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A BIDESMOSIDIC HEDERAGENIN HEXASACCHARIDE FROM THE ROOTS OF *SYMPHYTUM OFFICINALE*

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Key Word Index—*Symphytum officinale*; Boraginaceae; roots; triterpenoid saponin; symphytoxide B; hederagenin.

Abstract—A new bidesmosidic triterpenoidal saponin 3-*O*-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-α-L-arabinopyranosyl]-hederagenin-28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester, was isolated from the roots of *Symphytum officinale*. The structure was assigned by chemical methods and spectral analysis (^{1}H, ^{13}C, DEPT, NMR, EI-MS and FAB-MS) including ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HOHAHA. The prosapogenin of this saponin is also a new compound.

INTRODUCTION

Saponins constitute a pharmacodynamic group of natural products with a wide range of biological activities [1–4]. The pharmacological importance attached to these compounds has prompted us to investigate their natural occurrence in *Symphytum officinale* L. Our systematic phytochemical investigation on the roots of *S. officinale* has resulted in the isolation of two new saponins, belonging to the oleanane series [5, 6]. In continuation of our work on the chemical constituents of *S. officinale*, we report here the isolation and structure elucidation of a new triterpenoidal saponin containing six carbohydrate residues, designated symphytoxide B (**1**). This saponin was purified by reversed-phase semi-preparative HPLC (RP-8 column). The identification of the oligosaccharide unit poses serious problems in assigning a structure to any saponin. However, the concerted use of modern 2D NMR techniques, ^{1}H–^{1}H COSY, HOHAHA and ^{1}H–^{13}C COSY, readily allowed the elucidation of the saponin structure in general and that of the sugar components in particular. The structure was further confirmed by total acid hydrolysis of **1** followed by the identification of the monosaccharides and the aglycone. The latter is hederagenin (**2**) [3β, 23-dihydroxy-olean-12-en-28-oic acid]. The interglycosidic linkages, the position of attachment of the sugar chain to the aglycone and the sequence of the sugars have been established by ^{1}H and ^{13}C NMR spectra, interpreted with the aid of COSY and heteroCOSY spectra and negative ion FAB-mass spectroscopy, respectively.

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RESULTS AND DISCUSSION

The ethanolic extract obtained from the roots of *Symphytum officinale* was separated by repeated column chromatography on silica gel, followed by separation on Lobar RP-8 column and HPLC to give **1**.

Symphytoxide **B 1** gave a violet colour with Ce $(SO_4)_2$. The negative FAB-mass spectrum of compound **1** showed a pseudomolecular ion peak at *m/z* 1397 $[M-H]^-$ and fragment ions at *m/z* 1251 $[M-H-146]^-$, 1089 $[M-H-(146+162)]^-$, 927 $[M-H-(146+2\times162)]^-$, 765 $[M-H-(146+3\times162)]^-$, 603 $[M-H-(146+4\times162)]^-$, 471 $[M-H-(146+4\times162+132)]^-$. These fragment ions suggested the sequential loss of one deoxy hexose, four hexose and one pentose unit from the molecular ion. The negative ion FAB-mass spectrum together with ^{1}H and ^{13}C NMR data (Table 1) allowed us to propose the formula $C_{65}H_{106}O_{32}$ for **1**, indicating 13 double bond equivalents. The UV spectrum displayed only an end absorption at 200.6 nm showing the absence of conjugation. Its IR spectrum (KBr) exhibited characteristic absorption bands at 3400 (OH), 1740 (C=O), 1620 (C=C), and 1100–1000 (C–O–C) cm^{-1}. The ^{1}H NMR spectrum (CD_3OD, 500.14 MHz) of the intact saponin (**1**) showed the existence of six tertiary methyl groups for H_3-24, H_3-25, H_3-26, H_3-27, H_3-29 and H_3-30 characterized by the singlets at δ 0.71, 0.97, 0.79, 1.16, 0.90 and 0.93. These signals were correlated with C-24, C-25, C-26, C-27, C-29 and C-30 at δ 13.5, 16.7, 18.0, 26.6, 33.6 and 24.3 in the heteroCOSY spectrum [7]. A doublet at δ 1.27 (J = 6.2 Hz) was due to the methyl group of rhamnose. Compound **1** showed a distorted triplet of an olefinic proton (δ 5.24) in its ^{1}H NMR spectrum and signals of

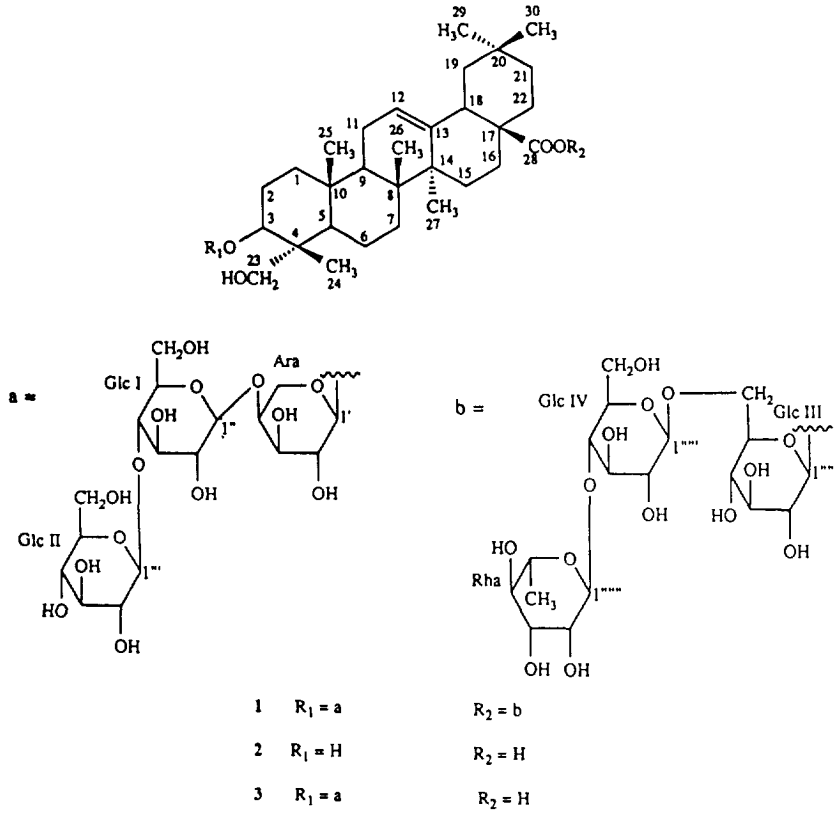

Table 1. ^{13}C (100.613 MHz) and ^{1}H (500.14 MHz) NMR spectral data of symphytoxide B (**1**) from one- and two-dimensional experiments (in CD_3OD)

C	^{13}C (δ)	DEPT	^{1}H–^{13}C Correlation	^{1}H (δ) chemical shift	Multiplicity	J (Hz)	^{1}H–^{1}H connectivity	HOHAHA
Aglycone								
1	39.6	CH_2	—	*	—	—		
2	26.4	CH_2	—	*	—	—		
3	84.1	CH	3.62 (H-3)	3.62	*m*	—		
4	44.1	C	No coupling	—	—	—		
5	48.4	CH	—	*	—	—		
6	19.0	CH_2	—	*	—	—		
7	33.5	CH_2	—	*	—	—		
8	40.8	C	No coupling	—	—	—		
9	49.1	CH	—	*	—	—		
10	37.8	C	No coupling	—	—	—		
11	24.7	CH_2	1.88 (H-11)	1.88	—	—		
12	123.8	CH	5.24 (H-12)	5.24	distorted *t*	—		
13	145.0	C	No coupling	—	—	—		
14	43.1	C	No coupling	—	—	—		
15	29.0	CH_2	—	*	—	—		
16	23.7	CH_2	—	*	—	—		
17	47.3	C	No coupling	—	—	—		
18	42.6	CH	—	*	—	—		
19	47.3	CH_2	—	*	—	—		
20	31.6	C	No coupling	—	—	—		
21	35.0	CH_2	—	*	—	—		
22	33.5	CH_2	—	*	—	—		
23	65.1	CH_2	—	*	—	—		
24	13.5	CH_3	0.71 (H-24)	0.71	*s*	—		
25	16.7	CH_3	0.97 (H-25)	0.97	*s*	—		

Table 1. (*Continued*)

C	^{13}C (δ)	DEPT	^{1}H–^{13}C Correlation	^{1}H (δ) chemical shift	Multiplicity	J (Hz)	^{1}H–^{1}H connectivity	HOHAHA
26	18.0	CH_3	0.79 (H-26)	0.79	*s*	—		
27	26.6	CH_3	1.16 (H-27)	1.16	*s*	—		
28	178.2	C	No coupling	—	—	—		
29	33.6	CH_3	0.90 (H-29)	0.90	*s*	—		
30	24.3	CH_3	0.93 (H-30)	0.93	*s*	—		
C-3-sugar								
Ara								
1′	104.3	CH	4.53 (H-1′)	4.53	unresolved *d*	—	Ara-2′ 3.86	
2′	73.5	CH	3.86 (H-2′)	3.86	*m*	—	Ara-1′ 4.53	
3′	71.8	CH	3.24 (H-3′)	3.24	*m*	—		
4′	79.2	CH	3.86 (H-4′)	3.86	*m*	—	Ara-5′ 3.64	
5′	63.0	CH_2	3.64 (H-5′)	3.64	*m*	—	Ara-4′ 3.86	
Glc I								
1″	104.5	CH	4.64 (H-1″)	4.64	*d*	7.70	Glc-2″ 3.22	4.64, 3.98, 3.84, 3.40, 3.28, 3.22.
2″	75.8	CH	3.22 (H-2″)	3.22	*m*	—	Glc-1″ 4.64	
3″	78.0	CH	3.40 (H-3″)	3.40	*m*	—	Glc-4″ 3.98	
4″	78.2	CH	3.98 (H-4″)	3.98	*m*	—	Glc-3″ 3.40	
5″	77.9	CH	3.28 (H-5″)	3.28	*m*	—		
6″	62.8	CH_2	3.84 (H-6″)	3.84	*m*	—		
Glc II (Terminal)								
1‴	105.5	CH	4.49 (H-1‴)	4.49	*d*	.7.65	Glc-2‴ 3.24	4.49, 3.84, 3.42, 3.40, 3.30, 3.24
2‴	75.4	CH	3.24 (H-2‴)	3.24	*m*	—	Glc-1‴ 4.49	
3‴	78.0	CH	3.40 (H-3‴)	3.40	*m*	—		
4‴	71.5	CH	3.30 (H-4‴)	3.30	*m*	—		
5‴	78.3	CH	3.42 (H-5‴)	3.42	*m*	—		
6‴	62.8	CH_2	3.84 (H-6‴)	3.84	*m*	—		
C-28-sugar								
Glc III								
1⁗	95.8	CH	5.33 (H-1⁗)	5.33	*d*	8.10	Glc-2⁗ 3.34	
2⁗	73.8	CH	3.34 (H-2⁗)	3.34	*m*	—	Glc-1⁗ 5.33	
3⁗	78.0	CH	3.40 (H-3⁗)	3.40	*m*	—		
4⁗	71.1	CH	3.42 (H-4⁗)	3.42	*m*	—		
5⁗	76.8	CH	3.30 (H-5⁗)	3.30	*m*	—	Glc-6⁗ 3.78	
6⁗	69.6	CH_2	3.78 (H-6⁗)	3.78	*m*	—	Glc-5⁗ 3.30	
Glc IV								
1⁗′	104.3	CH	4.40 (H-1⁗′)	4.40	*d*	7.85	Glc-2⁗′ 3.24	4.40, 3.64, 3.54, 3.46, 3.28, 3.24.
2⁗′	75.3	CH	3.24 (H-2⁗′)	3.24	*m*	—	Glc-1⁗′ 4.40	
3⁗′	76.8	CH	3.46 (H-3⁗′)	3.46	*m*	—		
4⁗′	79.7	CH	3.54 (H-4⁗′)	3.54	*m*	—		
5⁗′	77.9	CH	3.28 (H-5⁗′)	3.28	*m*	—	Glc-6⁗′ 3.64	
6⁗′	62.0	CH_2	3.64 (H-6⁗′)	3.64	*m*	—	Glc-5⁗′ 3.28	
Rha (Terminal)								
1⁗″	102.9	CH	4.84 (H-1⁗″)	4.84	*d*	1.00	Rha-2⁗″ 3.84	4.84, 3.96, 3.84, 3.64, 3.42, 1.27.
2⁗″	72.4	CH	3.84 (H-2⁗″)	3.84	*m*	—	Rha-1⁗″ 4.84	
3⁗″	72.3	CH	3.64 (H-3⁗″)	3.64	*m*	—	Rha-4⁗″ 3.42	
4⁗″	73.8	CH	3.42 (H-4⁗″)	3.42	*m*	—	Rha-3⁗″ 3.64	
5⁗″	70.7	CH	3.96 (H-5⁗″)	3.96	*m*	—	Rha-6⁗″ 1.27	
6⁗″	18.1	CH_3	1.27 (H-6⁗″)	1.27	*d*	6.20	Rha-5⁗″ 3.96	

* Peaks were not discernible.

one double bond at δ 123.8 and 145.0, respectively in its ^{13}C NMR spectrum indicated the presence of a triterpenoid aglycone of the olean-12-ene type.

Six anomeric proton signals were also observed in the ^{1}H NMR spectrum. The four doublets at δ 4.40 (*d*, J = 7.85 Hz, H-1''''), 4.49 (*d*, J = 7.65 Hz, H-1'''), 4.64 (*d*, J = 7.70 Hz, H-1'') and 5.33 (*d*, J = 8.10 Hz, H-1''''') are due to the anomeric protons of the D-glucose. Their coupling constant values confirmed the β-glycosidic linkages. The two doublets resonating at δ 4.53 (distorted *d*, H-1') and 4.84 (*d*, J = 1.00 Hz, H-1''''') were attributed to the anomeric protons of the L-arabinose and L-rhamnose confirming the α-linkage.

The ^{13}C NMR spectrum of **1** (CD_3OD, 100.613 MHz) summarized in Table 1 showed the presence of 57 carbon atoms in the molecule. Twenty-nine carbon signals were seen for the sugar moieties, the signal at δ 62.8 is assigned to C-6'' and C-6'''. The signal at δ 77.9 is assigned to C-5 of Glc I and Glc IV and the signal at δ 78.0 assigned to C-3'', C-3''' and C-3''''. The signal at δ 73.8 is assigned to C-2 of Glc III and C-4 of rhamnose. The anomeric signal at δ 104.3 is due to C-1' ad C-1'''', indicating the presence of six monosaccharide moieties, corresponding to five hexoses and one pentose which were in conformity with the appearance of anomeric signals at δ 104.3 (C-1' and C-1''''), 104.5, 105.5, 95.8 and 102.9, respectively. In the heteroCOSY experiment [7] these signals showed coupling with their respective anomeric protons at δ 4.53, 4.40, 4.64, 4.49, 5.33 and 4.84. The remaining 28 carbon signals were due to pentacyclic triterpenoid aglycone. The signal at δ 33.46 is assigned to C-7 and C-22 and the signal at δ 47.33 is assigned to C-17 and C-19. The assignments of all the carbon signals due to the aglycone were made by comparison with reported data of related compounds [8]. Multiplicities of the carbon were determined by employing DEPT pulse sequence [9, 10] with the last polarization angles 45°, 90° and 135°. This established that there were seven methyls, 16 methylene and 34 methine carbon atoms. The number of quaternary carbons were detected by subtracting the carbons of DEPT from BB, in agreement with structure **1**. The downfield C-3 signal at δ 84.1 and the upfield carbonyl signal at δ 178.2 of aglycone in the ^{13}C NMR spectrum suggested that the sugar moieties were attached at C-3 and C-28 of the aglycone [8, 11].

The acid hydrolysis of compound **1** with 20% hydrochloric acid in methanol–water (1:1) afforded an aglycone (**2**) that was identified as hederagenin by comparison with spectral and physical data reported in the literature [12, 13], along with three sugar moieties which were identified as D-glucose, L-arabinose and L-rhamnose by co-TLC and PC with authentic samples. The ^{13}C NMR spectral data (Table 1) indicated the β-D-pyranosyl configuration for glucose and the α-L-pyranosyl configuration for arabinose and rhamnose [14].

The anomeric carbon signal at δ 95.8 and the carbonyl signal at δ 178.2 revealed the presence of an ester glycoside linkage in **1** (Table 1) [15, 16], which was further confirmed by the ester group absorption at 1740 cm^{-1} by the IR. The simultaneous presence of 3-*O*-glycosidic linkage in **1** was easily seen by attendant downfield shift at δ 84.1 for C-3, whereas in hederagenin this carbon signal is observed at *ca* δ 76.4 [12]. Thus compound **1** is 3,28-bidesmoside. The presence of downfield methylene signal at δ 69.61 due to the C-6 of glucose in **1** revealed that one glucose was attached to the C-6 position of another glucose and the presence of downfield signal at δ 79.7 due to the C-4 of Glc IV in **1** revealed that the terminal rhamnose was attached to the C-4 of Glc IV. The ^{13}C NMR assignments also indicated that one glucose and one rhamnose are terminal sugars [14].

The alkaline hydrolysis [17] of **1** gave a prosapogenin (**3**) which exhibited anomeric signals at δ 104.3 (C-1', C-1'') and 105.5 (C-1''') indicating the presence of three sugar moieties. It also gave an apparent molecular ion peak in its negative FAB-mass spectrum at m/z 927 $[M - H]^-$, consistent with the molecular formula $C_{47}H_{76}O_{18}$. Characteristic ions that appeared at m/z 765 $[M - H - 162]^-$, 603 $[M - H - 162 - 162]^-$, and 471 $[M - H - 162 - 162 - 132]^-$ were generated by subsequent losses from the molecular ion of two hexose and one pentose units and clearly indicated that a hexose is the terminal sugar while a pentose is attached to the aglycone. The ^{1}H NMR spectrum of **3** (in CD_3OD, 300.13 MHz), displayed anomeric signals at δ 4.56 (distorted *d*, H-1'), 4.63 (*d*, J = 7.44 Hz, H-1'') and 4.49 (*d*, J = 7.60 Hz, H-1'''). A comparison of the ^{13}C NMR spectrum of **3** with that of saponin **1** revealed a loss of 15 resonance signals including the disappearance of three anomeric signals at δ 95.8, 104.3 and 102.9. Therefore, **1** may have a trisaccharide chain composed of two glucose and one rhamnose units bonded to the C-28 carbonyl group by an ester linkage, and the three sugar moieties linked to C-3 of hederagenin by a glycosidic bond are two glucoses and one arabinose. The disappearance of the downfield methylene signal at δ 69.6 and methine signal at δ 79.7 in the ^{13}C NMR spectrum of **3** confirmed the presence of (1 → 6) [18] and (1 → 4) [8] linkages between Glc III and Glc IV and terminal Rha attached to C-28 in **1**, while the anomeric signal at δ 95.8 showed the direct attachment of glucose to C-28 of the aglycone [19]. The structure of prosapogenin **3** has been established as 3-*O*-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-α-L-arabinopyranosyl]-hederagenin, a new sapogenin, isolated for the first time after alkaline hydrolysis of **1**.

The point of attachment of sugar units were also determined using the glycosidation rule [20, 21]. The downfield ^{13}C chemical shift of C-4 due to arabinose at δ 79.2 corresponding to the glycosidation shift of +10.1 ppm as compared to the methyl Ara [14, 20] thus disclosed that the inner β-D-glucopyranosyl (Glc I) is attached to C-4 of the α-L-arabinopyranosyl unit [8]. The linkage at C-4 was accompanied as expected by an upfield shift of the C-5 methylene peak (− 3.56 ppm). The downfield ^{13}C chemical shift of C-4 of Glc I at δ 78.17 and the small upfield shift of C-5 of Glc I at δ 77.9 showed that C-4 of Glc I is substituted and showed a (1 → 4) linkage between Glc II and Glc I [22]. The possibility of a (1 → 2) linkage between Glc II and Glc I was eliminated by comparing

the ^{13}C NMR data of saponins reported by Xu *et al.* [23]. It was demonstrated by these authors that when terminal glucose was attached to C-2 of inner glucose, the ^{13}C chemical shift was observed at *ca* δ 83–84. The glycosidic linkage at C-6 of Glc III produced a downfield shift of + 7.11 ppm of this carbon atom (methylene signal at δ 69.6) as compared to the methyl Glc [14, 20] and showed a (1 → 6) linkage between Glc IV and Glc III [8]. The very small upfield shift of C-5 of Glc III also showed that C-6 of Glc III is substituted. Comparison of the ^{13}C NMR spectrum of **1** showed that C-3 and C-5 signal of Glc IV appeared upfield and the C-4 signal of Glc IV appeared downfield which allowed us to place a (1 → 4) linkage between terminal Rha and Glc IV [8]. The chemical shift of Glc II and rhamnose almost corresponded to those of methyl Glc and methyl Rha [14, 20], indicating that they were terminal sugars.

A comparison of the ^{13}C NMR spectrum (Table 1) of **1** with that of the reported saponin [6], indicated that both saponins are identical with respect to the sugar sequence and linkage, except for the presence of three additional sugar moieties in the form of ester in **1**. The position of the sugar moieties linked in ester form was identified at C-28 of the hederagenin on the basis of ^1H and ^{13}C NMR spectral data which matched well with those reported for the 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester of hederagenin [8].

Saponin **1** exhibited no activity in the brine shrimp lethality assay with a $LD_{50} > 100\ \mu g\ \mu l^{-1}$. However, the crude saponin mixture showed activity with $LD_{50} = 53.650$ between the upper and lower confidence limits of 82.411 and 33.936, respectively.

The above spectral evidence led us to conclude that the structure of **1** is 3-*O*-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-α-L-arabinopyranosyl]-hederagenin 28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester. To the best of our knowledge the occurrence of **1** in nature has not been previously reported.

EXPERIMENTAL

Mps: uncorr. Optical rotations: in MeOH. UV (MeOH) and IR (KBr) spectra were measured on Hitachi U-3200 and Shimadzu IR-460 spectrophotometers, respectively. EI-MS was determined on a Finningan MAT-112 spectrometer. The negative ion FAB-MS was recorded on a Jeol JMS HX-110 spectrometer operating at an accelerating voltage of − 10 kV, using MeOH as a solvent and glycerol as a matrix. Samples were ionized by bombardment with Xe (gas) atoms. ^1H NMR spectra: CD_3OD on a Bruker Aspect AM-500 spectrometer operating at 500 MHz. ^{13}C NMR spectra (BB and DEPT) were recorded in CD_3OD on a Bruker Aspect AM-400 spectrometer operating at 100 MHz. The chemical shifts are expressed as ppm (δ), and coupling constants (J) are in Hz with TMS as an int. standard. The ^{13}C NMR spectral assignments were made partly through a comparison of the chemical shifts with the published data for a similar compound [8] and partly through the appearance of signals in DEPT and heteroCOSY spectra (Table 1). All the above proton signals and multiplicities were determined through 2D J-resolved spectra, and coupling interactions were established by COSY-45° spectra. The DEPT experiments were carried out with $\theta = 45°$, 90° and 135°, the quaternary carbons were determined by subtraction of these spectra from the broad band ^{13}C NMR spectrum. Values with identical superscripts are interchangeable. Lobar RP-8 reversed phase (40–63 μm Merck) and silica gel 60 (70–230 mesh) were used for CC. Precoated kieselgel 60, F_{254} cards (thickness 0.25 mm, Riedel de Haën Art No. 37360) were used for TLC using the following solvent systems $CHCl_3$–MeOH–H_2O (75:25:2), n-BuOH–HOAc–H_2O (12:3:5). Final purity of the compound was checked on RP-8 $F_{254}S$ precoated TLC plates (thickness 0.25 mm, E. Merck, Art No. 15684) using MeOH–H_2O (7:3). Spots were observed by spraying with 10% soln of $Ce(SO_4)_2$ in 1 M H_2SO_4 followed by heating at 80°C for 5 min. The HPLC consisted of a Shimadzu model LC-6A pump as a solvent delivery system, a Rheodyne sample injector with a 100 μl loop, a Hibar RP-8 column (24.4 cm × 10 mm i.d.) and a Shimadzu model RID-6A refractive index detector connected with a Kipp & Zonen BD-41 recorder.

The 2D COSY-45° spectra were acquired at 400 MHz with a sweep width of 2262 Hz (1 K data points in ω_2) and 1131 Hz (256 t_1 values) in ω_1. A 1.5 sec relaxation delay was used, and 16 transients were accumulated for each t_1 value. The heteronuclear 2D ^1H–^{13}C chemical shift correlation experiment were carried out at 300 MHz with a sweep width of 12 500 Hz (2 K data points in ω_2) and 883 Hz (512 t_1 value) in ω_1. A 1.5 sec relaxation delay was used, and 64 transients were performed for each t_1 value.

Refer to our earlier paper [5] for plant material, extraction and chromatography. The fractions eluted with $CHCl_3$–MeOH (39:11) yielded two major saponins with some minor impurities. This saponin mixture was rechromatographed on a Lobar column (Lichroprep RP-8 Merck) with MeOH–H_2O (1:1) and further purified by HPLC using MeOH–H_2O (3:2) on a semi-prep. reversed-phase (RP-8) column at a flow rate of 3.0 ml min^{-1}. to yield pure **1**.

Saponin **1**. Mp 192° (dec.), 75 mg; $[\alpha]_D^{25}$ − 2.86 (MeOH; *c* 0.14); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1740 (C=O), 1620 (C=C), 1100–1000 (C–O–C); UV λ_{max}^{MeOH} nm: 200.6 (end absorption); ^1H NMR (CD_3OD, 500.14 MHz): δ 0.71 (3H, *s*, H-24), 0.79 (3H, *s*, H-26), 0.90 (3H, *s*, H-29), 0.93 (3H, *s*, H-30), 0.97 (3H, *s*, H-25), 1.16 (3H, *s*, H-27), 1.27 (3H, *d*, J = 6.20 Hz, H-6''''''), 4.40 (1H, *d*, J = 7.85 Hz, H-1''''''), 4.49 (1H, *d*, J = 7.65 Hz, H-1'''), 4.53 (1H, distorted *d*, H-1'), 4.64 (1H, *d*, J = 7.70 Hz, H-1''), 4.84 (1H, *d*, J = 1.00 Hz, H-1'''''), 5.33 (1H, *d*, J = 8.10 Hz, H-1''''); ^{13}C NMR (CD_3OD, 100.613 MHz): see Table I; negative ion FAB-MS *m/z* $[M - H]^-$ 1397, $[M - H - 146]^-$ 1251, $[M - H - (146 + 162)]^-$ 1089, $[M - H - (146 + 2 \times 162)]^-$ 927, $[M - H -$ (146 +

$3 \times 162)]^-$ 765, $[M - H - (146 + 4 \times 162)]^-$ 603, $[M - H - (146 + 4 \times 162 + 132)]^-$ 471.

Acid hydrolysis of saponin **1**. Pure saponin **1** (10 mg) was refluxed with 10 ml HCl (20%) in aq. MeOH (5 ml) for 4 hr. MeOH was evapd under red. pres. The mixture was then diluted with H_2O (5 ml) and extracted $\times 3$ with EtOAc. The combined EtOAc layer was concd and the aglycone, hederagenin (**2**) was obtained which was crystallized from MeOH mp 325°, identified by direct comparison with an authentic sample (co-TLC, mmp, IR, MS, ^1H NMR) [24].

Identification of the sugar moieties of compound **1**. The aq. layer sepd above was evapd under red. pres. with repeated addition of H_2O to remove HCl. The residue was compared with standard sugars on silica gel plates (E. Merck, Art No. 5554) by using solvent system n-BuOH–i-PrOH–EtOAc–HOAc–H_2O (7:12:20:7:6). The TLC was developed twice in the same direction. The spots were detected with aniline phthalate sugar reagent, which indicated that the sugars were glucose, arabinose and rhamnose in saponin **1**. Moreover, the identity of the monosaccharides was further confirmed by comparison with standard sugar on paper chromatography (Whatman Filter paper No. 1, serrated edges along the lower descending ends) using the solvent system n-BuOH–pyridine–H_2O (10:3:3) and developing time 48 hr. Spots were detected by spraying with freshly prepared aniline phthalate sugar reagent followed by heating. Three spots were present, whose R_f's were identical to the R_f of arabinose, glucose and rhamnose.

Alkaline hydrolysis of saponin **1**. Compound **1** (25 mg) was refluxed with 2% KOH (25 ml) in MeOH for 1 hr [17]. After cooling, the reaction mixture was slightly acidified with dilute HCl, and extracted with n-BuOH. The n-BuOH extract was washed with H_2O, evapd under red. pres., and crystallized with MeOH to yield the prosapogenin **3**, as a powder, mp 262° (dec.); $[\alpha]_D^{24}$ 7.4 (MeOH; *c* 0.216); negative ion FAB-MS *m/z* 927 $[M - H]^-$, 765 $[M - H - Glc]^-$, 603 $[M - H - 2 \times Glc]^-$, 471 $[M - H - 2xGlc - Ara]^-$; ^1H NMR (CD_3OD, 300.13 MHz): δ 4.49 (*d*, $J = 7.60$ Hz, H-1‴), 4.56 (distorted *d*, H-1′), 4.63 (*d*, $J = 7.44$ Hz, H-1″), 5.23 (1H, distorted *t*, H-12); ^{13}C NMR (CD_3OD, 125.77 MHz): δ 39.4 (C-1), 26.2 (C-2), 83.6 (C-3), 44.0 (C-4), 48.1 (C-5), 18.8 (C-6), 33.1 (C-7), 40.5 (C-8), 49.6 (C-9), 37.6 (C-10), 24.5 (C-11), 123.7 (C-12), 145.2 (C-13), 43.9 (C-14), 29.0 (C-15), 23.9 (C-16), 48.1 (C-17), 42.2 (C-18), 47.1 (C-19), 31.5 (C-20), 34.8 (C-21), 33.8 (C-22), 64.7 (C-23), 13.2 (C-24), 16.3 (C-25), 17.8 (C-26), 25.3 (C-27), 181.9 (C-28), 33.4 (C-29), 23.9 (C-30), 104.3 (C-1′), 73.4 (C-2′), 71.7 (C-3′), 79.0 (C-4′), 62.9 (C-5′), 104.3 (C-1″), 75.7 (C-2″), 77.9 (C-3″), 78.1 (C-4″), 77.9 (C-5″), 62.6 (C-6″), 105.5 (C-1‴), 75.3 (C-2‴), 77.8 (C-3‴), 71.4 (C-4‴), 78.4 (C-5‴), 63.6 (C-6‴).

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