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A MONOTERPENE GLUCOSIDE FROM *PAEONIA PEREGRINA* ROOTS

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Key Word Index—*Paeonia peregrina*; *Paeoniaceae*; roots; "cage-like" monoterpene glucoside; paeonidanin; paeoniflorigenone; benzoylpaeoniflorin.

Abstract—A new "cage-like" monoterpene glucoside, named paeonidanin, has been isolated from *Paeonia peregrina* roots and its structure established on the basis of spectral evidence. In addition, five known substances, paeoniflorigenone, benzoylpaeoniflorin, benzoic, *p*-hydroxybenzoic and gallic acids, have also been identified.

INTRODUCTION

Paeoniae Radix, derived from the roots of several *Paeoniaceae* species, is one of the most important crude drugs in traditional Chinese medicine, used as an anticoagulant, antiinflammatory, analgesic and sedative agent. It is also frequently used as a remedy for female genital diseases [1, 2]. Roots of *P. peregrina* Mill. (*P. decora* Anders), growing in Bulgaria, are also recommended in Bulgarian folk medicine for similar purposes [3].

Paeonia species are a rich source of monoterpene compounds possessing a "cage-like" pinane skeleton [1, 4–6]. Recently, a new monoterpene paeonisuffrone (**1**) has been isolated from *P. suffriticosa* and its monoacetate (**1a**) and diacetate (**1b**) have been prepared [6].

In the course of our chemical studies on the ethanolic extract of *Paeonia peregrina*, we have isolated a new monoterpene glucoside, named paeonidanin (**2**), together with five known substances. In this paper, we describe the isolation and structural elucidation of the new compound.

RESULTS AND DISCUSSION

The total ethanolic extract of *P. peregrina* roots was fractionated, as described in the Experimental, to give the "cage-like" monoterpenes paeonidanin (**2**), paeoniflorigenone (**3**) and benzoylpaeoniflorin (**4**), as well as benzoic, *p*-hydroxybenzoic and gallic acids. This is the first report on the occurrence of the monoterpenes **3** and **4** and *p*-hydroxybenzoic acid in *P. peregrina*

Monoterpene glucoside **2**, named paeonidanin, could not be isolated as such. It was characterized as its tetraacetate (**2a**), $C_{32}H_{38}O_{15}$, obtained as amorphous powder. The positive FAB-mass spectrum showed a quasimolecular ion at *m/z* 685 $[M+Na]^+$ and fragment ion peaks at *m/z* 331 (a, formed from the glucosyl moiety) and 105 (b, C_6H_5–C≡O$^+$, due to cleavage of the benzoyl ester group). The assignment of all carbons and protons was achieved using 1D- (^{1}H, ^{13}C and DEPT) and 2D-NMR (DQCOSY, TOCSY, NOESY, HMQC and HMQC-TOCSY) techniques. Experimental data are summarized in Tables 1 and 2.

The NMR spectral data (Table 1) of **2a** confirmed the presence of glucosyl and benzoate moieties. The anomeric proton, H-1′, was clearly discerned as a doublet at δ 4.78 (J = 7.9 Hz, δ_C 96.2). In the DQCOSY spectrum, H-1′ correlated with the signal at δ 5.03 from the multiplet belonging to four protons, as seen from the ^{1}H spectrum. The TOCSY's cross-peaks of H-1′ were at δ 5.03 (H-2′), δ 5.14 (H-3′), δ 5.06 (H-4′), δ 3.65 (H-5′) and δ 4.17 (H-6′). In the HMQC spectrum, the first four signals showed correlations with the methine carbons at δ 71.4, δ 71.9, δ 68.3 and δ 72.9, respectively, and the last one, with the methylene carbon at δ 62.0. This assignment was also confirmed by the direct connectivities in the pathway H-6′ → H-5′ → H-4′ → H-3′ → H-2′, revealed in the DQCOSY spectrum. The down-field chemical shift of the glucosidic protons, H-1′–H-6′ implied that the four acetate groups, with resonances at δ 2.0, δ 2.04, δ 2.06 and δ 2.07 in the ^{1}H spectrum and their correlated ^{13}C methyl signals at δ 20.4 (two methyl groups) and δ 20.6, (two methyl groups), as well as

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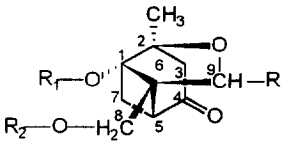
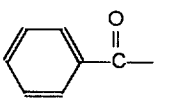
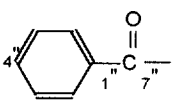
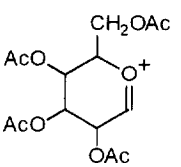
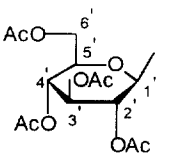


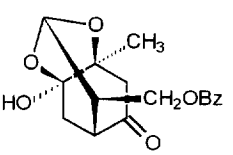
	R	R_1	R_2
1	H	H	H
1a	H	H	Ac
1b	H	Ac	Ac
2	OMe	Glu	
2a	OMe	Glu. 4 Ac	



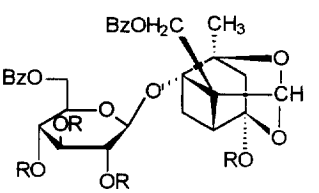
a, m/z 331

Glu. 4 Ac = 

Scheme 1



3



	R
4	H
4a	O Ac

Scheme 2

Table 1. ^1H and ^{13}C NMR chemical shifts (δ) of compound **2a** in $CDCl_3$*

Position	δH	δC	Position	δH	δC
1	—	87.7	1′	4.78 *d* (7.9)	96.2
2	—	85.8	2′	5.03 *m*	71.4
3α	2.63 *d* (18.6)†	48.7	3′	5.14 *t* (7.4, 8.1)	71.9
3β	2.69 *d* (18.6)		4′	5.06 *m*	68.3
4	—	204.8	5′	3.65 *m*	72.9
5	3.04 *d* (7.4)	46.7	6′	4.13 *dd* (5.7, 12.2)	62.0
6	—	62.8	6′	4.19 *dd* (2.6, 12.2)	
7α(ax)	1.95 *d* (overlapped)	26.2	1″	—	128.5
7β(eq)	2.70 *dd* (7.4, 11.1)		2″, 6″	8.01 *dd* (1.4, 8.1)	129.6
8	4.52 *s*	62.1	3″, 5″	7.50 *t* (7.4, 8.1)	128.5
9	5.02 *s*	105.9	4″	7.60 *t* (7.6)	133.4
10	1.41 *s*	20.2	7″	—	166.1
OMe	3.37 *s*	55.7			

* OCOMe—δ_C 169.3 (2 C), δ 170.2 (2 C).
OCOMe—δ_C 20.4 (2 Me), δ 20.6 (2 Me); δ_H 2.00*s*, 2.04*s*, 2.06*s*, 2.07*s*.
† Figures in parentheses, coupling constants in Hz.

Table 2. ^1H, ^1H NMR (TOCSY and NOESY) correlations and ^1H-NOE-difference data of compound **2a***

δ_H/Assignment	TOCSY	NOESY	NOE-difference
1.41/Me-10		2.66/H-3, 4.78/H-1′, 5.03/H-2′	
1.95/H-7ax(α)	2.70/H-7eq(β), 3.04/H-5	2.70/H-7eq(β), 3.04/H-5	
2.66/H-3		1.41/Me-10	1.41/Me-10 (44%)
2.70/H-7eq(β)	1.95/H-7, 3.04/H-5	1.95/H-7, 3.04/H-5, 4.52/H-8, 4.78/H-1′	1.95/H-7 (32%)
3.04/H-5	2.70/H-7	2.70/H-7, 4.52/H-8	4.52/H-8 (4%), 5.02/H-9 (2%)
3.37/OMe		5.02/H-9	1.41/Me-10 (28%), 2.00/OAc-2′ (24%)
3.65/H-5′	4.17/H-6′, 4.78/H-1′, 5.03/H-2′, 5.06/H-4′, 5.14/H-3′	4.17/H-6′, 4.78/H-1′, 5.14/H-3′	
4.52/H-8		2.70/H-7, 3.04/H-5, 4.78/H-1′, 5.02/H-9	2.00/OAc-2′ (26%), 2.70/H-7(2%), 3.04/H-5 (1%), 3.37/OMe (4%), 5.02/H-9 (2%)
4.78/H-1′	3.65/H-5′, 4.17/H-6′, 5.03–5.14/H-2′, H-3′, H-4′	2.70/H-7, 3.65/H-5′, 5.14/H-3′	1.41/Me-10 (42%), 2.00/OAc-2′ (44%), 2.70/H-7 (3%), 3.37/OMe (5%), 3.65/H-5′ (11%)
5.02/H-9		4.52/H-8, 3.37/OMe	3.37/OMe (8%)
5.03–5.14/H-2′, H-3′, H-4′	4.17/H-6′, 4.78/H-1′		1.41/Me-10 (23%), 2.0–2.1/OAc(22%), 2.70/H-7 (3%), 3.37/OMe (2%), 3.65/H-5′ (4%)
7.50/H-3″, 5″	7.60/H-4″, 8.01/H-2″, 6″	7.60/H-4″, 8.01/H-2″, 6″	

* Chemical shifts in δ.

the corresponding ester carbonyl signals at δ 169.3 (two CO) and δ 170.2 (two CO), belonged to the glucose moiety. This was in agreement with fragment ion a observed in the FAB-mass spectrum.

The benzoate moiety, suggested by ion b at *m/z* 105, was supported by the observation of multiplets at δ 8.01 (H-2″ and H-6″), δ 7.60 (H-4″) and δ 7.50 (H-3″ and H-5″). The signal at δ 166.1 was attributed to the benzoyl carbonyl carbon at C-7″.

The ^{13}C NMR spectrum (Table 1) of **2a** revealed the presence of 32 carbon atoms in the molecule. After substraction of the carbon atoms of the glucosyl and benzoyl fragments, 11 carbons were left for the monoterpene aglucone, viz, two methyls, three methylenes, two methines and four quaternary carbons, according to the DEPT experiment. The resonance at δ 1.41 was assigned to the protons of the C-10 methyl attached to quaternary C-2 at δ 85.8, by analogy with the monoterpenes found in *Paeonia* species [1, 2, 4–6]. The ^{13}C-signal at δ 204.8 was assigned to the ketonic carbonyl carbon at C-4. An isolated $CH\text{-}CH_2$ fragment was represented by the proton signals at δ 3.04, 2.70 and

1.95, and the C-5 and C-6 signals at δ 46.7 and 26.2 respectively, as determined by proton–proton and proton–carbon correlations.

The second methylene group (δ_C 48.7) appeared as an ABQ at δ 2.63 and 2.69 (J = 18.6 Hz) in the ^{1}H NMR spectrum, overlapping with the highfield part of the H-7 signal at δ 2.70. Its position at C-3 was fixed on the basis of the observed HMQC-TOCSY correlation from these protons to the C-5 methine carbon at δ 46.7. This position was further confirmed and, by the NOED enhancement observed at δ 1.41 on irradiation of H-3, as well as by the NOESY cross-peak Me-10/H-3 (Table 2). Therefore, the aglucone of paeonidanin tetraacetate appeared to contain one C-(Me)-CH_2-C(O)-CH-CH_2- fragment.

The third methylene group was indicated by the singlet at δ 4.52 (δ_C 62.1) and matched an oxymethylene group attached to a quaternary carbon. The two-bond correlation observed in the HMQC-TOCSY spectrum from the signal at δ 4.52 to the signal of the quaternary carbon at δ 62.8 (C-6) fixed the oxymethylene group at the C-8 position. The occurrence of a benzoyloxy unit attached to an 8-methylene group is a common feature of the natural monoterpenes of the paeoniflorin-type [1].

The presence of —O—CH—O— and OMe groups was suggested by the methine carbon at δ 105.9 (C-9, δ_H 5.02 *s*) and by the methyl carbon at δ 55.7 (δ_H 3.37 *s*, 3H). The three-bond HMQC-TOCSY correlation from the proton signals at δ 3.37 to the carbon signal at δ 105.9 fixed the attachment of OMe to C-9.

The location of the *O*-β-D-glucose unit at C-1 was established unambiguously by the NOE cross-peaks H-1′/H-7β(eq), H-1′/Me-10, H-8/H-1′ and Me-10/H-2′, found in the NOESY spectrum.

The proposed arrangement of all groups and units in the molecule of paeonidanin tetraacetate (**2a**) was supported by the NOE cross-correlations observed in the pairs of protons H-8/H-7β(eq), H-5/H-7, H-5/H-8 and H-8/H-9. Intensive NOEs for Me-10 (28%) and OAc-2′(24%), on irradiation of the OMe signal at δ 3.37, indicated that the points of OMe attachment to the *exo*-side of the molecule, as shown in **2a**. Based on these data, structure **2a** was proposed for paeonidanin tetraacetate and structure **2** for paeonidanin itself.

The absolute stereostructure of paeonisuffrone **1** was recently determined by Yoshikawa *et al.* [6]. The ^{13}C NMR data of **2a** are in agreement with those of paeonisuffrone **1** and its acetates, **1a** and **1b**. Paeonidanin (**2**) is a glucosylated and benzoylated 9-OMe derivative of **1** and, to the best of our knowledge, the first example of the occurrence of a "cage-like" monoterpene aglucone, as a methyl ether, in *Paeonia* species.

EXPERIMENTAL

General

Chemical shifts are expressed in δ relative to TMS as int. standard. NMR were recorded at 250 MHz for ^{1}H and 400 MHz for ^{13}C, using the standard versions of 2D NMR techniques. The mixing period (t_m) in HMQC-TOCSY expts used to detect direct- and long-range proton–carbon correlations was ca 30 ms and 50–65 ms, respectively. FAB-MS: monothioglycerol as matrix. TLC: aluminium sheets, silica gel 60 F_{254} (Merck), bands detected under UV light or by spraying with H_2SO_4 and heating. Prep.TLC: 20 × 20 cm plates coated with 1 mm of silica gel PF_{254} (Merck). CC: silica gel 60, Merck. Vacuum liquid chromatography (VLC): silica gel LS 5–40 μM (Chemapol).

Plant material

Paeonia peregrina Mill. roots were collected in the region of Konevska mountain, Bulgaria. Plant material was authenticated and a voucher specimen (No SO-98485) is deposited at the Department of Biology, Sofia University.

Extraction and isolation

Dried powdered roots (450 g) were extracted with 95% EtOH under reflux (3 × 900 ml, 2 h, 1 h and 1 h). The combined EtOH extracts were concd *in vacuo* to give a dark brown gum (66.5 g). A part of this residue (65.0 g) was subjected to solvent–solvent partition using petrol, $CHCl_3$, Et_2O, EtOAc and *n*-BuOH, and the corresponding petrol (3 g), $CHCl_3$ (0.3 g), Et_2O (3.9 g), EtOAc (4.7 g) and *n*-BuOH (6.0 g) extracts obtained.

VLC of the Et_2O extract (3.8 g) with hexane–Et_2O (1:1), Et_2O, Et_2O–EtOAc (1:1), EtOAc, EtOAc–EtOH (1:1) and EtOH, and TLC comparison of the eluates afforded 15 frs (F1–F15). From F2 (0.15 g, hexane–Et_2O, 1:1) benzoic acid (59.1 mg) was isolated by prep.TLC ($CHCl_3$–MeOH, 15:1) and recrystallization. Fraction F7 (0.17 g, Et_2O) was subjected to a CC on silica gel. Elution with $CHCl_3$-MeOH-H_2O, (7:3:1, lower layer) and combination of similar eluates after TLC comparison afforded 9 frs (P1–P9). Prep.TLC of P1 (0.02 g, $CHCl_3$–MeOH, 20:1) gave paeoniflorigenone **3** (1 mg) and *p*-hydroxybenzoic acid (2.1 mg). Prep.TLC of P2 (18.8 mg) in $CHCl_3$–MeOH, (10:1) produced impure benzoylpaeoniflorin **4** (5.1 mg), which was further purified by acetylation (Ac_2O–pyridine, room temp.) and identified as its tetraacetate **4a**. Prep.TLC of P7–P9 (0.07 g) using $CHCl_3$–MeOH–H_2O, (7:3:1, lower layer) yielded gallic acid (52.4 mg).

VLC of F15 (1.3 g) with $CHCl_3$, $CHCl_3$–MeOH–H_2O (60:15:4, 30:11:2, 6:4:1) and MeOH afforded 20 subfrs (T1–T20). T4 (115 mg) was further subjected to CC using $CHCl_3$–MeOH, (14:1) and similar subfrs containing one main spot combined to obtain an amorphous solid (S, 177 mg). Attempts to isolate a pure substance, paeonidanin (**2**), from S by prep.TLC failed. Acetylation of S (Ac_2O–pyridine, room temp.) and prep.TLC ($CHCl_3$–Me_2CO, 10:1) of the crude

reaction mixt., afforded paeonidanin tetraacetate (**2a**) as an amorphous powder (4.2 mg).

Paeonidanin tetraacetate (**2a**)

Amorphous. ν_{max}^{film} cm^{-1}: 1753–1726 (several bands, C=O), 1600, 1580, 1500, 1440, 1273, 1220. FAB-MS: *m/z* 685 $[M+Na]^+$, 331 (a), 105 (b, C_6H_5–C≡O^+). ^1H and ^{13}C NMR: Table 1.

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