Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 65 (2004) 1129–1135
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Lipoxygenase inhibiting and antioxidant oligostilbene and monoterpene galactoside from *Paeonia emodi*

Naheed Riaz, Abdul Malik*, Aziz-ur Rehman, Zaheer Ahmed, Pir Muhammad, Sarfraz Ahmad Nawaz, Juveria Siddiqui, Muhammad Iqbal Choudhary

International Centre for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

Received 30 June 2003; received in revised form 8 September 2003

Abstract

Paeoninol and paeonin C, oligostilbene and monoterpene galactoside, have been isolated from the methanolic extract of the fruits of *Paeonia emodi*. Their structures have been assigned on the basis of spectral analysis including 1D and 2D NMR techniques. In addition, 4-hydroxybenzoic acid **3**, gallic acid **4** and methyl gallate **5** have also been reported for the first time from this species. Compounds **1** and **2** have displayed potent inhibitory potential against enzyme lipoxygenase in a concentration-dependent fashion with the IC_{50} values 0.77 and 99.5 μM, along with $ABTS^{\cdot+}$ radical quenching activity with IC_{50} values of 147.5 and 498.2 μM, respectively.

Keywords: *Paeonia emodi*; Paeoniaceae; Oligostilbene; Monoterpene; Enzyme inhibition

1. Introduction

The genus *Paeonia* (Paeoniaceae) comprises 33 species, which are distributed in Pakistan, India and Afghanistan. It comprises perennial herbs or shrubs with alternate extipulate leaves. They have great medicinal importance. The underground tubers are used in nervous diseases, uterine diseases, colic, bilious obstructions, dropsy, epilepsy, convulsions and hysteria (Nasir and Ali, 1978). The dried flowers are used for stomach complaints (Zaheer, 1966). The whole plants of various *Paeonia* species are used for the treatment of vomiting, cholera, tuberculosis, and eye diseases and as anticoagulants and emmenagogues. The seeds are purgative and emetic (Perry and Metzger, 1980). Various constituents isolated from *Paeonia* species have shown sedative, anti-inflammatory activities and are used for blocking effect on neuromuscular junctions (Yu et al., 1990). *Paeonia emodi* is found in northern areas of Pakistan. Previously monoterpene glycosides (Muhammad et al., 1999; Riaz et al., 2003) and a triterpene (Nawaz et al., 2000) have been reported from the roots of this species. In the present investigation, a methanolic extract of the fruits of *P. emodi* showed positive activity in the brine shrimp lethality test (Meyer et al., 1982). 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. Herein we report the isolation and structure elucidation of Paeoninol, an oligostilbene and paeonin C, the monoterpene galactoside, from the methanolic extract. In addition 4-hydroxy benzoic acid **3**, gallic acid **4** and methyl gallate **5** have been reported for the first time from this species.

2. Results and discussion

The crude MeOH extract of the fruits of *P. emodi* was subjected to column chromatography over flash silica with different mobile phases. Compounds **1**–**5** were finally obtained and their structures were established by UV, IR, mass, and NMR spectroscopy. Paeoninol **1** was isolated as a brownish gummy solid. The molecular

* Corresponding author. Tel.: +92-21-924-3198; fax: +92-21-924-3190.
E-mail address: hejric@digicom.net.pk or hej@digicon.net.pk (A. Malik).

0031-9422/$ - see front matter © 2003 Published by Elsevier Ltd.
doi:10.1016/j.phytochem.2003.10.012

formula $C_{42}H_{32}O_9$, was determined by the data of the positive-ion FABMS, showing $[M+H]^+$ ion at m/z 681 and the negative-ion FABMS, showing $[M-H]^+$ ion at m/z 679. The IR bands (3395 and 1612 cm^{-1}) and UV absorptions (226, 277 and 283 nm) indicated the presence of phenolic chromophores, which are characteristic features for oligostilbenes (Kawabata et al., 1991). The ^1H NMR spectrum of **1** (Table 1); showed characteristic signals for a dihydrofuran moiety bearing 3,5-dihydroxyphenyl and 4-hydroxyphenyl groups which is a common feature of oligostilbenes (Oshima et al., 1990) [δ 5.40 (2H, *d*, $J=5.6$ Hz) and 4.40 (2H, *d*, $J=5.6$ Hz), four sets of *ortho*-coulpled protons for 4-hydroxyphenyl groups at δ 7.19 (4H, *d*, $J=8.5$ Hz), 6.79 (4H, *d*, $J=8.5$ Hz), 6.67 (2H, *d*, $J=8.5$ Hz) and 6.50 (2H, *d*, $J=8.5$ Hz)]. A broad singlet at δ 6.13 (6H) could be assigned to the olefinic protons of 3,5-dihydroxyphenyl group. A singlet at δ 6.41 and olefinic protons at δ 5.93 (1H, *d*, $J=11.9$ Hz) and 5.80 (1H, *d*, $J=11.9$ Hz) were also observed in the spectrum. The ^{13}C NMR spectrum (BB and DEPT) showed 20 carbon signals (Table 1); four oxygenated aromatic quaternary carbons (δ_C 158.3–162.9), five aromatic quaternary carbons (δ_C 120.3–147.5), nine olefinic methine carbons (δ_C 91.4–132.3), and two dihydrofuran methine carbons (δ_C 94.8) and (δ_C 58.9). The presence of a highly deshielded oxymethine ($\delta_H=5.40$, $\delta_C=94.8$) in the spectra was indicative of a dihydrofuran ring system in the molecule (Oshima and Ueno, 1993; Sarker et al., 1999; Sotheeswaran et al., 1985). The substitutions and linkages at various positions of the trimer was confirmed by long range HMBC experiments; the important correlations are illustrated in Table 1. The structure was further confirmed by ^1H–^1H COSY spectrum, it was possible to establish the proton sequences from H-2 to H-3, H-7 to H-8, H-2′ to H-3′, H-7′ to H-8′, H-2″ to H-3″ and H-7″ to H-8″. The relative configuration of the methine hydrogens on the dihydrofuran ring was established by ^1H NMR (through coupling constants) and NOESY experiments; the important NOESY interactions are shown in the Table 1. On the basis of these evidences, the structure 1 could be assigned to paeoninol.

Paeonin C **2** was isolated as colorless gummy solid. The molecular formula $C_{23}H_{28}O_{12}$, was determined by

Table 1
^1H and ^{13}C NMR data HMBC and NOESY correlations of compound **1** (CD_3OD)

Position	^1H multiplicity J (Hz)[a]	^{13}C (DEPT)[b]	HMBC (H→C)[c]	NOESY[d]
1		133.7 (C)		
2, 6	7.19 *d* (8.5)	128.0 (CH)	C-3/C-5, C-4, C-7	H-3, H-5
3, 5	6.79 *d* (8.5)	116.3 (CH)	C-1, C-2/C-6, C-4	H-2, H-6
4		158.5 (C)		
7	5.40 *d* (5.6)	94.8 (CH)	C-1, C-2/C-6, C-8, C-9, C-10′, C-11′	H-8
8	4.40 *d* (5.6)	58.9 (CH)	C-1, C-7, C-9, C-10/C-14, C-10, C-11	H-7
9		147.5 (C)		
10, 14	6.13 *br s*	107.3 (CH)	C-8, C-9, C-11/C-13, C-12	
11, 13		160.0 (C)		
12	6.13 *br s*	102.1 (C)	C-10/C-14, C-11/C-13	
1′		130.6 (C)		
2′, 6′	6.67 *d* (8.5)	128.6 (CH)	C-1′, C-3′/C-5′, C-4′, C-7′	H-3′, H-5′, H-12′
3′, 5′	6.50 *d* (8.5)	116.1 (CH)	C-1′, C-2′/C-6′, C-4′	H-2′, H-6′, H-12′
4′		158.3 (C)		
7′	5.80 *d* (11.9)	124.3 (CH)	C-1′, C-8′	
8′	5.93 *d* (11.9)	132.3 (CH)	C-1′, C-7′, C-9′	
9′		134.1 (C)		
10′, 14′		120.3 (C)		
11′, 13′		162.9 (C)		
12′	6.41 *s*	91.4 (CH)	C-11′, C-13′	H-6′, H-2′
1″		133.7 (CH)		
2″, 6″	7.19 *d* (8.5)	128.0 (CH)	C-3″/C-5″, C-4″, C-7″	H-3″, H-5″
3″, 5″	6.79 *d* (8.5)	116.3 (CH)	C-1″, C-2″/C-6″C-4″	H-2″, H-6″
4″		158.5 (C)		
7″	5.40 *d* (5.6)	94.8 (CH)	C-1″, C-2″/C-6″, C-8″, C-9″, C-13′, C-14′	H-8″
8″	4.40 *d* (5.6)	58.9 (CH)	C-1″, C-7″, C-9″, C-10″/C-14″, C-13′, C-14′	H-7″
9″		147.5 (C)		
10″, 14″	6.13 *br s*	107.3 (CH)	C-8″, C-9″, C-11″/C-13″, C-12″	
11″, 13″		160.0 (C)		
12″	6.13 *br s*	102.1 (CH)	C-10″/C-14″, C-11″, C-13″	

[a] ^1H NMR carried out at 400 MHz.
[b] ^{13}C NMR carried out at 100 MHz.
[c] HMBC carried out at 400 MHz.
[d] NOESY carried out at 400 MHz.

the data of the negative-ion FABMS, showing the $[M-H]^+$ ion at 495. The IR spectrum showed the absorption due to hydroxyl groups (3544–3405 cm^{-1}), ester carbonyl (1724 cm^{-1}) and UV absorptions at (277 and 236 nm). The ^1H and ^{13}C NMR spectra (Table 2) showed a striking resemblance to oxypaeoniflorin, (Lin et al., 1996), with common signals of monoterpene system and oxybenzoyl moiety. The signals of the sugar moiety appeared at δ 4.52 (1H, *d*, $J=7.5$ Hz), 3.29 (1H, *t*, $J=7.5$ Hz), 3.38 (1H, *t*, $J=7.5$ Hz), 3.33 (1H, *t*, $J=1.5$ Hz), and 3.60 (1H, *br t*, $J=7.1$ Hz) and methylene protons at δ 3.85 (1H, *dd*, $J=11.5$, 1.9 Hz) and 3.65 (1H, *dd*, $J=11.5$, 7.1 Hz). The acid hydrolysis of **2** 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 a standard in gas chromatography (GC). The ^1H NMR spectrum showed the presence of a tertiary methyl at δ 1.47 (s), two methylenes [δ 2.05 (*d*, $J=12.5$ Hz), 2.42 (*d*, $J=12.5$ Hz), 1.80 (*d*, $J=10.9$ Hz), 2.48 (*dd*, $J=10.9$, 6.6 Hz) and oxymethylene protons at δ 4.71 (1H, *d*, $J=12.1$ Hz) and 4.65 (1H, *d*, $J=12.1$ Hz). A doublet at δ 2.60 (1H, $J=6.6$ Hz) and a singlet at δ 5.38 (1H) were also observed in the spectrum. The remaining signals were due to a 4-hydroxybenzoyl moiety showing two doublets at δ 7.91 (2H, *d*, $J=8.6$ Hz) and 6.85 (2H, *d*, $J=8.6$ Hz). The presence of a 4-hydroxybenzoyl fragment was evident from the UV absorption at 282 nm and further confirmed by EIMS showing peak at *m*/*z* 138 due to the 4-hydroxybenzoic acid moiety. The ^{13}C NMR spectrum (BB and DEPT) showed 21 carbon signals (Table 2); including one methyl, four methylenes, nine methines and seven quaternary carbons of which the signal at δ 168.0 was assigned to an ester functionality, while other downfield signals at δ 163.7 and 106.4 were due to an oxygenated aromatic quaternary carbon and a dioxygenated quaternary carbon respectively. The position of the oxybenzoyl and galactose moieties was confirmed by HMBC experiments; the important correlations are illustrated in Fig. 1. The structure was further confirmed by ^1H–^1H COSY experiments. The stereochemistry at various stereocenters of the monoterpene unit was assigned on the basis of similarity of

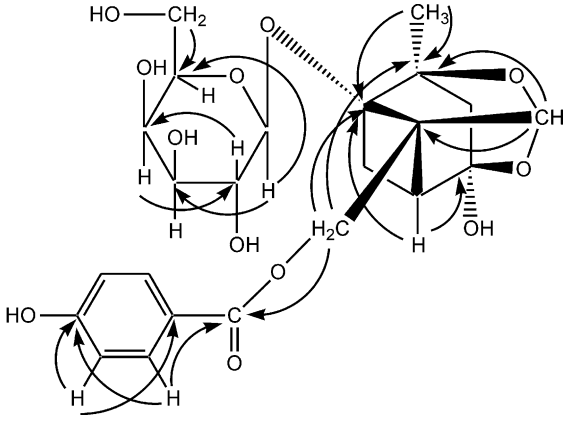

Fig. 1. Important HMBC correlations of **2**.

Table 2
^1H and ^{13}C NMR data and NOESY correlations of compound **2** (CD_3OD)

Position	^1H multiplicity *J* (Hz)[a]	^{13}C (DEPT)[b]	NOESY[c]
1		89.3 (C)	
2		87.2 (C)	
3	2.05 *d* (12.5), 2.42 *d* (12.5)	44.5 (CH_2)	H3a/H3b, 10 H3b/H3a, 6, 10
4		106.4 (C)	
5	2.60 *br d* (6.6)	43.9 (CH)	H5/H3b
6	1.80 *br d* (10.9), 2.48 *dd* (10.9, 6.6)	23.7 (CH_2)	
7		72.0 (C)	
8	4.71 *d* (12.1), 4.65 *d* (12.1)	61.2 (CH_2)	H8/H3b, H3a
9	5.38 *s*	102.1 (CH)	
10	1.47 *s*	19.7 (CH_3)	
1′	4.52 *d* (7.5)	99.8 (CH)	H1′/H8a, H8b
2′	3.29 *t* (7.5)	74.5 (CH)	
3′	3.38 *t* (7.5)	77.2 (CH)	H3′/H6a, H6b
4′	3.33 *t* (1.5)	70.9 (CH)	
5′	3.60 *t* (7.1)	77.3 (CH)	H5′/H1′, H6a, H6b
6′	3.85 *dd* (11.5, 1.9) 3.65 *dd* (11.5, 7.1)	61.5 (CH_2)	H6′a, H6′b/H3′, H5′
1″		120.5 (C)	
2″, 6″	7.91 *d* (8.6)	132.9 (CH)	H2″/H3″
3″, 5″	6.85 *d* (8.6)	116.9 (CH)	H5″/H6″
4″		163.7 (C)	
7″		168.0 (C)	

[a] ^1H NMR carried out at 400 MHz.
[b] ^{13}C NMR carried out at 100 MHz.
[c] NOESY carried out at 400 MHz.

spectral data with related compounds (Murakami et al., 1996; Ding et al., 1999) and NOESY correlations, which are described in Table 2. On the basis of these evidences the structure **2** could be assigned to paeonin C.

Lipoxygenases (EC 1.13.11.12) constitute a family of non-haem iron containing dioxygenases that are widely distributed in animals and plants. In mammalian cells these are key enzymes in the biosynthesis of variety of bioregulatory compounds such as hydroxy-eicosatetraenoic acids (HETEs), leukotrienes, lipoxins and hepoxylines (Lands, 1985). It has been found that these lipoxygenase products play a role in a verity of disorders such as bronchial asthma, inflammation (Steinhilber, 1999) and tumor angiogenesis (Nie and Honn, 2002). Lipoxygenases are therefore a potential target for the rational drug design and discovery of mechanism-based inhibitors for the treatment of a variety of disorders such as bronchial asthma, inflammation, cancer and autoimmune diseases.

Baicalein (Aldrich Chem. Co.) was used as positive control in the assay. From the results (Table 3) it is clear that both the compounds are potent inhibitors of enzyme lipoxygenase. Compound **1** posses approx. 130 times greater inhibitory potential than compound **2**, due to the presence of a greater number of phenolic groups in compound **1** as compared to compound **2** (Figs. 2–4). These phenolic groups donate electrons and change the Fe^{+3} active form of the enzyme to the inactive the Fe^{+2} form. A variety of antioxidants such as phenols, catechols, hydroquines and naphthols are inhibitors of 5-lipoxygenase (Ellis and Luscombe, 1992). Antioxidants interact non-specially with 5-lipoxygenase by scavenging radical intermediates and/or reducing the active heme site (Cao et al., 1996) (Table 4).

There is considerable recent evidence that the free radicals induce oxidative damage to biomolecules such as lipids, proteins and nucleic acids, which eventually

Table 3
In vitro quantitative inhibition of lipoxygenase by compounds **1** and **2**

S. no.	Compounds	IC_{50}±S.E.M (μM)[a]
1	**1**	0.77±0.004
2	**2**	99.5±2.5
3	Baicalein[b]	22.4±1.3

[a] Standard error of the mean of five assays.
[b] Standard inhibitor of the lipoxygenase enzyme.

Table 4
Antioxidant activities of the compounds **1** and **2** as compared with standard

S. No.	Compounds	IC_{50}±S.E.M (μM)[a]
1	**1**	147.0±1.8
2	**2**	498.2±2.6
3	Trolox[b]	87.5±.82

[a] Standard error of the mean of five assays.
[b] Positive control used in assay.

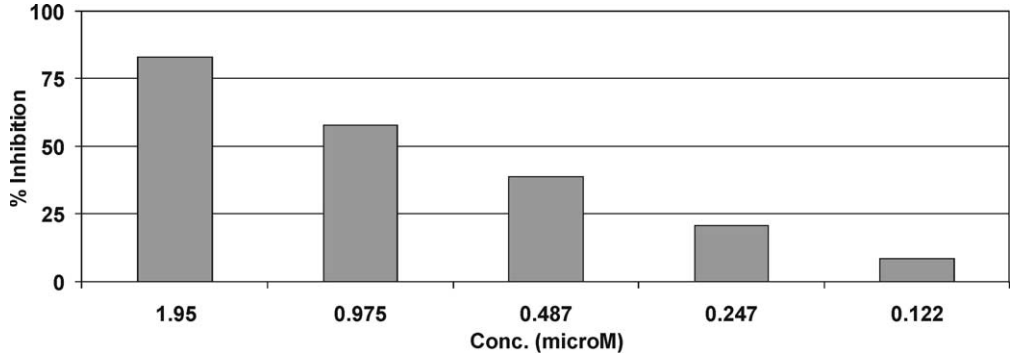

Fig. 2. Inhibition (%) of lipoxygenase by compound **1** at various concentrations.

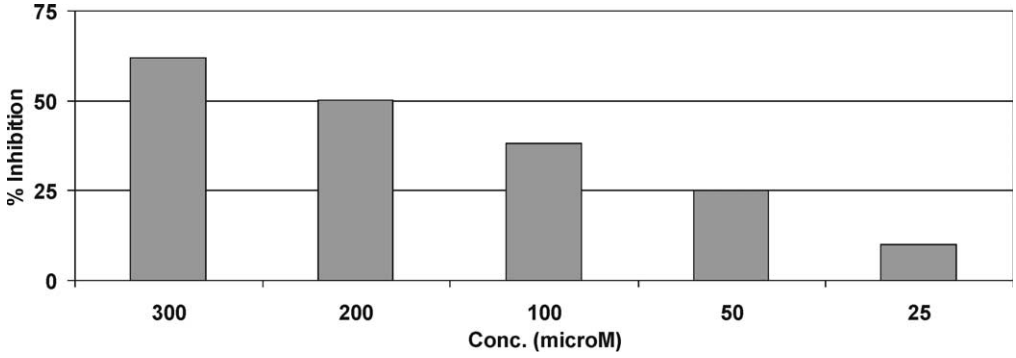

Fig. 3. Inhibition (%) of lipoxygenase by compound **2** at various concentrations.

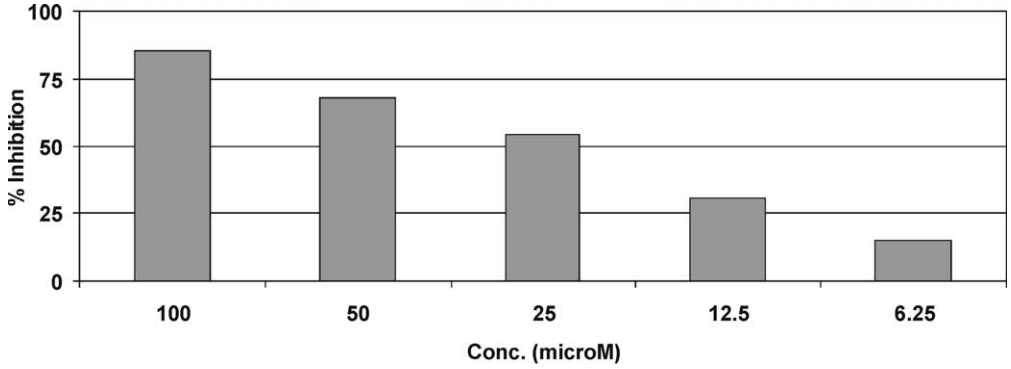

Fig. 4. Inhibition (%) of lipoxygenase by baicalein at various concentrations.

causes atherosclerosis, aging, cancer and several other diseases in humans (Musser and Kreft, 1992). Besides the antioxidant vitamins, such as vitamin E, C and β-carotene, the number of other compounds reported to have antioxidant activity is increasing. There is continuous search for "new" compounds and unidentified food ingredients with an antioxidant potential.

In a search for new bioactive substances from plant origin we have studied the chemical constituents of *P. emodi* for their antioxidant activity in the ABTS/HRP/H_2O_2 de-colorization assay.

3. Conclusion

In conclusion we can say that the search for new compounds from the plant *P. emodi* has resulted in the isolation of potent lipoxygenase inhibiting and antioxidant compounds which may find use in inflammation, asthma, aging, tumor angiogesis and cancer. However, there is a need for a further study of their in vitro and in vivo activities against these diseases.

4. Experimental

4.1. General

Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a 460 Shimadzu spectrometer. EIMS and HRFABMS were recorded on JMS-HX-110 with a data system and on JMS-DA 500 mass spectrometers. The ^1H and ^{13}C NMR, HMQC, and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for ^1H 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 silica gel 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, USA). 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).

4.2. Plant material

The fruits of *P. emodi* (2 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.

4.3. Extraction and isolation

The fruits of *P. emodi* (2 kg) were shade dried, ground, and extracted with methanol. The residue from the methanolic extract (85 g) was subjected to column chromatography over flash silica eluting with *n*-hexane–chloroform, chloroform, chloroform–methanol and methanol in increasing order of polarity. The fractions which were obtained from *n*-hexane–$CHCl_3$ (4:6) were combined and rechromatographed over flash silica to afford **3** (18 mg). The fraction which was obtained from $CHCl_3$ were rechromatographed over flash silica and eluted with *n*-hexane–EtOAc (3:7), to give compounds **4** (16 mg) and **5** (25 mg). The fractions obtained in $CHCl_3$–MeOH (9.5:0.5) were subjected to preparative TLC ($CHCl_3$–MeOH–H_2O; 80:19.5:0.5) to afford **1** (18 mg) and **2** (23 mg), respectively.

4.4. Paeoninol *1*

Brownish gummy solid: $[\alpha]_D^{25}$ +51.5° (CD_3OD; *c* 0.066); IR (KBr) ν_{max} 3395, 1612, 1518, 1452 cm^{-1}; UV (CD_3OD) λ_{max} (log ε) 226 (3.8), 277 (4.08), 283 (3.62);

^{13}C and ^{1}H NMR data, see Table 1; EIMS *m/z* 228 (2), 200 (8), 107 (20), 94 (100), 66 (81), 55 (37); HRFABMS *m/z* 680.2049 (calc. for $C_{42}H_{32}O_9$ 680.2046).

4.5. Paeonin C 2

Colorless gummy solid: $[\alpha]_D^{25}$ +14.5° (CD_3OD; *c* 0.03); IR (KBr) ν_{max} 3544–3405, 1724, 1606, 1592 cm^{-1}; UV (CD_3OD) λ_{max} (log ε) 236 (4.12), 277 (3.98); ^{13}C and ^{1}H NMR data, see Table 2; EIMS *m/z* 178 (25), 150 (17), 138 (45), 121 (100), 107 (9), 93 (32), 65 (43), 53 (34); HRFABMS *m/z* 496.1582 (calc. for $C_{23}H_{28}O_{12}$ 496.1580).

4.6. Acid hydrolysis of compound 2

A solution of **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 inseparable 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) with a standard 8 min, β-anomer 5.2 min.

4.7. In vitro lipoxygenase inhibition assay

Lipoxygenase inhibiting activity was conveniently measured by slightly modifying the spectrometric method developed by Tappel (1992). Lipoxygenase (1.13.11.12) type I-B and linoleic acid were purchased from sigma (St. Loius, MO, USA). All other chemicals were of analytical grade. Sodium phosphate (160 μl 100 mM) buffer (pH 8.0), 10 μl of test-compound solution and 20 μl of lipoxygenase solution were mixed and incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 μl linoleic acid (substrate) solution, with the formation of (9*Z*,11*E*)-(13*S*)-13-hydroperoxyoctadeca-9,11-dienoate, the change of absorbance at 234 nm was followed for 10 min. Test compounds and the control were dissolved in MeOH. All the reactions were performed in triplicate in 96-well micro-plate in SpectraMax 340 (Molecular Devices, USA). The IC_{50} values were then calculated using with the EZ-Fit Enzyme kinetics program (Perrella Scientific Inc., Amherst, USA).

4.8. Antioxidant activity

Horseradish peroxidase(HPR), 2,2′- azino-bis-(3-ethyl benzthiazoline 6-sulphonic acid, (ABTS), 6-hydroxy-2,57,8-tetrametylchroman-2-carboxlic acid (Trolox) were purchased from Sigma (St. Loius, MO, USA). All other chemicals were of analytical grade. The antioxidant was measured using ABTS/HRP/H_2O_2 decoloration method. The method is based on the capacity of the compound to scavenge the ABTS radical cation ($ABTS^{\cdot+}$) compared to the standard (Trolox) in an endpoint assay (Arnao et al., 2001). The reaction mixture (1 mM, ABTS, 35 μM H_2O_2, 0.25 μM HRP) was prepared in acidified Na-phosphate buffer (pH 7.5) at 25 °C for hydrophilic antioxidant activity. The enzymatically pre-generalized $ABTS^{\cdot+}$ radical solution is then added to 96-well plate containing 20 μl of the compound with various concentrations. This results in the decrease in absorbance at 730 nm (25 °C) i.e. disappearance of $ABTS^{\cdot+}$ radical cation. The antioxidant activity is then evaluated as moles of $ABTS^{\cdot+}$ quenched by 1 mol of compound (or standard). Trolox is used as standard antioxidant. The IC_{50} values were then calculated using with the EZ-Fit Enzyme kinetics program (Perrella Scientific Inc., Amherst, USA).

References

Arnao, M.B., Cano, A., Jesus, F., Acosta, M., 2001. Estimation of free radical-quenching activity of leaf pigment extracts. Phytochem. Anal. 1, 138–143.

Cao, G., Sofic, E., Prior, R.L., 1996. Antioxidant capacity of tea and common vegetables. J. Agric. Food Chem. 44, 3426–3431.

Ding, H.Y., Wu, Y.C., Lin, H.C., Chan, Y.Y., Wu, P.L., Wu, T.S., 1999. Glycosides from *Paeonia suffruticosa*. Chem. Pharm. Bull. 47, 652–655.

Ellis, G.P., Luscombe, D.K., 1992. Progress in Medicinal Chemistry, vol. 29. Elsevier Science.

Kawabata, J., Mishima, M., Kurihara, H., Mizutani, J., Kobophenol, B., 1991. A tetrastilbene from *Carex pumila*. Phytochemistry 30, 645–647.

Lands, W.E.M., 1985. Mechanism of action of anti-inflammatory drugs. Adv. Drug Res. 14, 147–167.

Lin, H.C., Ding, H.Y., Wu, T.S., Wu, P.L., 1996. Monoterpene glycosides from *Paeonia suffruticosa*. Phytochemistry 41, 237–242.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45, 31–34.

Muhammad, P., Ahmad, S., Rubnawaz, H., Ullah, N., Malik, A., 1999. New monoterpene glycosides from *Paeonia emodi*. Z. Naturforsch. 54b, 544–548.

Murakami, N., Saka, M., Shimada, H., Matsuda, H., Yamahara, J., Yoshikawa, M., 1996. New bioactive monoterpene glycosides from *Paeoniae radix*. Chem. Pharm. Bull. 44, 1279–1281.

Musser, J.H., Kreft, A.F., 1992. 5-Lipoxygenase: properties pharmacology and the quinolinyl (bridged) aryl class of inhibitors. J. Med. Chem. 35, 2501–2524.

Nasir, E., Ali, S.I., 1978. Flora of West Pakistan, vol. 121. Khursheed Printers, Islamabad.

Nawaz, H.R., Malik, A., Khan, P.M., Shujaat, S., Rahman, A.U., 2000. A novel β-glucoronidase inhibiting triterpene from *Paeonia emodi*. Chem. Pharm. Bull. 48, 1771–1773.

Nie, D., Honn, K.V., 2002. Cyclooxygenase, lipoxygenase and tumor angiogenesis. Cell. Mol. Life Sci. 59, 799–807.

Oshima, Y., Ueno, Y., 1993. Ampelopsins D, E, H and *cis*-ampelopsin E, oligostilbenes from *Ampelopsis brevipedunculata* var. *hancei* roots. Phytochemistry 33, 179–182.

Oshima, Y., Ueno, Y., Hikino, H., Yang, L.L., Yen, K.Y., 1990. Ampelopsins A, B and C, new oligostilbenes of *Ampelopsis brevipedunculata* var. *hancei*. Tetrahedron 46, 5121–5126.

Perry, L.M., Metzger, J., 1980. Medicinal Plants of East and Southeast Asia. MIT Press, Cambridge, London.

Riaz, N., Anis, I., Malik, A., Ahmed, Z., Rehman, A.U., Muhammad, P., Nawaz, S.A., Choudhary, M.I., 2003. Paeonins A and B, lipoxygenase inhibiting monoterpene galactosides from *Paeonia emodi*. Chem. Pharm. Bull. 51, 252–254.

Sarker, S.D., Whiting, P., Dinan, L., Sik, V., Rees, H.H., 1999. Identification and ecdysteroid antagonist activity of three resveratrol trimers (suffruticosols A, B and C) from *Paeonia suffruticosa*. Tetrahedron 55, 513–524.

Sotheeswaran, S., Sultanbawa, M.U.S., Surendrakumar, S., Balasubramaniam, S., Bladon, P.J., 1985. Polyphenols from *Dipterocarp* species. Vaticaffinol and ϵ-viniferin. J. Chem. Soc., Perkin Trans 1, 159–162.

Steinhilber, D., 1999. A target for anti-inflammatory drug revisited. Curr. Med. Chem. 6, 71–85.

Tappel, A.L., 1962. Methods of Enzymology, vol. 5. Academic Press, New York.

Yu, J., Elix, A.J., Iskander, M.N., 1990. Lactiflorin, a monoterpene glycoside from *Paeony* root. Phytochemistry 29, 3859–3863.

Zaheer, S.H., 1966. The Wealth of India, vol. 7. Publications and Information Directorate, CSIR, New Delhi.