Three Acylated Saponins and a Related Compound from *Pithecellobium dulce*

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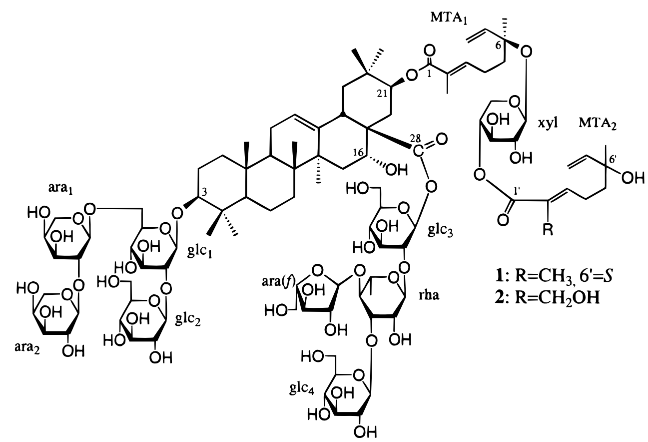
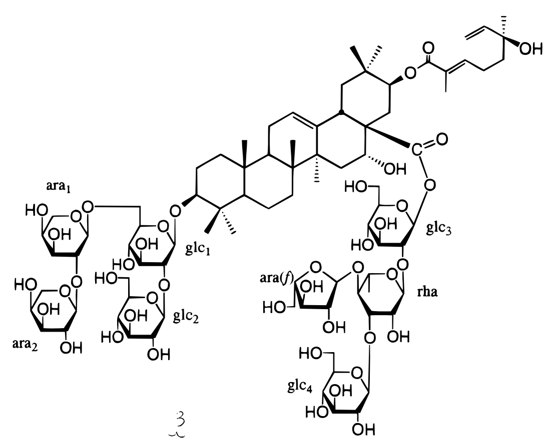
Received July 25, 1997⊗

Four new oleanane-type triterpene glycosides, pithedulosides H−K (**1**−**4**), were isolated from the seeds of *Pithecellobium dulce*. Their structures were established by extensive NMR experiments and chemical methods. Compounds **1**−**3** comprised acacic acid as the aglycon and either monoterpene carboxylic acid and its xyloside or monoterpene carboxylic acid as the acyl moiety at C-21. The oligosaccharide moieties linked to C-3 and C-28 were determined as α-L-arabinopyranosyl-(l→2)-α-L-arabinopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(l→2)]-*β*-D-glucopyranosyl and α-L-arabinofuranosyl-(l→4)-[*β*-D-glucopyranosyl-(l→3)]-α-L-rhamnopyranosyl-(l→2)-*β*-D-glucopyranosyl ester, respectively. Compound **4** was established as an echinocystic acid 3-*O*-glycoside having the same sugar sequences as **1**−**3**. Also obtained in this investigation was the known compound **5**, which was identified as echinocystic acid 3-*O*-*β*-D-xylopyranosyl-(l→2)-α-L-arabinopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(l→2)]-*β*-D-glucopyranoside.

Pithecellobium dulce Benth. (Leguminosae) is widely distributed throughout India and is also found in South Africa and Australia. This species has been used for hedges and street trees.[1,2] In a previous contribution, we reported the isolation and structure determination of seven saponins, termed pithedulosides A−G.[3] The further investigation of the saponins of this plant, observed as a very complex mixture, afforded four new saponins, pithedulosides H−K (**1**−**4**), along with one known compound (**5**), which was previously isolated from the seeds of *Albizzia lucida*.[4] We describe here the isolation and structure elucidation of pithedulosides H−K (**1**−**4**) by various NMR techniques, including COSY, HMQC, HMBC, TOCSY, and ROESY experiments and chemical degradation.





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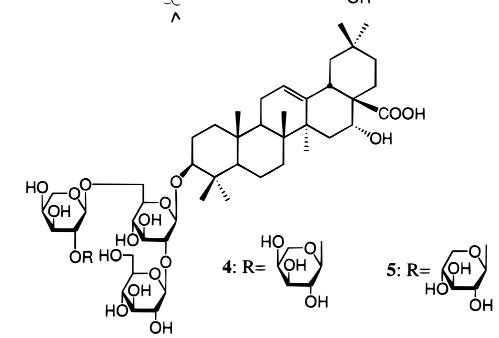

Results and Discussion

Pitheduloside H (**1**) was obtained as a major component from *P. dulce* seeds. The molecular formula was deduced as $C_{100}H_{158}O_{49}$ from a quasi-molecular ion observed at *m/z* 2166 $[M + Na]^+$ in the FABMS and from the ^{13}C-NMR spectrum. The IR spectrum showed carbonyl group (1740 cm^{-1}) and α,*β*-unsaturated carbonyl group (1690 cm^{-1}) absorptions. In the ^{13}C-NMR spectrum, the chemical shifts for the aglycon part of **1** exhibited some differences at positions C-17, C-20−C-22, and C-28−C-30 from those of pitheduloside A[3] and were in good agreement with those of acaciaside A, which was previously determined as acacic acid 3,21,28-*O*-trisdesmoside.[5] The acid hydrolysis of **1** afforded an acacic acid lactone (**6**), which was identified by comparison with published data,[6] and L-arabinose, D-glucose, L-rhamnose, and D-xylose were confirmed by specific rotation using chiral detection in HPLC analysis.[7] In the ^{1}H-NMR spectrum of **1**, nine anomeric proton signals appeared at δ 6.28 [1H, br s, ara(*f*)], 6.08 (1H, d, J = 8.0 Hz, glc$_3$), 5.92 (1H, br s, rha), 5.41 (1H, d, J = 7.7 Hz, glc$_2$), 5.35 (1H, d, J = 7.7 Hz, glc$_4$), 5.09 (1H, d, J = 5.2 Hz, ara$_2$), 5.05 (1H, d, J = 6.6 Hz, ara$_1$), 4.87 (1H, d, J = 7.1 Hz, glc$_1$), and 4.85 (1H, d, J = 7.7 Hz, xyl), respectively. The corresponding nine anomeric

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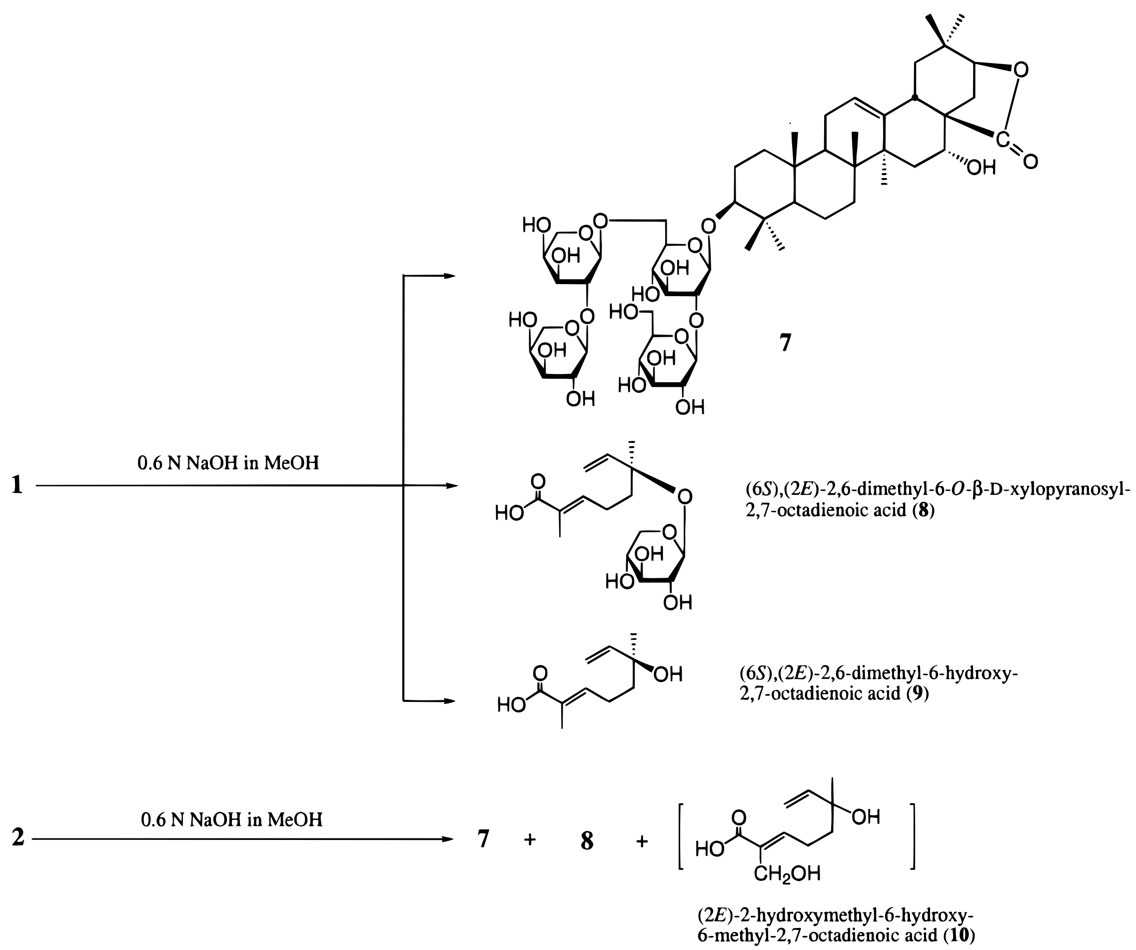
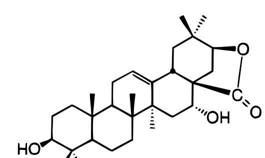
⊗ Abstract published in *Advance ACS Abstracts,* December 1, 1997.

Scheme 1



carbons were observed at δ 111.1 [ara(*f*)], 95.7 (glc$_3$), 101.8 (rha), 105.9 (glc$_2$), 105.8 (glc$_4$), 105.7 (ara$_2$), 102.4 (ara$_1$), 105.0 (glc$_1$), and 100.2 (xyl) in the ^{13}C-NMR spectrum. Among the three anomeric carbons of the arabinose units, the chemical shift at δ 111.1 demonstrated that one of those was in the α-furanose form.[8] The configuration of all of the other sugars in the pyranose form in **1** was fully defined from the chemical shift and the coupling constant of each of the remaining anomeric protons. Accordingly, four glucoses and one xylose were established to have the β configuration, and two arabinopyranoses and one rhamnose to have the α configuration. The ^{13}C-NMR spectrum of **1** showed 100 carbon signals, from which 30 signals were attributed to acacic acid and 50 signals were attributed to the sugar moieties. The remaining 20 signals were consistent with the presence of two monoterpene carboxylic acids.[9] Upon alkaline hydrolysis of the crude saponin with 0.6 N NaOH in MeOH, a prosapogenin (**7**), a monoterpene glycoside (**8**), and a monoterpene (**9**) were obtained as major components. The alkaline hydrolysis of **1** under the same conditions also afforded **7**−**9** as major products (Scheme 1). Compounds **8** and **9** were found to be identical with the known compounds, (6*S*),-(2*E*)-2,6-dimethyl-6-*O*-β-D-xylopyranosyl-2,7-octadienoic acid and its aglycon, (6*S*),(2*E*)-2,6-dimethyl-6-hydroxyl-2,7-octadienoic acid, which have been obtained by the alkaline hydrolysis of calliandra saponin E isolated from *Calliandra anomala*.[9]

The NMR spectra of the prosapogenin **7**, $C_{52}H_{82}O_{22}$, suggested that this reaction product was acacic acid



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lactone 3-*O*-tetraglycoside,[10] which contained two α-arabinopyranosyl and two β-glucopyranosyl units. The downfield-shifted ^{13}C-NMR resonances among the sugar units were observed at δ 83.1, 69.2, and 79.7, indicating the probable point of glycosidic linkage in the oligosaccharide as being at C-3.^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY experiments revealed the glycosdic positions of attachment at C-2 and C-6 for glucose (glc$_1$), and C-2 of arabinose (ara$_1$), respectively. Further, the HMBC spectrum showed connectivities between the H-1 proton (δ 4.89) of glc$_1$ and C-3 (δ 88.8) of the aglycon, the H-1 proton (δ 5.40) of glc$_2$ and C-2 (δ 83.1) of glc$_1$, the H-1 proton (δ 5.10) of ara$_1$ and C-6 (δ 69.2) of glc$_1$, and the H-1 proton (δ 5.05) of ara$_2$ and C-2 (δ 79.7) of ara$_1$. In this way, **7** was formulated as acacic acid lactone 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside.

The binding sites of the ester residues, that is, **8** and **9** in **1**, were revealed by two acylation shifts observed at δ 6.32 (1H, dt, J = 11.1, 5.6 Hz) and 5.50 (1H, dt, J = 9.7, 5.6 Hz). Using both ROESY and HMBC experiments, these signals were assigned to H-21 of aglycon and H-4 of xylose, respectively. Further, the HMBC spectrum exhibited significant corrlations between H-21

Table 1. ^{13}C-NMR Data of Aglycon Moieties and C-21 Portions of Compounds **1**−**4** and **7**−**9** in C_5D_5N

position	**1**	**2**	**3**	**4**	**7**	position	**1**	**2**	**3**	**8**	**9**
C−1	39.0	39.0	39.0	38.9	38.7	MTA$_1$ 1	167.8	167.8	167.8	170.6	170.7
2	26.8	26.8	26.8	26.8	26.8	2	127.6	127.6	128.3	129.1	128.9
3	89.1	89.2	89.1	89.1	88.8	3	143.9	143.8	142.8	142.2	142.6
4	39.6	39.6	39.6	39.6	39.6	4	24.1	24.1	24.0	23.9	24.2
5	56.0	56.0	56.0	56.0	55.9	5	41.4	41.4	41.6	40.7	41.8
6	18.7	18.7	18.7	18.6	18.4	6	79.8	79.8	72.2	79.7	72.2
7	33.6	33.6	33.6	33.5	32.5	7	143.9	143.9	146.6	144.1	146.7
8	40.1	40.1	40.1	40.0	40.3	8	115.2	115.2	111.7	115.0	111.7
9	47.1	47.1	47.1	47.3	47.2	9	12.5	12.5	12.6	12.9	12.8
10	37.1	37.1	37.1	37.1	36.9	10	23.6	23.7	28.6	23.9	28.6
11	23.9	23.9	23.9	23.9	23.8						
12	123.1	123.1	123.1	122.5	124.6	xyl 1	100.2	100.1		100.3	
13	143.3	143.4	143.4	145.2	140.1	2	75.4	75.3		75.3	
14	42.1	42.1	42.1	42.1	43.3	3	75.1	75.1		78.7	
15	35.9	35.9	35.9	36.2	38.2	4	73.1	73.1		71.2	
16	73.9	73.9	73.9	74.8	66.7	5	63.2	63.2		67.0	
17	51.7	51.7	51.7	48.9	50.0						
18	41.1	41.0	41.1	41.5	41.7	MTA$_2$1′	167.7	167.6			
19	47.9	47.9	47.9	47.3	42.9	2′	128.6	133.9			
20	35.3	35.5	35.3	31.1	34.1	3′	142.3	145.2			
21	77.1	77.1	77.1	36.3	83.4	4′	23.6	23.6			
22	36.4	36.4	36.4	32.9	27.2	5′	40.5	40.8			
23	28.2	28.2	28.2	28.2	28.0	6′	72.1	72.1			
24	16.9	16.9	16.9	16.9	16.8	7′	146.6	146.6			
25	15.9	15.9	15.9	15.7	15.7	8′	111.7	111.7			
26	17.4	17.4	17.4	17.6	16.2	9′	12.7	56.3			
27	27.3	27.3	27.3	27.3	28.8	10′	28.6	28.5			
28	174.5	174.5	174.5	180.1	181.2						
29	29.2	29.2	29.2	33.4	28.5						
30	19.2	19.2	19.2	24.8	24.2						

Table 2. ^{13}C-NMR Data of Sugar Moieties of Compounds **1**−**4** and **7** in C_5D_5N

C−3 Sugar	**1**	**2**	**3**	**4**	**7**	C−28 Sugar	**1**	2	3
glc$_1$ 1	105.0	105.0	105.0	105.0	105.0	glc$_3$ 1	95.7	95.8	95.7
2	82.9	82.9	82.9	83.0	83.1	2	76.8	76.9	76.9
3	78.0	78.0	78.0	78.0	77.9	3	78.0	78.0	78.0
4	71.8	71.8	71.8	71.7	71.8	4	71.2	71.2	71.2
5	77.1	77.1	77.0	77.2	77.2	5	79.1	79.2	79.1
6	69.3	69.2	69.3	69.3	69.2	6	62.0	62.0	62.0
ara$_1$ 1	102.4	102.4	102.3	102.4	102.4	rha 1	101.8	101.8	101.9
2	79.6	79.6	79.6	79.6	79.7	2	70.6	70.5	70.6
3	72.8	72.8	72.8	72.8	72.8	3	82.0	82.0	82.0
4	67.6	67.6	67.6	67.7	67.6	4	79.1	79.1	79.1
5	64.4	64.4	64.4	64.5	64.5	5	69.2	69.2	69.2
ara$_2$ 1	105.7	105.7	105.7	105.7	105.7	6	18.9	18.9	18.9
2	72.8	72.8	72.8	72.8	72.8	glc$_4$ 1	105.8	105.8	105.8
3	74.3	74.3	74.3	74.3	74.3	2	75.4	75.4	75.4
4	69.0	69.0	69.0	69.0	68.9	3	78.3	78.3	78.2
5	66.8	66.8	66.8	67.0	66.7	4	71.8	71.8	71.8
glc$_2$ 1	105.9	105.9	105.9	105.9	106.0	5	78.3	78.3	78.2
2	75.6	75.7	75.6	75.7	75.8	6	62.7	62.8	62.8
3	78.0	78.0	78.0	78.0	78.0	ara(*f*) 1	111.1	111.1	111.1
4	71.8	71.8	71.8	71.7	71.7	2	84.5	84.5	84.5
5	78.3	78.3	78.2	78.3	78.3	3	78.4	78.4	78.4
6	62.7	62.8	62.8	62.7	62.7	4	85.4	85.4	85.5
						5	62.5	62.6	62.5

of the aglycon and the carbonyl carbon (δ 167.8) of the monoterpene xyloside unit, and between H-4 of xylose and the carbonyl carbon (δ 167.7) of the outer monoterpene unit. Therefore, a (6*S*),(2*E*)-2,6-dimethyl-6-*O*-(4-*O*-(6′*S*),(2′*E*)-2′,6′-dimethyl-6′-hydroxyl-2′,7′-octadienoyl-β-D-xylopyranosyl)-2,7-octadienoyl residue was located at C-21 of the aglycon.

The FABMS of **1** showed a major fragmentation peak at *m/z* 1564, indicating that the sugar moiety linked to C-28 accounted for 602 mass units (four sugar residues).[11] The characteristic signal at δ 95.7 among the remaining four anomeric carbons (δ 95.7, 101.8, 105.8, 111.1) suggested that this sugar residue must be directly attached to C-28 through an ester bond. Indeed, the corresponding anomeric proton at δ 6.08 was correlated with C-28 at δ 174.5 in the HMBC spectrum, and this inner sugar was disclosed as a β-glucopyranosyl group. Starting from the anomeric proton at δ 6.08 of glucose, the extensive NMR experiments conducted in this investigation allowed the full assignment of the sugar moieties at C-28 (Table 2). The long-range correlations between C-28 (δ 174.5) of the aglycon and H-1 (δ 6.08) of glucose (glc$_3$), and C-2 (δ 76.8) of glucose (glc$_3$) and H-1 (δ 5.92) of rhamnose, and C-3 (δ 82.0) of rhamnose and C-1 (δ 5.35) of glucose (glc$_4$), and C-4 (δ 79.1) of rhamnose and H-1 (δ 6.28) of arabinose [ara(*f*)] were all definitely observed. Thus, the structure of sugar moiety at C-28 was determined to be α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(l→3)]-α-L-rhamnopyranosyl-(l→2)-β-D-glucopyranoside. Consequently, the whole structure of **1** (pitheduloside H) was concluded to be 3-*O*-{α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-*O*-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-21-*O*-[(6*S*),(2*E*)-(2,6-dimethyl-6-*O*-[4-*O*-(6′*S*),-(2′*E*)-2′,6′-dimethyl-6′-hydroxyl-2′,7′-octadienoyl-β-D-xylopyranosyl]-2,7-octadienoyl] acacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester.

Pitheduloside I (**2**) gave a $[M + Na]^+$ ion at *m/z* 2182 in the FABMS, 16 mass units higher than that of **1**, suggesting the presence of an additional oxygen-bearing function in **2**. Hydrolysis of **2** with 5% H_2SO_4 gave **6**, and the sugar units determined by chiral HPLC analysis were again, L-arabinose, D-glucose, L-rhamnose, and D-xylose. In the ^{13}C-NMR spectrum, the chemical shifts for the aglycon moiety and sugar moieties of **2** bore a close resemblance to those of **1**, indicating that both compounds had a common sugar-substitution pattern. The ^{13}C-NMR data showed also the presence of **8** and a second monoterpene carboxylic acid for the acyl moieties at C-21. Examination of the ^{1}H- and ^{13}C-NMR data obtained for the second acyl moiety in **2** revealed that it differed from **1** only in having a hydroxymethyl group (δ 56.3) and no methyl group (δ 12.7) at C-2. Thus, the second acid in **2** was shown to be (2*E*)-2-hydroxylmethyl-6-hydroxy-6-methyl-2,7-octadienoic acid (**10**).[12] Alkaline hydrolysis of **2** gave only prosapogenin **7** and the monoterpene xyloside **8**. Consequently, the absolute configuration of **10** was not established. HMBC correlations between H-21 and C-1 of MTA_1, H-1 (δ 4.84) of xylose and C-6 of MTA_1, and H-4 (δ 5.48) of xylose and C-1′ of MTA_2 established the presence of a substituent at C-21. From an analysis of all the data obtained, the structure of **2** was concluded to be 3-*O*-{α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-*O*-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-21-*O*-[(6*S*),(2*E*)-2,6-dimethyl-6-*O*-[4-*O*-(2′*E*)-2′,6′-dihydroxyl-6′-methyl -2′,7′-octadienoyl)-β-D-xylopyranosyl]-2,7-octadienoyl]-acacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester.

In the FABMS, pitheduloside J (**3**) showed a quasi-molecular ion at *m/z* 1844, 314 mass units lower than **2**. Acid hydrolysis of **3** afforded L-arabinose, D-glucose, and L-rhamnose by HPLC analysis, besides **6**. The ^{13}C-NMR chemical shifts due to the aglycon moiety and the sugar moieties attached at C-3 and C-28 of **3** were superimposable on those of **2**. In contrast, the acyl moiety at C-21 in **3**, the only monoterpene unit in the molecular, was not glycosylated at C-6 (δ 72.2) (Table 1). Alkaline hydrolysis of **3** afforded **7** and **9** as detected by TLC. Hence, the structure of **3** was concluded to be 3-*O*-{α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-*O*-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-21-*O*-(6*S*),(2*E*) −2,6-dimethyl-6-hydroxyl-2,7-octadienoyl acacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester.

Pitheduloside K (**4**) showed a quasi-molecular ion peak at *m/z* 1059 $[M - H]^-$ in the FABMS, indicating a molecular formula of $C_{52}H_{84}O_{22}$. Acid hydrolysis of **4** afforded echinocystic acid (**11**)[3] as an aglycon, and D-glucose and L-arabinose in a 1:1 ratio. The ^{13}C-NMR chemical shifts for the aglycon of **4** were very similar to those of pitheduloside A,[3] indicating that **4** was echinocystic acid 3-*O*-glycoside. The ^{13}C-NMR sugar signals at the C-3 position for **4** were very similar to analogous data for **1**. Therefore, **4** was formulated as echinocystic acid 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside.

Compound **5** showed the same quasi-molecular ion peak at *m/z* 1059 $[M - H]^-$ as **4** and gave L-arabinose, D-glucose, D-xylose, and **11** on acid hydrolysis. The carbon signals of **5** were superimposable on those of compound 2 from Orsini et al.[4] Therefore, **5** was identified with echinocystic acid 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside.

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were taken on a JASCO DIP-360 polarimeter. IR spectra were recorded on a JASCO FT/IR-5300, NMR spectra on Varian UNITY-600 and/or JEOL GSX-400 spectrometer in pyridine-d_5 solutions using TMS as internal standard. NMR experiments included ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, HMBC, TOCSY, and ROESY. Coupling constants (*J* values) are given in Hz. The FABMS (Xe gun, 10 kV, triethylene glycol as the matrix) was measured on a JEOL JMS-HX-100 mass spectrometer. HPLC separations were performed with a Hitachi HPLC system (L-6200 Pump, L-4000 UV).

Plant Material. The seeds of *P. dulce* were collected in August 1995. A voucher specimen is deposited in the Herbarium of the National Botanical Research Institute, Lucknow, India.

Extraction and Isolation. The powdered seeds (5.0 kg) of *P. dulce* were percolated with EtOH, and the alcoholic extract was partitioned, in turn, with petroleum ether and Et_2O. The EtOH-soluble residue was dissolved in the least amount of EtOH and the crude saponins precipitated by addition of a large excess of ether. The ether was decanted and the saponin mixture filtered over Si gel to yield a straw-colored powder (150 g). An aliquot (75 g) was subjected to Si gel column chromatography, eluting with CH_2Cl_2−MeOH−H_2O mixtures of increasing polarity to obtain fractions 1 to 6. The most polar fraction 6 (55 g) was passed through an Amberlite XAD-2 column, following elution with 80% and 100% MeOH. The 80% MeOH eluate (9.0 g) was chromatographed over Sephadex LH-20 with MeOH to give three fractions, A (3.81 g), B (1.12 g), and C (2.24 g). Fraction A was subjected to further Si gel column chromatography, eluting with CH_2Cl_2−MeOH−H_2O (6:4:1), and finally purified by HPLC on ODS with 33−30% CH_3CN in H_2O to furnish pithedulosides H (**1**, 80 mg), I (**2**, 20 mg), and J (**3**, 30 mg). Fraction C was subjected to HPLC on ODS with 31% CH_3CN in H_2O to yield pitheduloside K (**4**, 180 mg) and compound **5** (35 mg).

Pitheduloside H (1): colorless needles; mp 196−198 °C; $[\alpha]^{25}_D$ −23.6° (*c* 3.1, MeOH); FT-IR (dry film) ν_{max} 3410 (OH), 1740, 1690 (C=O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N) δ 0.96 (3H, s, H_3-25), 1.03 (3H, s, H_3-29), 1.07 (3H, s, H_3-30), 1.14 (3H, s, H_3-24), 1.18 (3H, s, H_3-26), 1.28 (3H, s, H_3-23), 1.79 (3H, d, J = 6.0 Hz, Me of rha), 1.86 (3H, s, H_3-27), 3.37 (1H, dd, J = 11.5, 4.9 Hz, H-3), 3.44 (1H, dd, $J = 14.4$, 5.2 Hz, H-18), 5.21 (1H, m, H-16), 5.65 (1H, m, H-12), 5.50 (1H, dt, $J = 9.7$, 5.6 Hz, H-4 of xyl), 6.32 (1H, dd, $J = 11.1$, 5.6 Hz, H-21), anomeric H 4.85 (1H, d, $J = 7.7$ Hz, xyl), 4.87 (1H, d, $J = 7.4$ Hz, glc_1), 5.05 (1H, d, $J = 6.6$ Hz, ara_2), 5.09 (1H, d, $J = 5.2$ Hz, ara_1), 5.35 (1H, d, $J = 7.7$ Hz, glc_4), 5.41 (1H, d, $J = 7.7$ Hz, glc_2), 5.92 (1H, br s, rha), 6.08 (1H, d, $J = 8.0$ Hz, glc_3), 6.28 [1H, br s, ara(*f*)]; MTA_1 1.52 (3H, s, H_3-10), 1.91 (3H, s, H_3-9), 5.26 (1H, dd, $J = 11.0$, 1.1 Hz, H_2-8), 5.43 (1H, dd, $J = 17.8$, 1.1 Hz, H_2-8), 6.22 (1H, dd, $J = 17.8$, 11.0 Hz, H_2-7), 7.08 (1H, t, $J = 7.6$ Hz, H-3); MTA_2 1.44 (3H, s, H_3-10), 1.87 (3H, s, H_2-9), 5.16 (1H, dd, $J = 10.7$, 2.1 Hz, H_2-8), 5.55 (1H, dd, $J = 17.3$, 2.1 Hz, H_2-8), 6.17 (1H, dd, $J = 17.3$, 11.0 Hz, H_2-7), 6.90 (1H, t, $J = 6.9$ Hz, H-3); ^{13}C-NMR data, see Tables 1 and 2; FABMS *m/z* $[M + Na]^+$ 2166, $[M + Na - 602]^+$ 1564.

Pitheduloside I (2): colorless needles; mp 222−224 °C; $[\alpha]^{25}_D$ −16.2 ° (*c* 2.0, MeOH); FT-IR (dry film) ν_{max} 3400 (OH), 1740, 1685 (C═O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N) δ 0.96 (3H, s, H_3-25), 1.06 (3H, s, H_3-29), 1.09 (3H, s, H_3-30), 1.14 (3H, s, H_3-24), 1.17 (3H, s, H_3-26), 1.28 (3H, s, H_3-23), 1.79 (3H, d, $J = 6.0$ Hz, Me of rha), 1.85 (3H, s, H_3-27), 3.37 (1H, dd, $J = 12.0$, 4.0 Hz, H-3), 3.44 (1H, dd, $J = 14.0$, 4.5 Hz, H-18), 5.22 (1H, m, H-16), 5.48 (1H, dt, $J = 9.6$, 5.5 Hz, H-4 of xyl), 5.64 (1H, m, H-12), 6.32 (1H, dd, $J = 11.1$, 5.6 Hz, H-21), anomeric H 4.84 (1H, d, $J = 7.7$ Hz, xyl), 4.87 (1H, d, $J = 7.1$ Hz, glc_1), 5.05 (1H, d, $J = 6.6$ Hz, ara_2), 5.09 (1H, d, $J = 5.2$ Hz, ara_1), 5.35 (1H, d, $J = 7.7$ Hz, glc_3), 5.41 (1H, d, $J = 6.9$ Hz, glc_2), 5.89 (1H, br s, rha), 6.06 (1H, d, $J = 8.0$ Hz, glc_3), 6.28 [1H, d, $J = 1.5$ Hz, ara(*f*)]; MTA_1 1.49 (3H, s, H_3-10), 1.86 (3H, s, H_3-9), 5.21(1H, dd, J = 11.0, 1.1 Hz, H_2-8), 5.38 (1H, dd, J = 17.5, 1.1 Hz, H_2-8), 6.20 (1H, dd, $J = 17.5$, 11.0 Hz, H_2-7), 7.08 (1H, dt, $J = 7.3$, 1.3 Hz, H-3); MTA_2 1.44 (3H, s, H_3-10), 4.73 (2H, s, H_2-9), 5.16 (1H, dd, $J = 10.6$, 2.0 Hz, H_2-8), 5.56 (1H, dd, $J = 17.3$, 2.0 Hz, H_2-8), 6.11 (1H, dd, $J = 17.3$, 10.6 Hz, H_2-7), 7.04 (1H, t, $J = 7.7$ Hz, H-3); ^{13}C-NMR data, see Tables 1 and 2; FABMS *m/z* $[M + Na]^+$ 2182, $[M + Na - 602]^+$ 1580.

Pitheduloside J (3): colorless needles; mp 199−201 °C; $[\alpha]^{25}_D$ −28.0° (*c* 2.2, MeOH); FT-IR (dry film) ν_{max} 3400 (OH), 1740, 1690 (C═O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N) δ 0.96 (3H, s, H_3-25), 1.03 (3H, s, H_3-29), 1.08 (3H, s, H_3-30), 1.14 (3H, s, H_3-24), 1.18 (3H, s, H_3-26), 1.28 (3H, s, H_3-23), 1.79 (3H, d, $J = 6.0$ Hz, Me of rha), 1.85 (3H, s, H_3-27), 3.37 (1H, dd, $J = 11.5$, 4.1 Hz, H-3), 3.44 (1H, dd, $J = 14.9$, 3.6 Hz, H-18), 5.23 (1H, m, H-16), 5.64 (1H, m, H-12), 6.24 (1H, dd, $J = 11.0$, 5.8 Hz, H-21), anomeric H 4.87 (1H, d, $J = 7.4$ Hz, glc_1), 5.05 (1H, d, $J = 6.6$ Hz, ara_2), 5.09 (1H, d, $J = 5.2$ Hz, ara_1), 5.35 (1H, d, $J = 7.7$ Hz, glc_4), 5.41 (1H, d, $J = 7.7$ Hz, glc_2), 5.91 (1H, br s, rha), 6.08 (1H, d, $J = 8.0$ Hz, glc_3), 6.28 [1H, d, $J = 1.4$ Hz, ara(*f*)]; MTA 1.45 (3H, s, H_3-10), 1.88 (3H, s, H_3-9), 5.16 (1H, dd, $J = 10.7$, 1.9 Hz, H_2-8), 5.57 (1H, dd, $J = 17.3$, 1.9 Hz, H_2-8), 6.13 (1H, dd, $J = 17.3$, 10.7 Hz, H_2-7), 7.02 (1H, dt, $J = 7.7$, 1.3 Hz, H-3); ^{13}C-NMR data, see Tables 1 and 2; FABMS *m/z* $[M + Na]^+$ 1868, $[M + Na - 602]^+$ 1266.

Pitheduloside K (4): colorless needles; mp 200−202 °C; $[\alpha]^{25}_D$ −4.1° (*c* 3.5, MeOH); FT-IR (dry film) ν_{max} 3410 (OH), 1690 (C═O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N) δ 0.88 (3H, s, H_3-25), 1.02 (3H, s, H_3-24), 1.05 (3H, s, H_3-26), 1.10 (3H, s, H_3-29), 1.17 (3H, s, H_3-30), 1.28 (3H, s, H_3-23), 1.85 (3H, s, H_3-27), 3.38 (1H, dd, $J = 11.2$, 4.0 Hz, H-3), 3.60 (1H, dd, $J = 13.0$, 4.5 Hz, H-18), 5.24 (1H, m, H-16), 5.61 (1H, m, H-12), anomeric H 4.87 (1H, d, $J = 7.3$ Hz, glc_1), 5.04 (1H, d, $J = 6.6$ Hz, ara_2), 5.08 (1H, d, $J = 5.1$ Hz, ara_1), 5.37 (1H, d, $J = 7.3$ Hz, glc_2); ^{13}C-NMR data, see Tables 1 and 2; FABMS *m/z* $[M - H]^-$ 1059, $[M - H - ara]^-$ 927, $[M - H - glc]^-$ 897.

Compound 5: colorless needles; mp 218−220 °C; $[\alpha]^{25}_D$ −10.0° (*c* 0.8, MeOH); FT-IR (dry film) ν_{max} 3410 (OH), 1690 (C═O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N) δ 0.88 (3H, s, H_3-25), 1.02 (3H, s, H_3-24), 1.04 (3H, s, H_3-26), 1.10 (3H, s, H_3-29), 1.17 (3H, s, H_3-30), 1.28 (3H, s, H_3-23), 1.86 (3H, s, H_3-27), 3.42 (1H, dd, $J = 12.0$, 4.0 Hz, H-3), 3.60 (1H, dd, $J = 13.0$, 4.5 Hz, H-18), 5.25 (1H, m, H-16), 5.60 (1H, m, H-12), anomeric H 4.90 (1H, d, $J = 7.3$ Hz, glc_1), 4.98 (1H, d, $J = 6.6$ Hz, xyl), 5.13 (1H, d, $J = 5.1$ Hz, ara), 5.39 (1H, d, $J = 7.3$ Hz, glc_2); ^{13}C-NMR data, see Orsini et al.;[4] FABMS *m/z* $[M - H]^-$ 1059.

Acid Hydrolysis of Pitheduloside H (1): a solution of **1** (30 mg) in 5% H_2SO_4−dioxane (1:1) was heated at 100 °C for 6 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was subjected to Si gel column chromatography with CH_2Cl_2−MeOH (30:1) to give acacic acid lactone (**6**, 5 mg).

Acacic acid lactone (6): colorless needles; mp 255−257 °C; $[\alpha]^{25}_D$ +1.7° (*c* 0.5, $CHCl_3$); ^{1}H-NMR (400 MHz, C_5D_5N) δ 0.84, 0.89, 0.96, 1.04, 1.08, 1.23, 1.35 (each 3H, s, Me), 3.44 (1H, t, $J = 8.0$ Hz, H-3), 4.26 (1H, dd, $J = 5.5$ Hz, H-21), 4.54 (1H, dd, $J = 12.4$, 5.0 Hz, H-16), 5.39 (1H, m, H-12); ^{13}C-NMR (400 MHz, C_5D_5N) δ 15.8 (C-25), 16.3 (C-26), 16.5 (C-24), 18.8 (C-6), 23.8 (C-11), 24.3 (C-30), 27.2 (C-22), 28.1 (C-2), 28.6 (C-23), 28.7 (C-29), 28.8 (C-27), 32.6 (C-7), 34.2 (C-20), 37.4 (C-10), 38.2 (C-15), 38.9 (C-1), 39.4 (C-4), 40.4 (C-8), 41.8 (C-18), 43.0 (C-19), 43.4 (C-14), 47.4 (C-9), 50.0 (C-17), 56.0 (C-5), 66.7 (C-16), 78.1 (C-3), 83.5 (C-21), 124.6 (C-12), 140.3 (C-13), 181.3 (C-28); FABMS *m/z* $[M - H]^-$ 469.

The aqueous layer was neutralized with Amberlite IRA-35 and evaporated *in vacuo* to dryness. The identification and the D or L configuration of sugar was determined by using RI detection (Waters 410) and chiral detection (Shodex OR-1) by HPLC (Shodex RSpak NH2P-50 4E, 95% CH_3CN, 1 mL/min, room temperature) by comparison with an authentic sugar (10 mmol each of L-ara, D-glc, L-rha, and D-xyl). The sugar portion gave the following peaks: L-(−)-rha 7.40 min; D-(+)-xyl 12.50 min; L-(+)-ara 14.00 min, and D-(+)-glc 29.10 min.

Alkaline Hydrolysis of Saponin Fraction. A solution of fraction A (500 mg) in 0.6 N NaOH (80 mL) in MeOH (20 mL) was heated at 30 °C for 4 days. The reaction mixture was adjusted to pH 1.0 with 10% H_2SO_4, and extracted with *n*-BuOH. The *n*-BuOH layer was subjected to HPLC on ODS (22−25% CH_3CN) to give a prosapogenin (**7**, 20 mg), a monoterpene xyloside (**8**, 35 mg), and a monoterpene (**9**, 40 mg).

Compound 7: colorless needles; mp 174−176 °C; $[\alpha]^{25}_D$ −22.3° (*c* 0.7, MeOH); FT-IR (dry film) ν_{max} 3380 (OH), 1760 (C═O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N) δ 0.79 (3H, s, H_3-26), 0.82 (3H, s, H_3-25), 0.92 (3H, s, H_3-29), 1.05 (3H, s, H_3-30), 1.12 (3H, s, H_3-24), 1.31 (3H, s, H_3-23), 1.36 (3H, s, H_3-27), 3.38 (1H, dd, $J = 11.8$, 4.4 Hz, H-3), 2.78 (1H, dd, $J = 12.3$, 6.5 Hz, H-18), 4.51 (1H, m, H-16), 5.32 (1H, m, H-12), anomeric H 4.89 (1H, d, J = 7.4 Hz, glc$_1$), 5.05 (1H, d, J = 6.6 Hz, ara$_2$), 5.10 (1H, d, J = 5.2 Hz, ara$_1$), 5.40 (1H, d, J = 7.7 Hz, glc$_2$); ^{13}C-NMR data, see Tables 1 and 2; FABMS m/z [M − H]$^-$ 1057.

Compound 8: colorless oil; $[\alpha]^{25}_D$ −14.2° (c 3.6, MeOH); ^{1}H-NMR (400 MHz, C_5D_5N) δ 1.54 (3H, s, H$_3$-10), 1.80 (2H, t, J = 7.8 Hz, H$_2$-5), 2.01 (3H, s, H$_3$-9), 2.47 (2H, q, J = 7.8, 7.3 Hz, H$_2$-4), 5.22 (1H, d, J = 10.8 Hz, H$_2$-8), 5.41 (1H, d, J = 17.8 Hz, H$_2$-8), 6.22 (1H, dd, J = 17.8, 10.8 Hz, H-7), 7.14 (1H, t, J = 7.3 Hz, H-3), 4.86 (1H, d, J = 7.3 Hz, H-1 of xyl); ^{13}C-NMR data, see Table 1; FABMS m/z [M − H]$^-$ 315.

Compound 9: colorless oil; $[\alpha]^{25}_D$ +11.7° (c 0.3, MeOH); ^{1}H-NMR (400 MHz, C_5D_5N) δ 1.46 (3H, s, H$_3$-10), 1.82 (2H, t, J = 7.8 Hz, H$_2$-5), 2.05 (3H, s, H$_3$-9), 2.50 (2H, q, J = 7.8, 7.3 Hz, H$_2$-4), 5.17 (1H, d, J = 10.8 Hz, H$_2$-8), 5.57 (1H, d, J = 17.8 Hz, H$_2$-8), 6.15 (1H, dd, J = 17.8, 10.8 Hz, H-7), 7.24 (1H, t, J = 7.3 Hz, H-3); ^{13}C-NMR data, see Table 1; FABMS m/z [M − H]$^-$ 183.

Alkaline Hydrolysis of Pithedulosides H−J (1−3). Compounds **1**−**3** (each 5 mg) was hydrolyzed same way as described for the crude saponin fraction to yield a prosapogenin (**7**), a monoterpene xyloside (**8**), and a monoterpene (**9**) from **1**, **7** and **8** from **2**, and **7** and **9** from **3**. TLC data: **7**, R_f 0.14 (CH_2Cl_2−MeOH−H_2O, 65:30:4); **8**, R_f 0.19; **9**, R_f 0.55 (CH_2Cl_2−MeOH−H_2O, 25:2:0.1).

Acid Hydrolysis of Pithedulosides I (2) and J (3). Acid hydrolysis of **2** and **3** (each 5 mg) was carried out in the same manner as described for **1** to yield acacic acid lactone (**6**) on TLC: **6**, R_f 0.23 (CH_2Cl_2−MeOH, 25:1). The aqueous layer was carried out in the same way as described for **1** to give L-ara, D-glc, L-rha, and D-xyl from **2**, and L-ara, D-glc, and L-rha from **3**.

Acid Hydrolysis of Pitheduloside K (4). Acid hydrolysis of **4** (40 mg) was carried out as described for **1** to yield echinocystic acid (**11**, 15 mg): colorless needles, mp 225−227 °C; $[\alpha]^{25}_D$ + 39.8° (c 3.6, EtOH); FT-IR (dry film) ν_{max} 3400 (OH), 1680 (C=O), 1080, 1050 (OH) cm^{-1}; EIMS m/z [M]$^+$ 472. The aqueous layer afforded L-ara and D-glc on HLPC analysis, as described for **1**.

Acknowledgment. We are grateful to Dr. T. Miyase, School of Pharmaceutical Sciences, University of Shizuoka, for mass spectral measurements.

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NP9703555