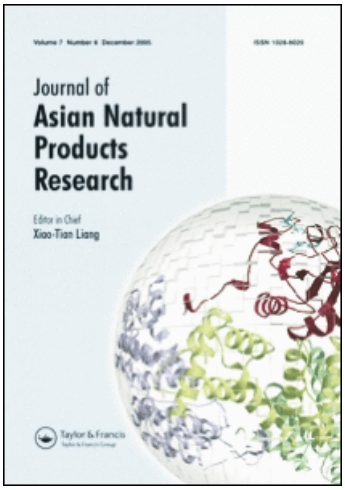

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Isolation and identification of a novel flavonoid from *Penthorum chinense* P.

H. -W. Wang[a]; Y. -Q. Liu[a]; C. -G. Feng[b]
[a] Department of Light Industry and Chemistry, Zhaoqing University, Zhaoqing, China [b] Centre for Research and Development of Medicine, Beijing Institute of Technology, Beijing, China

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Note

Isolation and identification of a novel flavonoid from *Penthorum chinense* P.

H.-W. WANG†*, Y.-Q. LIU† and C.-G. FENG‡

†Department of Light Industry and Chemistry, Zhaoqing University, Zhaoqing 526061, China
‡Centre for Research and Development of Medicine, Beijing Institute of Technology, Beijing 100081, China

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A novel flavonoid, pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (**1**) was isolated from the whole plant of *Penthorum chinense* P., along with four known compounds, pinocembrin-7-*O*-β-glucoside, quercitrin, quercetin-3-*O*-rhamnoside and gallic acid. The structures were established by spectroscopic analysis.

Keywords: *Penthorum chinense*; Pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose

1. Introduction

Penthorum chinense P. (Saxifragaceae) is a Miao nationality herbal drug used in Chinese folk medicine for cholecystitis, adiposis hepatica and infectious hepatitis. It is the only one of its kind in China [1]. Interest in this herb has been further increased by its efficacy against the hepatitis B, C and D viruses [2]. In clinical studies and traditional practices the polar fraction of the herb is of particular interest, but it has not been subjected to detailed chemical constitution analysis. Chromatographic separation of an MeOH extract of *P. chinense* resulted in the isolation of a novel flavonoid, pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (**1**). In addition, four known compounds, pinocembrin-7-*O*-(-glucoside (**2**), quercitrin (**3**), quercetin-3-*O*-rhamnoside (**4**) and gallic acid (**5**), were also obtained and identified by means of spectral and chemical evidence. This report describes the isolation and structural elucidation of these compounds.

*Corresponding author. E-mail: hwwang@zqu.edu.cn

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2. Results and discussion

Compound 1 was obtained as yellow powder, mp 268–269°C, $[\alpha]_D^{25}$ −31.8 (*c* 0.6, MeOH). It gave a strongly positive test for phenols (dark blue) with ferric chloride reagent. It also gave a strongly positive test with Mg–HCl reagent. Its UV spectrum in methanol showed characteristic absorption at 212 and 280 nm, indicating a 7-*O*-substituted flavonol skeleton [3], and analysis with the usual flavonoid shift reagents suggesting the presence of free hydroxyl groups at C-5 [4]. The IR spectrum showed absorption bands at 3369, 1732, 1639, 1562, 1522 and 1450 cm^{-1}, suggesting the existence of hydroxyl, carbonyl and aromatic groups. MALDI-TOF-MS gave quasi-molecular ions at *m/z* 873.1 $[M + 1]^+$, 895.1 $[M + 1 + Na]^+$ and 911.0 $[M + 1 + K]^+$. The molecular formula of $C_{42}H_{32}O_{21}$ was established by HRFAB-MS, at *m/z* 873.1520 $[M + H]^+$ and 895.1419 $[M + 1 + Na]^+$.

All the ^1H NMR and ^{13}C NMR signals of **1** in CD_3OCD_3 were assigned using ^1H–^1H COSY, NOESY, DEPT, HMQC and HMBC experiments (table 1). In the ^1H NMR spectrum of 1, the presence of a two-proton singlet at δ 7.04 and two one-proton singlet at δ 6.63 and 6.43 suggested the occurrence of a galloyl group and one hexahydroxyldiphenoyl (HHDP) group in the structure of 1 [5] (figure 1). This was further supported by the ^{13}C NMR spectrum of **1**, which showed three ester carbonyl signals at δ 166.6, 167.7 and 168.1. The chemical shift at δ 166.6 was due to the carbonyl signal of the galloyl group, and the signals at δ 167.7 and 168.1 were due to the signal of the HHDP group [5]. Acid hydrolysis of **1** yielded glucose and aglycone, which was identified as the known compound pinocembrin (5,7-dihydroxyflavanone) by comparison of the ^1H NMR and ^{13}C NMR with those of reported values [6,7]. The large coupling constant ($J = 7.6$ Hz) of the anomeric proton at δ 5.35 indicated that the sugar moiety should be a β-anomer [8]. The sequence of sugar moiety was determined by TCOSY spectroscopy. The sugar linkage to the aglycone in **1** was considered to be C-7 based on the HMBC experiments. The H-6 (δ 6.22) and H-8 (δ 6.28) showed a correlation with C-7 (δ 166.1), allowing assignment of the carbon signal for C-7

Table 1. NMR and DEPT spectral data of **1** in CD_3COCD_3.

No.	*^1H NMR*	*^{13}C NMR*	*DEPT*	*No.*	*^1H NMR*	*^{13}C NMR*	*DEPT*
2	5.63	80.2	CH	1‴	–	115.5	C
3	2.85, 3.26	43.7	CH_2	2‴	–	126.2	–
4	–	197.6	C	3‴	6.43	107.7	CH
5	–	164.5	C	4‴	–	145.1	C
6	6.22	97.7	CH	5‴	–	136.4	C
7	–	166.1	C	6‴	–	144.4	C
8	6.28	96.4	CH	7‴	–	167.7	C
9	–	164.0	C	1⁗	–	115.8	C
10	–	104.7	C	2⁗	–	126.6	C
1′	–	139.8	C	3⁗	6.63	108.1	CH
2′	7.57	127.5	CH	4⁗	–	145.1	C
3′	7.46	129.6	CH	5⁗	–	136.3	C
4′	7.40	129.5	CH	6⁗	–	144.4	C
5′	7.46	129.6	CH	7⁗	–	168.1	C
6′	7.57	127.5	CH	1⁗′	–	166.6	C
1″	5.35	101.0	CH	2⁗′	7.04	121.4	CH
2″	3.96	72.9	CH	3⁗′	–	110.2	C
3″	5.46	75.4	CH	4⁗′	–	145.7	C
4″	5.07	70.6	CH	5⁗′	–	138.7	C
5″	4.51	72.5	CH	6⁗′	–	145.7	C
6″	3.93, 5.32	63.4	CH_2	7⁗′	7.04	110.1	CH

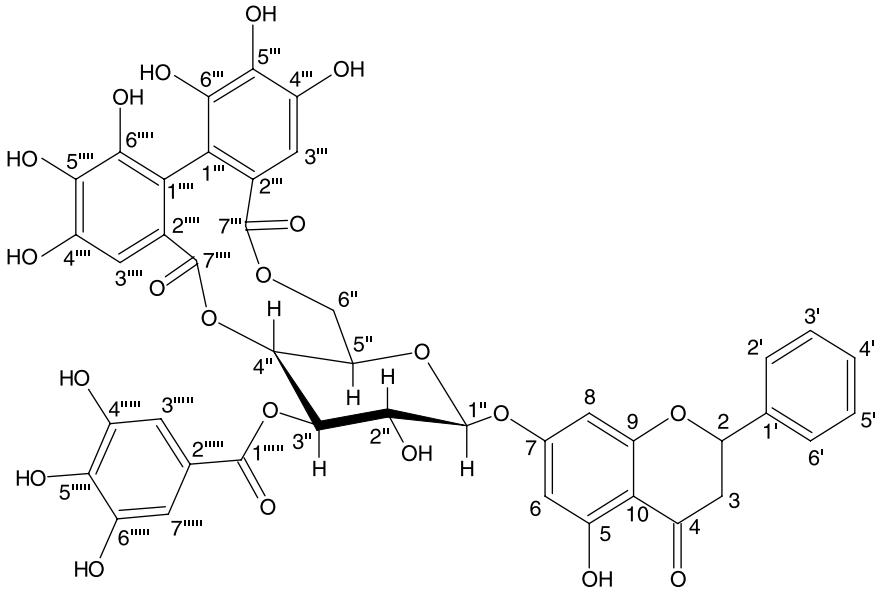

Figure 1. Chemical structure of compound **1**.

(δ 166.1), which also showed a correlation with the anomeric proton at δ 5.35. Correlations between H-4″ (δ 5.07), H-6″ (δ 3.93 and δ 5.32), and the two carboxyl signals (δ 167.7 and δ 168.1) of HHDP were also observed, indicating that the HHDP group was connected to the C-4″/6″ position. This was consistent with the appearance of two low-field-shift signals at δ 3.93 (1H, t, $J = 9.3$ Hz, H-4″) and 5.32 (1H, dd, $J = 6.2$, 13.2 Hz, H-6″) in the ^{1}H NMR spectrum of 1. The galloyl group was shown to be at C-3″ due to a downfield shift of the H-3″ (δ 5.46) and the HMBC correlation between the H-3″ and the carboxyl (δ 166.6) of the galloyl group. In addition, the configuration of the HHDP group was determined as *R* according to a positive Cotton effect at 261 nm and a negative one at 241 nm in the CD spectrum [9]. Thus, compound **1** was established as pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose.

The known compounds were identified by UV, FAB-MS, ^{1}H NMR and ^{13}C NMR spectral data as pinocembrin-7-*O*-(-glucoside (2), quercitrin (3), quercetin-3-*O*-rhamnoside (4) and gallic acid (5), in agreement with the literature data [10–13].

3. Experimental

3.1 General experimental procedures

Melting points were obtained with an XT_{4A} micro-melting-point apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 683 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter. UV spectra were recorded on a GBC model Cintra-10 spectrometer. MALDI-TOF-MS spectra were measured with a Bruker model Biflex III spectrometer. FAB-MS spectra were recorded on a VGZAB-2F instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded on a NVOA-500 instrument (500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively), using TMS as an internal standard.

3.2 Plant material

The whole plants of *Penthorum chinense* Pursh. were collected in October 2001 in Sichuan Province, China. A voucher specimen is deposited in the Chinese Medicine Research Institute of Sichuan.

3.3 Extraction and isolation

The air-dried whole plants of *P. chinense* (1000 g) were extracted with 95% EtOH. After evaporation of the solvent, the residue was chromatographed on a polyamide column eluting with gradient solvent systems of H_2O/EtOAc (10:1 to 5:1) and $CHCl_3$/MeOH (15:1 to 0:1) to yield eight fractions. Fraction 8 was rechromatographed over Sephadex LH-20, eluting with MeOH, to give mixtures A–E. Mixture B was similarly rechromatographed to offer ten fractions. Of these, fractions 5–8 were individually further purified by Sephadex LH-20 eluting with MeOH to give pinocembrin-7-*O*-(-glucoside (2, 10 mg) from fraction 5, quercitrin (3, 20 mg), quercetin-3-*O*-rhamnoside (4, 15 mg), and gallic acid (5, 10 mg) from fraction 6, and pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (1, 30 mg) from fractions 7–8. In addition, mixtures C and D were further rechromatographed by the same procedure as mixture B to afford pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (1, 20 mg) from mixture D.

3.4 Identification

3.4.1 Pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (1). Yellow powder; mp 268–269°C, $[\alpha]_D^{25}$ −31.8 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 280 (4.67), 212 (4.94) nm; UV (MeOH + $AlCl_3$) λ_{max} (log ϵ) 296 (4.60) nm; IR (KBr) ν_{max} 3369 (OH), 1732 (C = O), 1639(CO-CH = CH), 1562, 1522, 1450 cm^{-1}; MALDI-TOF-MS *m/z* 873.1 $[M + 1]^+$, 895.1 $[M + 1 + Na]^+$ and 911.0 $[M + 1 + K]^+$; positive FAB-MS *m/z* 873.1 $[M + H]^+$(15), 895.1 ($[M + 1 + Na]^+$, 25), 257 (100); HRFAB-MS *m/z* 873.1520 $[M + H]^+$, 895.1418 $[M + 1 + Na]^+$ (calcd for $C_{42}H_{33}O_{21}$, 873.1514); ^1H NMR (CD_3OD, 500 MHz) δ 2.85 (1H, dd, J = 3.0, 17.1 Hz, H-3), 3.26 (1H, dd, J = 12.8, 17.1 Hz, H-3), 3.96 (1H, br t, H-2″), 3.93 (1H, br t, J = 13.3 Hz, H-6″), 4.51 (1H, dd, J = 6.2, 9.6 Hz, H-5″), 5.07 (1H, t, J = 9.6 Hz, H-4″), 5.35 (1H, d, J = 7.6 Hz, H-1″), 5.32 (1H, dd, J = 6.2, 13.3 Hz, H-6″), 5.63 (1H, dd, J = 3.1, 12.8 Hz, H-2), 6.22 (1H, d, J = 2.2 Hz, H-6), 6.28 (1H, d, J = 2.2 Hz, H-8), 6.63 and 6.43 (each 1H, s, HHDP-H), 7.04 (2H, s, galloyl-H), 7.40 (1H, H-4′), 7.46 (2H, H-3′, H-5′), 7.57 (2H, m, H-2′, H-6′); ^{13}C NMR (CD_3OD, 125 MHz) δ 43.7 (C-3), 63.4 (C-6″), 70.6 (C-4″), 72.5 (C-5″), 72.9 (C-2″), 75.4 (C-3″), 80.2 (C-2), 96.4 (C-8), 97.7 (C-6), 101.0 (C-1″), 104.7 (C-10), 107.7 and 108.1 (HHDP C-3, 3′), 110.2 (galloyl C-2, 6), 115.5 and 115.8 (HHDP C-1, 1′), 121.4 (galloyl C-1), 126.2 and 126.6 (HHDP C-2, 2′), 127.5 (C-2′, 6′), 129.5 (C-4′), 129.6 (C-3′, 5′), 136.4 (HHDP C-5, 5′), 138.7 (galloyl C-4), 139.8 (C-1′), 144.4 (HHDP C-6, 6′), 145.1 (HHDP C-4, 4′), 145.7 (galloyl C-3, 5), 164.0 (C-9), 164.5 (C-5), 166.1 (C-7), 166.6 (galloyl -COO-), 167.7 and 168.1 (HHDP-COO-), 197.6 (C-4).

3.4.2 Acid hydrolysis of pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (1). Compound **1** (20 mg) in 2 mol/l HCl was refluxed for 2.5 h. After filtration,

the precipitate was dissolved in MeOH and rechromatographed through Sephadex LH-20 column chromatography with MeOH to give the aglucone (5 mg); it was discriminated as pinocembrin by comparison of its ^{1}H NMR and ^{13}C NMR spectra with those of literature data. The filtrate was neutralised with $Ba(OH)_2$ and then analysed by silica gel TLC (developing solvent systems of iPrOH/Me_2CO/H_2O [5:3:1]). It showed a brown spot (R_f 0.50) on TLC over spraying aniline phthalate solution and heating, which was coincident with that of glucose.

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