 d (2.2)
2″	3.89 dd (3.4, 1.4)	3.92 br s	3.89 d (2.4)	3.88 d (2.2)
3″	3.71 dd (9.6, 3.4)	3.64 br d (9.1)	—	—
4″	3.36 t (9.7)	3.37 dd (9.7, 9.3)	3.95 d (9.6), 3.76 d (9.6)	3.96 d (9.6), 3.76 d (9.6)
5″	4.14 dq (9.7, 6.2)	4.04 dq (9.4, 6.2)	3.56 s	3.58 s
6″	1.22 d (6.2)	1.20 d (6.2)		

[a] Measured in methanol-d_4, residue solvent peak at δ_H 3.31 ppm, J in Hz.
[b] Splitted signals caused by atropisomerism at 25 °C.

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**4**

No.	**1**[a]	**2**[a]	**3**[a]	**3**[b]	**4**[a]
1	41.5 (s)	38.8 (s)	36.8 (s)	36.1	40.4 (d)
2	47.6 (t)	49.3 (t)	49.7 (t)	50.1	148.2 (s)
3	67.3 (d)	65.7 (d)	63.6 (d)	62.3	121.2 (d)
4	40.7 (t)	42.9 (t)	48.3 (t)	47.7	37.3 (t)
5	35.1 (d)	125.6 (s)	78.3 (s)	77.8	82.0 (s)
6	76.8 (s)	138.2/138.1 (s)[c]	118.5 (s)	118.0	44.7 (s)
7	31.9 (t)	24.8/24.7 (t)[c]	212.9 (s)	211.6	39.2 (t)
8	32.1 (t)	36.6/36.4 (t)[c]	101.2 (d)	100.6	22.5 (q)
9	76.0 (d)	75.6/75.4 (d)[c]	200.7 (s)	197.6	20.1 (q)
10	21.1 (q)	20.92/20.88 (q)[c]	26.5 (q)	26.9	65.3 (t)
11	26.5 (q)	29.0 (q)	32.8 (q)	32.1	
12	25.6 (q)	30.4 (q)	30.0 (q)	29.7	
13	16.9 (q)	20.2 (q)	26.2 (q)	26.3	
1′	100.6 (d)	100.6/100.5 (d)[c]	98.5 (d)	98.4	99.8 (d)
2′	78.4 (d)	79.25/79.19 (d)[c]	75.0 (d)	75.0	75.0 (d)
3′	79.5 (d)	79.3 (d)	78.2 (d)	78.9	78.2 (d)
4′	71.8 (d)	71.9 (d)	71.3 (d)	71.4	71.9 (d)
5′	77.8 (d)	77.9 (d)	76.4 (d)	76.6	76.6 (d)
6′	62.8 (t)	62.8 (t)	68.7 (t)	68.8	68.9 (t)
1″	101.9 (d)	102.3 (d)	110.8 (d)	110.9	111.0 (d)
2″	72.0 (d)	72.1 (d)	77.9 (d)	77.7	78.1 (d)
3″	72.1 (d)	72.4 (d)	79.8 (s)	80.2	80.5 (s)
4″	74.1 (d)	73.8 (d)	74.8 (t)	74.9	75.0 (t)
5″	69.5 (d)	69.8 (d)	65.5 (t)	65.4	65.8 (t)
6″	17.9 (q)	17.9 (q)			

[a] Measured in methanol-d_4, residue solvent peak at δ_C 49.0 ppm.
[b] Measured in pyridine-d_5, residue solvent peaks at δ_C 149.64, 135.31, and 123.29 ppm.
[c] Splitted signals caused by atropisomerism at 25 °C.

and 35.1, and two quaternary carbons at δ_C 76.8 and 41.5, indicating a trihydroxylated megastigmane diglycoside. The three hydroxyls of the aglycone were assigned on C-3, C-6, and C-9 based on the NMR data comparison with those of dihydroalangionoside A (**1a**) and dihydrodendranthemoside A (**1b**), which both have 3*S*,5*R*,6*R*,9*R*-configuration established by X-ray diffraction and chemical conversion.[15] Besides an additional rhamnopyranosyl unit presented in **1**, the main differences between the ^{13}C NMR data of **1** and **1a** were observed at C-2′, C-8, and C-10 ($\Delta\delta_{\mathbf{1-1a}}$ = +3.3, −2.1 and +1.2, respectively), suggesting **1** to be 2′-α-L-rhamnopyranoside of C-9 diastereomer of **1a**.[15]

Previous studies disclosed an empirical rule that β-D-glucosylation on chiral secondary alcohols would cause non-equivalent shift changes on β-carbons, which was also appropriable to some diglycosides such as ginsenoside-R_d.[16] Namely, 10-methyl would subject to greater shielding effect than C-8 when β-D-glucosylation occurred on 9*R*-hydroxymegastigmanes, and vice versa.[15–18] The D-glucosylation shielding effects on C-8 and C-10 of **1** ($\Delta\delta_{\mathbf{1-1b}}$ = −3.7 and −2.5 ppm, respectively) were opposite trends to those of **1a** ($\Delta\delta_{\mathbf{1a-1b}}$ = −1.6 and −3.7 ppm for C-8 and C-10, respectively), indicating 9*S*-configuration of **1**. The absolute structure of pteleifoside A (**1**) was therefore determined to be (3*S*,5*R*,6*R*,9*S*)-megastigman-3,6,9-triol 9-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside.

Pteleifoside B (**2**) was also obtained as an amorphous powder whose elemental composition $C_{25}H_{44}O_{11}$ was deduced from the HR-ESI-MS. The acid hydrolysis, ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) disclosed **2** to have the same sugar moiety of α-L-rhamnopyranosyl(1→2)-β-D-glucopyranose with that of **1**. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of the aglycone of **2** showed 13 carbon atoms and 22 carbon-bearing proton signals for a doublet methyl at δ_H 1.25 (d, J = 5.6 Hz), three singlet methyls at δ_H 1.65, 1.07, and 1.05, four methylenes at δ_C 49.3, 42.9, 36.6, and 24.8, two oxygen-bearing methines at δ_C 75.6 and 65.7, and three quaternary carbons at δ_C 138.2, 125.6, and 38.8, suggesting a dihydroxymegastigman-5-ene diglycoside.[17] The ^{1}H and ^{13}C NMR data of the aglycone portion of **2** were nearly superposed with those of linarionoside B (**2a**) except for the side chain carbons (C-8 to C-10), suggesting a 9-isomer aglycone with that of **2a**.[17] The observed β-D-glucosylation trend on C-8 (−4.2) and C-10 (−2.4) of **2** in contrast with those of linarionoside A (**2b**) was opposite to

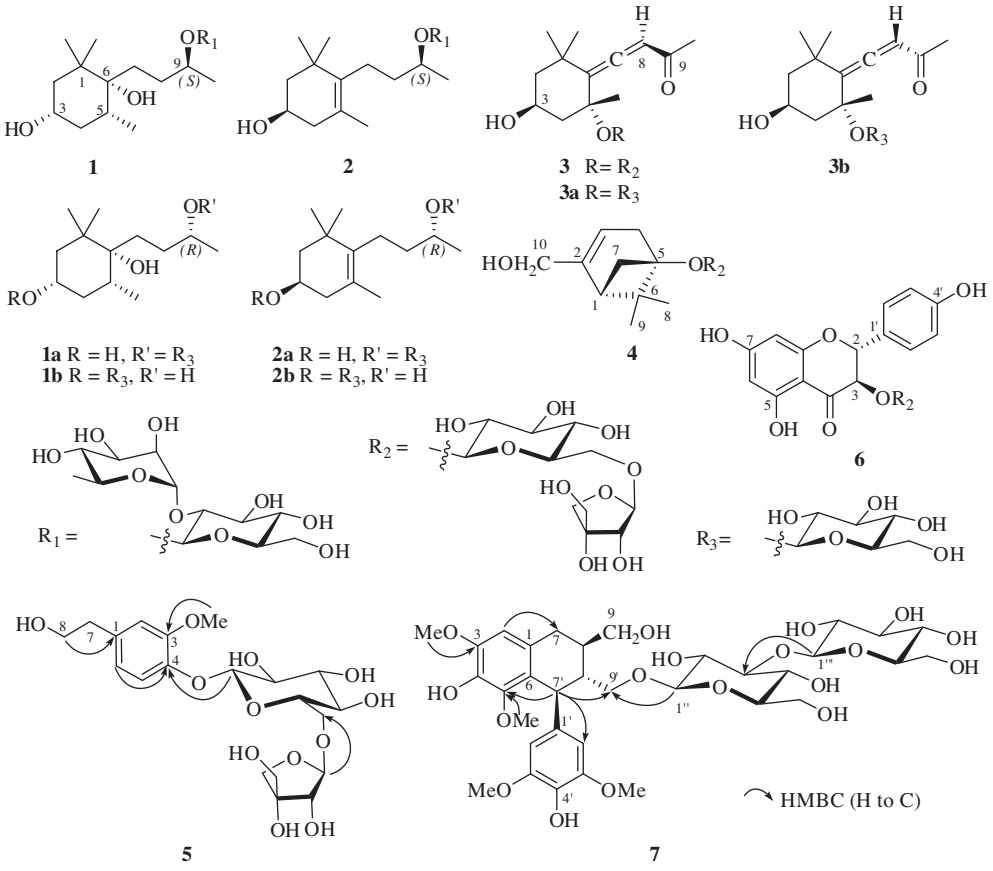

Figure 1. Structures of compounds **1**–**7**, **1**–**3a** and **1**–**3b**.

that of **2a** and **2b** ($\Delta\delta_{\mathbf{2a-2b}}$ = −1.8 and −3.5 ppm for C-8 and C-10, respectively). Thus, pteleifoside B (**2**) was assigned to be (3*R*,9*S*)-megastigman-5-ene-3,9-diol 9-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside.

It is interesting that some ^{1}H and ^{13}C signals of **2** exist in pairs (Table 2), whereas it is not found in the NMR spectra of **1**. A reasonable explanation was that double bond between C-5 and C-6 of **2** obstructed the rapid rotation of the sugar unit around the glucosidic linkage with the aglycone to result into equilibrium of two conformational isomers,[19] which was confirmed by the observation that the shift differences between those paired carbons tended to zero (Fig. 2) when ^{13}C NMR spectrum of **2** was measured at rising temperatures.

Pteleifoside C (**3**) was isolated as an amorphous powder, whose ESI-MS (negative-ion mode) spectrum exhibited quasi-molecular

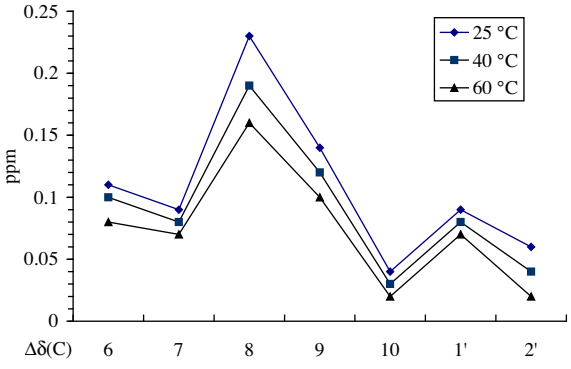

Figure 2. The shift differences of the paired carbons of **2** at different temperatures.

ion peaks at *m*/*z* 563 $[M+HCOO]^-$ and 1035 $([2M-H]^-$, indicating the molecular formula of $C_{24}H_{38}O_{12}$. The IR absorptions displayed the existences of hydroxyls (3417 cm^{-1}), an allenic moiety (1940 cm^{-1}) and a conjugated carbonyl group (1664 cm^{-1}).[20] After acid hydrolysis, **3** gave a D-apiose and a D-glucose as sugar moiety. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **3** exhibited signals for a β-apiofuranosyl(1→6)-β-glucopyranose at δ_H 4.93 (1H, d, J = 2.4 Hz) and 4.50 (1H, d, J = 7.7 Hz), and δ_C 98.5, 78.2, 76.4, 75.0, 71.3, and 68.7, as well as 110.8, 79.8, 77.9, 74.8, and 65.5, an allenic unit at δ_H 5.95 (s) and δ_C 212.9 (s), 118.5 (s) and 101.2 (d), an α,β-unsaturated carbonyl at δ_C 200.7, four tertiary methyls at δ_H 2.20, 1.47, 1.37, and 1.16, two sp^3 quaternary carbons at δ_C 78.3 and 36.8, one oxy-bearing methine at δ_C 63.6, and two methylenes at δ_C 49.7 and 48.3, diagnostic of grasshopper ketone.[20] The diglycosylation position of **3** was reasonably assigned on C-5 rather than C-3 on the basis of downfield shift of a quaternary carbon at 78.3 ppm. The ^{13}C NMR data (in CD_3OD) of **3** were very similar to those of citrosides A (**3a**) and B (**3b**)[20] of which represented two kinds of diastereomeric grasshopper ketone glucosides on C-8. The ^{13}C NMR data of **3a** and **3b** were nearly superposed except for slight differences on C-9 and C-13 (δ 197.6/199.0 and 26.5/27.6 for **3a**/**3b**, respectively, in pyridine-d_5). Furthermore, the original assignments for C-7 and C-9, C-2 and C-4, and four methyls of **3a** and **3b** in the literature[20] were reversed as indicated by Hao and co-workers.[21] The ^{13}C NMR data of the aglycone portion of **3** measured in pyridine-d_5 (Table 2) were identical with those of **3a**, especially C-9 and C-13 at δ 197.6 and 26.3, respectively, demonstrating **3** to be citroside A 6′-*O*-β-D-apiofuranoside.

Pteleifoside C (**4**) was obtained as an amorphous powder and its molecular formula was determined to be $C_{21}H_{34}O_{11}$ by the HR-ESI-MS. The acid hydrolysis, ^{1}H and ^{13}C NMR spectra (Tables 1 and 2)

Table 3
^{1}H and ^{13}C NMR spectroscopic data (400 and 100 MHz) for **5**–**7**[a]

No.	**5**		**6**		**7**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	135.5	—			130.1	—
2	114.5	6.88 d (1.8)	83.5	5.29 d (10.0)	107.7	6.58 s
3	150.7	—	77.2	4.91 d (10.0)	148.6	—
4	146.4	—	195.7	—	138.9	—
5	118.4	7.07 d (8.2)	165.5	—	147.6	—
6	122.6	6.78 dd (8.2, 1.8)	96.5	5.90 d (2.3)	126.3	—
7	39.8	2.76 t (7.0)	169.6	—	33.8	2.72 dd (15.0, 5.0) 2.62 dd (15.0, 11.4)
8	64.2	3.73 t (7.0)	96.5	5.87 d (2.3)	40.6	1.69 m
9			164.1	—	66.2	3.64 dd (11.9, 4.9) 3.54 dd (11.9, 6.2)
10			102.5	—		
1′			128.5	—	134.4	—
2′ & 6′			130.5	7.36 d (8.5)	106.8	6.43 s
3′ & 5′			116.3	6.81 d (8.5)	148.9	—
4′			159.3	—	139.3	—
7′					42.7	4.42 d (6.2)
8′					46.7	2.08 m
9′					71.4	3.88 dd (11.8, 5.3) 3.46 dd (11.8, 3.9)
3-OMe	56.7	3.85 s			56.5	3.86 s
5-OMe					60.1	3.35 s
3′&5′-OMe					56.8	3.74 s
1″	103.1	4.79 d (7.2)	102.3	3.84 d (7.7)	104.4	4.35 d (7.8)
2″	74.9	3.46 dd (7.7, 7.2)	74.6	3.22 dd (9.0, 7.8)	74.5	3.44 dd (9.0, 7.8)
3″	78.0	3.44 dd (9.2, 7.7)	77.6	3.10 dd (9.1, 8.9)	88.1	3.56 dd (8.9, 8.7)
4″	71.6	3.34 dd (9.8, 9.2)	71.4	3.23 dd (9.5, 9.1)	70.0	3.41 dd (9.8, 9.0)
5″	77.0	3.53 ddd (9.8, 6.7, 1.5)	77.1	3.10 m	77.6	3.28 m
6″	68.7	3.99 dd (11.1, 1.5) 3.61 dd (11.1, 6.5)	68.4	3.89 dd (11.0, 2.0) 3.54 dd (11.0, 5.0)	62.6	3.85 dd (11.9, 2.0) 3.67 dd (11.9, 5.4)
1‴	111.0	4.97 d (2.4)	110.8	4.98 d (2.5)	105.3	4.57 d (7.8)
2‴	77.8	3.89 d (2.4)	77.9	3.89 d (2.5)	75.5	3.26 dd (9.2, 7.8)
3‴	80.5	—	80.5	—	77.8	3.39 dd (9.0, 8.6)
4‴	74.9	3.94 d (9.7) 3.74 d (9.7)	74.9	3.94 d (9.7) 3.74 d (9.7)	71.5	3.28 dd (8.5, 8.7)
5‴	65.5	3.57 s	65.5	3.55 s	78.2	3.30 m
6‴					62.6	3.87 dd (11.5, 2.6) 3.63 dd (11.5, 6.6)

[a] Measured in methanol-d_4, residue solvent peak at δ_H 3.31 ppm and δ_C 49.0 ppm.

disclosed **4** to have the same sugar linkage of β-D-apiofuranosyl(1→6)-β-D-glucopyranose with that of **3**. The ^{1}H and ^{13}C NMR spectra (Table 1) of the aglycone of **4** showed signals for a trisubstituted double bond at δ_H 5.62 (br s) and δ_C 148.2 and 121.2, two quaternary carbons at δ_C 82.0 and 44.7, one methine at δ_C 40.4, three methylenes at δ_C 65.3, 39.2, and 37.3, and two methyls at δ_H 1.32 (s) and 0.93 (s), which were similar to those of 5-hydroxynopol (**4a**)[22] except for a hydroxymethyl signals at δ_H 3.91 (br d, J = 1.2 Hz, 2H) and δ_C 65.3 in **4** instead of the hydroxyethyl of **4a**, and downfield shift at C-5 ($\Delta\delta_{\mathbf{4-4a}}$ = 7.0 ppm). The above evidences suggested **4** to be a 5-*O*-diglycoside of 2-pinen-5,10-diol. In the HMBC spectrum of **4**, obvious cross-peaks of H-1′/C-5 and H_2-6′/C-1″ were observed, substantiating a disaccharide of β-D-apiofuranosyl(1→6)-β-D-glucopyranosyl to be connected with C-5 of the aglycone. Compound **4** was therefore elucidated to be 2-pinen-5,10-diol 5-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside.

Pteleifoside E (**5**) had the molecular formula of $C_{20}H_{30}O_{12}$ as suggested by the HR-ESI-MS. The acid hydrolysis experiment and ^{1}H and ^{13}C NMR data (Table 3) demonstrated a disaccharide sequence of β-D-apiofuranosyl(1→6)-β-D-glucopyranosyl in the molecule of **5**. The aglycone of **5** was determined to be homovanillyl alcohol based on the diagnostic ^{1}H signals for a 1,2,4-trisubstitued aromatic ring at δ_H 7.07 (d, J = 8.2 Hz), 6.88 (d, J = 1.8 Hz), and 6.78 (dd, J = 8.2, 1.8 Hz), a hydroxyethyl at δ_H 3.73 and 2.76 (each 2H, t, J = 7.0 Hz), and a methoxyl at δ_H 3.85 (s, 3H), as well as the HMBC ^3J correlations (Fig. 1) of H_2-8/C-1, MeO/C-3 and H-6/C-4. Furthermore, obvious HMBC cross-peaks of H-1′/C-4 and H-1″/C-6′ were also observed, which corroborated further **5** to be homovanillyl alcohol 4-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

Pteleifoside F (**6**) was isolated as an amorphous powder with the molecular formula of $C_{26}H_{30}O_{15}$ based on the HR-ESI-MS. The ^{1}H and ^{13}C NMR spectra of **6** (Table 2) exhibited signals for a β-D-apiofuranosyl(1→6)-β-D-glucopyranosyl group and a 2,3-*trans*-dihydrokaempferol characterized with a 1,2,3,5-tetrasubstituted aromatic ring at δ_H 5.90 and 5.87 (each 1H, d, J = 2.3 Hz), a 1,4-disubstituted phenyl at δ_H 7.36 and 6.81 (each 2H, d, J = 8.5 Hz), an isolated spin system of *trans*-1,2-diol at δ_H 5.29 and 4.91 (each 1H, d, J = 10.0 Hz). Compared the ^{1}H and ^{13}C NMR data of **6** with those of (2*R*,3*R*)-dihydrokaempferol 3-*O*-β-D-glucoside (**6a**),[23] the main differences were that **6** had a downfield shift at C-6′ and an additional apiofuranosyl, suggesting **6** to be a 6′-*O*-β-D-apiofuranosylated derivative of **6a**. The CD curve of **6** showed positive Cotton effect at 329 nm and negative absorption at 290 nm, in agreement with those of engeletin,[24] indicating the same 2*R*,3*R*-configuration of **6**. The structure of **6** was thus determined to be (2*R*,3*R*)-dihydrokaempferol 3-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside.

The molecular formula of pteleifoside G (**7**) was determined to be $C_{34}H_{48}O_{18}$ by the HR-ESI-MS. The ^{1}H NMR spectrum of **7** (Table 3) showed signals for two β-glucopyranosyls at δ_H 4.57 (d, J = 7.8 Hz) and 4.35 (d, J = 7.8 Hz), two aromatic rings at δ_H 6.43 (s, 2H) and 6.58 (s, 1H), and four methoxyls at δ_H 3.86, 3.74, 3.74, and 3.35 (each s). In the ^{13}C NMR spectrum (Table 3), the carbon signals of **7** were in accord with those of (±)-lyoniresinol

9′-*O*-β-D-glucopyranoside[25] except for a downfield shift at C-3′, together with the occurrence of an additional glucosyl unit, which suggested **7** to be lyoniresinol 9′-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranoside. The HMBC cross-peaks (Fig. 1) of H-7′ with C-5, C-2′, and C-9′, H-1″ with C-9′, and H-1‴ with C-3″ confirmed such a planar structure. The absolute configuration of **7** was established as 7′*S*,8*R*,8′*R* based on the same CD orientations at 242, 274, and 286 nm with that of (+)-lyoniresinol 9′-*O*-β-D-glucopyranoside (**10**).[25] Therefore, **7** was elucidated to be (+)-lyoniresinol 9′-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranoside.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined on a Perkin–Elmer 341 polarimeter. CD curves were obtained on a Jasco J-810 spectropolarimeter. IR spectra were recorded on a Nicolet Magna-750-FTIR spectrometer with KBr tablet. NMR spectra were acquired on a Bruker AM-400 or a Bruker AV-500 spectrometer. ESI-MS and HR-ESI-MS were obtained on an Esquire 3000plus and a Q-TOF-Ultima mass spectrometer, respectively. Semipreparative HPLC was carried out on a Waters 515 pump with a Waters 2487 (UV) detector and a Kromasil 100-5-C18 column (250 × 10 mm, 5 μm). D-1400 macroporous resin (Yangzhou Pharmaceutical Factory, Yangzhou, China), Rp-18 reversed phase silica gel (150–200 mesh, Fuji Silysia Chemical LTD, Aichi, Japan), MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd, Tokyo, Japan), and Sephadex LH-20 gel (Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography (CC), and precoated silica gel GF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, China) were used for TLC. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, China).

3.2. Plant material

The roots and rhizomes of *M. pteleifolia* were purchased from Bozhou Chinese Materia Medica Market, Bozhou, Anhui Province, P.R. China, in July 2009, which was identified by Professor Da-Yuan Zhu of Shanghai Institute of Materia Medica. A voucher specimen (No. 09-1008) was deposited with the Herbarium of Shanghai Institute of Materia Medica.

3.3. Extraction and isolation

Dried roots and rhizomes of *M. pteleifolia* (5 kg) were powdered and extracted with 95% ethanol at room temperature three times. After removal of the solvents by evaporation, the extract was suspended in H_2O and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble part (180 g) was subjected to macroporous resin column chromatography (CC, i.d. 10 × 80 cm) with gradient EtOH/H_2O (0%, 20%, 50%, 75%, and 95%, v/v) as eluant to give fractions A–E. Fraction C (30% EtOH fraction, 65 g) was separated by CC of MCI gel (MeOH/H_2O 0%, 20%, 40%, 60%, and 95%, v/v) to afford sub-fractions B1-B5. Fr. B4 was purified by CC of Rp-18 (MeOH/H_2O, 50%) and Sephadex LH-20 (MeOH) to obtain **8** (20 mg), **9** (18 mg), **10** (34 mg), **16** (18 mg), and **17** (52 mg); Fr. B3 was isolated by CC of Rp-18 (MeOH/H_2O, 20–60%) and Sephadex LH-20 (MeOH/H_2O, 75%) to furnish **3** (118 mg), **4** (36 mg), and **14** (30 mg); Fr. B2 was separated by CC of Rp-18 (MeOH/H_2O, 10–50%) and Sephadex LH-20 (MeOH/H_2O, 50%) to offer **7** (77 mg), **6** (19 mg), and **13** (18 mg). Semiprepared HPLC was applied to isolate **15** (9 mg, CH_3OH/H_2O, 50%) from Fr. B4, **2** (9 mg, CH_3OH/H_2O, 40%) from Fr. B3, and **1** (8 mg, CH_3OH/H_2O, 25%), **5** (9 mg, CH_3OH/H_2O, 20%), **11** (3 mg, CH_3OH/H_2O, 20%), and **12** (6 mg, CH_3OH/H_2O, 20%) from Fr. B2.

3.4. Identification

3.4.1. Pteleifoside A (1)

White amorphous powder (MeOH); $[\alpha]_D^{22}$ −61.5 (*c* 0.13, MeOH); IR (KBr) ν_{max} 3411, 2972, 2933, 1637, 1452, 1383, 1257, 1130, 1070, 1043, 987, 642 cm^{-1}; ^1H NMR (500 MHz, CD_3OD), and ^{13}C NMR (125 MHz, CD_3OD) data, see Tables 1 and 2; ESI-MS *m/z* (positive) 561 $[M+Na]^+$, (negative) 1075 $[2M-H]^-$; HR-ESI-MS *m/z* 561.2891 $[M+Na]^+$ (calcd for $C_{25}H_{46}O_{12}Na$, 561.2887).

3.4.2. Pteleifoside B (2)

White amorphous powder (MeOH); $[\alpha]_D^{22}$ −66.5 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3415, 2967, 2927, 1639, 1454, 1381, 1259, 1130, 1043, 814, 636 cm^{-1}; ^1H NMR (500 MHz, CD_3OD), and ^{13}C NMR (125 MHz, CD_3OD) data, see Tables 1 and 2; ESI-MS *m/z* (positive) 543 $[M+Na]^+$, (negative) 1039 $[2M-H]^-$; HR-ESI-MS *m/z* 543.2783 $[M+Na]^+$ (calcd for $C_{25}H_{44}O_{11}Na$, 543.2781).

3.4.3. Pteleifoside C (3)

White amorphous powder (MeOH); $[\alpha]_D^{25}$ −110.3 (*c* 0.31, MeOH); IR (KBr) ν_{max} 3417, 2927, 1940, 1664, 1457, 1365, 1245, 1070, 819 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 6.15 (1H, s, H-8), 5.69 (1H, d, *J* = 2.2 Hz, H-1″), 5.09 (1H, d, *J* = 7.7 Hz, H-1′), 4.74 (1H, d, *J* = 2.2 Hz, H-2″), 4.67 (1H, br d, *J* = 10.5 Hz, H-6′a), 4.60 (1H, d, *J* = 9.3 Hz, H-4″a), 4.37 (1H, d, *J* = 9.3 Hz, H-4″b), 4.19 (2H, s, H_2-5″), 4.18 (1H, dd, *J* = 9.3, 8.9 Hz, H-3′), 4.04 (1H, dd, *J* = 10.6, 6.2 Hz, H-6′b), 4.01–3.93 (3H, m, H-2′, H-4′ and H-5′), 3.00 (1H, br d, *J* = 13.3 Hz, H-4a), 2.27 (1H, br d, *J* = 12.6 Hz, H-2a), 2.21 (3H, s, Me-10), 1.77 (1H, dd, *J* = 13.5, 11.4 Hz, H-4b), 1.74 (3H, s, Me-12), 1.71 (3H, s, Me-13), 1.22 (3H, s, Me-11); ^1H NMR (400 MHz, CD_3OD), and ^{13}C NMR (100 MHz, CD_3OD or 125 MHz, pyridine-d_5) data, see Tables 1 and 2; ESI-MS *m/z* (positive) 541 $[M+Na]^+$, (negative) 563 $[M+HCOO]^-$, 1035 $[2M-H]^-$; HR-ESI-MS *m/z* 541.2270 $[M+Na]^+$ (calcd for $C_{24}H_{38}O_{12}Na$, 541.2261).

3.4.4. Pteleifoside D (4)

White amorphous powder (MeOH); $[\alpha]_D^{25}$ −20.4 (*c* 0.43, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 188 (−17.8), 205 (+54.1) nm; IR (KBr) ν_{max} 3405, 2933, 1646, 1384,1319, 1216, 1049, 823 cm^{-1}; ^1H NMR (400 MHz, CD_3OD), and ^{13}C NMR (100 MHz, CD_3OD) data, see Tables 1 and 2; ESI-MS *m/z* (positive) 485 $[M+Na]^+$, (negative) 461 $[M-H]^-$, 923 $[2M-H]^-$; HR-ESI-MS *m/z* 485.2001 $[M+Na]^+$ (calcd for $C_{21}H_{34}O_{11}Na$, 485.1999).

3.4.5. Pteleifoside E (5)

White amorphous powder; $[\alpha]_D^{25}$ −74.3 (*c* 0.075, MeOH); IR (KBr) ν_{max} 3404, 2932, 1603, 1516, 1272, 1051, 822 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) data, see Table 3; ESI-MS (positive) *m/z* (positive) 485 $[M+Na]^+$, (negative) 461 $[M-H]^-$, 923 $[2M-H]^-$; HR-ESI-MS *m/z* 485.1639 $[M+Na]^+$ (calcd for $C_{20}H_{30}O_{12}Na$, 485.1635).

3.4.6. Pteleifoside F (6)

White amorphous powder; $[\alpha]_D^{25}$ −22.1 (*c* 0.26, MeOH); IR (KBr) ν_{max} 3415, 2927, 1641, 1519, 1467, 1261, 1166, 1083, 833 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (+115.1), 231 (−4.8), 252 (+24.0), 290 (−113.9), 329 (+42.5) nm; ^1H and ^{13}C NMR (400 and 100 MHz, CD_3OD) data, see Table 3; ESI-MS (positive) *m/z* (positive) 605 $[M+Na]^+$, (negative) 581 $[M-H]^-$; HR-ESI-MS *m/z* 605.1468 $[M+Na]^+$ (calcd for $C_{26}H_{30}O_{15}Na$, 605.1482).

3.4.7. Pteleifoside G (7)

White amorphous powder; $[\alpha]_D^{25}$ +50.5 (*c* 0.05, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 206 (+248.6), 215 (−129.6), 243 (+110.7), 274 (+31.0), 286 (−10.0) nm; ^1H and ^{13}C NMR (400 and 100 MHz, CD_3OD) data, see Table 3; ESI-MS *m/z* (positive) 767 $[M+Na]^+$,

(negative) 743 $[M-H]^-$; HR-ESI-MS *m/z* 767.2732 $[M+Na]^+$ (calcd for $C_{34}H_{48}O_{18}Na$, 767.2738).

3.5. Acid hydrolysis

Acid hydrolysis of compounds **1**–**7** and sugar identification were performed according to a standard procedure.[26] In brief, each compound (1 mg) was refluxed in 1 mL 2 N HCl for 2 h. On cooling, the mixture was neutralized with $NaHCO_3$. After extraction with CH_2Cl_2, the aqueous layer was concentrated by blowing with N_2. The residue was dissolved in anhydrous pyridine (100 μL), 0.1 M L-cysteine methyl ester hydrochloride (200 μL) was added, and the mixture was warmed at 60 °C for 1 h. Then 20 μL *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (Acros Organics, Geel, Belgium) was added, and warming at 60 °C was continued for another 30 min. The supernatant was subjected to GC analysis to identify the sugars. Conditions for GC were: capillary column, DB5-MS (30 m × 0.25 mm × 0.25 μm), oven temperature program, 180–300 °C at 6 °C/min; injection temperature 350 °C; carrier gas, He at 1 mL/min. D-Glucose, L-rhamnose and D-apiose were detected from **1**–**7**, **1**–**3**, and **4**–**6**, respectively, by comparing its retention time with that of the authentic samples (t_R = 18.65, 13.20, 14.45 min for D-glucose, L-rhamnose, and L-apiose, respectively).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2012.08.011.

References

1. Abas, F.; Shaari, K.; Israf, D. A.; Syafri, S.; Zainal, Z.; Lajis, N. H. *J. Food Compos. Anal.* **2010**, *23*, 107–112.
2. Jiangsu New Medical College. *Dictionary of Chinese Materia Medica*. Shanghai Sci & Tech Press: Shanghai, 1986; p 68.
3. Wu, Z. Y.; Peter, H. R.; Hong, D. Y. In *Flora of China*; Scientific Press: Beijing, 2009; Vol. 11, p70.
4. Li, G. L.; Zhu, D. Y.; Pandey, R. K. In *Oriental Foods and Herbs*; Ho, C. T., Lin, J. K., Zheng, Q. Y., Eds.; American Chemical Society: Washington DC, 2003; Vol. 859, pp 247–257.
5. Zhu, S. H. Ph.D. Thesis, Guangzhou University of Chinese Medicine, 2009. Dissertation abstract is available via the Internet at http://www.res-medical.com/chinese-medicine/9107.
6. Inoshiri, S.; Sasaki, M.; Kohda, H.; Otsuka, H.; Yamasaki, K. *Phytochemistry* **1987**, *26*, 2811–2814.
7. Steinbeck, C.; Schneider, C.; Rotscheidt, K.; Breitmaier, E. *Phytochemistry* **1995**, *40*, 1313–1315.
8. Chang, J.; Case, R. *Phytochemistry* **2005**, *66*, 2752–2758.
9. Kanchanapoom, T.; Kasai, R.; Yamasaki, K. *Phytochemistry* **2002**, *61*, 461–464.
10. Zhang, S. W.; Xuan, L. J. *Helv. Chim. Acta* **2006**, *89*, 1241–1245.
11. Kanho, H.; Yaoya, S.; Kawahara, N.; Nakane, T.; Takase, Y.; Masuda, K.; Kuroyanagi, M. *Chem. Pharm. Bull.* **2005**, *53*, 361–365.
12. Nakanishi, T.; Konishi, M.; Murata, H.; Inada, A.; Fujii, A.; Tanaka, N.; Fujiwara, T. *Chem. Pharm. Bull.* **1991**, *39*, 2529–2533.
13. Kijima, H.; Ide, T.; Otsuka, H.; Ogimi, C.; Hirata, E.; Takushi, A.; Takeda, Y. *Phytochemistry* **1997**, *44*, 1551–1557.
14. Champavier, Y.; Comte, G.; Vercauteren, J.; Allais, D. P.; Chulia, A. J. *Phytochemistry* **1999**, *50*, 1219–1223.
15. Otsuka, H.; Kamada, K.; Ogimi, C.; Hirata, E.; Takushi, A.; Takeda, Y. *Phytochemistry* **1994**, *35*, 1331–1334.
16. Kasai, R.; Suzuo, M.; Asakawa, J.; Tanaka, O. *Tetrahedron Lett.* **1977**, *178*, 175–178.
17. Otsuka, H. *Phytochemistry* **1994**, *37*, 461–465.
18. Matsunami, K.; Otsuka, H.; Takeda, Y. *Chem. Pharm. Bull.* **2010**, *58*, 438–441.
19. Al-Abed, Y.; Sabri, S.; Abu, Z. *J. Nat. Prod.* **1990**, *53*, 1152–1161.
20. Umehara, K.; Hattori, I.; Miyase, T.; Ueno, A.; Hara, S.; Kageyama, C. *Chem. Pharm. Bull.* **1988**, *36*, 5004–5008.
21. Wang, J. S.; Shen, Y. M.; He, H. P.; Kang, W. Y.; Hao, X. J. *Planta Med.* **2005**, *71*, 96–98.
22. Miyazawa, M.; Suzuki, Y.; Kameoka, H. *Phytochemistry* **1995**, *39*, 337–340.
23. Baderschneider, B.; Winterhalter, P. *J. Agric. Food Chem.* **2001**, *49*, 2788–2798.
24. Gaffield, W.; Waiss, A. C.; Tominaga, T. *J. Org. Chem.* **1975**, *40*, 1057–1061.
25. Ohashi, K.; Watanabe, H.; Okumura, Y.; Uji, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 1924–1926.
26. Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501–506.