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Galloylpaeoniflorin, A New Acylated Monoterpene Glucoside from Paeony Root[1]

Sam Sik Kang*, Kuk Hyun Shin and Hyung-Joon Chi
Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea
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Abstract □ A new acylated monoterpene glucoside, galloylpaeoniflorin, was isolated from Paeony root. The structure was determined by chemical and spectroscopic methods.

Keywords □ Paeony root, Ranunculaceae, Acylated monoterpene glucoside, Galloylpaeoniflorin

Paeony root [roots of *Paeonia albiflora* Pallas var. *trichocarpa* Bunge (Ranunculaceae)] is one of the most important crude drugs used in traditional Chinese medicine and has been used as a circulatory tonic, weakness, night sweats and lumbar pain[1]. In the course of our study on the isolation of the well-known monoterpene glucoside, paeoniflorin[**1**], we isolated a new acylated monoterpene glucoside named galloylpaeoniflorin[**2**] and reported in a preliminary communication[2]. In this paper we report detailed evidence on the isolation and structure elucidation of this compound **2**.

Chromatographic separation of the EtOAc fraction from MeOH extract afforded galloylpaeoniflorin[**2**] as a pale yellow powder, mp 163-5°, together with paeoniflorin[**1**]. Galloylpaeoniflorin[**2**] showed IR absorption bands at 3420 (OH), 1710 (ester), 1615, 1538 (aromatic C=C), 1075 and 1035 cm^{-1} (glycosidic C-O), and UV absorption maxima at 222 and 277 nm. The ^{1}H-NMR spectrum of **2** showed the presence of a methyl singlet at δ 1.25, an acetal proton singlet at δ 5.37, and seven aromatic protons (δ 7.07, 2H, s; 7.45-7.55, 3H, m; 8.03, 2H, dd, J=7.8, 2.0 Hz). These spectral data closely resembled those of **1** and its derivatives[3-5], indicating that **2** was an acylated paeoniflorin. The SIMS spectrum of **2** exhibited a protonated molecular ion $[M+H]^+$ at *m/z* 633. Methanolysis of **2** gave D-glucose, benzoic acid and gallic acid. Acetylation of **2** yielded a heptaacetate [**3**], mp. 99-101°, which showed the presence of a methyl (δ 1.32), four aliphatic (δ 1.99, 2.02, 2.05 and 2.06), and three aromatic acetyl signals (δ 2.30) along with an acetal proton singlet (δ 5.49) in the ^{1}H-NMR spectrum. The assignment of the remaining protons in **3** was made on the basis of a ^{1}H-^{1}H COSY experiment. In the ^{1}H-^{1}H COSY spectrum of **3**, the proton at δ 1.95 (1H, br d, J=12.3 Hz) correlated to that at δ 2.32 (1H, d, J=12.3 Hz).

In a similar manner, the proton at δ 2.08 (1H, d J=11.0 Hz) correlated to that at δ 2.42 (1H, dd, J=11.0, 7.2 Hz) which in turn coupled to that at δ 2.81 (1H, br d, J=7.2 Hz). According to the chemical shifts and coupling constants, the proton at δ 2.81 was assigned to H-5, and the protons at δ 2.08 and 2.42 to H-7 methylene. The protons at δ 1.95 and 2.32 were attributed to H-3 methylene. Cross peaks arising from a W-type long-range coupling between H-5 and one of the H-3 methylene protons strongly supported the above assignments. The observation of the signals at δ 4.37 (1H, dd, J=12.5, 5.2 Hz), 4.44 (1H, dd, J=12.5, 3.2 Hz), and 4.45 and 4.58 (each 1H, d, J=12.1 Hz) indicated that the gallic acid is linked either at C-6′ of glucose or at C-8; the possibility at C-8 was excluded by the presence of a fragment ion for galloylglucosyl cation (**a**). The occurrence of a fragment ion at *m/z* 315 in **2** as well as at *m/z* 567 in **3** indicated that the gallic acid unit was located on the C-6′ position of glucose. Further evidence to exclude the possibility of a C-8-galloyl moiety was obtained from the ^{13}C-NMR spectrum

[1] Dedicated to Prof. Dr. Norman R. Farnsworth, Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, on the occasion of his 60th birthday.

of **2**, in which the chemical shift for C-8 at δ 61.17 was identical to that of **1**, indicating that a benzoyl moiety was present at C-8[5]. From the above findings, the structure of galloylpaeoniflorin could be formulated as **2**; this compound has not been reported previously. It was inactive in the NCI *in vitro* Anti-HIV screening test ($IC_{50}=4.00\times10^{-2}$ M)[2].

EXPERIMENTAL

General experimental procedures

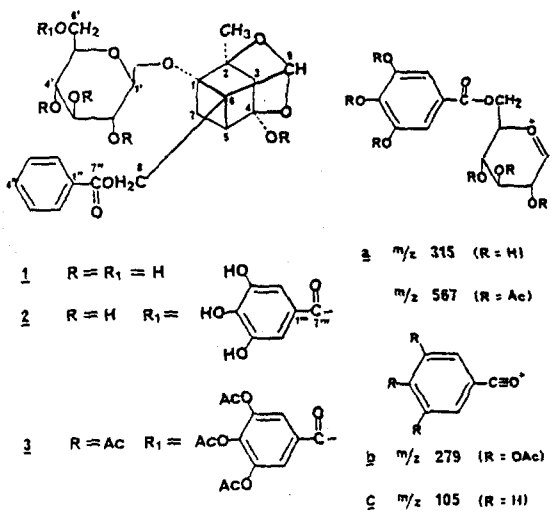
Melting points were determined on a Mitamura-Riken apparatus and are uncorrected. Optical rotations were measured on a Rudolph Autopol III automatic polarimeter. IR spectra were recorded on a Perkin-Elmer 283B spectrophotometer. ^{1}H-NMR spectra were obtained on either a Varian FT-80A (80 MHz) or a Bruker AM-300 (300 MHz) spectrometer using TMS as an internal standard. EIMS were determined on a Hewlett-Packard 5985B GC/MS System equipped with direct inlet system. For tlc, Kieselgel 60 F_{254} sheets (Merck) were used.

Plant material

Paeony root was purchased at Kyungdong market, Seoul, and authenticated by one of us (H.J.C.). Voucher specimens were deposited in our institute.

Isolation

The powdered Paeony root (500g) was extracted with MeOH in an ultrasonic apparatus for 1 h to yield a MeOH extract. The MeOH extract was concentrated *in vacuo* to give a residue which was suspended in H_2O and extracted sucessively with hexane, $CHCl_3$ and EtOAc. The EtOAc extract (8g) was chromatographed on a Si gel column and eluted with $CHCl_3$-MeOH(5:1) to give 8 fractions. Fraction No. 6 was rechromatographed over Si gel with EtOAc-EtOAc saturated with H_2O(1:1) to afford galloylpaeoniflorin (**2**, 65 mg). Precipitation from H_2O afforded **2** as pale yellow powder: mp 163-5°; $[\alpha]_D^{25}-10.0°$ (*c*=0.1, MeOH); UV λ_{max} (log ε) (MeOH) 222 (4.39), 277 (3.89) nm; λ_{max} (NaOH) 230 (sh, 4.25), 282 (3.70), 324 (3.72); IR ν_{max} (KBr) 3420, 1710, 1615, 1538, 1452, 1348, 1318, 1280, 1230, 1180, 1075, 1050, 1035, 766, 712 cm^{-1}; ^{1}H-NMR (80 MHz, CD_3OD): δ 1.25 (3H, s, CH_3), 5.37 (1H, s, H-9), 7.07 (2H, s, H-2‴, 6‴), 7.45-7.55 (3H, m, H-3″, 4″, 5″), 8.03 (2H, dd, J=7.8, 2.0 Hz, H-2″, 6″); ^{13}C-NMR (pyridine-d_5): δ 88.79 (C-1), 85.92 (C-2), 44.64 (C-3), 105.76 (C-4), 43.67 (C-5), 71.15 (C-6), 22.61 (C-7), 61.17 (C-8), 101.43 (C-9), 19.57 (C-10), 100.02 (C-1′), 74.64 (C-2′), 78.03 (C-3′′), 71.52 (C-4′), 75.06 (C-5′), 64.32 (C-6′), 130.48 (C-1″), 129.68 (C-2″, 6″), 128.53 (C-3″, 5″), 133.03 (C-4″), 166.42 (C-7″), 121.13 (C-1‴), 110.09 (C-2‴, 6‴), 147.32 (C-3‴, 5‴), 140.73 (C-4‴), 166.91 (C-7‴); SIMS *m/z* $(M+H)^+$633, [(M+H) $-H_2O]^+$ 615, [(M+H)−benzoic acid]$^+$ 511, [(M+H)-(benzoic acid+H_2O)]$^+$493, [(M+H)-(benzoic acid+2H_2O)]$^+$475, [(M+H)-gallic acid]$^+$ 463, (**a**)315; Anal. Calcd. for $C_{30}H_{32}O_{15}$, C 56.96, H 5.06; found C 56.88, H 5.21.



Methanolysis of 2

Compound **2** (10mg) was dissolved in 2% methanolic hydrogen chloride (10 m*l*) and allowed to stand for 30 h at room temperature. The solvent was removed by passing a stream of N_2 through the solution to give a residue which was acetylated (Ac_2O/pyridine) at room temperature overnight. After removal of the solvent the residue was subjected to GC. Methyl glucoside tetraacetate (t_R: 11.1, 12.9), methyl benzoate (t_R: 4.29) and methyl gallate triacetate (t_R: 20.1) were identified by comparison with authentic samples[column: 10% OV-17 on chromosorb WHP 100-120 (2.3 mm i.d. × 6ft); flow rate (N_2): 22 m*l*/min; column temp.: 240° for methyl glucoside tetraacetate, 160° for methyl benzoate, and 260° for methyl gallate triacetate].

[2] Personal communication from Dr. J.P. Bader, Rm. 837 C, Executive Plaza North, National Cancer Institute, Bethesda, MD 20892

Acetylation of 2

Compound **2** (20 mg) was dissolved in pyridine (1 *ml*) and treated with a slight excess of Ac_2O. The reaction mixture was allowed to stand at room temperature overnight and, after usual work-up, yielded a residue which was purified by repeated cc over Si gel eluting with hexane-EtOAc (8:5) to give a heptaacetate (**3**) as amorphous powder: mp 99-101°; $[\alpha]_D^{25}$ −17.1° (c 0.1, $CHCl_3$); IR ν_{max} (KBr) 1790, 1765, 1730, 1280, 1220, 1185, 1058, 719 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$); δ 1.32 (3H, s, CH_3), 1.95 (1H, br d, J=12.3 Hz, H-3A), 1.99, 2.02, 2.05, 2.06 (each 3H, s, OAc), 2.30 (9H, s, 3 × OAc), 2.08 (1H, d, J=11.0 Hz, H-7A), 2.32 (1H, d, J=12.3 Hz, H-3B), 2.42 (1H, dd, J=7.2, 11.0 Hz, H−7B), 2.81 (1H, br d, J=7.2 Hz, H-5), 3.76 (1H, m, H-5'), 4.37 (1H, dd, J=5.2, 12.5 Hz, H-6'), 4.44 (1H, dd, J=3.2, 12.5 Hz, H-6'), 4.45, 4.58 (each 1H, d, J=12.1 Hz, H-8), 4.78 (1H, d, J=7.7 Hz, H-1'), 4.99-5.14 (3H, m, H-2', 3', 4'), 5.49 (1H, s, H-9), 7.47 (2H, m, H-3'', 5''), 7.58 (1H, m, H-4''), 7.79 (2H, s, H-2''', 6'''), 8.03 (2H, dd, J=1.5, 8.7 Hz, H-2'', 6''); EIMS (30 eV) *m/z* (rel. int.) (**a**) 567 (3.0), (**a**-CH_2CO)$^+$ 525 (3.6), (**a**-2CH_2CO)$^+$483 (3.9), [(**a**-(CH_2CO+2HOAc)]$^+$405 (2.8), (**b**) 279 (14.0), (**b**-CH_2CO)$^+$237 (27.5), (**b**-2CH_2CO)$^+$195 (22.6), 178 (100), 169 (30), (**b**-3CH_2CO)$^+$153 (17.9), 152 (16.8), 150 (16.5), 122 (58.1), (**c**) 105 (92), (C_6H_5)$^+$77(45.2).

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