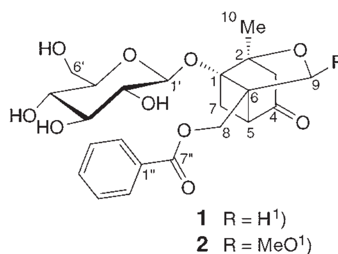
A New Monoterpene Glucoside from the Roots of *Paeonia lactiflora*

by **Ju Sun Kim**, **Min Hye Yean**, **Joo Young Lee**, **Yoon Jung Kim**, **Eun Ju Lee**, **So Young Lee**, and **Sam Sik Kang***

Natural Products Research Institute and College of Pharmacy, Seoul National University, 28 Yeongeon-dong, Jongno-gu, Seoul 110-460, Korea
(phone: +82-2-740-8925; fax: +82-2-743-3323; e-mail: sskang@snu.ac.kr)

A new monoterpene glucoside, 1-*O*-*β*-D-glucopyranosyl-8-*O*-benzoylpaeonisuffrone (**1**), was isolated from the roots of *Paeonia lactiflora* cultivated in Korea, together with two known compounds 1-*O*-*β*-D-glucopyranosylpaeonisuffrone and paeonidanin (**2**). Their structures were established on the basis of chemical and spectroscopic methods.

Introduction. – The roots of *Paeonia lactiflora* PALL. (Paeoniaceae) have been used in traditional Chinese medicine for a long time, with claims being made of its antispasmodic, tonic, astringent, and analgesic properties [1]. There have been many reports on the various chemical constituents of Paeoniae Radix, which are mainly monoterpenoid glycosides and phenol compounds [2][3]. It has been reported that paeoniflorin exhibits anticoagulant, neuromuscular blocking, cognition-enhancing, analgesia, anti-inflammatory, antiallergic, antihyperglycemic, antihyperlipidemic, and antithrombotic effects [4][5]. As part of our efforts to isolate the chemical constituents of *P. lactiflora* for the purpose of its standardization, we isolated a number of major and minor constituents from a *P. lactiflora* extract. In the present investigation, we report the isolation and structure elucidation of the new minor compound **1**, together with two known compounds, 1-*O*-*β*-D-glucopyranosylpaeonisuffrone and paeonidanin[1]) (**2**) from *P. lactiflora*.



Result and Discussion. – The dried roots of *P. lactiflora* were crushed and extracted with 70% EtOH. After evaporation, the residue was successively partitioned between H_2O and CH_2Cl_2, AcOEt, and BuOH. The BuOH extract was subjected to sequential

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

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column chromatography over silica gel and *RP-18* gel to yield the new monoterpene glucoside **1**, together with two known compounds, paeonidanin (**2**) [6][7] and 1-*O*-β-D-glucopyranosylpaeonisuffrone [8][9].

Compound **1** was obtained as a white amorphous powder. It showed a quasimolecular-ion peak at *m/z* 487 ($[M+Na]^+$) in the FAB-MS (positive mode). The HR-FAB-MS established its molecular formula as $C_{23}H_{28}O_{10}$. Other important fragment ions were observed at *m/z* 303 $[M+H-162]^+$, 179 ($C_6H_{11}O_6^+$) and *m/z* 105 ($C_6H_5CO^+$). The detailed analysis of the NMR data (*Table*, *Fig.*) revealed that the signals of **1** were very similar to those of 1-*O*-β-D-glucopyranosylpaeonisuffrone [8][9], and that it contained a monoterpenoid bearing glucose and benzoyl moieties in its structure. To determine the absolute configuration of **1**, a deacylation experiment was performed. After treatment with an anion-exchange resin (*Amberlite IRA-400*), compound **1** yielded 1-*O*-β-D-glucopyranosylpaeonisuffrone [8][9] which was identified by direct comparison with an authentic sample (^{1}H- and ^{13}C-NMR, [α]). Therefore, compound **1** had to have the same absolute configuration as the well-known paeoniflorin-type monoterpene glucosides. On the basis of all these data, compound **1** was characterized as 1-*O*-β-D-glucopyranosyl-8-*O*-benzoylpaeonisuffrone, which is a new natural product.

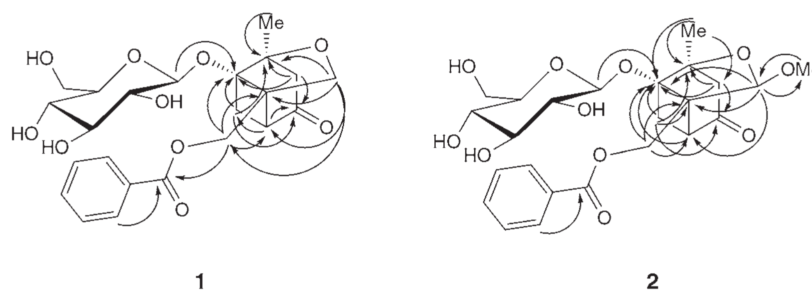

Figure. *Key HMBC correlations for* **1** *and* **2**

The monoterpenoid part of **1** exhibited ^{1}H-NMR signals at δ 2.38 and 3.09 (2*d*, $J=18.0$ Hz, 1 H each) for $CH_2(3)$, at δ 2.41 (*d*, $J=11.5$ Hz, 1 H) and 3.05 (*dd*, $J=7.5$, 11.5 Hz, 1 H) for $CH_2(7)$, at δ 2.99 (*d*, $J=7.5$ Hz, 1 H) for H–C(5), at δ 4.72 and 4.81 (2*d*, $J=11.5$ Hz, 1 H each) for $CH_2(8)$, at δ 3.72 and 3.97 (2*d*, $J=10.5$ Hz, 1 H each) for $CH_2(9)$, and at δ 1.43 (*s*) for Me(10). The glucose moiety appeared at δ 4.63 (*d*, $J=8.0$ Hz, 1 H), 3.61 (*dd*, $J=6.0$, 12.0 Hz, 1 H), and 3.88 (*dd*, $J=2.0$, 12.0 Hz, 1 H), with the signals of glucose H–C(3′), H–C(4′), and H–C(5′) overlapped by the solvent peak (δ 3.2–3.4), and was connected to the monoterpenoid by an acetal linkage between C(1′) of glucose and C(1) of the monoterpenoid. This was supported by the presence of an HMBC H–C(1′)/C(1) (*Fig.*). A set of benzoyl-group signals was found at δ 8.00 (br. *d*, $J=7.5$ Hz, 2 H), 7.49 (br. *t*, $J=7.5$ Hz, 2 H), and 7.62 (*tt*, $J=1.2$, 7.5 Hz, 1 H). The location of the benzoyloxy group was inferred to be C(8) due to the downfield shift of the signals for $CH_2(8)$ to δ 4.72 and 4.81 (2*d*, $J=11.5$ Hz, 1 H each).

During the course of the isolation of **1**, a second compound, **2**, was isolated as a minor constituent. Compound **2** was shown to have the molecular formula $C_{24}H_{30}O_{11}$ by HR-FAB-MS. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** suggested that its structure is very similar to that of compound **1**, except that the signals of the $CH_2(9)$

Table. *^{1}H- and ^{13}C-NMR Data.* (500 and 125 MHz, resp., CD_3OD) *of* **1** *and* **2**. δ in ppm, *J* in Hz.

	1[1])		**2**[1])	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		87.2		85.3
C(2)		88.3		88.3
$CH_2(3)$	2.38, 3.09 (2 br. *d*, *J* = 18.0)	50.1	2.41, 3.09 (2*d*, *J* = 17.1)	51.3
C(4)		211.9		211.3
H−C(5)	2.99 (*d*, *J* = 7.5)	50.3	3.04 (*d*, *J* = 8.7)	49.4
C(6)		62.2		64.9
$CH_2(7)$	2.41 (*d*, *J* = 11.5), 3.05 (*dd*, *J* = 7.5, 11.5)	29.3	2.43 (*dd*, *J* = 2.1, 13.2), 3.05 (*dd*, *J* = 6.9, 13.2)	30.2
$CH_2(8)$	4.72, 4.81 (2*d*, *J* = 11.5)	65.7	4.65, 4.89 (2*d*, *J* = 11.7)	62.5
$CH_2(9)$[a])	3.72, 3.97 (2*d*, *J* = 10.5)	71.6	4.81 (*s*)	106.0
Me(10)	1.43 (*s*)	20.4	1.47 (*s*)	21.0
MeO			3.37 (*s*)	57.2
H−C(1′)	4.63 (*d*, *J* = 8.0)	100.0	4.57 (*d*, *J* = 7.9)	100.2
H−C(2′)	[b])	75.0	[b])	75.0
H−C(3′)	[b])	78.1	[b])	78.1
H−C(4′)	[b])	71.8	[b])	71.7
H−C(5′)	[b])	78.1	[b])	77.9
$CH_2(6')$	3.61 (*dd*, *J* = 6.0, 12.0), 3.88 (*dd*, *J* = 2.0, 12.0)	62.9	3.62 (*dd*, *J* = 5.7, 11.7), 3.88 (*dd*, *J* = 1.5, 11.7)	62.8
C(1″)		131.0		131.1
H−C(2″,6″)	8.00 (br. *d*, *J* = 7.5)	130.6	7.99 (br. *d*, *J* = 7.2)	130.5
H−C(3″,5″)	7.49 (br. *t*, *J* = 7.5)	129.7	7.49 (br. *t*, *J* = 7.8)	129.7
H−C(4″)	7.62 (*tt*, *J* = 1.2, 7.5)	134.5	7.62 (*tt*, *J* = 1.2, 7.5)	134.4
C(7″)		167.8		167.8

[a]) In **2**, $CH_2(9)$ should be replaced by H−C(9). [b]) Overlapped with solvent peaks.

group of **1** were replaced by the signals of an acetal group (δ(H) 4.81 (*s*, 1 H) and 3.37 (*s*, 3 H); δ(C) 106.0 (C(9)) and 57.2 (MeO)). This was further supported by the presence of the HMBC H−C(9)/C(1), C(5), C(6), and a MeO C-atom (*Fig.*). Based on the above spectral analyses, the structure of **2** was deduced as paeonidanin which was previously isolated from the same genus, *P. peregrine* [6][7], *P. moutan* [10][11], and *P. parnassica* [12]. The spectroscopic data of **2** are given in the *Table* and the *Exp. Part* since data previously published were incomplete, and NMR assignments were revised.

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Experimental Part

General. Column chromatography (CC): silica gel (70–230 mesh; Art. No. 5715, *Merck*) and *LiChroprep*® *RP-18* gel (40–63 μm; *Merck*). TLC: precoated silica gel *60 F_{254}* (*Merck*) and cellulose plates (Art. No. 5716, *Merck*); visualization by spraying with 10% H_2SO_4 soln. for the silica gel and with aniline phthalate for the cellulose plates, followed by heating. Optical rotations: *Jasco-P-1020* polarimeter. UV/VIS Spectra: *Hitachi-U-3010* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco-FT/IR-5300* spectrometer; in cm^{-1}. NMR Spectra: *Varian-Unity-Inova-500* spectrometer; at 500 (^1H) and 125 MHz (^{13}C); CD_3OD soln.; δ in ppm, J in Hz. FAB-MS and HR-FAB-MS (positive-ion mode; 3-nitrobenzyl alcohol matrix): *Jeol-JMS-AX505WA* spectrometer; in *m/z*.

Plant Material. The dried root of *P. lactiflora* was purchased from the Asian Oriental Crude Drug Shop in Jeki-dong, Seoul, Korea, in May 2005, and authenticated by Dr. *J.-H. Lee*, College of Pharmacy, Kyung Hee University. A voucher specimen (LJH2005-12) was deposited in the herbarium of the College of Pharmacy, Kyung Hee University, Seoul.

Extraction and Isolation. The roots of *P. lactiflora* (18 kg) were chopped into small pieces and refluxed with 70% EtOH for 3 h (5 × 18 l) in a water bath. The extract was concentrated and then partitioned successively between H_2O (1.2 l) and CH_2Cl_2 (160 g), AcOEt (300 g), and then BuOH (680.1 g). The BuOH-soluble fraction was fractionated by CC (silica gel, CH_2Cl_2/MeOH/H_2O 7:2:0.5 → 52:28:8): *Fractions B-1–B-35*. *Fr. B-11* (0.9 g) was further purified by CC (silica gel, hexane/AcOEt gradient): *Fr. B-11-01–Fr. B-11-70*. *Fr. B-11-58* (100 mg) was subjected to CC (*RP-18*, 50% MeOH, then several times silica gel, $CHCl_3$/MeOH/H_2O 7:1:0.5): 1-*O*-*β*-D-glucopyranosylpaeonisuffrone (5 mg) and **2** (20 mg). *Fr. B-11-62* (100 mg) was further purified by CC (*RP-18*, MeOH/H_2O 6:4): **1** (45 mg).

*1-*O*-β-D-Glucopyranosyl-8-*O*-benzoylpaeonisuffrone* (=*(1*R*,3*R*,6*S*,9*S*)-9-[(Benzoyloxy)methyl]-1-(β-D-glucopyranosyloxy)-6-methyl-7-oxatricyclo[4.3.0.0^{3,9}]nonan-4-one*; **1**): $[\alpha]_D^{18} = -72.5$ ($c = 0.25$, MeOH). UV (MeOH): 229 (3.86), 272 (2.72). IR (KBr): 3432, 1723, 1657, 1640, 1603, 1277, 1074, 1044, 714. ^1H- and ^{13}C-NMR: *Table.* FAB-MS: 487 ($[M+Na]^+$), 303 ($[M+H-162]^+$), 179 ($C_6H_{11}O_6^+$), 105 ($C_6H_5CO^+$). HR-FAB-MS (pos.): 487.1573 ($[M+Na]^+$, $C_{23}H_{28}NaO_{10}^+$; calc. 487.1580), 465.1762 ($[M+H]^+$, $C_{23}H_{29}O_{10}^+$; calc. 465.1761).

Paeonidanin (=*(1*R*,3*R*,6*S*,9*S*)-9-[(Benzoyloxy)methyl]-1-(β-D-glucopyranosyloxy)-8-methoxy-6-methyl-7-oxatricyclo[4.3.0.0^{3,9}]nonan-4-one*; **2**): $[\alpha]_D^{27} = -113.0$ ($c = 0.12$, MeOH). IR (KBr): 3434, 1723, 1657, 1603, 1277, 1074, 1044, 714. ^1H- and ^{13}C-NMR: *Table.* FAB-MS: 517 ($[M+Na]^+$), 463 ($[M+H-MeOH]^+$), 179 ($C_6H_{11}O_6^+$), 105 ($C_6H_5CO^+$). HR-FAB-MS (pos.): 517.1674 ($[M+Na]^+$, $C_{24}H_{30}NaO_{11}^+$; calc. 517.1686).

Acid Hydrolysis of **1** *and Determination of the Absolute Configuration of the Sugar.* A soln. of **1** (2 mg) in 2N HCl/dioxane 1:1 (2 ml) was heated at 100° for 1 h. The mixture was neutralized with Ag_2CO_3, filtered, and then concentrated. The residue was treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.2 ml) at 60° for 1 h. The soln. was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.05 ml) at 60° for 1 h. The supernatant was applied to GC as described previously [13]: t_R 37.87 min (D-glucose).

Deacylation of **1**. To a soln. of **1** (15 mg) in 50% acetone (3 ml) was added *Amberlite IRA-400* (OH form, 300 mg). After stirring at r.t. for 3 h, the mixture was filtered, concentrated, and subjected to CC (silica gel, $CHCl_3$/MeOH/H_2O 7:1:0.5): *1-*O*-β-D-glucopyranosylpaeonisuffrone* (5 mg). Colorless solid. $[\alpha]_D$ and ^1H- and ^{13}C-NMR: identical to those of the natural product [9].

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