A New Phenolic Glucoside from *Paeonia lactiflora*

HE Xiao-yan, HAN Li, HUANG Xue-shi*

Department of Natural Products Chemistry, School of Pharmaceutical Science, China Medical University, Shenyang 110001, China

Abstract: **Objective** To study the chemical constituents from EtOAc extracts of *Paeonia lactiflora*. **Methods** Compounds were isolated by various chromatographic techniques and structures were elucidated on the basis of spectral analysis. **Results** Seventeen compounds were obtained and their structures were identified as 1,2,6-benzenetriol-1-*O*-α-*D*-glucoside (**1**), paeoniflorin (**2**), 4-methylpaeoniflorin (**3**), albiflorin (**4**), paeonidanin (**5**), benzoylpaeoniflorin (**6**), 4-methylbenzoylpaeoniflorin (**7**), benzoylalbiflorin (**8**), paeonidanin A (**9**), galloylalbiflorin (**10**), debenzoylalbiflorin (**11**), 4',5-dihydroxyflavanone-7-*O*-β-*D*-glucoside (**12**), 5,7-dihydroxy flavanone-4'-*O*-β-*D*-glucoside (**13**), (+)-catechin (**14**), gallic acid (**15**), vanillic acid (**16**), and 1,2,3-benzenetriol (**17**). **Conclusion** Compound **1** is a new compound named paeoniphenoside. Compounds **12** and **13** are firstly obtained from genus *Paeonia* L., and compounds **5** and **9** are isolated from *P. lactiflora* for the first time.

Key words: 1,2,6-benzenetriol-1-*O*-α-*D*-glucoside; (+)-catechin; *Paeonia lactiflora*; paeoniflorin; paeoniphenoside

DOI: 10.3969/j.issn.1674-6384.2011.02.002

Introduction

Paeoniaee Radix Alba (PRA) prepared by the roots of *Paeonia lactiflora* Pall. (Ranunculaceae) is one of the most important crude drugs in traditional Chinese medicine (TCM). It shows anti-inflammatory, anti-coagulative, analgesic, and spasmolytic activities (Braca *et al*, 2008; Wang *et al*, 2010). Previous phytochemical studies showed that monoterpenes and monoterpene glycosides were major components in the roots of *P. lactiflora* (Zhang, Wang, and Li, 2001; Gao and Tian, 2006; Wang *et al*, 2006; Duan *et al*, 2009; Wang *et al*, 2007). As searching for novel bioactive constituents from TCM, we investigated the chemical constituents in PAR. In the present paper, we described the isolation and structure elucidation of one new compound, 1,2,6-benzenetriol-1-*O*-α-*D*-glucoside, named paeoniphenoside (**1**), and sixteen known compounds as well.

Materials and methods

Materials

Optical rotation was obtained on SGW—1 automatic polarimeter. NMR spectra were obtained on Bruker—AV 600 MHz and Varian Mercury—VX 300 MHz spectrometer. ESI-MS were taken by Finnigan LCQ (Thermo) and Bruker APEX IV FT—MS mass spectrometer. Silica gel (100－200 and 200－300 mesh) for column chromatography (CC) and GF_{254} silica gel for TLC were provided by Qingdao Marine Chemistry Co., Ltd., and YMC*Gel ODS-A (S-50 μm, 12 nm) (YMC Co., Ltd., Japan) was used for CC. Sephadex LH-20 for CC was obtained from Amersham Biosciences Co., Ltd. (Shanghai, China). *D*-(+)-glucose was purchased from Sinopharm Chemical Reagent Co., Ltd.

PRA was purchased in Hebei Qixin Traditional Chinese Medicine Pellets Co., Ltd., in February, 2009 and authenticated by Prof. LU Jin-cai (Shenyang Pharmaceutical University, China). A specimen was deposited at Department of Natural Products Chemistry, School of Pharmaceutical Science, China Medical University.

Extraction and isolation

PRA (5 kg) was extracted with 95% hot ethanol for three times, 2 h each time. The combined ethanol extracts were evaporated under reduced pressure. A suspension of this crude extract in distilled water was

* Corresponding author: Huang XS Address: Department of Natural Products Chemistry, School of Pharmaceutical Science, China Medical University, 92 BeiEr Road, Heping District, Shenyang 110001, China Tel/Fax: +86-24-2325 6666-5250 E-mail: xshuang@mail.cmu.edu.cn
Received: March 1, 2011; Revised: March 20, 2011; Accepted: March 28, 2011
Fund: Fok Ying Tong Education Foundation, Ministry of Education of China (111046)

extracted with EtOAc to yield about 140 g EtOAc extract. The EtOAc extract was subjected to silica gel CC, eluting with CH_2Cl_2-MeOH (50:1→1:1→MeOH). The eluents were combined to afford 12 fractions (Fr. 1—12). Fr. 3 was subjected to Sephadex LH-20 CC (CH_3OH), then further separated by silica gel CC (petroleum ether-EtOAc = 1:1) to afford compounds **16** (10 mg) and **17** (45 mg). Fr. 4 was subjected to silica gel CC, then further separated by ODS CC (MeOH-H_2O = 40:60) to get compounds **6** (600 mg), **7** (70 mg), **8** (600 mg), and **9** (13 mg). Fr. 6 was subjected to Sephadex LH-20 CC (CH_3OH) to yield compound **14** (113 mg). Fr. 7 was subjected to ODS CC (MeOH-H_2O = 40:60) to yield compounds **15** (2.50 g) and **1** (12 mg). Fr. 8 was subjected to Sephadex LH-20 CC (CH_3OH) to get two sub-fractions (Fr. 8.1 and 8.2), Fr. 8.1 was further separated by ODS column chromatography (MeOH-H_2O = 40:60) to get compounds **2** (2.75 g), **3** (120 mg), and **5** (8 mg); Fr. 8.2 was separated by ODS CC (MeOH- H_2O = 80:20) to get compounds **4** (1.47 g), **10** (11 mg), **12** (14 mg), and **13** (9 mg). Fr. 10 was subjected to Sephadex LH-20 CC (CH_3OH), then further separated by silica gel CC (CH_2Cl_2-MeOH = 10:1) to yield compound **11** (10 mg).

Results and discussion

Compound **1** was obtained as white amorphous powder and its molecular formula was assigned as $C_{12}H_{16}O_8$ by HR-ESI-MS at *m/z* 311.0734 $[M + Na]^+$ (calcd 311.0737 for $C_{12}H_{16}NaO_8$). The ^{1}H-NMR data (Table 1) of compound **1** indicated three aromatic proton signals at δ 6.75 (1H, t, J = 8.4 Hz), and 6.32 (2H, d, J = 8.4 Hz), and a sugar moiety signals at δ 4.87 (1H, d, J = 3.6 Hz), 4.00 (1H, ddd, J = 9.9, 4.8, 2.4 Hz), 3.63 (1H, br d, J = 11.4 Hz), 3.62 (1H, m), 3.56 (1H, br d, J = 11.4 Hz), 3.40 (1H, dd, J = 9.6, 3.6 Hz), and 3.24 (1H, br t, J = 9.6 Hz). The ^{13}C-NMR spectrum of compound **1** showed all 12 signals indicated by the molecular formula (Table 1). Except for the signals corresponding to the ^{1}H-NMR data, three quaternary carbons were found. The NMR data of compound **1** were very similar to those of the known compound 1,2,6-benzenetriol-1-*O*-β-*D*-glucoside (Pridham and Saltmarsh, 1960), but the coupling constant of H-1'. The symmetrical C-signals of C-2 and C-6, C-3 and C-5, and H-signals of aromatic ring suggested that the glycosidic protein was at C-1. The HMBC correlation between δ_H 4.87 (Glu-H-1') and δ_C 134.2 (C-1) confirmed that the sugar was located at C-1 in benzenetriol. The configuration of glucose was determined as α-*D* by analyzing the coupling constant (J = 3.6 Hz) of the anomeric proton signal at δ 4.87. The ^{1}H-NMR and ^{13}C-NMR data of compound **1** were assigned based on HMQC and HMBC experiments (Table 1). Compound **1** was hydrolyzed and a sugar residue was got as white amorphous power. The sugar residue was determined as *D*-(+)-glucose by comparing the Rf value of sugar residue with those of authentic *D*-(+)-glucose on TLC plate under three defferent developing solvents, together with its positive optical rotation value. Therefore, the structure of compound **1** was assigned as 1,2,6-benzenetriol-1-*O*-α-*D*-glucoside, named paeoniphenoside.

The ^{1}H-NMR and ^{13}C-NMR data of paeoniflorin (**2**, Zhang, Wang, and Li, 2001), 4-methylpaeoniflorin (**3**, Braca *et al*, 2008), albiflorin (**4**, Zhou, Li, and Jiang, 2009), paeonidanin (**5**, Okasaka *et al*, 2008), benzoylpaeoniflorin (**6**, Zhang, Wang, and Li, 2001), 4-methylbenzoylpaeoniflorin (**7**, Duan *et al*, 2009), benzoylalbiflorin (**8**, Zhou, Li, and Jiang, 2009), paeonidanin A (**9**, Duan *et al*, 2009), galloylalbiflorin (**10**, Wang *et al*, 2006), debenzoylalbiflorin (**11**, Aimi *et al*, 1969), 4′, 5-dihydroxyflavanone-7-*O*-β-*D*-glucoside (**12**, Zhang *et al*, 2003), 5, 7-dihydroxy flavanone-4′-*O*-β-*D*-glucoside (**13**, Zhang *et al*, 2007), (+)-catechin (**14**, Zhang *et al*, 2007), gallic acid (**15**), vanillic acid (**16**), 1,2,3-benzenetriol (**17**, Tan *et al*, 2010) were consistent with those of references and so identified respectively.

Compound **1**: white amorphous power; $[\alpha]_D^{20}$ + 229.3° (*c* 0.5, MeOH); ESI-MS *m/z*: 311 $[M + Na]^+$, 599 $[2M + Na]^+$; HR-ESI-MS *m/z*: 311.0734 $[M + Na]^+$ (calcd for $C_{12}H_{16}NaO_8$, 311.0737); ^{1}H-NMR and ^{13}C-NMR data were shown in Table 1. Compound **1** (6 mg) was dissolved by 3 mL MeOH and hydrolyzed in 5 mL 2 mol/L HCl for 3 h at 75 ℃. After neutralization with 2 mol/L NaOH, the reactive solvent was evaporated under reduced pressure. The dried residue was subjected to silica gel CC, eluting with CH_2Cl_2-MeOH (5:1→1:1) to afford a sugar residue (2.8 mg). $[\alpha]_D^{20}$ + 65.7° (*c* 0.14, MeOH). Chemical structure is in Fig. 1.

Table 1 ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data of compound 1 (in DMSO-d_6)

No.	δ_C	δ_H
1	134.2	—
2	150.9	8.81 (1H, br s, 2-OH)
3	107.3	6.32 (1H, d, *J* = 8.4 Hz)
4	124.8	6.75 (1H, d, *J* = 8.4 Hz)
5	107.3	6.32 (1H, d, *J* = 8.4 Hz)
6	150.9	8.81 (1H, br s, 6-OH)
1'	104.2	4.87 (1H, d, *J* = 3.6 Hz)
2'	71.9	3.40 (1H, dd, *J* = 9.6, 3.6 Hz)
3'	73.3	3.62 (1H, m)
4'	69.5	3.24 (H, br t, *J* = 9.6 Hz)
5'	73.9	4.00 (1H, ddd, *J* = 9.6, 4.8, 2.4 Hz)
6'	60.4	3.64 (H, br d, *J* = 11.4 Hz) 3.56 (H, br d, *J* = 11.4 Hz)
		5.17 (1H, br s, Glu-OH)
		5.04 (1H, br s, Glu-OH)
		4.46 (1H, br s, Glu-OH)

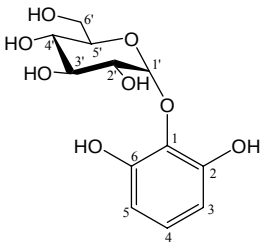

Fig. 1 Structure of compound 1

References

Aimi N, Inaba M, Watanabe M, Shibata S, 1969. Chemical studies on the oriental plant drugs-XXIII, paeoniflorin, a glucoside of Chinese paeony root. *Tetrahedron* 25(8): 1825-1838.

Braca A, Kiem PV, Yen PH, Nhim NX, Quang TH, Cuong NX, Minh CV, 2008. New monoterpene glycosides from *Paeonia lactiflora*. *Fitoterapia* 79(2): 117-120.

Duan WJ, Yang JY, Chen LX, Zhang LJ, Jiang ZH, Cai XD, Zhang X, Qiu F, 2009. Monoterpenes from *Paeonia albiflora* and their inhibitory activity on nitric oxide production by lipopoly-saccharide-activated microglia. *J Nat Prod* 72(9): 1579-1584.

Gao XY, Tian GY, 2006. Active principles of *Paeonia lactiflora* Pall. *Chin J New Drugs* 15(6): 416-418.

Okasaka M, Kashiwada Y, Kodzhimatov OK, Ashurmetov O, Takaishi Y, 2008. Monoterpene glycosides from *Paeonia hybrida*. *Phytochemistry* 69(8): 1767-1772.

Pridham JB, Saltmarsh MJ, 1960. The formation of phenolic glycosides by germinating broad-bean (*Vicia faba*) seeds. *Univ London Biochemical J* 74: 42.

Tan JJ, Zhao QC, Yang L, Shang ZP, Du ZQ, Yan M, 2010. Chemical constituents in roots of *Paeonia lactiflora*. *Chin Tradit Herb Drugs* 41(8): 1245-1248.

Wang Q, Guo HZ, Huo CH, Shi QW, Ye M, Bi KS, Guo DQ, 2007. Chemical constituents in root of *Paeonia lactiflora*. *Chin Tradit Herb Drugs* 38(7): 972-976.

Wang R, Lu L, Li YW, Hu YJ, Wang ZT, 2010. A comparison on pharmacological actions between *Radix Paeoniae Rubra* and *Radix Paeoniae Alba*. *Chin J Exp Tradit Med Form* 16(7): 112-114.

Wang XL, Jiao W, Liao X, Peng SL, Ding LS, 2006. Monoterpene glycosides from the roots of *Paeonia lactiflora*. *Chin Chem Lett* 17(7): 916-918.

Zhang CG, Li BG, Gu J, Zhang GL, 2003. Chemical study on *Exbucklandia populnea*. *Nat Prod Res Dev* 15(4): 308-309.

Zhang SJ, Wang YW, Liu L, Yu JY, Hu JP, 2007. Study on chemical constituents of *Herba Cynomorii*. *Chin Pharm J* 42(13): 975-977.

Zhang XY, Wang JH, Li X, 2001. A study on the chemical constituents of *Paeonia lactiflora* Pall. *J Shenyang Pharm Univ* 18(1): 30-32.

Zhou QX, Li YB, Jiang JQ, 2009. Chemical constituents of *Paeonia lactiflora*. *Strait Pharm J* 21(6): 92-94.