Asian Journal of Chemistry; Vol. 24, No. 2 (2012), 538-540

ASIAN JOURNAL OF CHEMISTRY

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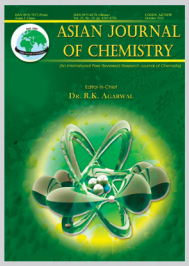

Isolation and Evaluation of Antinociceptive Activity of Sulfur-Containing Iridoid Glucosides from *Paederia scandens*

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(*Received*: 19 January 2011; *Accepted*: 5 October 2011) AJC-10476

Isolate and purify the sulfur-containing iridoid glucosides from *Paederia scandens*. Three sulfur-containing iridoid glucosides (**1-3**) were isolated and the structures of them were elucidated as paederosides (**1**), paederosidic acid (**2**) and paederosidic acid methyl ester (**3**) by spectroscopic analysis. The sulfur-containing iridoid glucosides were evaluated on antinociceptive activity in mice using thermal models of nociception. Both paederosidic acid (**2**, 120 mg/kg, i.g. or 70 mg/kg, i.p.) and paederosidic acid methyl ester (**3**, 70 mg/kg, i.p.) produced significant inhibitory activity on thermal nociception in the hot plate test. Paederoside (**1**, 70 mg/kg, i.p.) had not produced significant antinociceptive effects in the hot plate test.

Key Words: *Paederia scandens*, Sulfur-containing iridoid glucosides, Paederoside, Paederosidic acid, Paederosidic acid methyl ester, Antinociceptive activity, Spectroscopic analysis.

INTRODUCTION

Herbal drugs have been widely used for thousands of years in China for the treatment of human diseases. *Paederia scandens* (Lour.) Merri., a climbing plant, belonging to the family Rubiaceae, is widely grown around many countries including China, India, Japan, Vietnam, Philippines and USA[1]. In folklore medicine, the roots, leaves, barks and fruits of *P. scandens* have been used to treat toothache, chest pain, piles, inflammation of the spleen, diuretic, emetic, rheumatic arthritis and cure bacillary dysentery in the south-east Asia for thousands of years[2]. The leaves of the plant are used as an ingredient in various foods in Vietnam[3].

Chemically, it has been reported that the iridoid glycosides and the dimeric iridoid glycosides paederoside, asperuloside, paederosidic acid, deacetyl asperuloside, paederosidic acid methyl ester[1,4-7], were isolated from the MeOH extract from the stems and roots of *P. scandens*[8,9]. The sulfur-containing iridoid glucosides separated from this plant[1,10], members of this family of compounds have shown many pharmacological properties. It has been reported that the iridoid glycosides had significant antinociceptive effects[11]. Although *P. scandens* is a particularly useful pain-relief in folklore medicine. There has been no report on the antinociceptive activity of this plant and its mechanisms of analgesic activity so far.

In a continuation of our studies, we have reinvestigated the analgesic activity of the extracts of *P. scandens* and the antinociceptive activity of the volatile oils, the flavonids and the sulfur-containing iridoid glycosides from *P. scandens* and found that the sulfur-containing iridoid glucosides had a powerful antinociceptive activity in preliminary experiment. In this study, three sulfur-containing iridoid glucosides paederoside, paederosidic acid and paederosidic acid methyl ester was isolated from *P. scandens* and evaluated on nociception models mice induced by the thermal stimuli so as to elucidate the analgesic activity and the possible mechanism of the sulfur-containing iridoid glucosides.

EXPERIMENTAL

P. scandens was purchased from medicinal materials market of Bozhou (Bozhou City, Anhui Province, China) and identified by Professor Chenggang Huang. NMR spectra were measured on Varian Mercury-400 (Varian, USA), using CD_3OD as solvents and TMS as an internal standard. ESI-MS was recorded on a Finnigan LCQ_{DECA} mass spectrometer (Finnigan, USA). HR-ESI-MS was carried out on a Micromass Q-TOF Ultima (Micromass, UK). HPLC grade acetonitrile was purchased from Dikma Company (Dikma, USA). The silica gel was purchased from Qingdao Haiyang Chemical Plant (Qingdao Haiyang Chemical Plant, Qingdao, China) and other reagents used are of analytical grade (Sinopharm Chemical Reagent Co. Ltd., China).

Animals: Experimental groups consisted of 6 KM mice (18-22 g) per group. They were housed at 21 ± 1 ºC under a 12 h light/12 h dark cycle and had free access to a standard pellet diet (Purina Chow) and tap water. The animals were deprived of food for 15 h before the experiment, with free access to drinking water. Each animal was used only once in the experiment.

Sample preparation: The dried powders of whole plant of *P. scandens* (10 kg) were extracted with 80 % EtOH using Soxhlet apparatus at 80 ºC. The EtOH extract was concentrated under reduced pressure to obtain a residue (986 g). The EtOH extract was subsequently extracted with petroleum ether, chloroform, *n*-BuOH, respectively. The *n*-BuOH fraction was also concentrated under reduced pressure to obtain a residue (134.3 g).

The *n*-BuOH extract was subjected to silica gel column eluting with $CHCl_3$: MeOH (10:1) gradient to afford 4 fractions. Fraction 3 was purified by HPLC with Inertsil ODS-3 column (250 mm × 10 mm, 10 μm, GL Sciences Inc., Japan) eluting with MeOH:H_2O (50:50, flow rate 3 mL/min) to give **1** (52.5 mg) and **3** (92.5 mg). Fraction 2 was purified by HPLC with the same Inertsil ODS-3 column eluting with MeOH: H_2O (40:60, flow rate 3 mL/min) to give **2** (1836.9 mg).

Hot plate test: The hot-plate test[12] was carried out on groups of female mice using a hot-plate apparatus (model YLS-6B, China), maintained at 55 ± 1 ºC. Pre-treatment latencies were determined three times with intervals of 20 min. Only mice that showed initial nociceptive responses between 5 and 30 s were selected for the experiment. The latency to first sign of hind paw licking or jumping to avoid heat nociception was taken as an index of nociceptive threshold. In this test, pretreatment latencies were determined three times with intervals of 20 min. The cut-off time was 60 s in the hot-plate test in order to minimize skin damages.

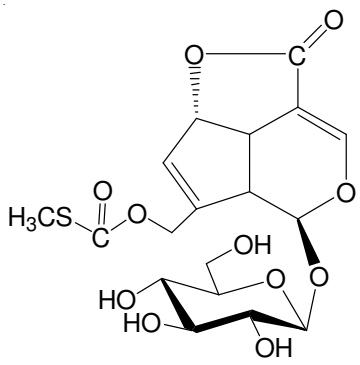
RESULTS AND DISCUSSION

Three sulfur-containing iridoid glucosides (**1-3**) were isolated from *P. scandens*. The structures of them were elucidated as paederoside (**1**), paederosidic acid (**2**) and paederosidic acid methyl ester (**3**) by spectroscopic analysis.

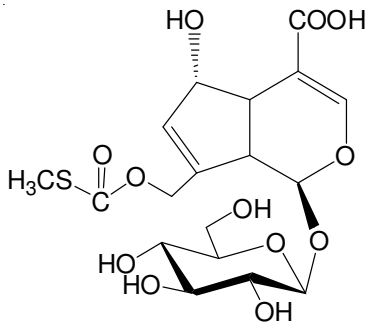
Data analysis of sulfur-containing iridoid glucosides

Compound 1: Yellow amorphous power, positive ion ESI-MS m/z (intensity %): 914.7 $[2M + Na]^+$ (77), 468.9 $[M + Na]^+$ (75); negative ion ESI-MS m/z (intensity %): 445.0 $[M-H]^-$ (42), 282.9 $[M-Glc-H]^-$ (19), 191.0 $[M-Glc-OCOSH_3-H]^-$ (62), 191.0 $[M-Glc-OCOSH_3-H]^-$ (62), 147.1 $[M-Glc-OCOSH_3-CO-O-H]^-$ (100); HR-ESI-MS m/z: 469.0769 $[M + Na]^+$ (calcd. for $C_{18}H_{22}O_{11}SNa^+$, 469.0775). ^1H NMR(CD_3OD) δ: 5.79 (1H, d, *J* = 1.2 Hz, H-1), 7.37 (1H, d, *J* = 2.0 Hz, H-3), 3.66 (1H, ddd, *J* = 8.8, 6.1, 2.0 Hz, H-5), 5.57 (1H, d, *J* = 6.1 Hz, H-6), 5.71 (1H, s, H-7), 3.26 (1H, dd *J* = 8.8, 1.2 Hz, H-9), 4.82 (1H, d, *J* = 14.2 Hz, H-10), 5.01 (1H, br d, *J* = 14.2 Hz, H-10), 4.48 (1H, d, *J* = 7.8 Hz, H-1'), 2.34 (3H, s, H-13). ^{13}C NMR (CD_3OD) date showed in Table-1.

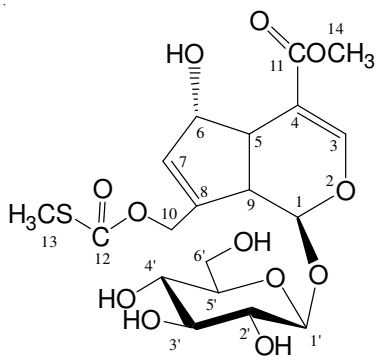
Compound 2: Yellow amorphous power, Positive ion ESI-MS m/z (intensity %): 950.9 $[2M + Na]^+$ (15), 487.0 $[M + Na]^+$ (39); ESI-MS m/z: 487$[M + Na]^+$, negative ion ESI-MS m/z (intensity %): 926.6 $[2M-H]^-$ (30), 463.0 $[M-H]^-$ (100);



Paederoside (**1**)



Paederosidic acid (**2**)



Paederosidic acid methyl ester (**3**)

HR-ESI-MS m/z: 487.0887 (calcd. (%) for $C_{18}H_{24}O_{12}SNa^+$, 487.0881). ^1H NMR (CD_3OD) δ: 5.07 (1H, d, *J* = 7.7 Hz, H-1), 7.66 (1H, d, *J* = 1.0 Hz, H-3), 3.06 (1H, ddd, *J* = 8.2, 6.0, 1.0 Hz, H-5), 4.86 (1H, dd, *J* = 6.0, 1.9 Hz, H-6), 6.08 (1H, d, *J* = 1.9 Hz, H-7), 2.63 (1H, dd, *J* = 8.2, 7.7 Hz, H-9), 4.96 (1H, dd, *J* = 15.4 Hz, H-10), 5.14 (1H, br d, *J* = 15.4 Hz, H-10), 4.74 (1H, d, *J* = 7.8 Hz, H-1'), 2.38 (3H, s, H-13). ^{13}C NMR (CD_3OD) date showed in Table-1.

Compound 3: Yellow amorphous power, ESI-MS m/z: 501$[M + Na]^+$, positive ion ESI-MS m/z (intensity %): 501.0 $[M + Na]^+$ (100), negative ion ESI-MS m/z (intensity %): 659.1 $[M + HAc-H]^-$ (36), 257.0 $[M-Glc-COOH_3-H]^-$ (100); HR-ESI-MS m/z: 501.1054 (calcd. (%) for $C_{19}H_{26}O_{12}SNa^+$, 501.1037). ^1H NMR (CD_3OD) δ: 4.82 (1H, d, *J* = 7.5 Hz, H-1), 7.54 (1H, d, *J* = 1.0 Hz, H-3), 3.06 (1H, ddd, *J* = 8.0, 6.0, 1.0 Hz, H-5), 5.26 (1H, dd, *J* = 6.0, 2.1 Hz, H-6), 6.15 (1H, d, *J* = 2.1 Hz, H-7), 2.63 (1H, dd, *J* = 8.0, 7.5 Hz, H-9), 4.96 (1H, dd, *J* = 15.1 Hz, H-10), 5.14 (1H, br d, *J* = 15.1 Hz, H-10), 4.85 (1H, d, *J* = 8.1 Hz, H-1'), 2.34 (3H, s, H-13). 3.81 (3H, s, H-14). ^{13}C NMR (CD_3OD) date showed in Table-1.

TABLE-2
EFFECTS OF COMPOUND **1-3** ON THERMAL-INDUCED ANTINOCICEPTION IN THE HOT PLATE TEST (n = 6)

Group	Duration on the hot-plate (s) (mean ± SEM)				
	0 min	30 min	60 min	90 min	120 min
1 (70 mg/kg, i.p.)	16.2 ± 2.1	18.8 ± 3.0	17.1 ± 3.9	14.4 ± 3.5	15.2 ± 5.6
2 70 mg/kg, i.p.)	18.9 ± 1.0	20.7 ± 4.5	20.6 ± 5.4	22.0 ± 6.4*	20.3 ± 5.2
2 (120 mg/kg, i.g.)	19.6 ± 0.9	21.1 ± 4.9	20.9 ± 3.1	24.8 ± 6.2*	19.4 ± 5.4
3 (70 mg/kg, i.p.)	13.1 ± 2.3	13.8 ± 3.0	16.6 ± 3.2**	15.9 ± 2.1*	15.4 ± 5.4

*$p < 0.05$, **$p < 0.01$, (ANOVA followed by Dunnett's test).

TABLE-1
^{13}C NMR OF COMPOUND **1-3** (CD_3OD)

No.	**1**	**2**	**3**
1	93.7	101.7	101.7
3	150.8	155.9	155.9
4	106.6	108.7	108.6
5	37.9	42.9	42.9
6	86.7	75.7	75.8
7	129.9	132.9	132.9
8	144.3	145.9	145.9
9	45.7	46.7	46.7
10	64.8	66.8	66.7
11	173.2	172.7	173.3
12	173.0	171.1	169.8
13	14.1	14.0	14.0
14	–	–	52.3
1'	100.5	101.1	101.2
2'	75.1	75.4	75.4
3'	78.4	78.4	78.4
4'	72.0	72.1	72.0
5'	78.8	79.1	79.1
6'	63.2	63.5	63.5

Effect of the hot-plate test: In the hot-plate test, the mean of the durations in the group of paederoside (**1**, 70 mg/kg, i.p.), paederosidic acid (**2**, 120 mg/kg, i.g. and 70 mg/kg, i.p.) and paederosidic acid methyl ester (**3**, 70 mg/kg, i.p.) showed in Table-2. The result showed that the group treated with paederosidic acid (**2**, 70 mg/kg, i.p. and 120 mg/kg, i.g.) and paederosidic acid methyl ester (**3**, 70 mg/kg, i.p.) had the powerful antinociceptive effect.

Conclusion

In this study, three sulfur-containing iridoid glucosides were isolated from *P. scandens* and the structures of them were elucidated as paederoside (**1**), paederosidic acid (**2**) and paederosidic acid methyl ester (**3**) by spectroscopic analysis. These three sulfur-containing iridoid glucosides were evaluated on antinociceptive activity in mice using thermal models of nociception. The results showed that the groups treated with paederosidic acid and paederosidic acid methyl ester both had significant antinociceptive effects. It provides scientific basis for the antinociceptive activity of the sulfur-containing iridoid glucosides from *P. scandens*. The result has great significance for the clinical use of *P. scandens* and the exploitation and utilization of the resources of *P. scandens*.

ACKNOWLEDGEMENTS

The authors thank the National Science & Technology Major Project "Key New Drug Creation and Manufacturing Program" (No. 2009ZX09301-001; 2009ZX093 08-005) for financial support of this work.

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