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ORIGINAL PAPER

A condensed phenylpropanoid glucoside and pregnane saponins from the roots of *Hemidesmus indicus*

Zhimin Zhao · Katsuyoshi Matsunami · Hideaki Otsuka · Nisha Negi · Ashok Kumar · Devendra Singh Negi

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Abstract From the roots of *Hemidesmus indicus*, one new condensed phenylpropanoid glucoside and three new pregnenolone glycosides, named hemidesmosides A–C, were isolated along with one known related compound, plocoside A.

Keywords *Hemidesmus indicus* · Asclepiadaceae · Condensed phenylpropanoid · Pregnenolone saponin

Introduction

Hemidesmus indicus (Linne) Robert Brown (Asclepiadaceae) is a well known ayurvedic plant called Indian sarsaparilla or sarsaparilla, and is used as a tonic, alterative, demulcent, diaphoretic, diuretic and blood purifier [1, 2]. *H. indicus* grows wild in South Asia, including India, Sri Lanka and Myanmar. It is a slender, laticiferous, twining, sometimes prostrate or semi-erect shrub, and its roots are woody and aromatic. It is one of the Rasayama plants of Ayurveda, as its effect is anabolic. Chemical analysis of its stems [3] and roots [4] has revealed the presence of triterpenes [3, 4] and pregnane glycosides [5–7]. This paper deals with reinvestigation of the constituents of roots of *H. indicus*.

Z. Zhao · K. Matsunami · H. Otsuka (✉)
Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan
e-mail: hotsuka@hiroshima-u.ac.jp

Z. Zhao
School of Pharmaceutical Sciences, Sun Yat-Sen University, 132 East Outer Ring Road, Guangzhou University City, 510006 Guangzhou, People's Republic of China

N. Negi · A. Kumar · D. S. Negi
Department of Chemistry, HNB Garhwal University (A Central University), Srinagar (Garhwal), Uttarakhand 246 174, India

Results and discussion

From the 1-BuOH-soluble fraction of a MeOH extract of the roots of *H. indicus*, one condensed phenylpropanoid (**1**) and three pregnane glycosides, named hemidesimosides (A–C) (**2**–**4**), were isolated, along with one known compound, plocoside A (**5**) [8]. Their structures were elucidated by analyses of spectroscopic data.

Compound **1**, $[\alpha]_D$ +69.7, was isolated as an amorphous powder and its elemental composition was determined to be $C_{16}H_{22}O_8$ by high-resolution (HR)–electrospray ionization (ESI)–mass spectrometry (MS). The IR spectrum exhibited absorptions for hydroxy groups (3390 cm^{-1}) and an aromatic ring (1606 and 1520 cm^{-1}). The presence of the aromatic ring was also supported by absorptions in the UV spectrum at 282 and 230 nm. In the ^{1}H-NMR spectra, distinct signals for three aromatic protons (δ_H 6.95, 6.82 and 6.77) coupled in an ABX system, an anomeric proton (δ_H 4.57, 1H, d, $J = 8$ Hz), a doublet methyl (δ_H 1.00, 3H, d, $J = 6$ Hz), and a singlet methyl (δ_H 3.86) were observed. The ^{13}C-NMR spectrum exhibited six signals assignable to a hexose moiety, six signals for the aromatic ring, and four signals for a methyl, two oxygenated methines and a methoxy group. On ^{1}H–^{1}H correlation spectroscopy (COSY), seven sequential protons from H-1′ to H_2-6′ were found to be correlated and also from the methyl signal (H_3-9) to the methine signal (H-7). Since coupling constants of H-1″ to H-5″ showed that these protons were all obviously in the axial positions, the hexose

was expected to be glucopyranose. However, the heteronuclear single quantum correlation spectrum (HSQC), together with the COSY spectrum, revealed that the C-2 position was fairly shifted downfield (δ_C 81.1) when compared with the common signal (ca. 75 ppm) for glucopyranose. In the heteronuclear multiple bond correlation spectrum (HMBC), the doublet methyl protons (δ_H 1.00) were correlated with two methine carbons (δ_C 85.4 and 78.0), and the methine proton (δ_H 4.11) on C-7 showed correlation cross-peaks with C-1, C-2, C-6 and C-8 (δ_C 130.6, 112.4, 122.1 and 78.0, respectively). These correlations together with other HMBC data allowed assignment of the structure of the aglycone moiety as 3-methoxy-4-hydroxyphenylpropane with two oxygen atoms at the C-7 and C-8 positions (Fig. 2). Based on the HMBC correlation between the H-1′ proton and C-8, the glucosidic connection was determined to be at this position. From the results of the HR-ESI–MS requiring 6° of unsaturation and the HMBC correlation cross-peak of H-7 with C-2 (δ_C 81.1), a cyclic system must be formed between C-7 and C-2′ through an ether bond. Judging from the correlations between H-7 and H-2′, and H-8 and H-1′ on phase-sensitive (PS) nuclear Overhauser exchange spectroscopy (NOESY), the absolute configurations at C-7 and C-8 were assigned as *S*, assuming that of glucose was the D-series. Therefore, the structure of compound **1** was elucidated to be as shown in Fig. 1. Similar and related compounds to **1** have been isolated from several plant sources, *Illicium oligandrum* [9], *Myrica rubra* [10], and *Melia toosendan* [11].

Hemidesmoside A (**2**), $[\alpha]_D$ −18.2, was isolated as an amorphous powder and its elemental composition was determined to be $C_{57}H_{90}O_{27}$ by HR-ESI–MS. The IR spectrum exhibited strong absorption bands at 3384 cm^{-1} for hydroxy groups and at 1746 cm^{-1} for a ketonic functional group. In the ^{1}H-NMR spectrum, three singlet methyl signals (δ_H 0.63, 0.88 and 2.34) and five anomeric proton signals (δ_H 4.77, 4.88, 5.16, 5.23 and 5.27) were observed (Table 2). Two further singlet methyls at δ_H 1.93 and 2.14 were expected to be for two acetyl groups, judging from the presence of two methyl carbon signals at δ_C 20.6 and 21.0, and carbonyl signals at δ_C 169.8 and 170.8 in the ^{13}C-NMR spectrum. The ^{13}C-NMR spectrum exhibited the presence of five corresponding anomeric carbon signals (δ_C 96.5, 103.0, 103.4, 104.9 and 106.7), and six signals were assignable as those of a terminal glucopyranose (Table 3). One of the anomeric protons appeared at δ_H 5.23 as a doublet of doublet ($J = 9$, 2 Hz) signal, implying the presence of a 2-deoxy sugar unit, which is frequently found in saponins isolated from Asclepiadaceous plants. In the ^{1}H–^{1}H COSY and HSQC spectra, protons and carbons of the sugar rings were assigned as shown in Fig. 3, and the remaining 21 ^{13}C-NMR spectral signals consisted of those of three methyls, seven

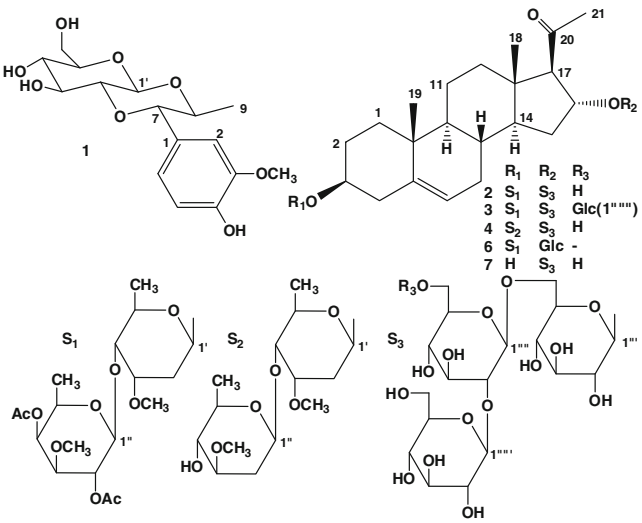
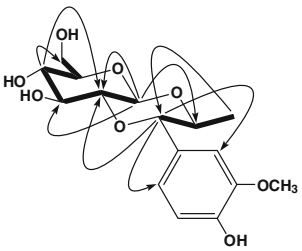


Fig. 1 Structures of isolated and reference compounds

Fig. 2 HMBC correlations for compound **1**



methylenes, four methines and two oxygenated methines, two quatenary carbons, one trisubstitued double bond and one carbonyl carbon. When the ^{13}C-NMR spectrum of **2** was compared with those of known pregnane glycosides, those for two sugar units, and rings A and B were essentially superimposable on those of a 3β,16α-dihydroxypreg-5-en-20-one derivative (**6**) isolated from *Streptocaulon tomentosums* (Asclepiadaceae) [12], and those of three sugar units, and rings C and D were with those of stelmatocryptonoside C (**7**) isolated from *Stelmatocrypton khasianum* (Asclepiadaceae) [13] (Table 2). In the HMBC spectrum, the anomeric proton of 2,4-diacetyldigitalose showed a correlation cross-peak with C-3 of cymaropyranose and that of cymaropyranose with C-3 of the aglycone, whereas the anomeric proton of the terminal glucopyranose was correlated with C-2 of an inner glucopyranose, whose anomeric proton was correlated with C-6 of the innermost glucopyranose (Fig. 3). Attachment of the innermost glucopyranose was also established by the HMBC correlation of H-1‴ (δ_H 4.88) with C-17 (δ_C 72.2) (Fig. 3). Compound **2** was then hydrolyzed under acidic conditions to give D-digitalose, D-cymarose and D-glucose [14].The structure of hemidesmoside A (**2**) was therefore elucidated to be

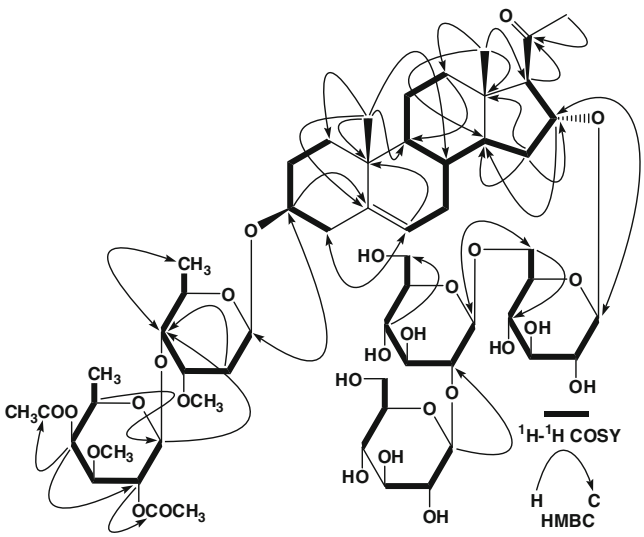


Fig. 3 ^{1}H–^{1}H COSY and diagnostic HMBC correlations for hemidesmoside A (**2**). *Dual arrows* denote that HMBC correlations were observed in both directions

3β,16α-dihydroxypreg-5-en-20-one 3-*O*-β-D-(2″,4″-di-*O*-acetyl-β-D-digitalopyranosyl)(1″→4′)cymaropyranoside, 16-*O*-β-D-glucopyranosyl(1‴″→2‴″)-*O*-β-D-glucopyranosyl (1‴″→6‴)-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Hemidesmoside B (**3**), $[\alpha]_D$ −34.1, was isolated as an amorphous powder and its elemental composition was determined to be $C_{63}H_{100}O_{32}$ by HR-ESI–MS. In the ^{13}C-NMR spectrum, signals for two sets of terminal glucopyranoses were observed and one of the C-6 signals of glucose moieties was shifted downfield. Thus, hemidesmoside B (**3**) was an analogous compound to the preceding hemidesmoside A (**2**), except for the presence of one more glucopyranose unit. The attachment position of the new glucopyraose unit was confirmed by the HMBC correlation between the anomeric proton (δ_H 5.01) and C-6‴″ (δ_C 69.5). The structure of hemidesmoside B (**3**) was therefore elucidated to be 3β,16α-dihydroxypreg-5-en-20-one 3-β-D-(2″,4″-di-*O*-acetyl-β-D-digitalopyranosyl)(1″→4′)cymaropyranoside 16-*O*-β-D-glucopyranosyl(1‴″→2‴″)-*O*-β-D-glucopyranosyl, (1‴″→6‴″)-*O*-β-D-glucopyranosyl(1‴″→6‴)-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Hemidesmoside C (**4**), $[\alpha]_D$ −34.9, was also isolated as an amorphous powder and its elemental composition was determined to be $C_{53}H_{86}O_{24}$ by HR-ESI–MS. The ^{13}C-NMR spectroscopic signals for three glucopyranose moieties, and the rings C and D region were superimposable on those of hemidesmoside A (**2**), and two anomeric protons at δ_H 4.78 and 5.28 appeared as doublet of doublets. Thus, two 2-deoxy sugars must be involved in sugar linkage on the hydroxy group at the C-3 position. The ^{13}C-NMR data for the β-D-oleandropyranosyl moiety were essentially the same as those of the outer 2-deoxy sugar of **4**, whereas

Table 1 NMR spectroscopic data for **1** (^{13}C: 100 MHz, ^{1}H: 400 MHz, CD_3OD)

	^{13}C	^{1}H
1	130.6	–
2	112.4	6.95, d, 2
3	149.0	–
4	148.0	–
5	116.1	6.77, d, 8
6	122.1	6.82, dd, 8, 2
7	85.4	4.11, br d, 10
8	78.0	3.89, overlapped
9	17.2	1.00, d, 6
1′	99.7	4.57, d, 8
2′	81.1	3.13, dd, 9, 8
3′	75.2	3.60, dd, 9, 9
4′	72.0	3.41, dd, 9, 9
5′	79.9	3.48, m
6′	62.7	3.73, dd, 12, 5
		3.90, dd, 12, 2
–OCH_3	56.6	3.86, s

those of the inner β-D-cymaropyranosyl moiety were the same as those of the inner 2-deoxy sugar of **4** (Table 3). In the HMBC spectrum, the anomeric proton of the outer sugar unit was correlated with C-4′ of the inner 2-deoxy sugar and that of the inner sugar unit with C-3 of the aglycone. The structure of hemidesmoside C (**4**) was therefore elucidated to be 3β, 16α-dihydroxypreg-5-en-20-one 3-*O*-β-D-(β-D-oleandropyranosyl)(1″→4′)cymaropyranoside, 16-*O*-β-D-glucopyranosyl (1‴″→2‴″)-*O*-β-D-glucopyranosyl(1‴″→6‴)-β-D-glucopyranoside.

Experimental

Optical rotations were measured on a JASCO P-1030 polarimeter. IR spectra were measured on a Horiba FT-710 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on JEOL JNM α-400 (H at 400 MHz and C at 100 MHz) and ECA-600 (H at 600 MHz and C at 150 MHz) spectrometers with tetramethylsilane as an internal standard. Positive-ion HR-MS were taken with an Applied Biosystems QSTAR XL system ESI (Nano Spray)-MS.

A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC and reversed-phase [octadecylsilanized silica gel (ODS)] open CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ = 50 mm, L = 25 cm, linear gradient: MeOH–H_2O (1:9, 1 L) → (1:1, 1 L), fractions of 10 g being collected], respectively. The droplet

Table 2 ^{1}H-NMR spectroscopic data for hemidesmosides A–C (**2**–**4**) (pyridine-d_5)

H	2	3	4
1	0.98 m	0.98 m	1.00 m
	1.70 m	1.71 m	1.73 m
2	1.71 m	1.70 m	1.71 m
	2.12 m	2.08 m	2.13 m
3	3.77 dddd 11, 11, 4, 4	3.74 dddd 11, 11, 5, 5	3.80 m
4	2.32 m	2.31 m	2.35 m
	2.50 dd 13, 4	2.50 m	2.51 m
6	5.29 br d 8	5.29 d 7	5.28 br d 8
7	1.42 m	1.40 m	1.40 m
	1.80 m	1.78 mz	1.77 m
8	1.28 dd 11, 5	1.30 m	1.27 m
9	0.86 dd 11, 5	0.86 m	0.87 m
11	1.24 m	1.26 m	1.24 m
	1.40 m	1.50 m	1.45 m
12	1.26 m	1.27 m	1.25 m
	1.81 m	1.91 m	1.84 m
14	1.35 dd 12, 4	1.35 m	1.35 m
15	1.71 ddd 12, 12, 4	1.71 ddd 11, 11, 3	1.83 m
	1.93 m	1.93 m	1.93 m
16	5.21 dd 7, 7	5.21 dd 7, 7	5.21 dd 8, 7
17	2.91 d 7	2.95 d 7	2.91 d 7
18	0.63 3H s	0.62 3H s	0.963 3H s
19	0.88 3H s	0.87 3H s	0.88 3H s
21	2.34 3H s	2.47 3H s	2.34 3H s
1′	5.23 dd 9, 2	5.24 dd 9, 2	5.28 m
2′	1.87 m	1.87 m	1.92 m
	2.32 m	2.35 m	2.35 m
3′	4.02 m	4.02 m	4.10 m
4′	3.51 m	3.53 m	3.56 dd 9, 3
5′	4.20 m	4.26 m	4.26 m
6′	1.45 3H d 6	1.46 3H d 6	1.45 3H d 6
1″	4.77 d 8	4.78 d 8	4.78 dd 8, 2
2″	5.58 d 10	5.58 dd 10, 8	1.73 m
			2.54 m
3″	3.66 dd 10, 3	3.68 dd 10, 3	3.68 ddd 9, 9, 4
4″	5.56 m	5.57 m	5.57 m
5″	3.92 m	3.94 m	3.94 m
6″	1.31 3H d, 6	1.31 3H d 6	1.31 3H d 6
1‴	4.88 d 8	4.88 d 8	4.89 d 8
2‴	4.30 m	4.30 m	4.03 m
3‴	4.19 m	4.19 m	4.18 m
4‴	4.44 m	4.42 m	4.35 m
5‴	4.38 m	3.92 m	4.02 m
6‴	4.33 dd 11, 4	4.28 m	4.31 m
	4.58 dd 11, 2	4.58 m	4.59 m
1⁗	5.16 d 8	5.06 d 8	5.17 d 8
2⁗	4.06 m	4.06 m	4.09 m
3⁗	4.36 m	4.27 m	4.37 m
4⁗	4.26 m	4.24 m	4.24 m
5⁗	3.95 m	4.02 m	4.00 m
6⁗	4.32 dd 11, 4	4.31 m	4.35 m
	4.48 m	4.73 dd 11, 2	4.48 m
1⁗′	5.27 d 8	5.16 d 8	5.28 d 8
2⁗′	4.03 m	4.03 m	4.06 m
3⁗′	4.12 m	4.25 m	4.12 m
4⁗′	4.14 m	4.11 m	4.17 m
5⁗′	3.90 m	3.95 m	3.91 m
6⁗′	4.37 m	4.34 dd 12, 6	4.33 m
	4.56 m	4.58 m	4.58 m
1⁗″		5.01 d 8	
2⁗″		4.06 m	
3⁗″		4.20 m	
4⁗″		4.18 m	
5⁗″		3.90 m	
6⁗″		4.37 m	
		4.58 m	
3′-CH_3O-	3.52 3H s	3.52 3H s	3.61 3H s
3″-CH_3O-	3.41 3H s	3.42 3H s	3.47 3H s
3‴-CH_3O-			
2″-CH_3CO-	2.14 3H s	2.15 3H s	
4″-CH_3CO-	1.93 3H s	1.94 3H s	

counter-current chromatograph (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns ($\Phi = 2$ mm, $L = 40$ cm), the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2) being used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; $\Phi = 6$ mm, $L = 25$ cm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor. Standard sugars were obtained from hydrolysis of cymarin (D-cymarose) (MP Biochemicals, Cedex, France), troleandromycine (L-oleandrose) (Wako Pure Chemical Co., Kyoto, Japan) and chartreusin (D-digitalose) (Santa Cruz Biotechnology, CA, USA).

Plant material

Roots of *H. indicus* were purchased by one of the authors (D.S.N.) from a market in Srinagar, India.

Extraction and isolation

Air-dried roots of *H. indicus* (985 g) were extracted three times with MeOH (15 L × 2) at room temperature for 2 weeks and then the extract was concentrated to 2 L in vacuo. The concentrated extract was washed with *n*-hexane (2 L × 2, 10.0 g) and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (1.5 L) and then extracted with EtOAc (2 L × 2) to give 13.4 g of an EtOAc-soluble fraction. The aqueous layer

Table 3 ^{13}C-NMR spectroscopic data for hemidesmosides A–C (**2**–**4**) (pyridine-d_5) and reference compounds

C	**2**[a]	**3**[a]	**4**[b]	**6**	**7**
1	37.6	37.4	37.3	37.3	37.62
2	30.5	30.4	30.4	30.2	32.46
3	77.6	77.5	77.5	77.3	71.22
4	39.5	39.9	39.5	39.2	43.34
5	141.0	140.9	140.0	140.8	141.74
6	121.8	121.6	121.6	121.6	120.86
7	32.1	32.0	32.0	31.9	31.90
8	31.6	31.5	31.5	31.4	31.43
9	50.4	50.2	50.3	50.1	50.17
10	37.1	36.9	36.9	36.8	36.74
11	21.1	20.9	20.9	20.9	20.89
12	38.9	38.9	38.7	38.7	38.67
13	45.0	44.9	44.8	44.9	44.77
14	54.4	54.5	54.4	54.4	54.41
15	33.8	33.7	33.6	33.7	33.56
16	80.8	80.8	80.6	81.0	80.53
17	72.2	72.1	72.0	72.1	71.89
18	14.8	14.6	14.6	14.6	14.59
19	19.5	19.3	19.3	19.3	19.44
20	208.1	208.2	207.9	208.2	207.85
21	32.6	32.6	32.4	32.2	32.46
1′	96.5	96.3	96.4	96.2	
2′	37.0	36.8	37.5	36.6	
3′	77.5	77.9[c]	78.0[c]	77.2	
4′	84.5	84.3	83.5	84.2	
5′	68.9	68.8	69.0	68.7	
6′	18.7	18.4	18.70	18.5	
1″	103.4	103.1	102.0	103.1	
2″	71.7	71.7	37.0	71.4	
3″	80.4	80.2[c]	81.5	80.1	
4″	69.5	69.3	76.3	69.2	
5″	69.7	69.6	73.0	69.5	
6″	16.7	16.6	18.68	16.6	
1‴	104.9	104.9	104.7	105.0	104.67
2‴	75.6	75.6	75.4	75.3	76.35
3‴	78.5[c]	78.3[c]	78.4[c]	78.5	78.23
4‴	71.2[d]	70.6	71.1	71.4	70.92
5‴	76.6	76.3	76.4	78.2	76.53
6‴	69.9	69.2[d]	69.7	62.5	69.57
1⁗	103.0	102.7	102.9		102.75
2⁗	85.2	84.8	85.0		85.01
3⁗	78.3[c]	78.5[c]	78.23[c]		78.00
4⁗	71.3[d]	70.7	71.0		70.81
5⁗	78.3[c]	78.4[c]	77.99[c]		78.12
6⁗	62.8[e]	69.5[d]	62.8[d]		62.40
1′′′′′	106.7	106.6	106.6		106.55
2′′′′′	76.7	76.5	76.6		76.38
3′′′′′	78.4[c]	78.2[c]	78.2[c]		78.12
4′′′′′	71.5	71.4	71.4		71.22
5′′′′′′	79.1	78.9	78.9		78.86
6′′′′′	62.6[e]	62.8	62.6[d]		62.24
1′′′′′′		105.5			
2′′′′′′		75.4			
3′′′′′′		78.4[c]			
4′′′′′′		71.8			
5′′′′′′		78.5[c]			
6′′′′′′		62.4			
3′-CH_3O-	58.5	58.3	58.8	58.2	
3″-CH_3O-	57.9	57.7	57.0	57.6	
3‴-CH_3O-					
2″-CH_3CO-	21.2	21.0		21.0	
2″-CH_3CO-	169.8	169.7		169.7	
4″-CH_3CO-	20.6	20.4		20.4	
4″-CH_3CO-	170.8	170.6		170.6	

[a] At 150 MHz

[b] At 100 MHz

[c,d,e] Figures with the same superscripts in each column may be interchangeable

was extracted with 1-BuOH (2 L × 3) to give a 1-BuOH-soluble fraction (50.4 g), and the remaining water layer was concentrated to furnish 104 g of a water-soluble fraction. The 1-BuOH-soluble fraction (49.7 g) was subjected to Diaion HP-20 CC ($\Phi = 50$ mm, $L = 50$ cm), using H_2O–MeOH (4:1, 2 L), (3:2, 2 L), (2:3, 2 L), and (1:4, 2 L), and MeOH (3 L), 300 mL fractions being collected. The residue (20.5 g) in fractions 4–6 from the 20 % MeOH eluate was subjected to silica gel (450 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (3 L), and $CHCl_3$–MeOH (49:1, 3 L), (24:1, 3 L), (23:2, 3 L), (9:1, 3 L), (7:1, 3 L), (17:3, 3 L), (4:1, 3 L), (3:1, 3 L), (7:3, 3 L), and (3:2, 3 L)], 500 mL fractions being collected. The residue (5.70 g) in fractions 16–27 was subjected silica gel (150 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (1.5 L), $CHCl_3$–MeOH (49:1, 1 L), (24:1, 1 L), (23:2, 1 L), (9:1, 1 L), (17:3, 1 L), (4:1, 1 L), (3:1, 1 L), and (7:3, 1 L)], $CHCl_3$–MeOH–H_2O (35:15:2, 1 L), and MeOH (800 mL), 100 mL fractions being collected. The residue (45.1 mg) in fractions 40–46 was purified by HPLC (H_2O–MeOH, 13:7) to give 4.5 mg of **1** from the peak at 7.5 min. The residue (1.05 g) in fractions 56–75 was subjected ODS open CC and the residue (147 mg) in fractions 103–113 was purified by HPLC (Inertsil; GL Science, Tokyo; $\Phi = 20$ mm, $L = 25$ cm; flow rate: 4 mL/min; H_2O–MeOH 13:7) to yield 76.3 mg of **2** from the peak at 60 min. The residue (1.47 g) in fractions 76–96

was subjected to ODS open CC and the residue (95.3 mg) in fractions 81–84 was purified by HPLC (H_2O–MeOH, 13.7) to afford **3** (16.6 mg), **5** (34.3 mg), and **4** (6.7 mg) from the peaks at 16, 20.5, and 23 min, respectively.

Compound **1**

Amorphous powder, $[\alpha]_D^{20}$ +69.7 ($c = 0.30$, MeOH). IR ν_{max} (film) cm^{-1}: 3390, 2935, 1606, 1520, 1279, 1130, 1035. UV λ_{max} (MeOH) nm (log ε): 282 (3.53), 230 (3.83). ^{1}H-NMR (400 MHz, CD_3OD): Table 1. ^{13}C-NMR (100 MHz, CD_3OD): Table 1. HR-ESI–MS (positive-ion mode) *m/z*: 365.1201 $[M + Na]^+$ (Calcd for $C_{16}H_{22}O_8Na$: 365.1206).

Hemidesmoside A (**2**)

Amorphous powder, $[\alpha]_D^{20}$ −18.2 ($c = 0.26$, MeOH). IR ν_{max} (film): 3384, 2935, 1746, 1371, 1233, 1088, 1066 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5): Table 2. ^{13}C-NMR (150 MHz, pyridine-d_5): Table 3. HR-ESI–MS (positive-ion mode) *m/z*: 1229.5579 $[M + Na]^+$ (Calcd for $C_{57}H_{90}O_{27}Na$: 1229.5561).

Hemidesmoside B (**3**)

Amorphous powder, $[\alpha]_D^{20}$ −34.1 ($c = 0.26$, MeOH). IR ν_{max} (film): 3395, 2934, 1747, 1370, 1232, 1065 cm^{-1}. ^{1}H-NMR (600 MHz, pyridine-d_5): Table 2. ^{13}C-NMR (150 MHz, pyridine-d_5): Table 3. HR-ESI–MS (positive-ion mode) *m/z*: 1391.6061 $[M + Na]^+$ (Calcd for $C_{63}H_{100}O_{32}Na$: 1391.6089).

Hemidesmoside C (**4**)

Amorphous powder, $[\alpha]_D^{20}$ −34.9 ($c = 0.45$, MeOH). IR ν_{max} (film): 3392, 2933, 1700, 1456, 1369, 1164, 1067 cm^{-1}. ^{1}H-NMR (400 MHz, pyridine-d_5): Table 2. ^{13}C-NMR (100 MHz, pyridine-d_5): Table 3. HR-ESI–MS (positive-ion mode) *m/z*: 1129.5387 $[M + Na]^+$ (Calcd for $C_{53}H_{86}O_{24}Na$: 1129.5401).

Sugar analysis

About 2.0 mg of each of compounds **2**, **3** and **4** was hydrolyzed with 1 M HCl in 50 % dioxane (0.1 mL) at 80 °C for 2 h. The water layers were neutralized with Amberlite IRA-96SB and analyzed with a chiral detector (JASCO OR-2090*plus*). Hydrolyzates of compounds **2** and **3** gave peaks for D-cymarose, D-digitalose and D-glucose with positive optical rotation signs, and that of compound **4** D-cymarose and D-glucose with positive optical rotation signs. A peak for D-oleandrose could not be detected in the hydrolyzate, due to a small optical rotation value ($[\alpha]_D$ +10.3 for L-oleandrose) [14]. Authentic D-digitalose and D-glucose showed peaks at 4.7 and 11.0 min, respectively, with positive signs on an amino column [Asahipak NH_2P-50 4E, $\Phi = 4.6$ mm, $L = 25$ cm, CH_3CN–H_2O (3:1), 1 mL/min]. Authentic L-oleandrose and D-cymarose showed peaks at 9.3 min with a positive sign and 10.5 min with a negative sign, respectively, on an ODS column [Inertsil ODS-3, $\Phi = 4.6$ mm, $L = 25$ cm, CH_3CN–H_2O (1:49), 1 mL/min].

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