Paeonins A and B, Lipoxygenase Inhibiting Monoterpene Galactosides from *Paeonia emodi*

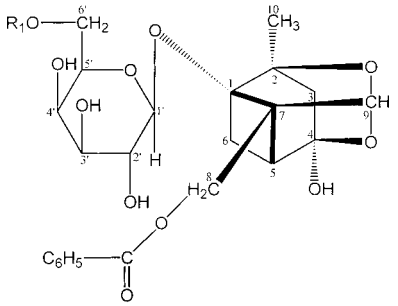
Naheed Riaz, Itrat Anis, Abdul Malik,* Zaheer Ahmed, Aziz-ur-rehman, Pir Muhammad, Sarfraz Ahmad Nawaz, and Muhammad Iqbal Choudhary

International Centre for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi; Karachi-75270, Pakistan. Received September 20, 2002; accepted November 8, 2002

Paeonins A and B, new monoterpene galactosides have been isolated from the chloroform-soluble fraction of the roots of *Paeonia emodi* and showed potent lipoxygenase inhibitory activity. The structures of 1 and 2 have been assigned on the basis of spectral analysis including one- and two-dimensional NMR techniques.

Key words *Paeonia emodi*; Paeoniaceae; monoterpene; enzyme inhibition

The genus *Paeonia* (Paeoniaceae) comprises 33 species, which are distributed in Pakistan, India, and Afghanistan. They have great medicinal importance. The underground tubers are used in nervous diseases, uterine diseases, colic, bilious obstructions, dropsy, epilepsy, convulsions, and hysteria. The dried flowers are used against diarrhea. The seeds are purgative and emetic.[1,2] The whole plants of various *Paeonia* species are used for the treatment of vomiting, cholera, tuberculosis, and eye diseases and as anticoagulants and emmenagogues.[3,4] *Paeonia emodi* is found in northern areas of Pakistan. Previously monoterpene glycosides[5] and a triterpene[6] have been reported from this species. In the present investigation, a methanolic extract of the roots of *P. emodi* showed positive activity in the brine shrimp lethality test.[7] Further biological screening of the methanolic extract revealed significant inhibitory activity against the enzyme lipoxygenase. This prompted us to carry out bioassay-directed isolation studies on this plant. Here we report the isolation and structure elucidation of two new monoterpene galactosides called paeonins A and B.



1 R_1 = COPh
2 R_1 = H

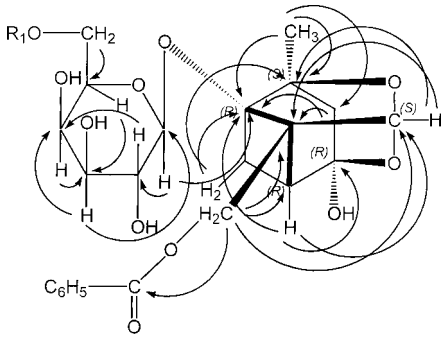
Results and Discussion

Paeonin A (**1**) was isolated as colorless gummy solid. The high-resolution (HR) FAB-MS established the molecular formula to be $C_{30}H_{32}O_{12}$, showing a $[M-H]^+$ peak at *m/z* 583.1898 (Calcd for $C_{30}H_{31}O_{12}$, 583.1893). The IR spectrum showed the absorption band due to hydroxyl groups (3545—3402 cm^{-1}) and ester carbonyl (1724 cm^{-1}). The UV absorption showed λ_{max} at 276 and 236 nm. The ^{1}H- and ^{13}C-NMR spectra showed a striking resemblance to those of benzoyl paeoniflorin,[8] with common signals of a monoterpene system and the benzoyl moiety. The signals of the sugar moiety appeared at δ 4.57 (1H, d, J=7.5 Hz), 3.29 (1H, t, J=7.5 Hz, H-2′), 3.38 (1H, t, J=7.5 Hz, H-3′), 3.30 (1H, t, J=1.5 Hz, H-4′), and 3.60 (1H, br t, J=7.1 Hz, H-5′) and methylene protons at δ 4.65 (1H, dd, J=11.5, 1.9 Hz) and 4.48 (1H, dd, J=11.5, 7.1 Hz). The acid hydrolysis of **1** provided various products, among which the glycone could be separated and identified as D-galactose through its optical rotation sign and comparison of the retention time of its trimethylsilyl (TMS) ether with that of the standard in gas chromatography (GC). The ^{1}H-NMR spectrum showed the presence of a tertiary methyl at δ 1.46 (s, 10-H_3), two methylenes [δ 2.05 (d, J=12.5 Hz), 2.42 (d, J=12.5 Hz) (3-H_2); 1.80 (d, J=10.9 Hz), 2.48 (dd, J=10.9, 6.6 Hz) (6-H_2)] and oxymethylene protons at δ 4.72 (1H, d, J=12.1 Hz) and 4.59 (1H, d, J=12.1 Hz). A doublet at δ 2.60 (1H, J=6.6 Hz) was ascribed to H-5, while the singlet at δ 5.43 (1H) was attributed to H-9. The remaining signals were due to two benzoyloxy groups showing triplets at δ 7.46 (4H, J=7.5 Hz) for H-3″ and H-3‴ and H-5″ and H-5‴, while another triplet at δ 7.60 (2H, J=7.5 Hz) was assigned to H-4″ and H-4‴. The doublet at δ 8.02 (4H, J=7.5 Hz) was due to H-2″, H-2‴, H-6″, and H-6‴. The presence of benzoyloxy moieties was evident from UV absorption at 276 nm and further confirmed by electron ionization (EI)-MS showing peaks at *m/z* 122 due to the benzoic acid moiety and *m/z* 301 due to the loss of the benzoylated sugar. The broadband and distortionless enhancement by polarization transfer (DEPT) ^{13}C-NMR spectra of **1** corroborated the presence of one methyl, four methylenes, 17 methines and eight quaternary carbons. The downfield signals at δ 167.7 and 168.0 were assigned to ester functionalities, while another downfield signal at δ 106.2 was assigned to C-4. The position of the benzoyloxy and galactose moieties was confirmed by heteronuclear multiple-bond connectivity (HMBC) experiments; the important correlations are illustrated in Fig. 1. The structure was further confirmed by correlation spectroscopy (COSY) experiments. The stereochemistry at various stereocenters of the monoterpene unit was assigned on the basis of similarity of spectral data with related compounds[9—13] and further confirmed through nuclear Overhauser enhancement and exchange spectroscopy (NOESY) correlations, which are described in Table 1 and showed close agreement with those of paeoniflorin.[10] On the basis of this evidence paeonin A was assigned the structure **1**.

Paeonin B (**2**) was also isolated as colorless gummy solid. The negative FAB-MS showed a peak at *m/z* 479 $[M^+-H]$

* To whom correspondence should be addressed. e-mail: hejric@digicom.net.pk

which confirmed the molecular formula to be $C_{23}H_{28}O_{11}$. The IR, UV, ^{1}H- and ^{13}C-NMR spectra of **2** were almost identical to those of **1** except for the absence of one benzoyl moiety at the C-6′ position of galactose in **1**. This was confirmed by ^{13}C-NMR spectroscopy in which the methylene carbon appeared comparatively upfield at δ 62.2. The ^{13}C-NMR spectrum also showed only one signal of the carbonyl group at δ 168.2 (C-7″). These assignments were further confirmed by COSY, ^{1}H-detected hetronuclear multiple-quantum coherence (HMQC), and HMBC experiments. The HMBC interactions were similar to those of **1**, allowing us to assign structure **2** to paeonin B.



1 R_1 = COPh
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Fig. 1. Important HMBC Interactions of Compounds **1** and **2**

Arachidonic acid metabolism through lipoxygenase pathways generates various biologically active lipids that play important roles in thrombosis and tumor progression. Angiogenesis, the formation of new capillary vessels from preexisting ones, underpins a number of physiological processes and participates in the development of several pathological conditions such as arthritis and cancer.[14] Lipoxygenases are therefore attractive targets for rational drug design and the discovery of mechanism-based inhibitors for the treatment of a variety of disorders such as bronchial asthma, inflammation, cancer, and autoimmune diseases.

The inhibitory activity of **1** and **2** against lipoxygenase was determined using the method described in the Experimental section. The IC_{50} values of **1** and **2** were found to be 66.1 μM and 56.9 μM, respectively. Both compounds **1** and **2** are potent inhibitors of lipoxygenase. Compound **2** has greater inhibitory potential than **1**. Baicalein (Aldrich Chemical Co.) was used as a positive control, and the IC_{50} values are shown in Table 2.

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data and NOESY Correlations of Compounds **1** and **2**

Compound **1**				Compound **2**			
C	δ_C	δ_H [a]	NOESY	C	δ_C	δ_H [a]	NOESY
1	89.3			1	89.4		
2	87.1			2	87.3		
3	44.5	2.05 d (12.5)	H3a/H3b, 10	3	44.6	2.06 d (12.7)	H3a/H3b, 10
		2.42 d (12.5)	H3b/H3a, 6, 10			2.43 d (12.7)	H3b/H3a, 6, 10
4	106.2			4	106.4		
5	43.9	2.60 br d (6.6)	H5/H3b	5	44.0	2.59 br d (6.7)	H5/H3b
6	23.1	1.8 br d (10.9)		6	23.5	1.82 br d (11.0)	
		2.48 dd (10.9, 6.6)				2.50 dd (11.0, 6.7)	
7	72.1			7	72.3		
8	61.6	4.59 d (12.1)	H8/H3b, H9	8	61.8	4.58 d (12.2)	H8/H3b, H9
		4.72 d (12.1)				4.73 d (12.2)	
9	102.3	5.43, s		9	102.5	5.41, s	
10	19.5	1.46, s		10	19.7	1.48, s	
1′	100.1	4.57 d (7.5)	H1′/H8a, H8b	1′	100.2	4.53 d (7.6)	H1′/H8a, H8b
2′	74.8	3.29 t (7.5)		2′	75.0	3.28 t (7.5)	
3′	77.9	3.38 br t (7.5)	H3′/H6′a, H6b′	3′	78.1	3.35 br t (7.5)	H3′/H6′a, H6b′
4′	71.8	3.30 t (1.5)		4′	71.8	3.29 t (1.6)	
5′	75.3	3.60 br t (7.1)	H5′/H1′, H6′a, H6b′	5′	75.2	3.60 br t (7.2)	H5′/H1′, H6′a, H6b′
6′	65.2	4.48 dd (11.5, 7.1)	H6′a, H6′b/H3′, H5′	6′	62.2	4.48 dd (12.1, 7.2)	H6′a, H6′b/H3′, H5′
		4.6 dd (11.5, 1.9)				4.65 dd (12.1, 1.9)	
1″	131.4			1″	131.2		
2″	130.7	8.02 d [b] (7.5)	H2″/H3″	2″	130.8	8.05 d [e] (7.6)	H2″/H3″
3″	129.7	7.46 t [c] (7.5)		3″	129.6	7.50 t [f] (7.6)	
4″	134.5	7.60 t [d] (7.5)	H4″/H3″, 5″	4″	134.8	7.62 t (7.6)	H4″/H3″, 5″
5″	129.7	7.46 t [c] (7.5)	H5″/H6″	5″	129.6	7.50 t [f] (7.6)	H5″/H6″
6″	130.7	8.02 d [b] (7.5)		6″	130.8	8.05 d [e] (7.6)	
7″	168.0			7″	168.2		
1‴	131.2						
2‴	130.5	8.02 d [b] (7.5)	H2‴/H3‴				
3‴	129.6	7.46 t [c] (7.5)					
4‴	134.4	7.60 t [d] (7.5)	H4‴/H3‴, 5‴				
5‴	129.6	7.46 t [c] (7.5)	H5‴/H6‴				
6‴	130.5	8.02 d [b] (7.5)					
7‴	167.7						

a) Coupling constants (*J* in Hz) are in parentheses. *b*—*f*) Signals were overlapping.

Table 2. *In Vitro* Quantitative Inhibition of Lipoxygenase by Compounds **1** and **2**

Compound	IC_{50} (μM)±S.E.M.[a)]
1	66.1±5.0
2	56.9±3.0
Paeoniflorin	95.1±5.0
Baicalein	22.4±1.3

a) S.E.M.±standard mean error of five assays.

Experimental

General Optical rotations were measured on 'a' JASCO DIP-360 polarimeter. IR spectra were recorded on 'a' 460 Shimadzu spectrometer. EI-MS and HR-FAB-MS were recorded on JMS-HX-110 with a data system and on JMS-DA 500 mass spectrometers. The ^{1}H- and ^{13}C-NMR, HMQC, and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for ^{1}H- and 100.6 MHz for ^{13}C-NMR, respectively. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz. Aluminum sheets precoated with silicagel 60 F_{254} (20×20 cm, 0.2 mm thick; E-Merck) were used for TLC and flash silica (230—400 mesh) was used for column chromatography. Visualization of the TLC plates was carried out under UV at 254 and 366 nm and by spraying with ceric sulfate reagent solution (with heating). For enzyme inhibition assay, all chemicals used and lipoxygenase (1.13.11.12) type I-B were purchased from Sigma (St. Louis, MO, U.S.A.). The GC was performed on a Shimadzu gas chromatograph (GC-9A) (3% OV-1 silanized *chromosorb W*, column temperature 180 °C, injection port and detector temperature 275—300 °C, flow rate 35 ml/min, flame-ionization detector).

Plant Material The roots of *P. emodi* (15 kg) were collected from Swat (Pakistan) in July 2001 and identified by Mr. Habib Ahmed, Plant Taxonomist, Government Post Graduate College Swat, where a voucher specimen is deposited.

Extraction and Isolation The roots of *P. emodi* (15 kg) were shade-dried, ground, and extracted with methanol. The residue from the methanolic extract was partitioned between hexane and H_2O. The H_2O fraction was further extracted with $CHCl_3$ and EtOAc. The $CHCl_3$ fraction (45 g) was subjected to column chromatography over flash silica eluted with hexane–$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH, and MeOH in increasing order of polarity. The fractions, which were obtained from $CHCl_3$–MeOH (90 : 10), were combined and rechromatographed over flash silica eluted with $CHCl_3$–MeOH in increasing order of polarity. The fractions obtained in $CHCl_3$–MeOH (88 : 12) were subjected to preparative TLC ($CHCl_3$: MeOH : H_2O; 80 : 19.8 : 0.2) to afford the pure compounds **1** (28 mg) and **2** (33 mg).

Paeonin A (**1**): Colorless gummy solid. HR-FAB-MS: *m*/*z* 583.1898 (Calcd for $C_{30}H_{31}O_{12}$, 583.1893) $[M-H]^+$. UV λ_{max} (MeOH) nm (log ε): 276 (3.92), 236 (3.98). IR ν_{max} cm^{-1}: 3545—3402, 1725. EI-MS *m*/*z*: 301 (5), 178 (30), 122 (53), 105 (100), 77 (73). $[\alpha]_D^{25}$ +10° (*c*=0.012, MeOH). ^{1}H- and ^{13}C-NMR data of **1** are shown in Table 1.

Paeonin B (**2**): Colorless gummy solid. HR-FAB-MS: *m*/*z* 479.1556 (Calcd for $C_{23}H_{27}O_{11}$, 479.1553 $[M-H]^+$. UV λ_{max} (MeOH) nm (log ε): 275 (4.08), 228 (4.12). IR ν_{max} cm^{-1}: 3545—3402, 1725. EI-MS *m*/*z*: 301 (8), 178 (52), 122 (40), 105 (100), 77 (81). $[\alpha]_D^{25}$ +2.5° (*c*=0.016, MeOH). ^{1}H- and ^{13}C-NMR data of **2** are shown in Table 1.

Acid Hydrolysis of Compounds 1 and 2 A solution of **1** or **2** (8 mg) in MeOH (5 ml) containing 1 N HCl (4 ml) was refluxed for 4 h, concentrated under reduced pressure, and diluted with H_2O (8 ml). It was extracted with EtOAc and the residue recovered from the organic phase was found to be an inseperatable mixture of products. The aqueous phase was concentrated and D-galactose was identified by the sign of its optical rotation ($[\alpha]_D^{20}$ +80.1°). It was also confirmed based on the retention time of its TMS ether (α-anomer 3.8 min, β-anomer 5.2 min) with a standard.

Assay of Lipoxygenase Inhibition Lipoxygenase-inhibiting activity was conveniently measured by slightly modifying the spectrometric method developed by Tappel.[15)] Lipoxygenase (1.13.11.12) type I-B and linoleic acid were purchased from Sigma Chemicals. A mixture of 160 μl of 0.1 mM sodium phosphate buffer (pH 7.0), 10 μl of test compound solution, and 20 μl of lipoxygenase solution was incubated for 5 min. at 25 °C. The reaction was then initiated by the addition of linoleic acid 10 μl (substrate) solution, with the formation of (9*Z*,11*E*,13*S*)-13-hydroperoxyoctadeca-9,11-dienoate. The change in absorbance was followed for 10 min. Test compounds and the control were dissolved in 50% EtOH. All the reactions were performed in triplicate. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, MA, U.S.A.).

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