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SYNTHESIS OF ESTERS OF THE MONOTERPENE GLYCOSIDE PAEONIFLORIN

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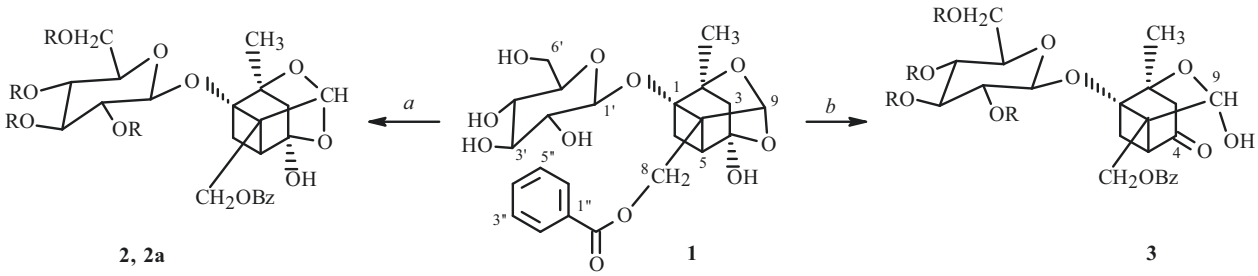
The monoterpene glycoside paeoniflorin (PF, **1**) is a unique biologically active secondary metabolite from roots of *Paeonia*, which are widely used in traditional Chinese medicine in combination with *Glycyrrhiza uralensis* roots [1–4]. The PF content in roots of Siberian wild *P. anomala* is 1–2%. It is easily isolated from root extract by chromatographic methods [5, 6]. PF possesses anti-inflammatory and antiarrhythmic [7], analgesic [8], hepatoprotective [9], antihyperglycemic [10], and antioxidant [11] activity, exhibits neuroprotective activity, and enhances cognitive capabilities [12, 13]. The high biological activity and poorly understood reactivity of PF make its chemical transformations a critical problem.



2: R = Ac; **2a, 3:** R = Bz

a. Ac_2O, Py, 20–22°C, 24 h; *b*. C_6H_5COCl, Py, 20–22°C, 48 h

PF was isolated in 1.2% yield using the literature method [5] from ground air-dried roots of *P. anomala* collected in the Altai. The physicochemical and spectral characteristics of PF agreed with those in the literature [5, 6, 14]. Acetylation of PF by Ac_2O–Py at room (20–22°C) temperature formed paeoniflorin tetra-*O*-acetate (**2**) [15] (83% yield), which was characterized by us for the first time using the ^{13}C NMR spectrum. The C-4 hemi-ketal resonance had chemical shift (CS) 108.4 ppm, which was close to the CS of this C atom in the spectrum of starting PF.

PF 6-*O*-monobenzoate was synthesized earlier from **1** by treating it with benzoylchloride in Py at 0°C for 1 h [16]. Herein, PF was acylated by benzoylchloride in Py at 20–22°C.

The reaction product was isolated in 58% yield by column chromatography (CC) over silica gel. NMR spectral data showed that the reaction did not form the expected benzoate **2a** but a product with a rearranged aglycon (**3**). The PMR spectrum of **3** showed strong resonances for aromatic protons at weak field (δ 8.1–7.4 ppm) and a broad singlet for at C-9 OH (δ 12.2 ppm). The C-9 proton had CS 4.87 ppm (singlet). The C-5 proton was observed at δ 2.96 ppm as a doublet with spin–spin coupling constant (SSCC) J = 7.4 Hz. The ^{13}C NMR spectrum of **3** exhibited weak-field resonances for aromatic C atoms (δ 133.8–128.3 ppm) and five benzoyl C=O C atoms (172.5–164.5 ppm). Furthermore, the ^{13}C NMR spectrum contained a new carbonyl C atom resonance (δ 205.4 ppm) that belonged to C-4 and was missing in the spectrum of starting PF. The C-9 resonance was shifted to strong field (δ 105.7 ppm) compared with the spectrum of starting PF (102.0 ppm). A similar aglycon rearrangement was observed previously [15] during the reaction of PF with MeOH or EtOH in the presence of *p*-toluenesulfonic acid with heating to form a mixture of 4-*O*-methyl(ethyl)- and 4-oxo-9-*O*-methyl(ethyl)-derivatives of PF.

IR spectra were recorded from mineral-oil mulls on a Prestige 21 spectrophotometer (Shimadzu). PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and CD_3OD with TMS internal standard on a Bruker AMX-300 spectrometer at operating

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frequency 300 and 75.5 MHz with broad-band and off-resonance proton suppression. Optical activity was measured on a PerkinElmer 341 MC polarimeter in a 1-dm cuvette. Melting points were determined on a Boetius apparatus.

TLC was performed on Sorbfil plates (ZAO Sorbpolimer). Spots were detected using H_2SO_4 (5%) in EtOH followed by heating at 110–120°C for 2-3 min. Column chromatography was carried out over KSK silica gel (50–150 μm fraction) (Sorbpolimer). Molecular ions were registered using liquid-chromatography–mass-spectrometry (LC-MS) on a Shimadzu LCMS-2010 instrument and chemical ionization at atmospheric pressure as MeOH solutions. Elemental analyses data agreed with those calculated.

Py was distilled over BaO and stored over 4-Å molecular sieves. Other solvents were purified as usual [17]. Plant raw material consisted of wild *P. anomala* roots collected in Altai. PF was isolated from ground roots by the literature method [5]; $[\alpha]_D^{20}$ –13.5° (*c* 1.0, MeOH); lit. [5] $[\alpha]_D^{20}$ –15.6° (*c* 4.5, EtOH); [6] $[\alpha]_D^{20}$ –13.17° (*c* 3.23, EtOH). The PMR and ^{13}C NMR spectra agreed with those in the literature [5, 14].

Paeoniflorin tetra-*O*-Acetate (2). PF (0.48 g, 1 mmol) was acetylated by a mixture of Ac_2O (5 mL) and Py (5 mL) at 20–22°C for 48 h. The mixture was diluted with cold H_2O. The precipitate was filtered off, dried, and recrystallized from EtOH. Yield 0.54 g (83%). R_f 0.7 ($CHCl_3$–MeOH, 5:1), mp 165–167°C, $[\alpha]_D^{20}$ –13.8° (*c* 0.08; EtOH). IR spectrum (ν, cm^{-1}): 3400 (br), 1750, 1370, 1220. ^1H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 8.03 (2H, d, J = 8, H-2″, 6″), 7.62 (1H, t, J = 8, H-4″), 7.49 (2H, t, J = 8, H-3″, 5″), 5.52 (1H, s, H-9), 5.11 (1H, t, J = 9.2, H-3′), 5.02 (1H, t, J = 9.2, H-4′), 5.00 (1H, dd, J_1 = 9.2, J_2 = 7.8, H-2′), 4.76 (1H, d, J = 7.8, H-1′), 4.62 (1H, d, J = 12.0, H-8b), 4.48 (1H, d, J = 12.0, H-8a), 4.15 (2H, d, J = 4.2, 2H-6′), 3.63 (1H, dt, J_1 = 9.2, J_2 = 4.2, H-5′), 2.81 (1H, dd, J_1 = 4.0, J_2 = 1.3, H-5), 2.52 (1H, d, J = 12.4, H-3b), 2.42 (1H, dd, J_1 = 10.8, J_2 = 4.0, H-6b), 2.19 (1H, dd, J_1 = 10.8, J_2 = 1.3, H-6a), 1.98, 2.00, 2.02, 2.10 (4OAc, all s, 12H), 1.38 (3H, s, CH_3-2). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 170.6, 170.3, 169.5, 169.4 ($4CH_3\underline{C}$=O), 166.4 (C-7″), 133.6 (C-4″), 129.7 (C-3″, 5″), 129.5 (C-1″), 128.7 (C-2″, 6″), 108.4 (C-4), 101.4 (C-9), 96.4 (C-1′), 88.2 (C-1), 86.2 (C-2), 73.0 (C-3′), 71.9 (C-5′), 71.3 (C-2′), 69.5 (C-4′), 68.5 (C-7), 62.0 (C-6′), 59.8 (C-8), 43.6 (C-5), 41.3 (C-3), 23.3 (C-6), 21.4, 20.8, 20.6, 18.9 ($4\underline{C}H_3CO$, C-10)). $[M + H]^+$ 649. $C_{31}H_{36}O_{15}$. M = 648.6.

Paeoniflorin tetra-*O*-Benzoate (3). A solution of PF (0.48 g, 1 mmol) in Py (5 mL) was treated with benzoylchloride (5 mL), stored at 20–22°C for 48 h, diluted with cold H_2O (20 mL), and extracted with $CHCl_3$ (2 × 20 mL). The $CHCl_3$ extract was washed with H_2O, Na_2CO_3 solution (5%), and H_2O; dried over $MgSO_4$; and evaporated. The residue was chromatographed over a silica gel column with elution by C_6H_6–EtOH (200:1, 100:1, 500:1, v/v, stepwise gradient). Fractions that were pure according to TLC were combined and evaporated. Yield **3** 0.52 g (58%). R_f 0.48 (toluene–EtOH, 5:1), mp 128–130°C (C_6H_6–EtOH), $[\alpha]_D^{20}$ +6° (*c* 0.04, $CHCl_3$). IR spectrum (ν, cm^{-1}): 3600–3200 (OH), 1730, 1650, 1643, 1593. ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, J/Hz): 12.2 (1H, br.s, OH), 8.10–8.20 (10H, m, $5C_6H_5$), 7.45–7.55 (5H, m, $5C_6H_5$), 7.30–7.40 (10H, m, $5\,C_6H_5$), 5.95 (1H, t, J = 9.5, H-4′), 5.62 (2H, m, H-2′, 3′), 5.19 (1H, d, J = 7.8, H-1′), 4.87 (1H, s, H-9), 4.62 (1H, dd, J_1 = 10.7, J_2 = 2.5, H-6′b), 4.59 (1H, dd, J_2 = 10.7, J_2 = 6.0, H-6′a), 4.52 (1H, d, J = 11.8, H-8b), 4.41 (1H, d, J = 11.8, H-8b), 4.23 (1H, m, H-5′), 2.96 (1H, d, J = 7.4, H-5), 2.80 (1H, dd, J_1 = 7.4, J_2 = 5.7, H-6b), 2.52 (1H, d, J = 5.7, H-6a), 2.15 (1H, d, J = 10.6, H-3b), 1.95 (1H, d, J = 10.6, H-3a), 1.47 (3H, s, CH_3). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 205.4 (C-4), 172.5, 166.1, 166.0, 165.0, 164.9 (5 C=O), 133.8–133.4, 131.0–130.0, 129.8–129.4, 128.8–128.3 ($5C_6H_5$), 105.7 (C-9), 96.7 (C-1′), 87.8 (C-1), 86.1 (C-2), 73.1 (C-2′), 72.5 (C-3′), 71.9 (C-5′), 69.7 (C-4′), 63.2 (C-7), 63.0 (C-6′), 62.0 (C-8), 48.6 (C-3), 46.7 (C-5), 26.4 (C-6), 20.3 (CH_3). $[M + H]^+$ 898. $C_{51}H_{44}O_{15}$. M = 896.9.

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