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Triterpenoids from the Roots of *Dipsacus asper*

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Four triterpenoids were isolated from the roots of *Dipsacus asper*. On the basis of chemical and spectral evidence, the structures of these compounds have been elucidated to be hederagenin (**1**), hederagenin 3-*O*-α-L-arabinoside (**2**). 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester (**3**) and hederagenin 28-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl ester (**4**). The new glycoside, **4**, was named dipsacus saponin A.

Key words : *Dipsacus asper*, Dipsacaceae, Hederagenin, Hederagenin glycosides, Dipsacus saponin A

INTRODUCTION

Dipsacus asper (Dipsacaceae) is a perennial herb which has been used in traditional Chinese medicine as analgesic, the enhancement of liver activity, anti-inflammatory agent and for the treatment of fractures (Shanghai Sci. and Tech. Pub. and Shougakukan, 1985; Namba, 1986). Recently, several saponins and iridoid glycosides were isolated from this plant (Kouno *et al.*, 1990; Zhang and Xue, 1991a; Zhang and Xue, 1991b). As a part of our phytochemical study on the roots of *Dipsacus asper*, one new triterpene glycoside together with three known triterpenoids were isolated. The present paper describes isolation and structural characterization of these compounds.

MATERIALS AND METHODS

General experimental procedures

The mps were taken on a Yanaco micro-melting point apparatus and are uncorrected. The optical rotation was measured with Jasco DIP 360 automatic polarimeter. Elemental analysis was performed on a Perkin-Elmer 240C instrument. The MS spectrum was measured on a Kratos MS 25 RFA spectrometer. The IR spectra were determined in KBr tablets on a Perkin-Elmer 841 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded with Brucker Am-300 spectrometer with TMS as an internal standard and chemical shifts are given as ppm. TLC chromatography was performed on precoated Kieselgel 60 F_{254} plates (Merck, 5715).

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Plant material

The dried roots of *Dipsacus asper* were purchased from Gyeongdong market (Seoul) in 1991 and were authenticated by Dr. Han, Dae Suk. A voucher specimen is deposited in College of Pharmacy, Yeungnam University.

Extraction and isolation

The dried and chopped roots of *D. asper* (1 kg) was refluxed with MeOH for 12 h (3 times) and concentrated *in vacuo*. The MeOH extract (257 g) was suspended in H_2O and successively partitioned with $CHCl_3$ (24 g), EtOAc (15 g) and then *n*-BuOH (68 g). The $CHCl_3$ extract was chromatographed on silica gel with a gradient $CHCl_3$-MeOH (0 to 1%) to give crude **1**, which was recrystallized with MeOH to give white needles. The EtOAc portion was subjected to SiO_2 column chromatography with EtOAc-MeOH-H_2O (100 : 16.5 : 13.5) to give 12 subfractions. Subfraction No. **4** was subjected to flash column chromatography eluting with $CHCl_3$-MeOH (7 : 1) to afford compound **2**. Subfraction No. **6** was rechromatographed on a SiO_2 column, eluted with $CHCl_3$-MeOH-H_2O (8 : 2 : 0.5, lower layer) to yield compound **3**. Subfraction No. **8** was subjected to a SiO_2 column with $CHCl_3$-MeOH-H_2O (26 : 14 : 5, lower layer) to afford compound **4**.

Compound 1

White needles from MeOH: mp. 300℃ over; $[\alpha]_D^{20}$

Table I. ^{13}C-NMR chemical shifts of **1-4** in pyridine-d_5[a]

Carbon No.	1	2	3	4	Carbon No.		2	3	4
C-1	38.8	38.8	38.9	38.9	Ara	C-1	106.6	106.6	
C-2	27.6	26.1	26.1	27.6		C-2	73.1	73.1	
C-3	73.5	82.0	82.0	73.6		C-3	74.7	74.7	
C-4	42.9	43.5	43.5	42.9		C-4	69.6	69.6	
C-5	48.7	47.6	47.7	48.8		C-5	66.9	66.9	
C-6	18.6	18.2	18.2	18.7					
C-7	33.0	32.9	32.9	33.0	Glc	C-1		95.7	95.7
C-8	39.8	39.8	40.0	40.0		C-2		74.2	73.9
C-9	48.2	48.2	48.2	48.2		C-3		79.3	78.4
C-10	37.2	37.0	37.0	37.3		C-4		71.2	71.1
C-11	23.8[b]	23.7[b]	23.9	23.9		C-5		78.9	78.0
C-12	122.6	122.6	122.9	122.8		C-6		62.3	69.5
C-13	144.8	144.9	144.1	144.2					
C-14	42.2	42.2	42.2	42.2	Glc^6-Glc	C-1			105.3
C-15	28.3	28.3	28.3	28.3		C-2			75.2
C-16	23.7[b]	23.9[b]	23.4	23.4		C-3			78.8
C-17	46.7	46.7	47.0	47.1		C-4			71.6
C-18	42.0	42.0	41.8	41.8		C-5			78.4
C-19	46.5	46.5	46.2	46.2		C-6			62.8
C-20	30.9	30.9	30.8	30.8					
C-21	34.2	34.2	34.0	34.0					
C-22	33.2	33.2	32.6	32.6					
C-23	68.1	64.5	64.4	68.2					
C-24	13.1	13.6	13.6	13.1					
C-25	16.0	16.1	16.2	16.1					
C-26	17.5	17.5	17.6	17.7					
C-27	26.2	26.2	26.1	26.1					
C-28	180.1	180.3	176.4	176.6					
C-29	33.2	33.2	33.1	33.1					
C-30	23.8[b]	23.8[b]	23.7	23.7					

[a]Chemical shifts are reported in ppm from TMS. Multiplicites were obtained by DEPT spectra.
[b]Assignment may be reversed in each column.

$=+85°$ (c=0.7, pyridine); IR ν_{max}^{KBr} cm^{-1} 3420 (OH), 1690 (acid), 1640, 810 (tri-substituted double bond); El-MS *m/z* (rel. int.) 472 (M^+, 0.8), 454 (M-H_2O, 1.0), 426 (M-HCOOH, 0.5), 409 (M-COOH-H_2O, 0.4), 248 (D/E rings, 59.9), 224 (A/B rings, 6.8), 206 (A/B rings-H_2O, 13.7), 203 (D/E rings-COOH, 55.4); ^{1}H-NMR (pyridine-d_5) δ 0.92, 0.96, 0.99, 1.02, 1.03, 1.22 (3H each, s, CH_3), 3.27 (1H, dd, *J*=13.7 and 4.0 Hz, H-18), 3.69 (1H, d, *J*=10.3 Hz, H-3α), 5.47 (1H, brs, H-12); ^{13}C-NMR see Table I.

Compound 2

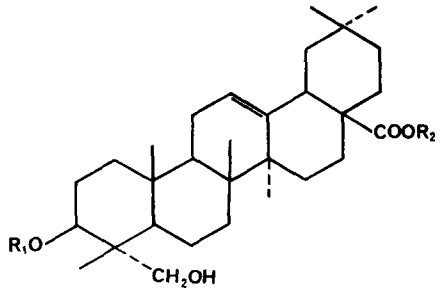
White needles from EtOH: mp. 237-239℃; $[\alpha]_D^{17}=+48°$ (c=1.0, MeOH); Anal. Calcd. for $C_{35}H_{56}O_8$, C 69.50, H 9.33, Found C 69.24, H 9.46. IR ν_{max}^{KBr} cm^{-1} 3398 (OH), 1693 (acid), 1649 (C=C), 1100-1000 (glycosidic C-O); FAB-MS *m/z* (rel.int.) $[M+Na]^+$ 627 (4.7); ^{1}H-NMR (pyridine-d_5) δ 0.92, 0.94, 0.95, 1.00, 1.02, 1.25 (3H each, s, CH_3), 4.98 (1H, d, *J*=7.1 Hz, anomeric H), 5.48 (1H, brs, H-12); ^{13}C-NMR see Table I.

Compound 3

White amorphous from MeOH: mp. 198-200℃; $[\alpha]_D^{17}=+35°$ (c=0.5, MeOH); Anal. Calcd. for $C_{41}H_{66}O_{13}\cdot 2H_2O$, C 61.32, H 8.79, Found C 60.98, H 8.69. IR ν_{max}^{KBr} cm^{-1} 3409 (OH), 1735 (ester), 1646 (C=C), 1100-1000 (glycosidic C-O); FAB-MS *m/z* (rel. int.) $[M+Na]^+$ 789 (4.3); ^{1}H-NMR (pyridine-d_5) δ 0.83 (6H, s, 2×CH_3), 0.87, 0.92, 1.07, 1.14 (3H each, s, CH_3), 4.90 (1H, d, *J*=7.0 Hz, anomeric H), 5.38 (1H, brs, H-12), 6.25 (1H, d, *J*=7.9 Hz, anomeric H); ^{13}C-NMR see Table I.

Compound 4

White needles from EtOAc saturated with H_2O: mp. 200-203℃; $[\alpha]_D^{17}=+40°$ (c=0.04, pyridine); Anal. Calcd. for $C_{42}H_{68}O_{14}\cdot H_2O$, C 61.89, H 8.66, Found C 61.55, H 8.41. IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1732 (ester), 1644 (C=C), 1100-1000 (glycosidic C-O); FAB-MS *m/z* (rel. int.) $[M+Na]^+$ 819 (34.5), $[M+H]^+$ 797 (3.6); ^{1}H-NMR (pyridine-d_5) δ 0.86, 0.88, 1.02, 1.05, 1.14,



1 R_1=H, R_2=H
2 R_1=−Ara, R_2=H
3 R_1=−Ara, R_2=−Glc
4 R_1=H, R_2=−Glc^6-Glc

1.18 (3H each, s, CH_3), 5.01 (1H, d, J=7.7 Hz, anomeric H), 5.43 (1H, brs, H-12), 6.23 (1H, d, J=7.9 Hz, anomeric H); ^{13}C-NMR see Table I.

Acid hydrolysis of 2, 3 and 4

Acid hydrolysis of 2-4 was separately performed by refluxing each glycoside with 4% H_2SO_4 in MeOH for 1 h. Hederagenin (**1**) was identified as the common aglycone by direct comparison with an authentic sample. Arabinose from 2, arabinose and glucose from 3, and glucose from 4 were detected by TLC.

Alkaline hydrolysis of 3 and 4

Each solution of 3 and 4 in a mixture of 3% KOH (MeOH) was refluxed for 30 min. Each reaction mixtures were neutralized with 0.1 N H_2SO_4 and then extracted with *n*-BuOH. The *n*-BuOH layer from 3 was washed with water and concentrated to give a product, which was identified as 2 by direct comparison with an authentic sample. In the same manner, the reaction product from **4** was identified as hederagenin (**1**).

RESULTS AND DISCUSSION

Repeated column chromatography of the $CHCl_3$ and EtOAc soluble portions of the MeOH extract and purification by crystallization led to the isolation of compounds **1**, **2**, **3** and **4**.

Compound **1** was identified as a well-known triterpene, hederagenin, on the basis of spectroscopic evidence of IR, El-MS, ^{1}H-NMR and ^{13}C-NMR (Kang, 1987) (see experimental).

Acid hydrolysis of compounds **2-4** gave the common aglycone, hederagenin (**1**), identified by direct comparison with an authentic sample as well as arabinose from **2**, arabinose and glucose from **3** and glucose from **4** identified by TLC.

The FAB-MS spectrum of **2** exhibited a cationized molecular ion $[M+Na]^+$ at *m/z* 627, suggesting that **2** was a hederagenin monosaccharide. The ^{1}H-NMR spectrum of **2** showed one anomeric proton signal at δ 4.98 (1H, d, J=7.1 Hz). The relative large coupling constant of one anomeric proton of **2** indicated α configuration for arabinose (Zhang and Xue, 1991b). The glycosidation shift around C-3 as well as one anomeric carbon signal at δ 106.6 in the ^{13}C-NMR spectrum of **2** indicated that this compound is hederagenin 3-*O*-α-L-arabinopyranoside which had been isolated from *Akebia quinata (Chandel et al.*, 1980) and *Fatsia japonica* (Mahato *et al.*, 1988).

Compound **3** showed an ester absorption band at 1735 cm^{-1} in its IR spectrum and gave **2** on alkaline hydrolysis with 3% KOH. **3** showed a cationized molecular ion $[M+Na]^+$ at *m/z* 789 in the FAB-MS spectrum, indicating that **3** was a diglycoside of hederagenin. The ^{13}C- and ^{1}H-NMR spectra of **3** showed signals due to two anomeric carbons (δ 95.7, 106.6) and two anomeric protons (δ 4.90, d, J=7.0 Hz; δ 6.25, d, J=7.9 Hz). These results indicated that **3** is a bisdesmoside of hederagenin carrying a α-L-arabinopyranosyl moiety at C-3 and a β-D-glucopyranosyl ester moiety at C-28. Accordingly, **3** is 3-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester which had been isolated from *Hedera nepalensis (Kizu et al.*, 1985a) and *Chenopodium quinoa* (Mizui *et al.*, 1988).

Compound **4** showed an ester absorption band in its IR spectrum and alkaline hydrolysis **4** gave hederagenin which was identified by direct comparison with an authentic sample. The FAB-MS spectrum of **4** exhibited a cationized molecular ion $[M+Na]^+$ at *m/z* 819, suggesting that **4** was a hederagenin diglycoside. The ^{1}H-NMR spectrum of **4** exhibited two anomeric proton signals at δ 5.01 (d, J=7.7 Hz) and 6.23 (d, J=7.9 Hz), and ^{13}C-NMR spectrum of **4** showed two anomeric carbon signals at 95.7 and 105.3 ppm. These data suggested that **4** was a hederagenin monodesmosyl ester with a disaccharide moiety at the C-28 position. In the ^{13}C-NMR spectrum, there was a set of signals assignable to a terminal glucosyl unit and the chemical shifts for the inner glucose is virtually identical to those of gentiobiosyl ester moiety (Matsumoto *et al.*, 1987). Thus the glycosidic linkage in the glucosyl-glucose moiety is 1→6. Based on the above mentioned evidence, the structure of **4** is hederagenin 28-*O*-β-D-glucopyranosyl ester. Kizu *et al.* previously obtained **4** by chemical degradation of kizuta saponin K_{12}, but isolation of **4** from a natural source has not been reported previously (Kizu *et al.*, 1985b). Accordingly, **4** was named dipsacus saponin A.

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