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Iridoid Compounds from the Whole Plant of *Galium verum* var. *asiaticum*

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Abstract – One new iridoid glycoside, 10-*p*-dihydrocoumaroyl-6-α-hydroxygeniposide (**1**), and six known iridoid glycoside derivatives, 10-*p*-dihydrocoumaroyl deacetylasperuloside (**2**), asperulosidic acid methylester (**3**), asperuloside (**4**), asperulosidic acid (**5**), deacetylasperuloside (**6**), and scandoside (**7**) were isolated from the methanolic extract of the whole plant of *Galium verum* var. *asiaticum* Nakai (Rubiaceae) through repeated column chromatography. Their chemical structures were characterized by spectroscopic analysis. This is the first report of the characterization of compounds **1** - **7** from this plant.
Keywords – *Galium verum* var. *asiaticum,* Rubiaceae, Iridoid glycoside

Introduction

Galium verum var. *asiaticum* Nakai (Rubiaceae) is a perennial plant, widely distributed in Korea (Lee, 2003; 1996). This plant has been used as a traditional medicinal plant for the treatment of hepatitis, tonsillitis and dermatitis in Korea (Kang, 2008). Previous phytochemical investigations of the genus *Galium* resulted in the isolation of iridoid glycosides, anthraquinones, and triterpenes (Morimoto *et al*., 2002; de Rosa *et al.*, 2000; Handjieva*etal*., 1996; El-Gamal *et al*., 1995; Uesato *et al*., 1984; Böjthe-Horváth *et al*., 1980). From this plant, several phenolic compounds were reported from the *n*-butanol soluble fraction as antioxidative constituents (Kim, 2011). Except for those phenolic compounds, phytochemical and pharmacological studies of *G. verum* var. *asiaticum* have not been performed yet. Therefore phytochemical studies were performed to investigate their components. This paper presents information on the isolation and structure elucidation of compounds **1** - **7**.

Experimental

General experimental procedures – ^{1}H and ^{13}C NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. UV spectra were recorded on Shimadzu UV-1601 UV-Visible spectrophotometer, and Mass spectra were acquired on a JEOL JMSAX 505-WA. TLC was carried out on Merck (Darmstadt, Germany) precoated silica gel F_{254} plates, and silica gel for column chromatography was Kiesel gel 60 (230 - 400 mesh, Merck). Spots were detected under UV and by spraying with 10% H_2SO_4 in ethanol followed by heating at 100 - 120 ºC for 3 min. Sephadex LH-20 was used for column chromatography (25 - 100 μm; GE Healthcare, Uppsala, Sweden). Prep-HPLC was carried out on a Jaigel GS310 column (Tokyo, Japan). Methanol-d_4 (CD_3OD) for NMR experiment was obtained from Merck (Darmstadt, Germany). Solvents used for analyses were of HPLC grade and purchased from Fisher Scientific Korea (Seoul, Korea). Methanol, *n*-hexane, ethyl acetate, methylene chloride, and *n*-butanol for extraction and solvent fractionation were purchased from Samchun Chemical (Pyeongtack, Korea).

Plant materials – The whole plant *of G. verum* var. *asiaticum* was collected in August 2011 at Wanju, Jeonbuk, Korea, and identified by Dae Keun Kim, College of pharmacy, Woosuk University. A voucher specimen was deposited in the herbarium of the College of Pharmacy, Woosuk University (WSU-11-040).

Extraction and isolation – The shade dried plant material (550 g) was extracted three times with methanol at 50 ºC and filtered. The extracts were combined and evaporated *in vacuo* at 50 ºC. The resultant methanol extract (43 g) was successively partitioned as *n*-hexane (5.5 g), methylene chloride (2.0 g), *n*-BuOH (12.7 g) and H_2O soluble fractions. Sephadex LH-20 (MeOH) column chromatography of *n*-BuOH soluble (3 g) extract gave six fractions (B1-B6). Fraction B1 (1.6 g) was chromatographed on silica gel column chromatography (EtOAc-

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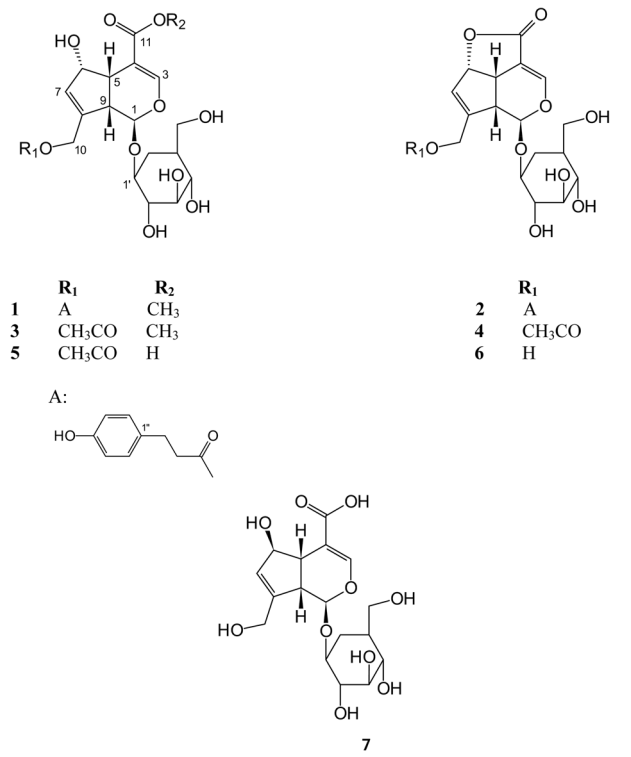

Fig. 1. Structures of isolated compounds.

MeOH-H_2O, 50 : 10 : 1) as an eluent to give five subfractions (B11-B15). Subfraction B11 was purified by HPLC column (MeOH) to give compounds **1** (6.8 mg) and **2** (11.2 mg), respectively. Subfraction B12 was purified by HPLC column (MeOH) to give compound **3** (25 mg) and compound **4** (30 mg), respectively. Subfraction B13 was purified by HPLC column (MeOH) to give compounds **5** (7 mg) and **6** (15 mg), respectively. Subfraction B15 was purified by HPLC column (MeOH) to give compound **7** (8 mg).

10-*p*-dihydrocoumaroyl-6-α-hydroxygeniposide (1) – Amorphous solid; HR-FABMS 575.1577 $[M + Na]^+$ ($C_{26}H_{32}NaO_{13}^+$, calc. 575.1579); UV λ_{max} (MeOH) nm 225, 315; ^1H and ^{13}C NMR Table 1 and Table 3.

10-*p*-dihydrocoumaroyl deacetylasperuloside (2) – Amorphous solid; UV λ_{max} (MeOH) nm 223, 281; ^1H and ^{13}C NMR Table 1 and Table 3.

Asperulosidic acid methylester (3) – Amorphous solid; UV λ_{max} (MeOH) nm 233; ^1H and ^{13}C NMR Table 2 and Table 3.

Asperuloside (4) – Amorphous solid; UV λ_{max} (MeOH) nm 232; ^1H and ^{13}C NMR Table 2 and Table 3.

Asperulosidic acid (5) – Amorphous solid; UV λ_{max} (MeOH) nm 230; ^1H and ^{13}C NMR Table 2 and Table 3.

Deacetylasperuloside (6) – Amorphous solid; UV λ_{max} (MeOH) nm 233; ^1H and ^{13}C NMR Table 2 and Table 3.

Table 1. ^1H-NMR spectral data of compounds **1** and **2**

C	1	2
1	5.03 (1H, d, *J* = 9.2)	5.96 (1H, d, *J* = 0.8)
3	7.64 (1H, s)	7.26 (1H, d, *J* = 2.0)
5	4.70 (1H, m)	4.70 (1H, m)
6	4.75 (1H, m)	5.51 (1H, d, *J* = 6.4)
7	5.88 (1H, s)	5.93 (1H, s)
9	2.98 (1H, m)	3.38 (1H, m)
10	4.93 (1H, d, *J* = 15.4)	4.74 (1H, d, *J* = 14.4)
	4.73 (1H, d, *J* = 15.4)	4.62 (1H, d, *J* = 14.4)
1'	4.70 (1H, d, *J* = 8.0)	4.66 (1H, d, *J* = 8.0)
2",6"	7.02 (2H, d, *J* = 8.4)	7.01 (2H, d, *J* = 8.4)
3",5"	6.68 (2H, d, *J* = 8.4)	6.68 (2H, d, *J* = 8.4)
α	2.84 (2H, t, *J* = 7.6)	2.82 (2H, t, *J* = 7.2)
β	2.64 (2H, d, *J* = 7.6)	2.63 (2H, d, *J* = 7.2)
OCH_3	3.73 (3H, s)	

Recorded at 400 MHz in CD_3OD

Scandoside (7) – Amorphous solid; UV λ_{max} (MeOH) nm 235; ^1H and ^{13}C NMR Table 2 and Table 3.

Results and Discussion

The methanol extract was suspended in water and partitioned successively with *n*-hexane, methylene chloride and *n*-BuOH. After observation of TLC patterns of each fraction, the *n*-BuOH soluble fraction was subjected to column chromatography. Through several repeated chromatographies using Silica gel, Sephadex LH-20, and HPLC, seven iridoid glycoside derivatives were isolated from this fraction.

Compounds **2, 4,** and **6** have similar patterns in their NMR spectra except for the dihydrocoumaroyl group of **2**, and the acetyl signal of **4**. Compounds **3** and **5** have similar patterns in their NMR spectra except for the methoxyl group of **3**. Structure characterization of compounds **2 - 7** was carried out by interpretation of their spectral data and comparison with the data previously reported in the literature. Compounds **2 - 7** were identified as 10-*p*-dihydrocoumaroyl deacetylasperuloside (**2**) (Böjthe-Horváth *et al*., 1982), asperulosidic acid methylester (**3**) (El-Naggar and Beal, 1980; Takeda *et al*., 2002), asperuloside (**4**) (El-Naggar and Beal, 1980; Lee *et al.,* 2004), asperulosidic acid (**5**) (El-Naggar and Beal, 1980; Chaudhuri *et al*., 1980), deacetylasperuloside (**6**) (Myagoshi *et al*., 1987; Peng *et al.,* 1997), and scandoside (**7**) (Chaudhuri *et al*., 1980), respectively.

Compound **1** was obtained as an amorphous powder, and molecular composition of compound **1** was determined to be $C_{26}H_{32}O_{13}$ by FABMS. Its UV spectrum showed maximum absorption at 225 nm from an aromatic ring

Table 2. ^{1}H-NMR spectral data of compounds **3 - 7**

C	3	4	5	6	7
1	5.05 (1H, d, J = 8.8)	5.96 (1H, br.d, J = 0.8)	5.05 (1H, d, J = 8.8)	5.94 (1H, br.d, J = 0.8)	4.85 (1H, d, J = 7.2)
3	7.65 (1H, s)	7.30 (1H, d, J = 2.4)	7.59 (1H, s)	7.28 (1H, d, J = 2.0)	7.33 (1H, s)
5	2.63 (1H, t, J = 8.4)	3.75 (1H, m)	3.02 (1H, m)	3.65 (1H, m)	3.65 (1H, m)
6	4.76 (1H, m)	5.57 (1H, br.d, J = 6.4)	4.82 (1H, br. s)	5.55 (1H, br.d, J = 6.8)	4.53 (1H, m)
7	6.02 (1H, s)	5.73 (1H, s)	6.01 (1H, s)	5.63 (1H, s)	5.82 (1H, s)
9	3.03 (1H, m)	3.38 (1H, m)	2.61 (1H, m)	3.37 (1H, m)	2.89 (1H, m)
10	4.89 (1H, d, J = 16.0)	4.78 (1H, d, J = 14.4)	4.93 (1H, d, J = 15.5)	4.18 (2H, s)	4.36 (1H, d, J = 15.5)
	4.75 (1H, d, J = 16.0)	4.66 (1H, d, J = 14.4)	4.80 (1H, d, J = 15.5)		4.17 (1H, d, J = 15.5)
1'	4.71 (1H, d, J = 7.6)	4.67 (1H, d, J = 8.0)	4.72 (1H, d, J = 8.0)	4.67 (1H, d, J = 7.6)	4.71 (1H, d, J = 8.4)
OCH_3	3.74 (3H, s)				
CH_3	2.08 (3H, s)	2.08 (3H, s)	2.08 (3H, s)		

Recorded at 400 MHz in CD_3OD

Table 3. ^{13}C-NMR spectral data of compounds **1 - 7**

C	1	2	3	4	5	6	7
1	100.7	100.0	100.6	100.0	101.1	99.9	99.4
3	155.4	150.3	155.4	150.3	154.4	150.3	150.3
4	108.1	106.1	108.1	106.1	109.8	106.5	116.0
5	42.4	37.0	42.4	37.4	42.8	37.5	47.3
6	75.4	86.3	75.4	86.3	75.5	86.7	83.1
7	131.9	129.1	131.8	128.9	131.9	125.7	129.9
8	145.9	144.2	146.0	144.2	146.0	149.8	147.4
9	46.2	45.1	46.3	45.2	46.4	45.0	48.3
10	63.6	62.8	63.8	62.8	63.8	62.8	61.6
11	169.4	172.6	169.3	172.3	172.5	172.9	173.1
1'	101.4	93.3	101.3	93.3	100.5	93.3	100.3
2'	74.9	74.7	74.9	74.6	75.0	74.7	75.0
3'	78.6	78.3	78.6	78.3	78.6	78.4	78.4
4'	71.6	71.6	71.6	71.6	71.6	71.6	71.5
5'	77.9	77.9	77.9	77.8	77.9	77.9	77.8
6'	63.0	61.7	63.0	61.9	63.0	60.1	62.6
α	37.1	37.4					
β	31.2	31.0					
1"	132.6	129.1					
2"	130.3	130.4					
3"	116.3	116.3					
4"	156.8	156.8					
5"	116.3	116.3					
6"	130.3	130.4					
C=O	174.5	174.2	172.5	172.6	172.6		
OCH_3	51.8		51.8				
CH_3			20.7	20.6	20.7		

Recorded at 100 MHz in CD_3OD

conjugated with a ketone function. In the ^{13}C-NMR spectrum of compound **1**, an acyl moiety composed of aromatic signals (δ 156.8, 132.6, 130.3, 116.3) and a carbonyl carbon signal (δ 174.5) were observed. Except for the acyl portion of compound **1** and acetyl group of compound **3**, NMR chemical shifts of compounds **1** and **3** showed very similar patterns to each other. The characteristic peak of α-configuration of hydroxyl group at C-6 of the iridoid compound was observed at δ 75.4 in the ^{13}C-NMR spectrum of compound **1**. In the ^{1}H-NMR spectra, an A_2B_2 system appears between δ 7.02 and 6.68 (each 2H, d, J = 8.4) of compound **1** indicating a *p*-disubstituted benzene derivative. In addition, a set of signals intergrating for four hydrogens and characteristic of an A_2B_2 system appears between δ 2.84 and 2.64 (each 2H, t, J = 7.6). These data implied the acyl portion is a dihydrocoumaroyl. According to the down shifted chemical shift values and signal patterns of the 2H-10 protons, compound **1** is acylated at this point (Böjthe-Horváth *et al*., 1982), and long-range coupling signal between carbonyl carbon of dihydrocoumaroyl group and H-10 protons was observed in the HMBC NMR spectrum of

compound **1**. The characteristic peak of α-configuration of hydroxyl group at C-6 was observed at δ 75.4 in the ^{13}C-NMR spectrum of compound **1** (β configuration of hydroxyl group is usually observed around at δ 83.0). NMR spectra indicate that compound **1** is a 10-*O*-acylated derivative of asperulosidic acid methyl ester. On the basis of the above evidences, the structure of compound **1** was determined to be 10-*p*-dihydrocoumaroyl-6-α-hydroxygeniposide.

To our best knowledge, this is the first report on the elucidation of compound **1** in the nature, and compounds **2-7** were characterized for the first time from this plant.

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