Paeonicluside, a New Salicylic Glycoside from the Greek Endemic Species *Paeonia clusii* [§]

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A new glycoside of salicylic aldehyde, paeonicluside, was isolated from the roots of the Greek endemic species *Paeonia clusii* subsp. *clusii* and identified as α-L-arabinopyranosyl-(1→6)-*O*-β-D-glucopyranoside of salicylic aldehyde (**1**). In addition, one characteristic monoterpene and two monoterpene glycosides were identified as paeoniflorigenone, paeoniflorin and benzoyl paeoniflorin, respectively. The structure of **1** was elucidated on the basis of its spectroscopic data and chemical correlation. It is the first time that a derivative of salicylic aldehyde is isolated from the well-studied *Paeonia* genus.

Introduction

Paeonia plants and especially their roots are known for their medicinal properties since antiquity. The name *Paeonia (Paionia)* commemorating Paeon (the doctor of the ancient Greek gods) was given to the peony by the ancient Greeks (Baumann *et al*., 1996). Their roots are one of the most important source of crude drugs in traditional Chinese medicine (Zhu, 1998). They have been described to possess analgesic, sedative and anti-inflammatory properties, and they have been used as a remedy for cardiovascular and female genital diseases (Miyazawa *et al*., 1984; Lin *et al*., 1999; Müller *et al*., 1999). Twelve species of the genus *Paeonia* are found in Europe while two of them, *Paeonia clusii* F. C. Stearn and *Paeonia parnassica* Tzanoud., are endemic in Greece (Tutin, 1968; Stearn and Davis, 1984).

The broad pharmacological activities of the paeoniaceae roots led us to investigate the chemical constituents of the roots of the Greek endemic species *P. clusii* subsp. *clusii.* The species *P. clusii* comprises two subspecies: subsp. *clusii*, which is endemic in the islands of Crete and Karpathos and subsp. *rhodia*, which is endemic in the island of Rhodos. *P. clusii* subsp. *clusii* is a perennial herb (up to 30 cm) with fleshy roots and is characterized by its white or occasionally flushed pink flowers.

The phytochemical analysis of the extracts of the roots led to the isolation and structure elucidation of paeonicluside (**1**), a new salicylic glycoside. Moreover, one monoterpene and two monoterpene glycosides, characteristic of the genus were identified as paeoniflorigenone (**2**), paeoniflorin (**3**), and benzoyl paeoniflorin (**4**), respectively.

Results and Discussion

Compound **1** was obtained as an amorphous yellowish powder. Its molecular formula was determined by HRFABMS as $C_{18}H_{24}O_{11}$. The ^{1}H NMR spectrum of **1** exhibited a deshielded proton corresponding to an aromatic aldehyde, four characteristic signals belonging to an *ortho* bisubstituted aromatic ring, two anomeric protons and several highly overlapped signals corresponding to two sugar moieties. The ^{13}C NMR spectrum confirmed the above observations and showed eleven carbon signals in the region of the sugar carbons, revealing the presence of a hexose and a pentose. In the HMBC spectrum, the first anomeric proton was correlated with an oxygenated aromatic carbon while the second anomeric proton was correlated

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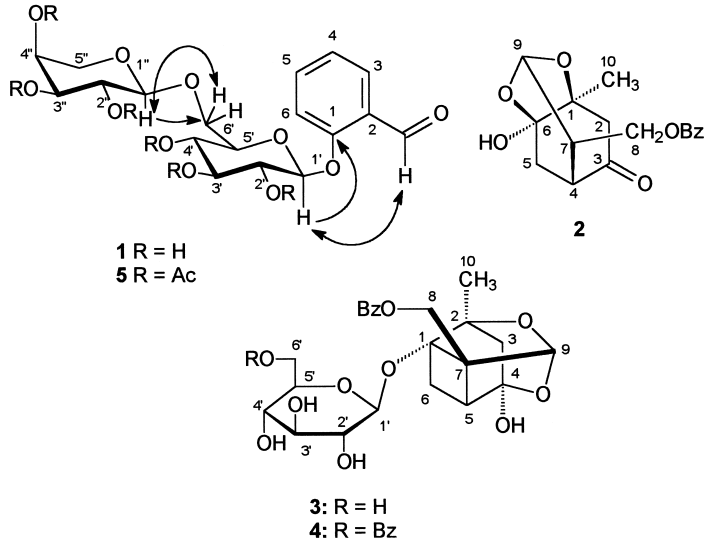

Fig. 1. The structures of paeonicluside (**1**), paeoniflorigenone (**2**), paeoniflorin (**3**), benzoyl paeoniflorin (**4**), hexaacetyl paeonicluside (**5**) and characteristic HMBC (→) and NOESY (↔) correlations for **1**.

Table I. ^{1}H NMR (CD_3OD/TMS, 400 MHz, δ ppm, *J* in Hz) and ^{13}C NMR (CD_3OD/TMS, 50 MHz, δ ppm) of **1** and **5** (C_6D_6).

Position	^{13}C NMR		^{1}H NMR	
	1	**5**	**1**	**5**[a]
Aglycon				
1	161.3	159.5	–	–
2	126.9	126.9	–	–
3	128.5	128.0	7.80 (d, *J* = 8.0)	7.95 (dd, *J* = 7.5, 1.5)
4	123.6	123.7	7.17 (t, *J* = 8.0)	6.82 (t, *J* = 7.5)
5	137.7	136.2	7.70 (t, *J* = 8.0)	7.53 (ddd, *J* = 8.2, 7.5, 1.5)
6	118.0	116.9	7.44 (d, *J* = 8.0)	7.07 (d, *J* = 8.2)
CHO	191.8	188.3	10.54 (s)	10.65 (s)
Glucose				
1′	102.3	99.7	5.05 (d, *J* = 7.5)	4.61 (d, *J* = 7.8)
2′	77.7	71.2	3.38–3.83 [b]	5.49 (dd, *J* = 9.5, 7.8)
3′	77.4	72.8	3.38–3.83 [b]	5.36 (t, *J* = 9.5)
4′	74.7	69.2	3.38–3.83 [b]	5.06 (t, *J* = 9.5)
5′	74.1	74.0	3.38–3.83 [b]	3.47 [b]
6′	71.3	67.4	4.15 (d, *J* = 10.9); 3.80[b]	3.85 (d, *J* = 8.6); 3.47 [b]
Arabinose				
1′′	105.1	100.9	4.31 (d, *J* = 6.8)	4.16 (d, *J* = 7.0)
2′′	69.5	69.4	3.38–3.83 [b]	5.58 (dd, *J* = 9.5, 7.0)
3′′	72.4	70.7	3.38–3.83 [b]	5.11 (dd, *J* = 9.5, 3.2)
4′′	74.7	68.1	3.38–3.83 [b]	5.23 (dd, *J* = 3.2, 3.0)
5′′	66.7	63.4	3.85 (dd, *J* = 12.3, 3.1); 3.47 [b]	3.62 (dd, *J* = 13.1, 3.0); 2.90 (d, *J* = 13.1)

[a] COOC**H**$_3$: δ 1.78, 1.70, 1.66, 1.63, 1.61 (x 2), COO**C**H$_3$: δ 20.0–20.4, **C**OOCH$_3$: δ 169.1–169.8; [b] Overlapped signals.

with a carbon corresponding to an oxygenated methylene. All these data made obvious that **1** was a disaccharide of salicylic aldehyde. The highly overlapped ^{1}H NMR spectrum did not permit the direct discrimination of the disaccharide structure and thus **1** was further studied in its peracetylated form **5**.

The FABMS spectrum of the peracetylated compound showed a molecular weight increased by six acetoxy groups, relatively to **1**, confirming the presence of a hexose and a pentose. Its ^{1}H-NMR spectrum in C_6D_6 (but not in $CDCl_3$) permitted the clear observation of all the sugar protons and of their splitting pattern. The TOCSY experiment

showed that the first sugar that was directly attached to the aromatic skeleton was an hexose and contained one anomeric proton (doublet with J = 7.8 Hz) and three deshielded protons (due to acetylation), which were observed as one doublet of doublets with J = 9.5 and 7.8 Hz and two triplets with J = 9.5 Hz. This splitting pattern was compatible only with H-1,2,3,4 of β-glucopyranose. The same experiment showed that the second sugar contained one anomeric proton (doublet with J = 7.0 Hz) and also three deshielded protons which were observed as one doublet of doublets with J = 9.5, and 7.0 Hz, one doublet of doublets with J = 9.5 and 3.2 Hz and one doublet of doublets with J = 3.2 and 3.0 Hz. In this case, the splitting pattern and the coupling constants were compatible only with H-1,2,3,4 of α-arabinopyranose. Characteristically, the coupling constants of H-4 are very important for the discrimination between β-D-xylose and α-L-arabinose. The NOESY experiment confirmed the observations of the HMBC experiment of the non acetylated compound and showed a clear correlation between the anomeric proton of glucose and the proton of the aldehyde group and additionally a correlation between the anomeric proton of arabinose and H-6 of glucose.

All these observations made clear that salicylic aldehyde was linked with α-arabinopyranosyl-(1→6)-*O*-β-glucopyranose. The absolute stereochemistry of the sugars was elucidated after chemical hydrolysis, which afforded D-glucose and L-arabinose identified by their ^{1}H NMR data and optical rotation.

Consequently **1** is the α-L-arabinopyranosyl-(1→6)-*O*-β-D-glucopyranoside of salicylic aldehyde, for which we propose the trivial name paeonicluside. It should be noted that although the genus *Paeonia* is well studied, due to its pharmacological properties, no derivatives of salicylic aldehyde have been identified up today.

Paeoniflorigenone (Shimizu *et al.*, 1983; Hattori *et al.*, 1985), paeoniflorin (Lin *et al.*, 1996; Opitz *et al.*, 1999) and benzoyl paeoniflorin (Lin *et al.*, 1996) were in complete agreement with the already published data, but some additional NMR data for paeoniflorigenone and benzoyl paeoniflorin are described in the experimental section.

Experimental

General experimental procedures

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Shimadzu-160A spectrophotometer. IR spectra were taken on a Perkin-Elmer Paragon 500 instrument. ^{1}H NMR spectra were measured on a Bruker DRX-400, (400 MHz) spectrometer and ^{13}C NMR on a Bruker AC-200, (50 MHz). Chemical shifts are given in δ values with TMS as an internal standard. Coupling constants (J) are given in Hz. The signals in the ^{1}H and ^{13}C spectra were assigned unambiguously using 2D NMR techniques: COSY, TOCSY, NOESY, HMQC, and HMBC. These 2D experiments were performed using standard Bruker microprograms. FABMS were obtained using a ZAB HF instrument, in glycerol matrix with NaCl as additive for positive ion mode. HRFABMS were obtained on a AEI MS-902 mass spectrometer. Column chromatography was conducted using Si flash gel 60 Merck (40–63 μm), with an overpressure of 300 mbar. Medium-pressure liquid chromatography (MPLC) was performed with a Büchi model 688 apparatus on columns containing Si gel 60 Merck (20–40 μm) or RP-18 Si gel 60 Merck (20–40 μm).

Plant material

The roots of *P. clusii* subsp. *clusii* were collected at mount Dhikti (1100 m. height) in the island of Crete in May 1999. A voucher specimen (PROK 010A) is deposited in the herbarium of the Division of Pharmacognosy, University of Athens.

Extraction and isolation

The air dried roots of *P. clusii* subsp. *clusii* (400 g) were extracted with CH_2Cl_2 (3 × 2 l) and then with MeOH (3 × 2 l). A part of the dichloromethane extract was evaporated to dryness yielding 1.8 g dry wt and then fractionated by column chromatography over Si gel 60 Merck (40–63 μm), using a cHex-EtOAc gradient to afford paoniflorigenone (40 mg), β-sitosterol (98 mg) and vanilline (20 mg). A part of the methanolic extract (8.0 g) was evaporated to dryness and then fractionated by column chromatography over Si gel 60 Merck (40–63 μm), using a CH_2Cl_2-MeOH gradient. The less polar fractions were rechromatographed by MPLC [Si

gel 60 Merck (20–40 μm), CH_2Cl_2-MeOH gradient] to afford methyl gallate (22 mg), benzoyl paeoniflorin (61 mg), and paeoniflorin (1.150 g). The more polar fractions were rechromatographed by MPLC [RP-18 Si gel 60 Merck (20–40 μm), H_2O-MeOH gradient] to afford paeonicluside (**1**) (50 mg).

Paeonicluside (**1**), $[\alpha]_D$ -28.0° (0.5 g/100 ml, MeOH); UV (MeOH) λ_{max}: 307, 250 nm; IR ν_{max}: 3500, 2900, 1720, 1600, 1300, 1100 cm^{-1}; ^1H and ^{13}C NMR, see Table I; HRFABMS found: 417.1391 (calcd for $C_{18}H_{25}O_{11}$, 417.1397); FABMS *m/z* 417 $[M+H]^+$.

Hexaacetyl paeonicluside (**5**). Treatment of **1** (20 mg) with Ac_2O (1 ml) and pyridine (1 ml) at room temperature overnight gave an hexaacetate (**5**, 90%); ^1H and ^{13}C NMR, see Table I; FABMS *m/z* 669 $[M + H]^+$.

Hydrolysis of paeonicluside A solution of **1** (20 mg) in HCl (1 N) was refluxed for 60 min under an Ar atmosphere. On cooling, the reaction mixture was neutralized with an Amberlite IR-50 resin. The solvent was removed under reduced pressure and the residue was chromatographed by MPLC [RP-18 Si gel 60 Merck (20–40 μm), H_2O-MeOH gradient] to afford D-glucose, L-arabinose and salicylic aldehyde.

Paeoniflorigenone (**2**), ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 1.30 (3H, s, H-10), 2.21 (1H, dd, *J* = 13.4, 2.4 Hz, H-5b), 2.39–2.40 (2H, overlapped, H-5a, H-7), 2.65 (1H, d, *J* = 12 Hz, H-2a), 2.69 (1H, d, *J* = 12 Hz, H-2b), 2.88 (1H, br s, H-4), 4.07 (1H, dd, *J* = 11.6, 9.1 Hz, H-8b), 4.36 (1H, dd, *J* = 11.6, 6.1 Hz, H-8a), 5.46 (1H, s, H-9), 7.41 (2H, t, *J* = 7.3 Hz, H-3′,5′), 7.54 (1H, t, *J* = 7.3 Hz, H-4′), 7.99 (2H, d, *J* = 7.3 Hz, H-2′,6′). ^{13}C NMR ($CDCl_3$, 50MHz, δ ppm): 21.10 (C-10), 34.18 (C-5), 42.96 (C-7), 46.23 (C-4), 46.78 (C-2), 62.55 (C-8), 78.57 (C-1), 99.63 (C-9), 101.65 (C-6), 128.40 (C-3′,5′), 129.58 (C-2′,6′), 130.06 (C-1′), 133.18 (C-4′), 166.11 (**C**OPh), 209.65 (C-3).

Benzoyl paeoniflorin (**4**). ^1H NMR (CD_3OD, 400MHz, δ ppm): 1.22 (3H, s, H-10), 1.66 (1H, d, *J* = 12 Hz, H-3b), 1.67 (1H, d, *J* = 10.5 Hz, H-6b), 1.81 (1H, d, *J* = 12 Hz, H-3a), 2.46 (1H, dd, *J* = 10.5, 7 Hz, H-6a), 2.48 (1H, d, *J* = 7 Hz, H-5), 3.26, 3.32, 3.36 (3H, overlapped, H-2′, H-4′, H-3′), 3.58 (1H, m, H-5′), 4.50 (1H, dd, *J* = 12, 6, H-6b'), 4.55 (1H, d, *J* = 8 Hz, H-1′), 4.62 (1H, dd, *J* = 12, 2, H-6a′), 4.71 (2H, s, H-8), 5.39 (1H, s, H-9), 7.50 (4H, t, *J* = 7.3 Hz, H-3″, 3‴, 5″, 5‴), 7.62 (2H, t, *J* = 7.3 Hz, H-4″, 4‴), 8.02 (2H, d, J = 7.3 Hz, H-2″, 6″), 8.04 (2H, d, J = 7.3 Hz, H-2‴, 6‴). ^{13}C NMR (CD_3OD, 50MHz, δ ppm): 19.52 (C-10), 22.98 (C-6), 43.74 (C-5), 44.37 (C-3), 61.57 (C-8), 65.10 (C-6′), 72.01 (C-7,4′), 74.91 (C-5′), 75.13 (C-2′), 77.81 (C-3′), 87.03 (C-2), 89.24 (C-1), 99.97 (C-1′), 102.18 (C-9), 106.19 (C-4), 129.60 (C-3″, 3‴,5″,5‴), 130.52 (C-2″,6″), 130.63 (C-2‴,6‴), 131.11 (C-1″,1‴), 134.41 (C-4″,4‴), 167.60 (**C**OPh), 167.93 (**C**OPh).

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