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TWO ACYLATED MONOTERPENE GLUCOSIDES FROM *PAEONIA PEREGINA*

IVANKA N. KOSTOVA,* MARIO F. SIMEONOV, DANIELA I. TODOROVA and PETJA L. PETKOVA

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

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Key Word Index—*Paeonia peregrina*; Paeoniaceae; roots; "cage-like" monoterpenes; paeonidaninols A and B; paeoniflorin; benzoylpaeoniflorin.

Abstract—The roots of *Paeonia peregrina* afforded two new acylated "cage-like" monoterpene glucosides, named paeonidaninols A and B, the known monoterpenes, paeoniflorin and benzoylpaeoniflorin, as well as glucose, galactose and sucrose. The structures of the new compounds were elucidated on the basis of spectral data. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus Paeonia has been reported to produce unique "cage-like" monoterpene glucosides. Recently, various compounds of this type have been isolated, including paeonisuffrone (**1**), paeonidanin (**2**) and many derivatives of paeoniflorin (**3**) [1–3]. The occurrence of these interesting metabolites in this genus prompted us to continue our studies [2] on the roots of *P. peregrina* native to Bulgaria. Our recent investigations resulted in the isolation of two new epimeric "cage-like" monoterpene glucosides, along with the two known monoterpenes, paeoniflorin (**3**) and benzoylpaeoniflorin (**4**), as well as glucose, galactose and sucrose. Here, we describe the isolation and structural elucidation of the new compounds paeonidaninol A (**5a**) and paeonidaninol B (**5b**).

RESULTS AND DISCUSSION

Roots of *P. peregrina* worked-up as described in the Experimental, yielded four "cage-like" monoterpene glucosides, sucrose, glucose and galactose. Two of the monoterpenes proved to be the known compounds paeoniflorin (**3**) and benzoylpaeoniflorin (**4**), whereas **5a** and **5b** have not been described previously.

Paeonidaninol A (**5a**) and paeonidaninol B (**5b**) were isolated as an epimeric mixture of their acetates **6a** and **6b** (see Experimental) in a ratio 1.5:1.0 as deduced from their ^{1}H NMR spectrum. A molecular formula of $C_{38}H_{40}O_{16}$ was established for **6a, b** on the basis of the FAB mass spectrometry ($[M+H]^+$ *m/z* 753) and NMR (^{1}H and ^{13}C) data. Elucidation of the structures of these compounds was achieved by one- and two- dimensional (COSY, HMQC, HMBC and NOESY) NMR studies.

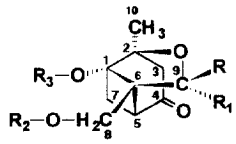
Comparison between the ^{1}H and ^{13}C NMR spectra (Table 1) of **6a, 6b** and paeonidanin tetraacetate (**2a**) [2] showed a close similarity regarding the monoterpene moiety. However, instead of the C-9 methoxyl group in **2a**, an additional OAc appeared in **6a** and **6b**. The presence of OAc at C-9 in the epimeric acetates was suggested by the downfield shift of H-9 (δ 6.28 and 6.12) and confirmed by the HMBC correlation from these protons to the carbonyl signal at δ 170.2 ($\underline{OCO}CH_3$).

The proposed arrangement of the monoterpene nucleus of **6a** and **6b** was further supported by the following long-range heteronuclear shift correlations: (a) from H-9 to C-2 (δ 87.5), C-5 (δ 47.5) and C-6 (δ 62.9), (b) from H-8 to C-1 (δ 87.9), C-5 (δ 47.5), C-6 (δ 62.9) and C-9 (δ 98.3), (c) from Me-10 to C-2 (δ 87.5) and C-3 (δ 48.5), and (d) from H-3, H-5 and H-7 to C-4 (δ 204.9).

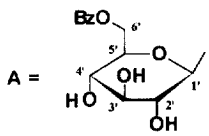
The remaining ^{1}H and ^{13}C NMR signals disclosed the presence of glucose and two benzoyloxy units (Table 1). The HMBC correlation from H-1′ to C-1 (δ_C 87.9) gave evidence for the location of glucose at C-1. The three-bond correlations from H-2′, 3′ and 4′ to the carbon signals at δ 169.7 and 170.2 ($\underline{OCO}CH_3$) suggested the attachment of OAc groups to C-2′, 3′ and 4′, and indicated the position of the benzoyloxy units at C-8 and C-6′.

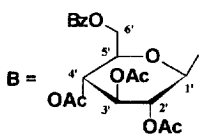
The NOE cross-peak H-9 (δ 6.28)/H-1′ (δ 4.83) observed in the NOESY spectrum of **6a, b** suggested that, in the epimer **6a**, H-9 is directed towards the *exo*-side of the molecule. The observation of this cross-peak also indicated that for **6a** a conformation having

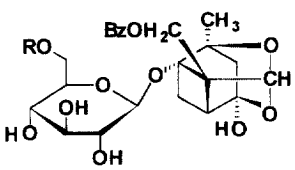
* Author to whom correspondence should be addressed.



	R	R_1	R_2	R_3
1	H	H	H	H
2	OMe	H	Bz	Glu
2a	OMe	H	Bz	Glu.4Ac
5a	H	OH	Bz	A
5b	OH	H	Bz	A
6a	H	OAc	Bz	B
6b	OAc	H	Bz	B







	R
3	H
4	Bz

the 6′-benzoylated glucose unit oriented towards H-9 is predominant. This implied that in the epimer **6b** the OAc-9 points to the *exo*-side of the molecule and, consequently, a change of the preferred conformation of the glucose moiety. The different predominant conformations of the 6′-benzoylated glucose units in **6a** and **6b** could to some extent explain the observed difference in the chemical shifts of H-7 and H-8 for the two epimers.

Based on the above data, structures **6a** and **6b** were deduced for the acetylated epimeric derivatives and structures **5a** and **5b** for the corresponding parent epimers paeonidaninols A and B, respectively.

EXPERIMENTAL

Chemical shifts are expressed in δ relative to TMS as int. standard. Spectra were recorded at 250 MHz and 400 MHz, respectively, using standard versions of 2D NMR techniques. The mixing period (tm) 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: silica gel 60 F_{254} (Merck), bands detected under UV or by spraying with H_2SO_4 and heating. Prep. TLC: 1 mm of silica gel PF_{254} (Merck). CC: silica gel 60, Merck. Vacuum liquid chromatography (VLC): silica gel LS 5–40 μ (Chemapol).

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

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 the crude ethanolic extract as a dark brown gum (66.5 g).

VLC of the crude EtOH extract (8.6 g) using hexane, hexane–EtOAc (1 : 1), EtOAc, EtOAc–EtOH (1 : 1) and EtOH as eluates afforded 12 fractions (F1–F12). F8 (0.32 g, EtOAc) was purified by repeated CC over silica gel and pure paeoniflorin **3** (0.10 g) was

Table 1. ^1H and ^{13}CNMR chemical shifts of compounds **2a**, **6a**, and **6b** in $CDCl_3$

Position	δ_H **6a**	δ_H **6b**	δ_C **6a**	δ_C **6b**	**2a** δ_H	**2a** δ_C
1	—	—	87.9		—	87.7
2	—	—	87.5		—	85.8
3	2.58 *s*	2.57 *s*	48.5		2.63	48.7
4	—	—	204.9		—	204.8
5	3.02 *d* (7.4)	3.05 *d* (7.4)	47.5		3.04 *d* (7.4)	46.7
6	—	—	62.9		—	62.8
7αax	1.95 *d* (overlapped)	2.15 *d* (11.1)	26.2		1.96 *d* (overlapped)	26.2
7βeq	2.74 *dd* (7.4, 11.1)	2.83 *dd* (7.4, 11.1)			2.70 *dd* (7.4, 11.1)	
8a	4.61 *d* (11.8)	4.71 *d* (11.8)	61.9		4.52 *s*	62.1
8b	4.48 *d* (11.8)	4.49 *d* (11.8)				
9	6.28 *s*	6.12 *s*	98.3	95.2	5.02 *s*	105.9
10	1.37 *s*	1.42 *s*	20.1		1.41	20.2
OMe					3.37 *s*	55.7
1′	4.83 *d* (7.8)	4.82 *d* (7.8)	96.2		4.78 *d* (7.9)	96.2
2′	5.05 *m* (overlapped)	5.09 *m*	71.3		5.03 *m*	71.4
3′	5.23 *t* (9.3)	5.30 *t* (9.3)	72.0		5.14 *t* (7.4, 8.1)	71.9
4′	5.12 *m*	5.17 *m*	68.6		5.06 *m*	68.3
5′	3.82 *m*	3.87 *m*	72.7		3.65 *m*	72.9
6′a	4.38 *dd* (6.0, 11.9)	4.41 *dd* (6.0, 11.9)	62.3		4.13 *dd* (5.7, 12.2)	62.0
6′b	4.51 *dd* (2.5, 11.9)	4.54 *dd* (2.5, 11.9)			4.19 *dd* (2.6, 12.2)	
1″	—	—	128.5			128.5
2″, 6″	8.01 *dd* (1.5, 8.2)	8.02 *dd* (1.2, 8.2)	129.5		8.01 *d* (1.4, 8.1)	129.6
3″, 5″	7.44 *bt* (8.1)	7.44 *bt* (8.1)	128.5		7.50 *t* (7.4, 8.1)	128.5
4″	7.59 *bt* (8.1)	7.59 *bt* (8.1)	133.6		7.60 *t* (7.6)	133.4
7″	—	—	166.1			166.1
1‴	—	—	128.5			
2‴, 6‴	7.92 *dd* (1.2, 8.2)	7.92 *dd* (1.2, 8.2)	129.5			
3‴, 5‴	7.44 *bt* (8.1)	7.44 *bt* (8.1)	128.5			
4‴	7.59 *bt* (8.1)	7.59 *bt* (8.1)	133.6			
7‴	—	—	166.1			
OAc	2.06 *s*, 2.05 *s*, 2.01 *s*, 1.94 *s*	2.17 *s*, 2.07 *s*, 2.04 *s*, 1.98 *s*			2.07 *s*, 2.06 *s*, 2.04 *s*, 2.0 *s*	

For **6a** and **6b**: OCOMe-δ_C 170.2, 169.7; OCOMe-δ_C 20.9, 20.6.

obtained. F9 (0.37 g, EtOAc–EtOH, 1 : 1) was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (3 : 11 : 2) and 13 subfrs (S1–S13) collected. S5 (96.6 mg) proved to be a mixt. (45.6 mg) of glucose and galactose in a ratio 1 : 1.5 by ^1H NMR and direct comparison with authentic samples. S7 (15.0 mg) on prep. TLC ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1; multiple development) yielded pure sucrose (5.2 mg).

A part of the crude EtOH extract (20.0 g) was subjected to a solvent–solvent partition using petrol, $CHCl_3$, Et_2O, EtOAc and BuOH, and the corresponding extracts obtained. The Et_2O extract (3.8 g) was worked-up as previously described [2] by LVC and 14 frs (L1–L14) collected. L7 (Et_2O, 0.17 g) on CC over silica gel and elution with $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer) afforded 9 subfrs (M1–M9). Prep. TLC of M3 (14.3 mg) in $CHCl_3$–MeOH (10 : 1) produced benzoylpaeoniflorin **4** (2.0 mg) and impure **5a, b** (5.3 mg).

Attempts to purify **5a, b** by prep. TLC failed. Acetylation of **5a, b** (Ac_2O-pyridine), room temp. and prep. TLC of the reaction product resulted in the isolation of the tetraacetate **6a, b** (1.7 mg) as an amorphous powder.

Mixture of paeonidaninol-A tetraacetate and paeonidaninol-B tetraacetate (**6a, b**)

Amorphous. IR ν_{max}^{film} cm^{-1} 1751–1723 (several bands, C=O), 1600, 1580, 1490, 1450, 1250. FAB-MS *m/z*: 753 $[M+H]^+$. ^1H and ^{13}C NMR: Table 1.

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