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Keun Young Jung, Jae Chul Do, and Kun Ho Son

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TRITERPENE GLYCOSIDES FROM THE ROOTS OF *DIPSACUS ASPER*

KEUN YOUNG JUNG, JAE CHUL DO,

College of Pharmacy, Yeungnam University, Kyongsan 712-749, Korea

and KUN HO SON*

Department of Food and Nutrition, Andong National University, Andong 760-749, Korea

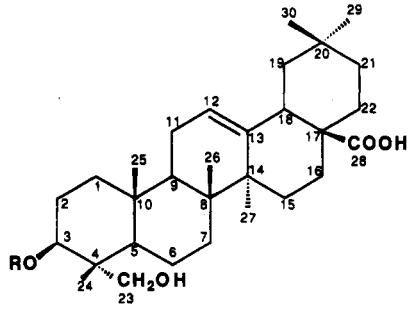
ABSTRACT.—The roots of *Dipsacus asper* have yielded two new triterpenoid saponins, dipsacus saponins B [**1**] and C [**2**], which have been characterized by chemical and spectral means as hederagenin-3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside [**1**] and hederagenin-3-*O*-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[α-L-rhamopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside [**2**].

Dipsacus asper Wall (Dipsacaceae) is a perennial herb that is distributed in China. Its roots are used in traditional Chinese medicine as an analgesic, for enhancement of liver activity, as an anti-inflammatory agent, and for the treatment of fractures (1,2). Previous authors reported the isolation of several triterpenoid saponins and iridoid glycosides from this plant (3–5). In the preceding paper, we reported the occurrence of hederagenin and three hederagenin glycosides, including dipsacus saponin A, from the roots of *D. asper* (6). This paper deals with the isolation and structural characterization of two additional new saponins.

RESULTS AND DISCUSSION

Repeated cc of the *n*-BuOH-soluble fraction of the MeOH extract and purification by crystallization led to the isolation of compounds **1** and **2** in the order of increasing polarity.

On acid hydrolysis, **1** and **2** afforded hederagenin [**3**] as a common aglycone, identified by comparison with a standard sample (mmp and co-tlc). The sugars obtained from the hydrolysates were identified by tlc as arabinose, rhamnose, and glucose for **1**, arabinose, xylose, rhamnose, and glucose for **2**. The ^{13}C-nmr spectra of **1** and **2** showed the signals of C-3 (δ 81.1) and C-28 (δ 180.5). This evidence suggested that **1** and **2** were monodesmosidic glycosides. The negative ion fabms spectrum of **1** gave the mol



1 R=glc(1→4)[rha(1→6)]glc(1→3)rha(1→2)ara
2 R=xyl(1→4)glc(1→4)glc(1→3)rha(1→4)rha(1→2)ara
3 R=H
4 R=ara
5 R=rha(1→2)ara
6 R=glc(1→3)rha(1→2)ara
7 R=glc(1→4)glc(1→3)rha(1→2)ara
8 R=xyl(1→4)glc(1→4)glc(1→3)rha(1→2)ara

wt and established the sugar sequence. The fragmentation pattern for **1** showed an $[M]^-$ ion at *m/z* 1220. Fragment ions at *m/z* 1074 $[M-146]^-$ and 1058 $[M-162]^-$, corresponding to the elimination of 1 mol of rhamnose and of 1 mol of glucose residue from the $[M]^-$ ion, respectively, showed the presence of two terminal sugar residues. The remaining fragments at *m/z* 912 $[M-(146+162)]^-$, *m/z* 749 $[912-162-H]^-$, and *m/z* 603 $[749-146]^-$ suggested that the sugar sequence of **1** was hederagenin-arabinose-rhamnose-glucose(-rhamnose)-glucose. Mild acid hydrolysis of **1** supported this result. Prosapogenins **4–7**, obtained after partial hydrolysis of **1**, yielded arabinose for **4**, arabinose and rhamnose for **5**, and arabinose, rhamnose, and glucose for **6** and **7** on acid hydrolysis. Prosapogenins **4**, **5**, and **6** were identified by direct comparison (mp, ^1H and ^{13}C nmr) with literature data as hederagenin-3-*O*-α-L-arabinopyranoside (6), hederagenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside [previously isolated from the leaves of *Kalopanax pictus* (7)], and hederagenin-3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside [previously isolated from the leaves of *Aralia elata* (8)], respectively. The ^1H-nmr spectrum of another prosapogenin, **7**, showed the presence of four anomeric proton signals at δ 6.11 (1H, s), 5.32 (1H, d, J=7.6 Hz), 5.06 (1H, d, J=7.7 Hz), and 5.00 (1H, d, J=6.4 Hz). The ^{13}C-nmr signals ascribable to a terminal glucopyranosyl moiety (9), indicated that the sugar sequence of **7** was linear. Furthermore, the signals due to the inner glucopyranosyl moiety of **7** revealed significant glycosidation shifts at C-3 (−2 ppm), C-4 (+10 ppm), and C-5 (−2.2 ppm) signals compared to those of **6**, suggesting that the terminal glucose unit was attached at C-4 of inner glucoside. From this observation, the structure of **7** was established as hederagenin-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside. The ^1H-nmr spectrum of **1** showed five anomeric proton signals at δ 6.31 (1H, s), 6.14 (1H, s), 5.40 (1H, d, J=7.8 Hz), 5.30 (1H, d, J=7.8 Hz), and 5.02 (1H, d, J=6.5 Hz). On comparison of the ^{13}C-nmr spectrum of **1** with that of **7**, the C-5 and C-6 chemical shifts of the inner glucopyranosyl moiety of **1** revealed glycosidation shifts at C-5 (−1.3 ppm) and C-6 (+7.1 ppm), indicating that the terminal rhamnose unit was attached at C-6 of the inner glucose moiety. Moreover, the chemical shifts of inner glucose moiety of **1** were superimposable on those of smilaxin B isolated from *Smilax sieboldii* (**10**). Thus, **1** was hederagenin-3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

The fabms spectrum of **2** exhibited the fragment ions at *m/z* 1352 $[M]^-$, 1220 $[M-132]^-$, 1206 $[M-146]^-$, and 1074 $[M-(132+146)]^-$, indicating the presence of two terminal sugar units, one pentose and one rhamnose. Partial hydrolysis of **2** afforded five prosapogenins and the aglycone, hederagenin. Four prosapogenins were identified as **4–7** by comparison with authentic samples that were obtained by partial hydrolysis of **1**. Prosapogenin **8** yielded arabinose, xylose, rhamnose, glucose, and hederagenin on acid hydrolysis. The ^1H-nmr spectrum of **8** showed the five anomeric proton signals at δ 6.17 (1H, s), 5.36 (1H, d, J=7.6 Hz), 5.11 (1H, d, J=7.8 Hz), 5.02 (1H, d, J=5.9 Hz), and 5.00 (1H, d, J=7.3 Hz), suggesting an α configuration for one arabinose and two rhamnose and a β configuration for one xylose and two glucose. On comparison of the ^{13}C-nmr spectrum of **8** with that of **7**, the C-4 signal of the terminal glucopyranosyl moiety of **8** was seen to be deshielded (+9.0 ppm). Therefore, **8** was hederagenin-3-*O*-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside. In the ^1H- and ^{13}C-nmr spectra of **2**, the six anomeric proton and carbon signals revealed the presence of six sugar units. The ^{13}C-nmr spectrum of **2** showed the signals of one terminal xylose and one terminal rhamnose unit (9). On comparison of the ^{13}C-nmr spectrum of **2** with that of **8**, the

signals due to C-3, C-4, and C-5 of inner rhamnose moiety were displaced upfield by 0.8 ppm, downfield by 4.8 ppm, and upfield by 0.8 ppm, respectively, while other signals remained almost unshifted. This evidence indicated that the terminal rhamnopyranosyl unit was located at the 4-hydroxyl group of the inner rhamnosyl unit of **2**. Based on the above results, **2** was hederagenin-3-*O*-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

The new glycosides **1** and **2** were named dipsacus saponin B and dipsacus saponin C, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Yanaco apparatus and are uncorrected. Optical rotations were measured on a Rudolph Autopol III or Jasco DIP 360 automatic polarimeters. Ir spectra were determined in KBr on a Perkin-Elmer 840 spectrophotometer. Elemental analysis was performed on a Perkin-Elmer 240C instrument. Nmr spectra were recorded on a Bruker AM-300 (300 MHz for ^{1}H nmr and 75.5 MHz for ^{13}C nmr) spectrometer using TMS as an internal standard and measured at room temperature. Chemical shifts are given as ppm. Fabms and eims spectra were recorded on a VG Analytical 70-250S or Kratos MS 25 RFA mass spectrometers. Tlc was carried out on precoated Si gel 60 F_{254} sheets (Merck), and detection was achieved by spraying 10% H_2SO_4 followed by heating. Sugars were run on precoated cellulose plates (Merck) and detected by aniline phthalate. Cc utilized Merck Si gel.

TABLE 1. Partial ^{1}H-nmr Spectral Data for **1–8** in Pyridine-d_5.[a]

Compound	Protons			
	Me	Rha-Me	H-12	Anomeric protons
1	1.20 s, 1.06 s 0.99 s, 0.99 s 0.93 s, 0.92 s	1.51 d (5.7) 1.49 d (5.9)	5.44 br s	6.31 s, 6.14 s 5.40 d (7.8) 5.30 d (7.8) 5.02 d (6.5)
2	1.20 s, 1.05 s 0.98 s, 0.98 s 0.93 s, 0.91 s	1.52 d (5.2) 1.50 d (5.2)	5.44 br s	6.28 s, 6.11 s 5.32 d (7.7) 5.26 d (7.4) 5.03 d (6.5) 5.01 d (7.4)
3	1.22 s, 1.03 s 1.02 s, 0.99 s 0.96 s, 0.92 s		5.47 br s	
4	1.25 s, 1.02 s 1.00 s, 0.95 s 0.94 s, 0.92 s		5.48 br s	4.98 d (7.1)
5	1.22 s, 1.04 s 0.99 s, 0.99 s 0.94 s, 0.92 s	1.61 d (6.0)	5.45 br s	6.18 s 5.10 d (6.0)
6	1.25 s, 1.12 s 1.02 s, 1.00 s 0.94 s, 0.93 s	1.56 d (6.0)	5.47 br s	6.30 s 5.33 d (7.3) 5.08 d (6.0)
7	1.20 s, 1.03 s 0.95 s, 0.93 s 0.88 s, 0.88 s	1.51 d (5.9)	5.41 br s	6.11 s 5.32 d (7.6) 5.06 d (7.7) 5.00 d (6.4)
8	1.23 s, 1.08 s 0.99 s, 0.99 s 0.94 s, 0.92 s	1.51 d (5.9)	5.41 br s	6.17 s 5.36 d (7.6) 5.11 d (7.8) 5.02 d (5.9) 5.00 d (7.3)

[a]Only assignable signals are listed. Data are δ (ppm), multiplicity, and *J* (in parentheses) in Hz.

PLANT MATERIAL.—The dried roots of *D. asper* were purchased from Gyeongdong market (Seoul) in 1991 and were authenticated by Dr. Dae Suk Han, an emeritus professor of Seoul National University. A voucher specimen is deposited in the college of Pharmacy, Yeungnam University.

ISOLATION PROCEDURE.—The dried chopped roots of *D. asper* (5 kg) were refluxed with hot MeOH (3 times, 6 h for each extraction) and evaporated in vacuo to give a residue (1.28 kg), which was suspended in H_2O and extracted with $CHCl_3$, EtOAc, and *n*-BuOH, succesively. The *n*-BuOH solution was concentrated to give the *n*-BuOH-soluble fraction (340 g). A portion of the *n*-BuOH-soluble fraction (100 g) was subjected to cc over Si gel eluted with $CHCl_3$-MeOH-H_2O (52:28:8, lower layer) to give a mixture of compounds **1** and **2**, which was further subjected to repeated cc over Si gel using EtOAc-MeOH-H_2O (100:16.5:13.5) to obtain **1** (1.8 g) and **2** (3.3 g).

TABLE 2. ^{13}C-nmr Chemical Shifts of Hederagenin [**3**] and its Glycosides in Pyridine-d_5.[a]

Carbon		Compound							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Aglycone moiety									
	C-3	81.1	81.1	73.5	82.0	81.1	81.2	81.1	81.2
	C-23	64.0	64.3	68.1	64.5	64.0	64.4	64.2	64.1
	C-28	180.5	180.5	180.1	180.3	180.3	180.2	180.3	180.2
3-*O*-Sugar moieties									
Ara	C-1	104.6	104.5		106.6	104.3	104.6	104.7	104.6
	C-2	75.5[b]	75.6[b]		73.1	75.8	75.9	75.6	75.5[b]
	C-3	74.6	74.7		74.7	74.6	74.4	74.6	74.8
	C-4	69.6[c]	69.5		69.6	69.7	69.6	69.6	69.7
	C-5	66.2	66.0		66.9	65.6	66.0	65.8	66.0
Rha	C-1	101.4	101.4			101.6	101.4	101.3	101.5
($\rightarrow^2$Ara)	C-2	71.6	71.6			72.4	72.0	71.8	71.8
	C-3	82.7	82.7			72.5	83.1	83.0	83.5
	C-4	73.0	77.8			74.1	73.0	72.6	73.0
	C-5	70.5	68.7			69.3	69.8	69.3	69.5
	C-6	18.4	18.5			18.5	18.5	18.4	18.4
Glc*	C-1	104.3	104.0				106.8	104.5	104.6
($\rightarrow^3$Rha)	C-2	75.0[b]	75.0[b]				75.1	75.2	75.5[b]
	C-3	76.8	76.3				78.5	76.4[b]	76.7
	C-4	79.3	80.0				71.1	81.1	81.0
	C-5	75.0	76.3				78.4	76.3[b]	76.1
	C-6	68.8	61.0[c]				62.5	61.7	61.5[c]
Glc**	C-1	106.2	106.1					106.1	106.6
($\rightarrow^4$Glc*)	C-2	75.4[b]	75.6[b]					75.2	75.6[b]
	C-3	78.5	76.9					78.3	76.7
	C-4	71.6	79.0					71.4	80.4
	C-5	78.2	75.7					78.1	75.6[b]
	C-6	60.6	60.6[c]					62.3	61.9[c]
Rha	C-1	101.8	101.8						
($\rightarrow^6$Glc*	C-2	72.4	72.4						
or	C-3	72.7	72.6						
$\rightarrow^4$Rha)	C-4	74.6	74.6						
	C-5	69.5[c]	69.6						
	C-6	18.5	18.4						
Xyl	C-1		105.3						105.5
($\rightarrow^4$Glc**)	C-2		74.6						74.5
	C-3		78.2						78.3
	C-4		70.8						70.8
	C-5		67.2						67.4

[a]Chemical shifts are reported in ppm from TMS. Assignments were made by comparison with model compounds and DEPT spectra.

[b,c]Assignments may be reversed in each column.

Dipsacus saponin B [**1**].—White amorphous powder from MeOH: mp 238–241°; $[\alpha]^{20}D$ −34.7° (c=0.15, pyridine); ir ν max (KBr) 3410, 1693, 1645, 1044, 808 cm^{-1}; fabms *m/z* $[M]^-$ 1220, $[M-146]^-$ 1074, $[M-162]^-$ 1058, $[M-(146+162)]^-$ 912, $[912-162-H]^-$ 749, $[749-146]^-$ 603; ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{59}H_{96}O_{26}\cdot 2H_2O$, C 56.35, H 8.02; found C 56.47, H 7.94.

Dipsacus saponin C [**2**].—White amorphous powder from MeOH: mp 256–260°; $[\alpha]^{20}D$ −52.7° (c=0.15, pyridine); ir ν max (KBr) 3396, 1696, 1637, 1043, 811 cm^{-1}; fabms *m/z* $[M]^-$ 1352, $[M-132]^-$ 1220, $[M-146]^-$ 1206, $[M-(132+146)]^-$ 1074, $[M-(132+162)]^-$ 1058, $[1058-146]^-$ 912, $[1058-162]^-$ 896, $[912-162-H]^-$ 749, $[749-146]^-$ 603; ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{64}H_{104}O_{30}\cdot H_2O$, C 56.04, H 7.79; found C 56.16, H 7.75.

ACID HYDROLYSIS OF **1** AND **2**.—Each saponin (10 mg) was refluxed with 4% H_2SO_4 (5 ml) in MeOH for 1 h. The reaction mixture was then concentrated under reduced pressure to remove MeOH, diluted with H_2O and filtered. Each precipitate was purified by recrystallization from MeOH to afford the same aglycone, hederagenin [**3**], as colorless needles: mp>300°; $[\alpha]^{20}D$ +85° (c=0.7, pyridine); ir ν max (KBr) 3420, 1690, 1640, 810 cm^{-1}; eims *m/z* (rel. int.) 472 (0.8), 454 (1.0), 426 (0.5), 409 (0.4), 248 (59.9), 224 (6.8), 206 (13.7), 203 (55.4); ^1H nmr see Table 1; ^{13}C nmr see Table 2. Each filtrate was adjusted to pH 7 with $BaCO_3$ and filtered. The filtrates were concentrated and examined by tlc. Arabinose, glucose, and rhamnose from **1** and arabinose, glucose, rhamnose, and xylose from **2** were identified by comparison with authentic samples.

PARTIAL HYDROLYSIS OF **1**.—Compound **1** (500 mg) was refluxed with 0.5 N ethanolic HCl (5 ml) for 30 min. After cooling, the reaction mixture was diluted with ice H_2O, and the precipitate was collected by filtration and dried. The residue (350 mg) was subjected to cc over Si gel with $CHCl_3$-MeOH (50:1), $CHCl_3$-MeOH-H_2O (8:2:0.5, lower layer), and then $CHCl_3$-MeOH-H_2O (52:28:8, lower layer) to afford hederagenin [**3**], four prosapogenins **4**–**7** and recovered **1**. The prosapogenins were refluxed with acid in the same manner as described above. Arabinose from **4**, arabinose and rhamnose from **5**, and arabinose, rhamnose, and glucose from **6** and **7** were detected by tlc. Compound **4** was recrystallized from EtOH as colorless needles: mp 237–239°; $[\alpha]^{17}D$ +48° (c=1.0, MeOH); ^1H nmr see Table 1; ^{13}C nmr see Table 2. Compound **5** was recrystallized from MeOH as amorphous powder: mp 249–251°; $[\alpha]^{24}D$ +17.5° (c=0.5, MeOH); ^1H nmr see Table 1; ^{13}C nmr see Table 2. Compound **6** was recrystallized from MeOH as amorphous powder: mp 240–243°; $[\alpha]^{22}D$ +7.6° (c=1.3, pyridine); ^1H nmr see Table 1; ^{13}C nmr see Table 2. Compound **7** was recrystallized from MeOH as amorphous powder: mp 252–255°; $[\alpha]^{20}D$ +28.7° (c=0.15, pyridine): ^1H nmr see Table 1; ^{13}C nmr see Table 2.

PARTIAL HYROLYSIS OF **2**.—Compound **2** (1 g) was hydrolyzed in the same manner as described above. The precipitate was chromatographed to give hederagenin [**3**], prosapogenins **4**–**8**, and recovered **2** in the same order of elution as those of **1**. Hederagenin and prosapogenins **4**–**7** were identified by direct comparison with the previously obtained samples. Compound **8** was refluxed with acid, and arabinose, xylose, rhamnose, and glucose were detected in the hydrolysate. Compound **8** was recrystallized from MeOH as white amorphous powder: mp 259–262°; $[\alpha]^{20}D$ −25.0° (c=0.14 pyridine); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

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