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Iridoid glycosides from *Scrophularia ningpoensis*

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Abstract

Three new minor iridoids were isolated from the roots of *Scrophularia ningpoensis*. They were elucidated as 8-O-feruloylharpagide, 8-O-(2-hydroxy-cinnamoyl)harpagide and 6-O-α-D-galactopyranosylharpagoside. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Scrophularia ningpoensis*; Scrophulariaceae; Iridoid glycosides; 8-O-feruloylharpagide; 8-O-(2-hydroxycinnamoyl)harpagide; 6-O-α-D-galactopyranosylharpagoside

1. Introduction

The roots of *Scrophularia ningpoensis* Hemsl. are used as a famous Chinese medicine named ''Xuanshen'' for the treatment of various inflammatory diseases Jiangsu New Medical College (1977); Duck & Ayensu (1985). In previous papers seven iridoid glycosides: harpagide, harpagoside, aucubin, 6-O-methylcatalpol, scropoliside A and ningpogosides A and B were reported from this plant Kajimoto, Hidaka, Shoyama, & Nohara (1989); Qian, Hunkler, & Rimpler (1992); Zhang et al. (1994). In this paper, we report on the isolation of three new minor iridoid glycosides from the roots of *S. ningpoensis*.

2. Results and discussion

The water suspension of the hot EtOH extract of the powdered roots of the plant was partitioned successively against ether and *n*-BuOH. The *n*-BuOH-soluble part was subjected to Sephadex LH20, ODS and silica gel CC. Five iridoid glycosides were obtained: 8-O-feruloyl harpagide (**1**), 8-O-(2-hydroxycinnamoyl) harpagide (**2**), 6-O-α-D-galactopyranosylharpagoside (**3**), harpagoside (**4**) and harpagide (**5**). The known compounds **4** and **5** were identified by comparison with the published data for authentic samples Zhang et al. (1994). The structures of the new compounds **1**–**3** were established mainly by spectroscopic methods.

The ^{1}H and ^{13}C NMR data of compound **1** were similar to those of **4** Zhang et al. (1994) except for the signals of the cinnamic acid residue. The ^{1}H NMR spectrum of **1** contained the signals for two *trans* olefenic protons (δ 7.42 and 6.18; $2 \times 1H$, d, $J = 15.8$ Hz), three protons of an aromatic ring (δ 7.01, d, $J = 1.6$ Hz, H-2′; 6.90, dd, $J = 8.4$ and 1.6 Hz, H-6′; 6.64, d, $J = 8.4$ Hz, H-5′) and a methoxyl group (δ 3.72, s, 3H) linked to the aromatic ring. The ^{13}C NMR spectrum of **1** revealed two oxygen atoms linked to the aromatic ring (δ 149.9 and 147.1). These data established that in **1** a ferulic or isoferulic acid residue had replaced the cinnamic acid residue found in harpagoside. Since NOEs were observed between H-2 and the methoxyl group, we decided it was a ferulic acid residue so **1** was 8-O-feruloylharpagide.

Compound **2** was obtained as a gum. Its ^{1}H and ^{13}C NMR spectra were also similar to those of **4** except for the signals belonging to the cinnamic acid residue. The ^{1}H NMR spectrum of **2** contained the signals for two *trans* olefenic protons (δ 7.78, 6.53; $2 \times 1H$, d, $J = 16.0$ Hz) and four aromatic protons linked together (δ 7.57, 1H, d, $J = 7.8$ Hz; 7.23, 1H, t, $J = 7.8$ Hz; 6.91, 1H, d, $J = 7.6$ Hz; 6.82, 1H, t, $J = 7.6$ Hz). The ^{13}C NMR spectrum of **2** showed

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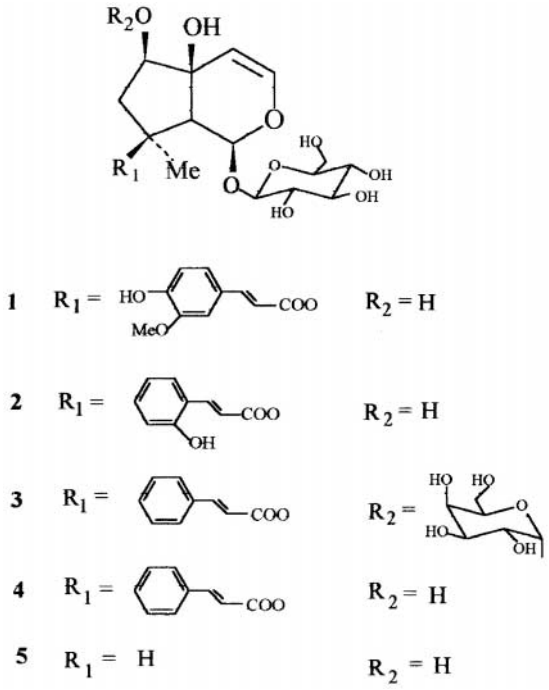

there was only one oxygen atom linked to the aromatic ring (δ 156.8), so the hydroxyl group had to be linked to C-2′ of the aromatic ring and, therefore, in **2** a 2-hydroxy cinnamic acid residue replaced the cinnamic acid residue grand in harpagoside. This was confirmed by the ^{13}C NMR data and the HMBC spectrum. Therefore, **2** was 8-O-(2-hydroxycinnamoyl)harpagide.

Compound **3** was an amorphous powder. Its molecular formula was established as $C_{30}H_{40}O_{16}$ by its FABMS and the NMR spectral data. The NMR spectra showed that **3** was a glycoside of **4**. On acid hydrolysis and TLC with authentic samples, **3** gave glucose and galactose, but did not yield any disaccharide. These facts implied that the galactose was linked to either the C-5 or the C-6 hydroxyl group of the aglycone. By using HMBC, HMQC, TOCSY and ^1H–^1H COSY methods, every proton and carbon NMR signal was assigned unambiguously. With the clear coupling data for every proton, we knew that the sugar moieties were α-D-galactopyranosyl and β-D-glucopyranosyl. In HMBC, the anomeric proton and carbon of the galactosyl moiety were correlated with C-6 and H-6 of the aglycone. Furthermore, the resonance of C-6 in **3** was downfield (δ_C 87) compared with those of **4**(δ_C 78.1) and **5** (δ_C 77.9). The anomeric proton of the glucosyl residue was correlated with C-1 of the aglycone just as in harpagoside and harpagide. Therefore, the structure of **3** was proven to be 6-O-α-D-galactopyranosylharpagoside.

3. Experimental

3.1. General

Mps: uncorr.; FABMS: glycerol as matrix on a MAT-95 double focusing mass spectrometer; ^1H and ^{13}C NMR: Bruker AM-400 spectrometer with TMS as int. standard and MeOH or DMSO as solvent.

3.2. Plant material

Roots of *Scrophularia ningpoensis* were collected from the Lingbo area (Zhejiang province of China). The plant was identified by vice-professor Xiaoqiang Ma of our institute. A voucher specimen (No. 95-10) had been deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3. Extraction and isolation

8 kg of powdered roots of the plant were extracted with hot 95% EtOH (twice), 60% EtOH (twice) for 1 h each time under refluxing. The combined EtOH extract was concentrated in vacuo. The residue was suspended in H_2O and shaken successively with Et_2O and *n*-BuOH. A portion of the *n*-BuOH layer residue (140 g) was subjected to CC on the macro-porous resin DA-201 (which is similar to amberlite XAD-2) eluted with H_2O and increasing amounts of EtOH. A portion of the 60% EtOH fraction (1.3 g) was subjected to CC on silica gel eluted repeatedly with $CHCl_3$–MeOH–H_2O (7:1:0.1) to afford compound **4** (0.95 g). The 40% EtOH fraction was subjected to CC on silica gel eluted with $CHCl_3$–MeOH–H_2O (6:1:0.1) and combined by TLC to obtain three main parts (A–C). Frs A (13.5 g) was applied to Sephadex LH20 column with 10% EtOH followed by CC on silica gel with EtOAc–MeOH–H_2O (10:1:0.5). Frs 8–14 (310 mg) were combined and rechromatographed on silica gel with CH_2Cl_2–CH_3COCH_3–H_2O (10:12.5:0.1) as eluant. Frs 65–70 were combined and subjected to ODS CC with 50% MeOH to obtain two main parts. Frs 17–21 (part 1) were combined and purified by CC on silica gel with EtOAc–MeOH–H_2O (10:2:1) to afford compound **1** (8 mg). Frs 27–29 (part 2) were combine and purified by the same method to afford compound **2** (11 mg). Fr B on repeated CC on silica gel with $CHCl_3$–MeOH–H_2O(6:1:0.1) and EtOAc–MeOH–H_2O (40:5:3) gave compound **5** (54 mg). Fr C (10.2 g) was subjected to CC on silica gel with EtOAc–MeOH–H_2O (40:5:3) and Frs 5–6 combined and subjected to ODS CC with 50% MeOH and further purified by silica gel CC with EtOAc–MeOH–H_2O (40:5:3) to obtain compound **3** (14 mg).

3.4. 8-O-feruloylharpagide (**1**)

Amorphous powder; mp 150–152°C, $[\alpha]_D$ −24.20° (MeOH; *c* 0.231). Anal. calcd. for $C_{25}H_{32}O_{13}\cdot 1/2H_2O$: C 54.65, H 6.05, Found: C 54.13, H 6.14. FABMS *m/z*: 563[M + Na]$^+$, 579[M + K]$^+$, 541[M + H]$^+$. ^{1}H NMR (400 Mz, CD_3OD): δ 1.35 (3H, s, H-10), 1.85 (1H, dd, *J* = 15.3/4.0 Hz, H-7a), 2.08 (1H, d, *J* = 15.3 Hz, H-7b), 2.76 (1H, s, H-9), 3.59 (1H, d, *J* = 4.0 Hz, H-6), 4.77 (1H, dd, *J* = 6.4/1.4 Hz, H-4), 6.01 (1H, s, H-1), 6.25 (1H, d, *J* = 6.4 Hz, H-3), 7.01 (1H, d, *J* = 1.6 Hz, H-2′), 6.64 (1H, d, *J* = 8.4 Hz, H-5′), 6.90 (1H, dd, *J* = 8.4/1.6 Hz, H-6′), 6.18 (1H, d, *J* = 15.8 Hz, H-α), 7.42 (1H, d, *J* = 15.8 Hz, H-*β*), 4.46 (1H, d, *J* = 7.9 Hz, H-1″), 3.05 (1H, t, *J* = 9.1 Hz, H-2″), 3.24 (1H, t, *J* = 8.8 Hz, H-3″), 3.14 (1H, t, *J* = 7.9 Hz, H-4″), 3.19 (1H, dd, *J* = 5.7/2.0 Hz, H-5″), 3.55 (1H, dd, *J* = 12.1/5.7 Hz, H-6a″), 3.77 (1H, dd, *J* = 12.1/2.0 Hz, H-6b″); ^{13}C NMR: Table 1.

Table 1
^{13}C NMR data of compounds **1**–**5**

C	**1** (CD_3OD)	**2** (DMSO-d_6)	**3** (CD_3OD)	**4** (CD_3OD)	**5**(CD_3OD)
Aglycone					
1	95.2	92.5	95.1	95.1	93.6
3	144.4	141.3	145.4	144.3	142.9
4	107.4	107.3	106.0	107.3	108.8
5	73.9	71.5	75.2	73.8	72.9
6	78.2	75.7	87.8	78.1	77.9
7	46.8	44.6	45.7	46.6	47.6
8	88.9	86.6	89.5	89.2	78.7
9	56.2	54.3	56.7	56.0	60.0
10	23.4	22.3	23.1	23.0	25.3
Cinnamoyl					
1′	128.1	120.8	136.1	136.2	
2′	112.2	156.8	130.6	130.4	
3′	147.1	116.1	129.8	129.6	
4′	149.9	131.6	132.1	131.9	
5′	117.0	119.5	129.8	129.6	
6′	124.6	129.2	130.6	130.4	
α	117.3	118.7	120.3	120.5	
β	144.4	139.9	146.8	146.5	
C=O	169.6	166.5	168.9	169.2	
CH_3O	57.0				
Glucose					
1‴	100.6	97.2	101.0	100.4	99.8
2‴	75.2	73.1	75.0	75.0	74.9
3‴	78.2	76.1	78.0	78.5	78.7
4″	72.3	70.1	72.5	72.0	72.2
5″	78.7	77.2	78.6	78.0	78.6
6″	63.6	61.0	63.7	63.4	63.2
Galactose					
1‴			104.0		
2‴			71.3		
3‴			72.3		
4‴			71.3		
5‴			73.4		
6‴			62.9		

3.5. 8-O-(2-hydroxycinnamoyl)harpagide (**2**)

Gum, $[\alpha]_D$ −35.73° (MeOH; *c* 0.187). Anal. calcd. for $C_{24}H_{30}O_{12}\cdot 1/2H_2O$: C 55.49, H 6.01, Found: C 54.87, H 6.17. FABMS *m/z*: 533[M + Na]$^+$, 549[M + K]$^+$, 511[M + H]$^+$. ^{1}H NMR (400 Mz, DMSO-d_6): δ 1.44 (3H, s, H-10), 1.83 (1H, dd, *J* = 14.8/4.0 Hz, H-7a), 2.16 (1H, d, *J* = 14.6 Hz, H-7b), 2.67 (1H, s, H-9), 3.61 (1H, d, *J* = 4.0 Hz, H-6), 4.90 (1H, d, *J* = 6.3 Hz, H-4), 5.99 (1H, s, H-1), 6.39 (1H, d, *J* = 6.3 Hz, H-3), 6.91 (1H, d, *J* = 7.6 Hz, H-3′), 7.23 (1H, t, *J* = 7.8 Hz, H-4′), 6.82 (1H, t, *J* = 7.6 Hz, H-5′), 7.57 (1H, d, *J* = 7.8 Hz, H-6′), 6.53 (1H, d, *J* = 16.0 Hz, H-α), 7.78 (1H, d, *J* = 16.0 Hz, H-*β*), 4.41 (1H, d, *J* = 8.0 Hz, H-1″), 2.99 (1H, t, *J* = 8.4 Hz, H-2″), 3.14 (1H, m, H-3″), 3.08 (1H, t, *J* = 9.0 Hz, H-4″), 3.14 (1H, m, H-5″), 3.49 (1H, dd, *J* = 11.8/5.7 Hz, H-6a″), 3.70 (1H, brd, *J* = 11.8 Hz, H-6b″); ^{13}C NMR: Table 1.

3.6. 6-O-α-D-galactopyranosylharpagoside (**3**)

Amorphous powder, mp 169–173°C, $[\alpha]_D$ −7.43° (MeOH; *c* 0.336). Anal. calcd. for $C_{30}H_{40}O_{16}\cdot H_2O$: C 53.41, H 6.27, Found: C 53.10, H 6.39. FABMS *m/z*: 679[M + Na]$^+$, 695[M + K]$^+$, 657[M + H]$^+$; ^{1}H NMR (400 Mz, CD_3OD): δ 1.38 (3H, s, H-10), 1.88 (1H, dd, *J* = 15.4/4.0 Hz, H-7a), 2.31 (1H, d, *J* = 15.3 Hz, H-7b), 2.75 (1H, s, H-9), 3.55 (1H, d, *J* = 4.0 Hz, H-6), 4.79 (1H, dd, *J* = 6.4/1.0 Hz, H-4), 6.02 (1H, s, H-1), 6.29 (1H, d, *J* = 6.4 Hz, H-3), 7.46 (1H, m, H-2′), 7.24 (1H, m, H-3′), 7.24 (1H, m, H-4′), 7.24 (1H, m, H-5′), 7.46 (1H, m, H-6′), 6.36 (1H, d, *J* = 16.1 Hz, H-α), 7.51 (1H, d, *J* = 16.1 Hz, H-*β*), 4.44 (1H, d, *J* = 7.8 Hz, H-1″), 3.06 (1H, t, *J* = 8.3 Hz, H-2″), 3.24 (1H, t, *J* = 8.8 Hz, H-3″), 3.14 (1H, dd, *J* = 8.7/6.4 Hz, H-4″), 3.20 (1H, dd, *J* = 6.4/2.0 Hz, H-5″), 3.55 (1H, m, H-6a″), 3.78 (1H, dd, *J* = 12.0/2.0 Hz, H-6b″); 4.85 (1H, d, *J* = 3.8 Hz, H-1‴), 3.63 (1H, dd, *J* = 10.0/3.7 Hz, H-2‴), 3.50 (1H, dd, *J* = 9.9/3.2 Hz, H-3‴), 3.67 (1H, d, *J* = 2.9 Hz, H-4‴), 4.08 (1H, t, *J* = 6.3 Hz, H-5‴), 3.55 (2H, m, H-6‴); ^{13}C NMR: Table 1.

3.7. Harpagoside (**4**)

Amorphous powder, mp 128–130°C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1600, 1570, 1505, 1445, 770; ^{1}H NMR (400 Mz, CD_3OD): δ 1.37 (3H, s, H-10), 1.85 (1H, dd, *J* = 14.5/4.0 Hz, H-7a), 2.11 (1H, d, *J* = 14.5 Hz, H-7b), 2.77 (1H, s, H-9), 3.60 (1H, d, *J* = 4.0 Hz, H-6), 4.77 (1H, dd, *J* = 6.4/1.4 Hz, H-4), 6.02 (1H, s, H-1), 6.25 (1H, d, *J* = 6.4 Hz, H-3), 7.42 (1H, m, H-2′), 7.24 (1H, m, H-3′), 7.24 (1H, m, H-4′), 7.24 (1H, m, H-5′), 7.42 (1H, m, H-6′), 6.35 (1H, d, *J* = 16.0 Hz, H-α), 7.49 (1H, d, *J* = 16.0 Hz, H-*β*), 4.46 (1H, d, *J* = 8.0 Hz, H-1′), 3.05 (1H, t, *J* = 8.4 Hz, H-2″), 3.24 (1H, t,

J = 8.6 Hz, H-3″), 3.14 (1H, t, J = 8.1 Hz, H-4″), 3.18 (1H, dd, J = 5.8/2.1 Hz, H-5″), 3.55 (1H, dd, J = 12.5/5.8 Hz, H-6a″), 3.77 (1H, dd, J = 12.5/2.1 Hz, H-6b″); ^{13}C NMR: Table 1.

3.8. Harpagide (**5**)

Amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650; ^{1}H NMR (400 Mz, CD_3OD): δ 1.08 (3H, s, H-10), 1.63 (1H, dd, J = 13.7/5.1 Hz, H-7a), 1.71 (1H, d, J = 14.5 Hz, H-7b), 2.77 (1H, s, H-9), 3.60 (1H, d, J = 4.0 Hz, H-6), 4.77 (1H, dd, J = 6.4/1.4 Hz, H-4), 6.02 (1H, s, H-1), 6.25 (1H, d, J = 6.4 Hz, H-3), 4.46 (1H, d, J = 8.0 Hz, H-1″), 3.05 (1H, t, J = 8.4 Hz, H-2″), 3.24 (1H, t, J = 8.6 Hz, H-3″), 3.14 (1H, t, J = 8.1 Hz, H-4″), 3.18 (1H, dd, J = 5.8/2.1 Hz, H-5″), 3.55 (1H, dd, J = 12.5/5.8 Hz, H-6a″), 3.77 (1H, dd, J = 12.5/2.1 Hz, H-6b″); ^{13}C NMR: Table 1.

3.9. Acid hydrolysis of **3**

Compound **3** (4 mg) was refluxed with 2 M HCl in MeOH (2 ml) for 4 h. The reaction mixture was evaporated under reduced pressure and the residue was extracted with Et_2O. The H_2O layer was neutralized with alkali solution and concentrated under reduced pressure. The residue was compared with standard sugars by silica gel TLC with *n*-propanol–MeOH–H_2O (16:1:3), which showed the sugars to be glucose and galactose.

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