

Article

Suffruiyabiosides A and B, Two New Monoterpene Diglycosides from Moutan Cortex

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Abstract: Two new monoterpene diglycosides, suffruiyabiosides A (**1**) and B (**2**), and seven known compounds **3–9** were isolated from Moutan Cortex (root cortex of *Paeonia suffruticosa* Andrews). The structures were elucidated on the basis of 2D NMR spectral data. Suffruiyabiosides A (**1**) and B (**2**) are rare monoterpene diglycosides, including a cellobiose in the molecules. Salicylpaeoniflorin (**4**) had a antiproliferation effect similar to paeoniflorin (**3**) on human lung adenocarcinoma epithelial A549 cells. Galloylpaeoniflorin (**8**) and salicylpaeoniflorin (**4**) revealed a more pronounced radical scavenging effect than α -tocopherol (positive control). An increase in the number of phenolic hydroxyl groups produced a more effective radical scavenging effect [**8** > mudanpioside E (**6**) > oxypaeoniflorin (**5**)]. Comparison of the effects of **4** and **5** showed that *o*-substitution with a phenolic hydroxyl group was more effective than *p*-substitution. The results suggest that salicylpaeoniflorin (**4**) may be useful as a cytotoxic and a radical scavenging agent.

Keywords: monoterpene diglycoside; suffruiyabiosides A and B; human lung adenocarcinoma epithelial A549 cells; radical scavenging effect

1. Introduction

Moutan Cortex (root cortex of *Paeonia suffruticosa* Andrews) is one of the most important natural medicines in China, Korea, and Japan. The main production places of Moutan Cortex are Anhui, Sichuan, Gansu, Shaanxi, Hubei, Hunan, Shandong, Guizhou and other places in China. It is clinically

used for its analgesic and anti-inflammatory properties, as well as a remedy for female genital diseases [1–4]. Moreover, the monoterpene glycosides isolated from Moutan Cortex are known for their radical scavenging effect [5]. In a recent study, paeoniflorin (**3**) exhibited antiproliferative activity against human lung adenocarcinoma epithelial A549 cells by arresting cell cycle progression in the G₀/G₁ phase and inducing apoptosis [6]. However, the structure-activity relationships of the related monoterpene glycosides, including paeoniflorin (**3**), have not been studied in A549 cells. In this study, we isolated two new monoterpene diglycosides, suffruiabiosides A (**1**) and B (**2**), and known compounds **3–9** from Moutan Cortex. In addition, we examined the structure-activity relationships of cytotoxic activity on A549 cell and radical scavenging effect of compounds **1–9**.

2. Results and Discussion

The EtOH extract of Moutan Cortex (1.0 kg) was partitioned between EtOAc and H₂O, and then the H₂O-layer was partitioned with *n*-BuOH. The *n*-BuOH layer was separated by column chromatography on silica gel and ODS, and using HPLC to give two new monoterpene diglycosides, suffruiabiosides A (**1**, 0.00028%) and B (**2**, 0.00013%), together with seven known compounds **3–9**. The known compounds **3–9** were identified as paeoniflorin (**3**) [3], salicylpaeoniflorin (**4**) [7], oxypaeoniflorin (**5**) [8,9], mudanpioside E (**6**) [10], mudanpioside D (**7**) [10], galloylpaeoniflorin (**8**) [2,9], and mudanpioside I (**9**) [11].

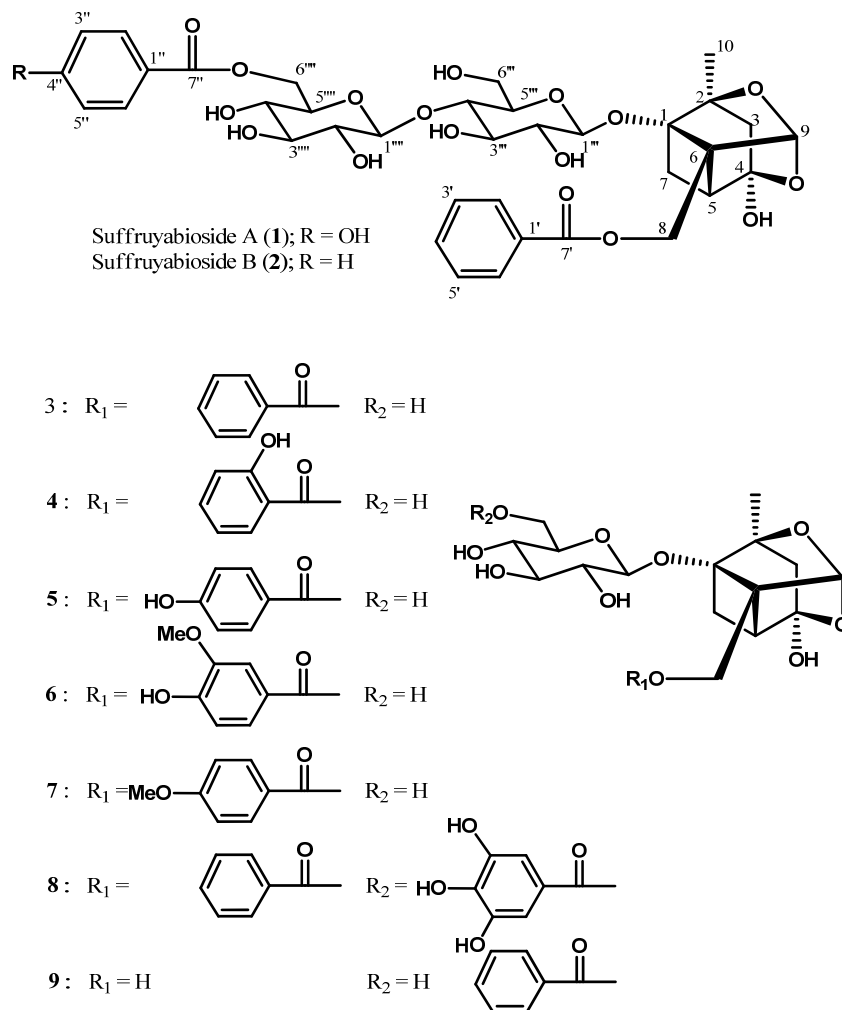
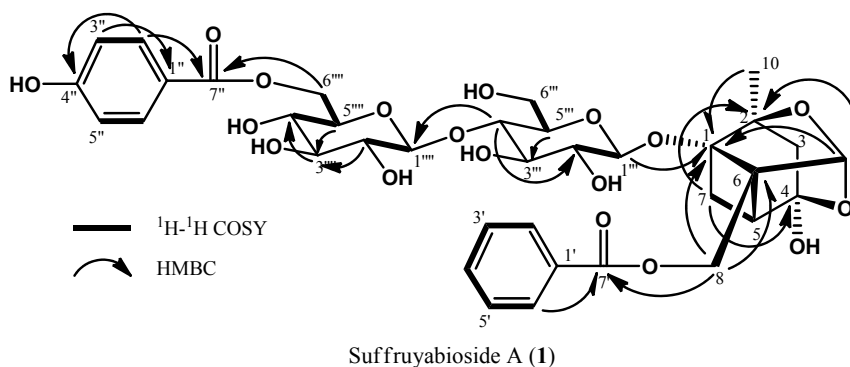
The molecular formula of suffruiabioside A (**1**) was assigned as C₃₆H₄₂O₁₈ by HRESIMS [*m/z* 785.2212 (M+Na)⁺, Δ −5.7 mmu]. The IR spectrum indicated the presence of hydroxyl (3412 cm^{−1}) and ester (1729 cm^{−1}) moieties. The ¹H- and ¹³C-NMR spectra of **1** (Table 1) revealed signals assignable to a monoterpene pinane type skeleton similar to that of paeoniflorin (**3**) [12] with signals for three methylenes at δ_H 2.21 (1H, d, *J* = 13.0 Hz, H-3), 1.73 (1H, d, *J* = 13.0 Hz, H-3), 1.73 (1H, brd, *J* = 12.3 Hz, H-7), 2.50 (1H, m, H-7), and 4.87 (2H, s, H-8), two methines at δ_H 2.52 (1H, m, H-5) and 5.40 (1H, s, H-9), and one methyl at δ_H 1.25 (3H, s, H-10). HMBC correlations (Table 1, Figures 1 and 2) of H-5, H-7, H-8, H-9, and H-10 to C-1 (δ_C 90.1), H-7, H-9, and H-10 to C-2 (δ_C 88.8), H-10 to C-3 (δ_C 44.7), H-3, H-5, and H-7 to C-4 (δ_C 106.1), H-3 to C-5 (δ_C 41.1), H-8 to C-6 (δ_C 72.5), H-5 to C-7 (δ_C 27.1), and H-8 to C-9 (δ_C 103.0) revealed the presence of a pinane type skeleton. The ¹H-¹H COSY connectivities of H-1''' to H-2'', H-5''' to H-6'', H-1''' to H-2'', and H-4''' to H-6'' and HMBC correlations of H-2''' to C-4''' (δ_C 82.1), H-4''' to C-2''' (δ_C 75.4), H-5''' to C-3''' (δ_C 78.7), H-1''', H-4'', and H-5''' to C-3''' (δ_C 72.4), and H-2''' and H-6''' to C-4''' (δ_C 74.7) implied the presence of two sugar moieties. NOESY correlations of H-1''' to H-3'', H-1''' to H-5'', H-1''' to H-3'', H-1''' to H-5'', and H-2''' to H-4'' and two anomeric proton signals of H-1''' and H-1''' observed a doublet at δ_H 4.64 (1H, *J* = 7.8 Hz) and 4.44 (1H, *J* = 7.8 Hz), suggesting the presence of two β-glucose moieties. Moreover the presence of benzoyl and *p*-hydroxybenzoyl groups was revealed by the ¹H-¹H COSY connectivities of H-2' to H-6', H-2'' to H-3'', and H-5'' to H-6'' and HMBC correlations of H-2' and H-6' to C-4' (δ_C 135.3) and C-7' (δ_C 168.3), H-3'' and H-5'' to C-1'' (δ_C 120.0), and H-2'' and H-6'' to C-7'' (δ_C 168.8) and C-4'' (δ_C 163.6). HMBC correlations of H-8 (δ_H 4.87) to C-7', H-4''' (δ_H 3.64) to C-1''' (δ_C 105.7), and H-6''' (δ_H 4.86) to C-7'' (δ_C 168.8) revealed that the benzoyl group was attached to C-8 (δ_C 63.8) by the ester linkage, the first sugar was linked to C-1''' (δ_C 100.6) with the other sugar

attached to C-4''' (δ_C 82.1) by ether linkages, and the *p*-hydroxybenzoyl group was connected to C-6''' (δ_C 65.4) of the other sugar through an ester linkage.

Table 1. ^{13}C - and ^1H -NMR data of suffruiabiosides A (1) and B (2) in CD_3OD .

Suffruiabioside A (1)			Suffruiabioside B (2)		
	δ_C	δ_H, J in Hz	HMBC ^a		
1	90.1, C	-		90.1, C	-
2	88.8, C	-		87.8, C	-
3	44.7, CH_2	1.73, d, 13.0 2.21, d, 13.0	4, 5	41.4, CH_2	1.76, d, 13.9 2.18, d, 13.9
4	106.1, C	-		104.0, C	-
5	41.1, CH	2.52, m	1, 4, 7	40.7, CH	2.56, m
6	72.5, C	-		72.5, C	-
7	27.1, CH_2	1.73, bd, 12.3 2.50, m	1, 2, 4	24.2, CH_2	1.67, d, 9.5 2.56, m
8	63.8, CH_2	4.87, s	1, 6, 9, 7'	62.8, CH_2	4.80, s
9	103.0, CH	5.40, s	1, 2	102.9, CH	5.50, s
10	22.5, CH_3	1.25, s	1, 2, 3	20.4, CH_3	1.22, s
1'	131.1, C	-		132.1, C	-
2'	130.7, CH	8.08, dt, 7.8, 1.2	3', 4', 6', 7'	131.5, CH	8.09, dt, 7.6, 1.4
3'	130.6, CH	7.53, t, 7.8	1', 2', 5'	130.6, CH	7.54, t, 7.6
4'	135.3, CH	7.64, tt, 7.4, 1.2	2', 3', 5', 6'	135.3, CH	7.66, tt, 7.6, 1.4
5'	130.6, CH	7.53, t, 7.8	1', 2', 3'	130.6, CH	7.54, t, 7.6
6'	130.7, CH	8.08, dt, 7.8, 1.2	2', 3', 4', 7'	131.5, CH	8.09, dt, 7.6, 1.4
7'	168.3, C	-		168.3, C	-
1''	120.0, C	-		132.0, C	-
2''	133.8, CH	7.93, dd, 8.8, 1.9	4'', 6'', 7''	131.4, CH	8.07, dt, 8.3, 1.3
3''	112.0, CH	6.86, dd, 8.8, 1.9	1'', 5''	130.4, CH	7.51, t, 8.3
4''	163.6, C	-		135.2, CH	7.65, tt, 8.3, 1.3
5''	112.0, CH	6.86, dd, 8.8, 1.9	1'', 3''	130.4, CH	7.51, t, 8.3
6''	133.8, CH	7.93, dd, 8.8, 1.9	2'', 4'', 7''	131.4, CH	8.07, dt, 8.3, 1.3
7''	168.8, C	-		168.8, C	-
1'''	100.6, CH	4.64, d, 7.8	1, 5'''	100.6, CH	4.65, d, 7.8
2'''	75.4, CH	3.34, m	4'''	75.5, CH	3.35, m
3'''	78.7, CH	3.35, m	1''', 5'''	79.1, CH	3.30, m
4'''	82.1, CH	3.64, m	2''', 1''''	82.2, CH	3.64, m
5'''	77.1, CH	3.39, m	3'''	77.1, CH	3.39, m
6'''	63.3, CH_2	3.69, dd, 11.9, 5.7 3.93, dd, 11.9, 2.0	5''' 5'''	63.3, CH_2	3.70, dd, 12.0, 6.3 3.93, dd, 12.0, 2.0
1''''	105.7, CH	4.44, d, 7.8	3''''	105.7, CH	4.44, d, 7.8
2''''	75.6, CH	3.38, m	1''', 4''''	75.6, CH	3.40, m
3''''	72.4, CH	3.33, m	1''', 5''''	72.8, CH	3.30, m
4''''	74.7, CH	3.68, m	2''', 3''''	74.7, CH	3.60, m
5''''	78.7, CH	3.86, m	3''''	78.7, CH	3.81, m
6''''	65.4, CH_2	4.65, dd, 12.8, 5.1 4.86, dd, 12.8, 2.0	7'', 4'''' 7''	65.4, CH_2	4.66, dd, 11.9, 3.8 4.86, dd, 11.9, 2.0

^a HMBC correlations are from carbon stated to the indicated proton(s).

Figure 1. Structures of suffruiyabiosides A (**1**) and B (**2**), and seven known compounds **3–9**.**Figure 2.** 2D NMR correlations of suffruiyabioside A (**1**).

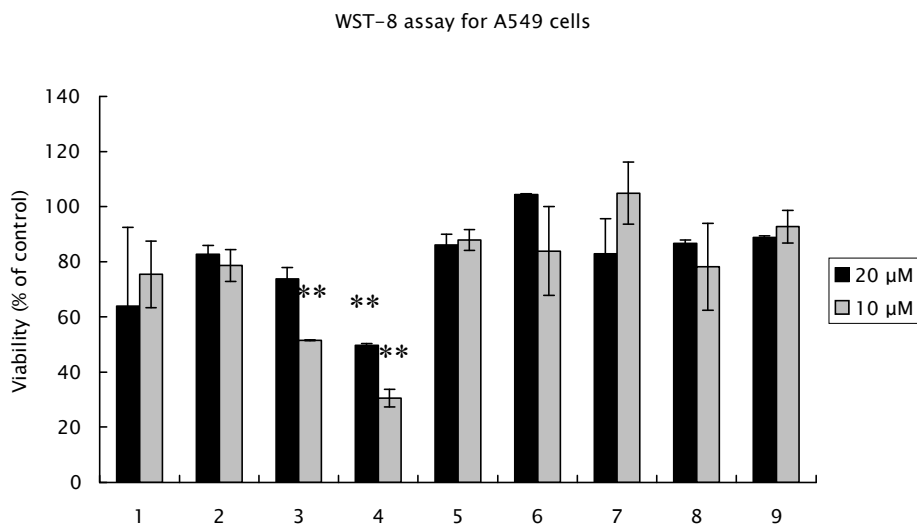
The stereochemistry at various stereocenters of the monoterpene unit was assigned on the basis of similarity of the reported spectral data [13]. On the basis of these observations **1** was established as 6'''-*O*-*p*-hydroxybenzoyl-6'''-*O*-β-glucopyranosyl- paeoniflorin.

The ¹H and ¹³C-NMR spectra (Table 1) of suffruiyabioside B (**2**) also resembled that of paeoniflorin (**3**), except for the additional appearance of sugar and benzoyl proton signals. The molecular formula was established as C₃₆H₄₂O₁₇ by HRESIMS data [*m/z* 769.2281 (M+Na)⁺, Δ −3.9 mmu]. Analysis of

the IR spectrum of **2** suggested the presence of hydroxyl (3388 cm^{-1}) and ester (1711 cm^{-1}) groups in the molecule. The ^1H and ^{13}C -NMR data indicated the presence of the same monoterpene pinane type skeleton as in **3** (Table 1). The two sugar NMR signals at H-1''' (δ_{H} 4.65, $J = 7.8\text{ Hz}$; δ_{C} 100.6) and H-1'''' (δ_{H} 4.44, $J = 7.8\text{ Hz}$; δ_{C} 105.7) and NOESY correlations of H-1''' to H-3''', H-1''' to H-5''', H-1''' to H-3''', and H-1''' to H-5''' revealed the presence of two β -glucose moieties. Furthermore, analysis of the HMBC spectrum (Table 1) clearly indicated that the anomeric carbon C-1'''' (δ_{C} 105.7) was coupled with H-4''' (δ_{H} 3.64) through the ether linkage. HMBC correlations of H₂-8 (δ_{H} 4.80) to C-7' (δ_{C} 168.3) indicated that the benzoyl group was attached to C-8 (δ_{C} 62.8) of the aglycone by the ester linkage, while the other benzoyl group was connected to C-6'''' (δ_{C} 65.4) of Glc2 by the ester linkage on the basis of HMBC correlation of H-6'''' (δ_{H} 4.66 and 4.86) to C-7'' (δ_{C} 168.8). Therefore, **2** was identified as 6''''-*O*-benzoyl-6'''-*O*- β -glucopyranosylpaeoniflorin.

The cytotoxic activities of suffruiyabiosides A (**1**) and B (**2**) and known compounds **3–9** against human lung adenocarcinoma epithelial A549 cells were assayed (Figure 3). Salicylpaeoniflorin (**4**) and paeoniflorin (**3**) showed moderate cytotoxicities (viabilities: 31% and 52%, respectively), while other compounds including suffruiyabiosides A (**1**) and B (**2**), showed very weak activities (viabilities: 63–100%).

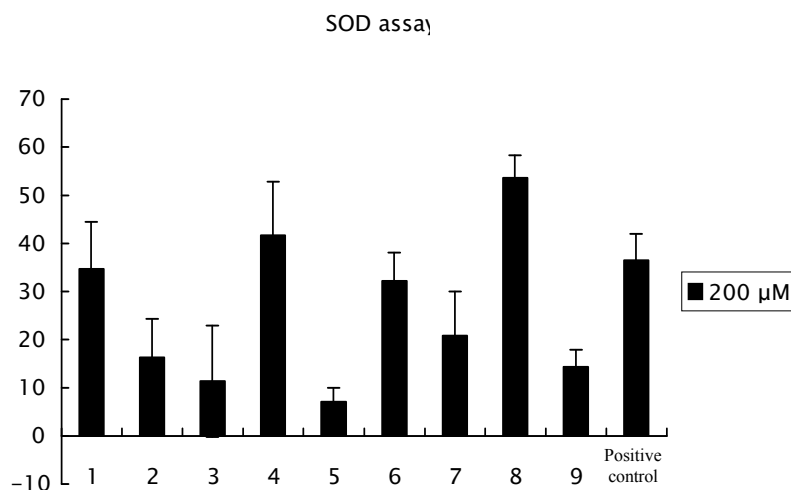
Figure 3. Antiproliferative effects of suffruiyabiosides A (**1**) and B (**2**), and known compounds **3–9** for A549 cells. Cells were incubated with different concentration of each compound for 48 h in 96-well plates. Proliferation was measured by WST-8 Assay. Results are expressed as percentage cell proliferation relative to the control group. This assay was repeated two times. Data are the mean $\pm$ SD of three determinations. ** significant difference ($p < 0.01$) vs. control cells. (Dunnett's test).



The structure-activity relationships for radical scavenging with isolated compounds **1–9** are shown in Figure 4. Galloylpaeoniflorin (**8**) was the most effective among the nine compounds **1–9**. Galloylpaeoniflorin (**8**) and salicylpaeoniflorin (**4**) revealed a more pronounced radical scavenging effect than α -tocopherol (positive control). Increase in the number of phenolic hydroxyl groups produced a more effective of radical scavenging effect (**8** > **6** > **5**). Comparing the effect of **4** with **5** showed that *o*-substitution with a phenolic hydroxyl group was more effective than *p*-substitution. In

addition, mudanpioside I (**9**) was more effective than paeoniflorin (**3**), indicating that a benzoyl group connected to a C-6 of the β -glucose by the ester linkage was more effective for radical scavenging than a benzoyl group attached to the C-8 monoterpene pinane type skeleton.

Figure 4. Radical scavenging effects of suffruiyabiosides A (**1**) and B (**2**) and known compounds **3–9**. After the incubation of the plate at 37 °C for 20 min, read the absorbance at 450 nm using an ELISA reader. This assay was repeated two times. Data are the mean $\pm$ SD of three determinations.



In conclusion, two new monoterpene glycosides, suffruiyabiosides A (**1**) and B (**2**) were isolated from Moutan Cortex. It was the first reported as having a cellobiose in paeoniflorin related compounds [9]. Salicylpaeoniflorin (**4**) exhibited both cytotoxic activity against A549 cells and a radical scavenging effect in an SOD assay, suggesting that salicylpaeoniflorin (**4**) may be useful as a cytotoxic and a radical scavenging agent.

3. Experimental

3.1. General

Optical rotations were measured with a JASCO DIP-370 polarimeter. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) spectra were measured and recorded in CD_3OD on a Bruker Avance 500 spectrometer. Chemical shift values (δ) were reported in parts per million (ppm) relative to CD_3OD (δ_{H} 3.35, δ_{C} 49.8). HRESIMS were recorded on Waters Xevo Q-Tof.

3.2. Plant Material

Moutan Cortex (the roots cortex of *Paeonia suffruticosa* Andrews.) was purchased from Beijing Tong Ren Tang Group Co., Ltd. in November 2007, which was collected from Anhui Province in China and dried by sunlight. This sample was paeoniaceae plant peony root bark, and the root was removed the wooden heart, washed, sliced, and dried. A voucher specimen has been deposited at the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan.

3.3. Extraction and Isolation

Moutan Cortex (1.0 kg) was crushed with homogenizer and extracted three times with EtOH (2L $\times$ 3) overnight at room temperature, followed by removal of solvent under reduced pressure, to yield a dried EtOH extract (340 g). The EtOH extract was dissolved in H₂O (900 mL) and extracted with EtOAc (900 mL $\times$ 3). The H₂O-soluble portion was partitioned between *n*-BuOH (900 mL $\times$ 3) and aqueous layers. The *n*-BuOH layer (16.4 g) was chromatographed on a silica gel using CHCl₃-MeOH-H₂O (65:25:4 $\rightarrow$ 5:5:1) to afford MC-BU-1~11. Further MC-BU-4 was separated using chromatograph on an ODS with MeOH-H₂O (1:9 $\rightarrow$ 3:7 $\rightarrow$ 1:1), to give 8 fractions MC-BU-4-1~8. MC-BU-4-4 was subjected to passage over an ODS HPLC column (TSK-gel ODS-80Ts, TOSOH, ϕ 7.8 $\times$ 300 mm, MeOH-H₂O = 20:80 $\rightarrow$ 100:0, 1.0 mL/min) to give oxypaeoniflorin (**5**, 25.3 mg). MC-BU-4-4-12 was further purified using an ODS HPLC column (TSK-gel ODS-80Ts, TOSOH, ϕ 4.6 $\times$ 250 mm, MeOH: H₂O = 20:80 $\rightarrow$ 100:0, 1.0 mL/min) to give mudanpioside E (**6**, 1.4 mg). MC-BU-4-5 (548.5 mg) was subjected to passage over an ODS HPLC column (TSK-gel ODS-80Ts, TOSOH, ϕ 7.8 $\times$ 300 mm, MeOH-H₂O = 20:80 $\rightarrow$ 100:0, 1.0 mL/min) to give paeoniflorin (**3**, 127.6 mg) and mudanpioside D (**7**, 0.9 mg). MC-BU-4-5-8 was further purified by an ODS HPLC column (TSK-gel ODS-80Ts, TOSOH, ϕ 7.8 $\times$ 300 mm, MeOH-H₂O = 30:80 $\rightarrow$ 100:0, 1.0 mL/min) to give salicylpaeoniflorin (**4**, 2.6 mg). MC-BU-4-6 (79.2 mg) subjected to passage over an ODS HPLC column (TSK-gel ODS-80Ts, TOSOH, ϕ 7.8 $\times$ 300 mm, MeOH-H₂O = 35:65 $\rightarrow$ 100:0, 1.0 mL/min) to give 9 fractions MC-BU-4-6-1~9. MC-BU-4-6-6 and MC-BU-4-6-7 were purified to give galloylpaeoniflorin (**8**, 4.7 mg) and mudanpioside I (**9**, 3.3 mg), respectively. MC-BU-4-6-8 was then purified by separation over an ODS HPLC column (TSK-gel ODS-80Ts, TOSOH, ϕ 4.6 $\times$ 250 mm, MeOH-H₂O = 40:60 $\rightarrow$ 100:0, 0.8 mL/min) to give suffruiyabioside A (**1**, 2.8 mg). MC-BU-4-6-9 further purified using an ODS HPLC column (TSK-gel ODS-80Ts, TOSOH, ϕ 4.6 $\times$ 250 mm, MeOH-H₂O = 50:50 $\rightarrow$ 100:0, 1.0 mL/min) to give suffruiyabioside B (**2**, 1.3 mg).

Suffruiyabioside A (1). Colorless amorphous solid; $[\alpha]_D^{27}$ -18.0 ($c = 1.00$, MeOH); IR (KBr) $\nu_{\max}$ 3412, 1729, 1525, 1404, and 1069 cm^{-1} ; UV (MeOH) $\lambda_{\max}$ nm (ϵ) 206 (26500), 226 (18400), and 258 (13700); ¹H and ¹³C-NMR see Table 1; ESIMS m/z 785 $[\text{M}+\text{Na}]^+$; HRESIMS m/z 785.2212 $[\text{M}+\text{Na}]^+$, calcd for C₃₆H₄₂O₁₈Na, 785.2269.

Suffruiyabioside B (2). Pale yellow amorphous solid; $[\alpha]_D^{28}$ -14.0 ($c = 1.00$, MeOH); IR (KBr) $\nu_{\max}$ 3388, 1711, 1459, 1357, and 1078 cm^{-1} ; UV (MeOH) $\lambda_{\max}$ nm (ϵ) 202 (18700), 226 (26300), and 258 (4500); ¹H and ¹³C-NMR: see Table 1; ESIMS m/z 769 $[\text{M}+\text{Na}]^+$; HRESIMS m/z 769.2281 $[\text{M}+\text{Na}]^+$, calcd for C₃₆H₄₂O₁₇Na, 769.2320.

3.4. Preparation of Paeoniflorin and Other Compounds

Suffruiyabiosides A (**1**) and B (**2**) and other seven known compounds **3–9** were dissolved in water and stored at -30 °C. For all cell proliferation assay, the final concentrations of the test compounds were prepared by diluting the stock with RPMI-1640. Control cultures also received the carrier solvent RPMI-1640.

3.5. Cell Culture

Human lung adenocarcinoma epithelial A549 cells (ATCC CCL 185, RIKEN Cell Bank) was maintained in monolayer culture at 37 °C and 5% CO₂ in RPMI-1640 supplemented with 10% FBS, 100 units/mL penicillin G and 100 µg/mL streptomycin.

3.6. Cell Proliferation Assay

Inhibition of cell proliferation for A549 cell by suffruiabiosides A (1) and B (2) and other seven known compounds 3–9 was measured using the WST-8 assay. Briefly, cells were placed in 96-well culture plates (1 × 10⁴ cells/well). After 24 h incubation, cells were then incubated for 48 h in the presence or absence of compounds 1–9 (20 and 10 µM) dissolved in culture medium and an appropriate solvent. The WST-8 test solution (10 µL) was added to each well. After 4 h incubation, absorbance was measured on ELISA reader (Microplate Reader Model 550, Bio-RAD) at the test wavelength of 450 nm. RPMI-1640 supplemented with 10% FBS was used as the negative control.

3.7. Radical Scavenging Effect with SOD

Suffruiabiosides A (1) and B (2) and other seven known compounds 3–9 were subjected to a superoxide dismutase (SOD) assay using the SOD Assay Kit-WST according to the technical manual. Briefly, in a 96-well plate, 20 µL of sample solution was added to each sample and blank 2 well, and 20 µL of double distilled water was added to each blank 1 and blank 3 well. Then 200 µL of WST working solution was added to each well. After mixing, 20 µL of dilution buffer was added to each blank 2 and blank 3 well, and 20 µL of enzyme working solution (15 µL of enzyme mixed with 2.5 µL dilution buffer) was added to each sample and blank 1 well. The plate was incubated at 37 °C for 20 min and the O.D. was determined at 450 nm using an ELISA reader (Microplate Reader Model 550, Bio-RAD). The SOD-like activity was calculated by the following equation:

$$\text{SOD activity (inhibition rate \%)} = \{[(A \text{ blank } 1 - A \text{ blank } 3) - (A \text{ sample} - A \text{ blank } 2)] / (A \text{ blank } 1 - A \text{ blank } 3)\} \times 100$$

where A blank 1, A blank 2, A blank 3, and A sample were the absorbance of blank 1, blank 2, blank 3, and the sample, respectively.

4. Conclusions

Two new monoterpene diglycosides, suffruiabiosides A (1) and B (2), and seven known compounds 3–9 were isolated from Moutan Cortex (root cortex of *Paeonia suffruticosa* Andrews). Suffruiabiosides A (1) and B (2) are rare monoterpene diglycosides, including a cellobiose in the molecules. Salicylpaeoniflorin (4) had a cytotoxic effect on human lung adenocarcinoma epithelial A549 cells and radical scavenging effect.

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Sample Availability: Samples of the compounds **3** and **5** are available from the authors.