NEW BIOACTIVE MONOTERPENE GLYCOSIDES FROM PAEONIAE RADIX

Nobutoshi MURAKAMI, Masami SAKA, Hiromi SHIMADA, Hisashi MATSUDA, Johji YAMAHARA, and Masayuki YOSHIKAWA*

Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan

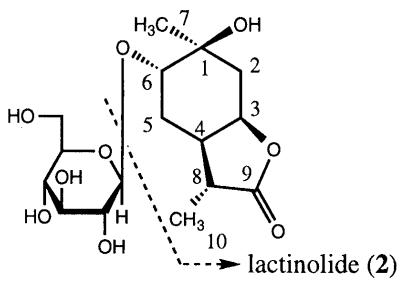
Bioassay-guided separation of MeOH extract of Japanese Paeoniae Radix inhibiting contractile responses of guinea pig ileum stimulated with electric field disclosed a new monoterpene glycoside, 6-*O*-β-D-glucopyranosyl-lactinolide (**1**), as an active constituent together with two new monoterpene glycosides (**3** and **4**) and two new monoterpenes (**2** and **5**). Furthermore, 1-*O*-β-D-glucopyranosyl-paeonisuffrone (**2**) was found to inhibit histamine release from rat peritoneal exudate cells induced by antigen-antibody reaction.

KEY WORDS Paeoniae Radix; *Paeonia lactifolia*; 6-*O*-β-D-glucopyranosyl-lactinolide; 1-*O*-β-D-glucopyranosyl-paeonisuffrone; histamine release inhibitor; electric field stimulated twitch contraction inhibitor

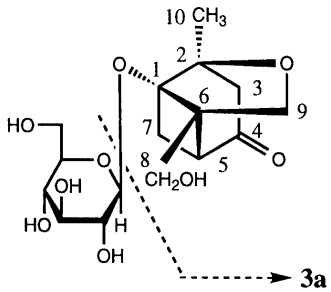
Paeoniae Radix (Shakuyaku in Japanese) is one of the most important natural medicines and is prescribed in various Chinese medicinal preparations as an anodyne, sedative, antispasmodic, and astringent. Chemical studies on this natural medicine carried out by many investigators revealed the various constituents, represented by monoterpenes and monoterpene glycosides such as paeoniflorin, albiflorin, and paeoniflorigenone.[1] During the course of our studies searching for new biologically active constituents from natural medicines,[2] the MeOH extract of Japanese Paeoniae Radix was found to inhibit twitch contraction of guinea pig ileum induced by electric field stimulation. Thus, bioassay-guided separation of the extract led us to a new water-soluble monoterpene glycoside, 6-*O*-β-D-glucopyranosyl-lactinolide (**1**), as an active constituent together with two new monoterpene glycosides (**3**, **4**) and two new monoterpenes (**2**, **5**). On the other hand, a new monoterpene glycoside, 1-*O*-β-D-glucopyranosyl-paeonisuffrone (**3**), inhibited histamine release from rat peritoneal exudate cells induced by the antigen-antibody reaction. Here, we deal with the structural elucidation of the new constituents from Paeoniae Radix and biological activities of **1** and **3**.

The MeOH extract (prepared below 25°C) of Japanese Paeoniae Radix cultivated in Nara Prefecture was dissolved in MeOH, and then the soluble portion was subjected to successive normal- and reversed-phase SiO_2 chromatography and reversed-phase HPLC to furnish 6-*O*-β-D-glucopyranosyl-lactinolide (**1**, 0.0099%), lactinolide (**2**, 0.0028%), 1-*O*-β-D-glucopyranosyl-paeonisuffrone (**3**, 0.020%), oxybenzoyl-paeoniflorin (**4**, 0.0015%), and paeonilactinone (**5**, 0.00015%) along with the eight known constituents, 10-hydroxyverbenone[3] (0.00017%), (+)-1-*p*-menthane-7,8-diol[4] (0.00023%), paeoniflorin[1] (1.8%), lactiflorin[1] (0.0047%), benzoyl-oxypaeoniflorin[5] (0.0019%), benzoylpaeoniflorin[1] (0.0065%), albiflorin[1] (0.031%), and oxypaeoniflorin[1] (0.011%).

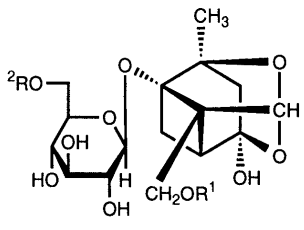
The FAB-MS spectrum of **1**[6] showed a quasimolecular ion peak at m/z 361 $(M-H)^-$ (neg. mode), m/z 363 $(M+H)^+$, and m/z 385 $(M+Na)^+$ (pos. mode) and the molecular formula was established to be $C_{16}H_{26}O_9$ by high-resolution FAB-MS measurement. In the IR spectrum, absorption bands attributable to hydroxy groups (3432 cm^{-1}) and the γ-lactone portion (1765 cm^{-1}) appeared. The ^1H- and ^{13}C-NMR (Table 1) spectra exhibited the presence of one lactone carbonyl (δc 181.9), one *tert.*-CH_3 group [δ 1.24 (3H, s, 7-H_3)], one *sec.*-CH_3 group [δ 1.22 (3H, d, J=7.3, 10-H_3)], two oxymethines [δ 4.64 (1H, ddd, J=5.9, 6.4, 9.2, 3-H), δc 78.2; δ 3.75 (1H, dd, J=4.5, 10.1, 6-H), δc 83.2], two methylenes, two methines, and one quaternary carbon bearing oxygen function [δc 72.5 (1-C)] as well as a β-D-glucopyranosyl moiety [δ 4.49 (1H, d, J=7.6, 1'-H), δc 106.1]. Detailed analysis of H-H, C-H COSY spectra suggested the aglycone of **1** for the *p*-menthane framework including an α-methyl-γ-lactone. Enzymatic hydrolysis of **1** using β-



6-*O*-β-D-glucopyranosyl-lactinolide (**1**)

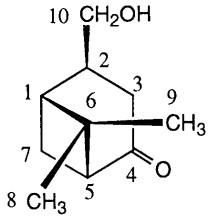


1-*O*-β-D-glucopyranosyl-paeonisuffrone (**3**)

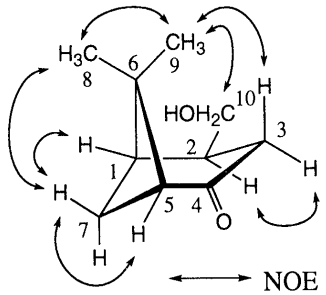


oxybenzoyl-paeoniflorin (**4**: R^1=Bz, R^2=4-OH-Bz)
benzoyl-oxypaeoniflorin (**4a**: R^1=4-OH-Bz, R^2=Bz)
debenzoylpaeoniflorin (**4b**: R^1=R^2=H)

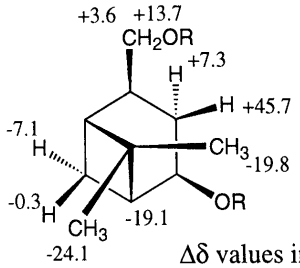
* To whom correspondence should be addressed.



paeonilactinone (**5**)



NOE



6 : R=H
6a: R=(*R*)-MTPA
6b: R=(*S*)-MTPA

Δδ values in Hz (δ*S*-δ*R*, 270 MHz)

glucosidase yielded lactinolide (**2**)[7] isolated in the present study, which was converted into paeonilactone A[8] by Swern oxidation. Thus, **1** was deduced to be 6-dihydropaeonilactone A 6-*O*-β-D-glucopyranoside. This assumption was confirmed by the following spectral features: 1) Detailed comparison of ^{13}C-NMR data for **1** and **2** indicated a glycosylation shift around 6-C. 2) In the HMBC spectrum of **1**, a H-C long-range correlation was observed between 1'-H and 6-C. The absolute configuration at 6-C was determined by the NOESY spectrum of **2**, in which NOE enhancement between 2β-H and 6-H was observed. Consequently, the chemical structure of **1** with a 6*S*-configuration was elucidated as shown.

Another active constituent (**3**),[9] white powder, $[\alpha]_D$ -59.6° (MeOH), $C_{16}H_{24}O_9$, showed absorption bands due to a ketonic carbonyl group (1710 cm^{-1}) and hydroxyl groups (3432 cm^{-1}) in the IR spectrum. The ^{1}H- and ^{13}C-NMR spectra of **3** indicated the presence of one ketonic carbonyl group (δc 212.4), one *tert.*-CH_3 group [δ 1.38 (3H, s, 10-H_3)], two oxymethylenes [δ 3.54, 3.90 (2H, ABq, J=10.2, 9-H_2), δc 70.6; δ 3.86, 3.96 (2H, ABq, J=11.9, 8-H_2), δc 61.5], two methylenes, one methine [δ 2.80 (1H, d, J=7.3, 5-H)] linked to the carbonyl group, and three quaternary carbons, one of which bore an oxygen function (δc 87.2), along with the β-glucopyranosyl portion [δ 4.64 (1H, d, J=7.6, 1'-H), δc 99.6]. Detailed analysis of the NMR data involving the H-H, C-H COSY spectra revealed that the signals of **3** were similar to those of paeonisuffrone (**3a**)[10] isolated from Moutan Cortex except for the region around 1-C. Treatment of **3** with β-glucosidase gave **3a** in 98.0% yield. The glycosylation shift around 1-C between **3** and **3a** as well as the long-range H-C correlation due to 1'-H and 1-C in the HMBC spectrum caused the β-D-glucopyranosyl residue to connect with the hydroxyl group on 1-C. The absolute structure of **3** was, therefore, constructed based on the above-mentioned findings.

Oxybenzoyl-paeoniflorin (**4**),[11] white powder, $[\alpha]_D$ -17.4° (MeOH), $C_{30}H_{32}O_{13}$, UV[λmax, nm (log ε)]: 260 (4.0), 227 (4.1), IR (KBr): 3453, 1719, 1701, 1638, 1610 cm^{-1}, positive FAB-MS (m/z) : 623 $(M+Na)^+$, negative FAB-MS (m/z) : 599 $(M-H)^-$, showed the presence of 4-hydroxybenzoyl and benzoyl groups, and the β-glucopyranosyl portion in the ^{1}H- and ^{13}C-NMR spectra. Intensive analysis of the NMR data of **4** indicated that the two acyl groups were linked to debenzoylpaeoniflorin (**4b**), but **4** was not identical with benzoyl-oxypaeoniflorin (**4a**). Thus, it was suggested that the 4-hydroxybenzoyl and the benzoyl group were, respectively, attached to 6'-OH and 8-OH in **4**. This was confirmed by the correlations observed between 6'-H_2 and 4-hydroxybenzoyl carbonyl carbon and between 8-H_2 and benzoyl carbonyl carbon in the HMBC spectrum. Anion-exchange resin (Amberlite IRA-400) treatment of **4** in acetone-H_2O (1:1) gave **4b**. On the basis of these findings, the absolute structure of **4** was elucidated as shown.

Paeonilactinone (**5**),[12] colorless oil, $[\alpha]_D$ +52.8° ($CHCl_3$), $C_{10}H_{16}O_2$, showed hydroxyl (3432 cm^{-1}) and a ketonic carbonyl (1709 cm^{-1}) absorption bands in the IR spectrum. The ^{1}H- and ^{13}C-NMR spectra of **5** showed the signals arising from two *tert.*-CH_3 groups [δ 0.94 (3H, s, 9-H_3), δ 1.36 (3H, s, 8-H_3)], one oxymethylene [δ 3.62 (2H, m, 10-H_2), δc 65.9], two methylenes, three methines, and one quaternary carbon together with one ketonic carbonyl group (δc 213.3). Precise analysis of the H-H, C-H COSY, and HMBC spectra placed **5** in the pinane framework. Additionally, respective observed correlations between ketoic carbonyl carbon and 3-H_2, and 5-H between oxymethylene carbon and 2-H, and 3-H_2 located the oxygen functions at 4-C. The relative structure of **5** was established by the NOESY spectrum giving NOEs as shown by arrows. The absolute structure was elucidated as follows. Treatment of **5** with $NaBH_4$ in MeOH exclusively yielded a diol (**6**) through stereo-specific hydride attack from the less hindered α-side. NOE enhancements between 4-H and 7-H defined the relative stereochemistry of **6**. The modified Mosher's method was applied to both α-methoxy-α-trifluoromethylphenylacetyl (MTPA) esters (**6a** and **6b**) prepared from **6** with the corresponding acid using 4-dimethylaminopyridine and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide·hydrochloride (EDC·HCl). The signals due to 3-H_2 and

Table 1. ^{13}C-NMR Data for the Aglycone Moieties of Monoterpene Glycosides (**1**, **3**, **4**) and the Monoterpenes (**2**, **5**)

	1	**2**	**3**	**4**	**5**
C-1	72.5	73.1	88.3	89.3	59.6
C-2	40.6	41.3	87.2	87.1	44.0
C-3	78.2	78.8	50.0	44.7	37.7
C-4	42.4	42.8	212.4	106.3	213.3
C-5	29.9	31.2	49.8	43.8	41.0
C-6	83.2	73.5	63.7	72.1	41.3
C-7	21.5	21.6	28.9	23.1	28.6
C-8	39.2	40.3	61.5	61.1	27.1
C-9	181.9	182.6	70.6	102.3	25.0
C-10	13.9	14.6	20.3	19.6	65.9

The spectra were taken in CD_3OD at 68 MHz.

10-H_2 in the (*R*)-MTPA ester (**6a**) appeared in higher fields than those of the (*S*)-MTPA ester (**6b**), while the signals ascribable to 5-H, 7-H_2, 8-H_3, and 9-H_3 of **6b** were observed in lower fields as compared to those of **6a**. Consequently, the absolute configuration at 4-C in **6** was elucidated to be *R* and the absolute structure of **5** was determined.

Table 2 illustrates that 6-*O*-β-D-glucopyarnosyl-lactinolide (**1**) inhibits the contractile response of guinea pig ileum induced by electric field stimulation, dependent on its concentration.

Table 2. Inhibitory Effects of 6-*O*-β-D-Glucopyranosyl-lactinolide (**1**) on Contractile Responses of Guinea Pig Ileum Induced by Electric Field Stimulation.

	Conc. (M)	n	Contractile response
6-*O*-β-D-glucopyranosyl-lactinolide (**1**)	$3x10^{-5}$	5	100.2±3.6
	10^{-4}	5	94.3±4.4
	$3x10^{-4}$	5	82.6±7.6
	10^{-3}	5	56.3±9.9

Paeoniae Radix is prescribed in some Chinese medicinal preparations, treat allergosis, so histamine release inhibitory activity, one of the *in vitro* anti-allergic assays, was primarily examined for the three new monoterpene glycosides (**1**, **3**, and **4**) and the monoterpene (**2**). Among the compounds tested, 1-*O*-β-D-glucopyranosyl-paeonisuffrone (**2**) only inhibited histamine release from the rat peritoneal exudate cell-induced antigen-antibody reaction (Table 3).

Table 3. Inhibitory Effects of 1-*O*-β-D-Glucopyranosyl-paeonisuffrone (**3**) on Histamine Release from Rat Peritoneal Exudate Cells Induced by Antigen-Antibody Reaction.

	Conc. (M)	n	Inhibition
1-*O*-β-D-glucopyranosyl-paeonisuffrone (**3**)	10^{-6}	4	23.3± 9.2
	10^{-5}	4	31.0±10.4
	10^{-4}	3	52.0± 8.8

Taking into consideration that the biologically active two monoterpene glycosides (**1** and **3**) were not only fairly water-soluble and stable in hot water but also were contained in comparatively high yields of Paeoniae Radix, they may be associated with traditional efficacy of this natural medicine. Although Paeoniae Radix is classified into "Sekishaku" and "Hakushaku" by the processing in China, they have been properly used as different natural medicines. However, chemical characterization on the processing of Paeoniae Radix has not been extensively examined. Therefore, the above two constituents also may be useful for elucidating chemical characterization during the processing of Paeoniae Radix.

REFERENCES AND NOTES

1) Morita N., Shimizu M., Hayashi T., *The Journal of Traditional Sino-Japanese Medicine*, **12**, 86-92 (1991), and the literature cited therein. 2) Yoshikawa M., Murakami T., Ueno T., Kadoya M., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **43**, 2115-2122 (1995). 3) Bohlmann F., Jakupovic J., Schuster A., King R. M., Robinson H., *Planta Med.*, **50**, 202-203 (1984). 4) Suga T., Hirata T., Ym S. L., *Chem. Lett.*, **1982**, 1595-1598. 5) Kitagawa I., Yoshikawa M., Tsunaga K., Tani T., *Shoyakugaku Zasshi*, **33**, 171-177 (1979). 6) **1**: white powder, $[\alpha]_D^{26}$ +16.8° (MeOH), ^{1}H-NMR (CD_3OD) δ: 1.68 (1H, dd, J=9.2, 13.5, 2β-H), 1.81 (1H, ddd, J=6.3, 10.1, 14.5, 5α-H), 2.11 (1H, dd, J=5.9, 13.5, 2α-H), 2.20 (1H, dd, J=4.5, 14.5, 5β-H), 2.37 (1H, m, 4-H), 2.64 (1H, dq, J=7.3, 10.2, 8-H), ^{13}C-NMR (CD_3OD) δ: 106.1 (1'-C), 75.7 (2'-C), 77.7 (3'-C), 71.6 (4'-C), 78.0 (5'-C), 62.8 (6'-C). 7) In the course of structure determinination of paeonilactone A, the dihydro derivative (lactinolide, **2**) was prepared from paeonilactone A by $NaBH_4$ reduction, but physicochemical properties have not been reported in detail.[8] This is the first example of isolation of **2** from natural sources. **2**: white powder, $[\alpha]_D^{26}$+48.3° (MeOH), $C_{10}H_{16}O_4$, IR (KBr, cm^{-1}): 3410, 1752, ^{1}H-NMR (CD_3OD) δ: 1.17 (3H, s, 7-H_3), 1.22 (3H, d, J=6.9, 10-H_3), 1.66 (1H, dd, J=9.2, 13.9, 2β-H),1.69 (1H, dd, J=9.9, 14.5, 5α-H), 1.97 (1H, ddd, J=4.6, 4.6, 14.5, 5β-H), 2.09 (1H, dd, J=5.9, 13.9, 2α-H), 2.33 (1H, m, 4-H), 2.59 (1H, dq, J=6.9, 9.9, 8-H), 3.65 (1H, dd, J=4.6, 9.9, 6-H), 4.65 (1H, ddd, J=5.9, 6.6, 9.2, 3-H), FAB-MS (m/z) : 233 $(M+Na)^+$, 199 $(M-H)^-$. 8) Hayashi T., Shinbo T., Shimizu M., Arisawa M., Morita N., Kimura M., Matsuda S., Kikuchi T., *Tetrahederon Lett.*, **26**, 3699-3702 (1985). 9) **3**: ^{1}H-NMR (CD_3OD) δ: 1.38 (3H, s, 10-H), 2.30 (1H, d, J=11.2, 7α-H), 2.32, 2.98 (1H each, both d, J=17.5, 3-H_2), 2.80 (1H, d, J=7.3, 5-H), 2.95 (1H, dd, J=7.3, 11.2, 7β-H), 3.54, 3.90 (2H, ABq, J=10.2, 9-H_2), 3.62 (1H, dd, J=5.6, 11.9, 6'-H), 3.85 (1H, d, J=11.9, 6'-H), 3.86, 3.96 (2H, ABq, J=11.9, 8-H_2), 4.64 (1H, d, J=7.6, 1'-H), ^{13}C-NMR (CD_3OD) δc: 99.6 (1'-C), 74.9 (2'-C), 78.1 (3'-C), 71.7 (4'-C), 77.9 (5'-C), 62.9 (6'-C). FAB-MS (m/z) : 361 $(M+H)^+$, 359 $(M-Na)^-$. 10) Yoshikawa M., Harada E., Kawaguchi A., Yamahara J., Murakami N., Kitagawa I., *Chem. Pharm. Bull.*, **41**, 630-632 (1993). 11) **4**: ^{1}H-NMR (CD_3OD) δ: 1.23 (3H, s, 10-H_3), 1.68, 1.84 (2H, ABq, J=12.2, 3-H_2), 1.69 (1H, d, J=11.0, 7α-H), 2.47 (2H, m, 5, 7β-H), 2.46 (1H, d, J=11.2, 7α-H), 4.56 (1H, d, J=7.6, 1'-H), 4.65 (2H, s, 8-H_2), 5.35 (1H, s, 9-H), 6.82 (2H, d, J=8.9, 3''',5'''-H), 7.48 (2H, dd, J=6.9, 7.6, 3'',5''-H), 7.59 (1H, d, J=7.6, 4''-H), 7.89 (2H, d, J=8.9, 2''',6'''-H), 8.04 (2H, d, J=6.9, 2'',6''-H), ^{13}C-NMR (CD_3OD) δc: 100.1 (1'-C), 75.0 (2'-C), 77.9 (3'-C), 72.1 (4'-C), 75.2 (5'-C), 65.2 (6'-C), 131.4 (1''-C), 130.6 (2'',6''-C), 129.7 (3'',5''-C), 134.5 (4''-C), 167.7 (COPh), 122.3 (1'''-C), 133.0 (2''',6'''-C), 116.3 (3''',5'''-C), 163.6(4'''-C), 168.0. 12) **5** : ^{1}H-NMR (CD_3OD) δ: 1.49 (1H, d, J=10.6, 7α-H), 2.23 (1H, dd, J=4.5, 19.5, 3α-H), 2.38 (1H, m, 1-H), 2.47 (1H, m, 2-H), 2.50 (1H, d, J=5.3, 5-H), 2.70 (1H, dd, J=5.3, 10.6, 7β-H), 2.80 (1H, dd, J=10.2, 19.5, 3β-H), $CD[\theta]^{25}$ (MeOH, nm) : +5400 (277), -1700 (219), FAB-MS (m/z) : 169 $(M+H)^+$.

(Received March 25, 1996; accepted May 12, 1996)