Pergamon

PII: S0031-9422(96)00725-X

Phytochemistry, Vol. 44, No. 7, pp. 1329–1334, 1997

0031-9422/97 $17.00+0.00

PITHEDULOSIDES A–G, OLEANANE GLYCOSIDES FROM *PITHECELLOBIUM DULCE*

S. K. NIGAM, MISRA GOPAL,* RAIS UDDIN,† KAZUKO YOSHIKAWA,*‡ MIWAKO KAWAMOTO‡ and SHIGENOBU ARIHARA‡§

Upgraded Department of Pharmacology and Therapeutics, K. G's Medical College, *National Botanical Research Institute, and †Institute of Unani Medicinal Plants, Lucknow, India; ‡Faculty of Pharmaceutical Sciences, Yamashiro-Cho, Tokushima Bunri University, Tokushima 770, Japan

(*Received in revised form* 23 *September* 1996)

Key Word Index—*Pithecellobium dulce*; Leguminosae; seeds; oleanane glycoside; echinocystic acid; pithedulosides A–G.

Abstract—Seven saponins named pithedulosides A–G were isolated from the seeds of *Pithecellobium dulce*. Their structures were established through spectral analyses as echinocystic acid 3-*O*-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside, echinocystic acid and oleanolic acid 3-*O*-α-L-arabinopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosides and 3-*O*-β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosides, oleanolic acid 3-*O*-α-L-arabinopyranosy-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside, and 3-O-β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside.

INTRODUCTION

Though indigenous to America, *Pithecellobium dulce* Benth is an evergreen tree widely distributed in the greater part of India and is also found in southeast Asia. *Pithecellobium dulce* is now commonly grown as a hedge plant throughout India and is locally called 'Jungal jalebi' [1]. A phytochemical survey of saponin components in this plant was limited to the isolation of oleanolic and echinocystic acids as sapogenins of the crude saponin, of echinocystic acid bisdesmoside, dulcin, and saponin P_E as an anti-inflammatory saponin [2, 3]. Chemical characterization of the other saponins has not been achieved. Our attention was drawn to the saponins occurring as a very complex mixture. We have reinvestigated the seeds and isolated seven new monodesmosides, named pithedulosides A–G (**1**–**7**). In the present work, we report the isolation and structural elucidation of seven saponins from the seeds of the title plant.

RESULTS AND DISCUSSION

By a combination of silica gel and ODS column chromatography, seven novel saponins, named pithedulosides A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**) and G (**7**), were isolated from the ethanolic extract of seeds of *P. dulce*. Analysis by ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, HMBC, TOCSY and ROESY led to determination of the structures of **1**–**7**, including the sequence of the sugar moieties and position of attachment of the sugar chains to the aglycone.

Compound **2** was obtained as needles and deduced to be $C_{46}H_{74}O_{16}$ from the observation of a quasimolecular ion at m/z 881 $[M-H]^-$ in the negative FAB mass spectrum and carbon number in the ^{13}C NMR spectrum. The IR absorption band at 1690 cm^{-1} and the signal at δ 180.3 in the ^{13}C NMR spectrum showed the presence of a COOH group. The molecular formula $C_{46}H_{74}O_{16}$ implied 10 degrees of unsaturation. Seven can be assigned to one carbonyl group (δ 180.3), one olefinic bond (δ 144.9 and 122.7) and five due to the pentacyclic triterpene ring system indicated by the positive Salkowsky reaction. The remaining three are due to hemiacetal linkages of the sugar parts. The acid hydrolysis of **2** afforded oleanolic acid (**8**), L-arabinose and D-glucose in a 2:1 ratio confirmed by specific rotation using chiral detection in HPLC. The ^{1}H and ^{13}C NMR spectra of **2** indicated the presence of two α-arabinopyranosyl units [H-1:δ 5.09 (*d*, J = 5.4 Hz), C-1:δ 102.6 (Ara-1); H-1:δ 5.03 (*d*, J = 6.6 Hz), C-1:δ 105.7 (Ara-2)], one β-glucopyranosyl unit [H-1:δ 4.89 (*d*, J = 8.1 Hz),

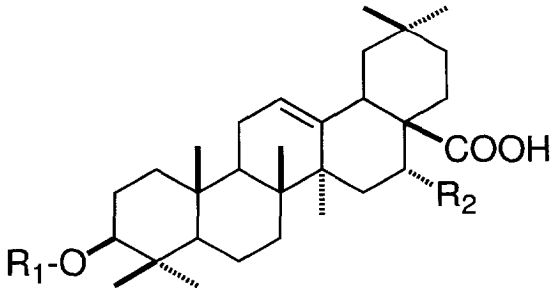
§Author to whom correspondence should be addressed.

C-1: δ 106.9]. A ^{13}C NMR spectral comparison of **2** with **8** revealed a glycosylation shift [4] of +10.7 ppm for the C-3 signal (δ 88.9), indicating that the sugar moieties were located at position C-3. The negative FAB mass spectrum of **2** showed fragment ion peaks at *m/z* 749 $[M-Ara-H]^-$, 617 $[M-2Ara-H]^-$, and 455 $[M-2Ara-Glc-H]^-$, disclosing the sugar sequence to be C-3-*O*-Ara-Ara-Glc. In the ^{13}C NMR spectrum of **2**, the C-6 signal of glucose and the C-2 signal of arabinose were shifted to δ 69.7 and 79.7 by the glycosylation shifts, respectively, establishing the site of glycosylation. Furthermore, in the HMBC spectrum of **2**, long-range correlations were observed between H-1 (δ 4.89) of glucose and C-3 (δ 88.9) of the aglycone, H-1 (δ 5.09) of arabinose (Ara-1) and C-6 (δ 69.7) of glucose, and H-1 (δ 5.03) of arabinose (Ara-2) and C-2 (δ 79.7) of arabinose (Ara-1). Hence, compound **2** was formulated as oleanolic acid 3-*O*-α-L-arabinopyranosyl (1 → 2)-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside.

Compound **3** gave an $[M-H]^-$ peak at *m/z* 881, suggesting **3** to have the same molecular formula ($C_{46}H_{76}O_{16}$) as **2**. Acid hydrolysis of **3** afforded D-xylose in addition to D-glucose and L-arabinose and **8**. A ^{13}C NMR spectral comparison of **3** with **2** showed that **3** was also a 3-*O*-glycoside of oleanolic acid and differed structurally from **2** only in its saccharide moieties: i.e. a terminal xylosyl group in **3** instead of a terminal arabinosyl group in **2**. The sugar linkages at C-3 were determined by means of an HMBC experiment in the same way as for **2**. The anomeric proton signals at δ 4.91 (glucose), 5.15 (arabinose) and 4.99 (xylose) showed long-range correlations with the ^{13}C signal at δ 88.9 (C-3), 69.6 (C-6 of glucose) and 80.6 (C-2 of arabinose), respectively, in the HMBC spectrum. Therefore, compound **3** was identified as oleanolic acid 3-*O*-α-L-arabinopyranosy-(1 → 2)-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside.

Compound **6** showed a quasimolecular ion peak at *m/z* 1043 $[M-H]^-$, 162 mass units more than that of **2**. Acid hydrolysis of **6** afforded D-glucose and L-arabinose in a 1:1 ratio, and **8**. The ^1H and ^{13}C NMR signals were similar to those of **3**, except only in those due to the sugar moiety linked with O-3. In an HMBC experiment, long-range correlations were observed between the C-3 signal (δ 88.9) of the aglycone and H-1 (δ 4.90) of glucose (Glc-1), the C-2 signal (δ 83.0) of glucose (Glc-1) and H-1 (δ 5.42) of glucose (Glc-2), the C-6 signal (δ 69.3) of glucose (Glc-1) and H-1 (δ 5.11) of arabinose (Ara-1), and the C-2 signal (δ 79.7) of arabinose (Ara-1) and H-1 (δ 5.07) of arabinose (Ara-2). Therefore, compound **6** was identified as oleanolic acid 3-*O*-α-L-arabinopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside.

Compound **7** had the same molecular formula $C_{52}H_{84}O_{21}$ (FAB mass spectrum, *m/z* 1043 $[M-H]^-$) as **6**. On acid hydrolysis, compound **7** gave D-glucose, L-arabinose and D-xylose in a 2:1:1 ratio, and **8**. A ^{13}C NMR spectral comparison of **7** with **6** showed

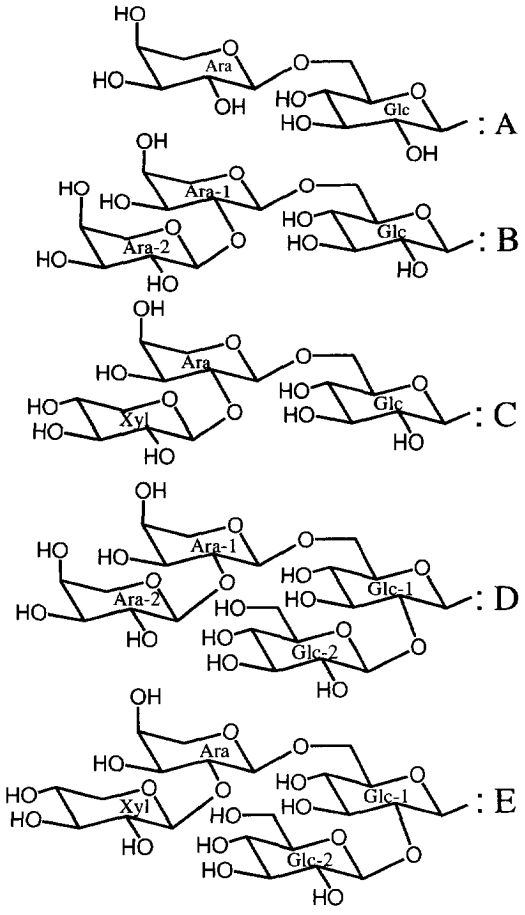


1 : R_1=A, R_2=OH
2 : R_1=B, R_2=H
3 : R_1=C, R_2=H
4 : R_1=B, R_2=OH
5 : R_1=C, R_2=OH
6 : R_1=D, R_2=H
7 : R_1=E, R_2=H
8 : R_1=H, R_2=H
9 : R_1=H, R_2=OH

that **6** was also a 3-*O*-glycoside of oleanolic acid and differed structurally from **6** only in its saccharide moiety: there was a terminal xylosyl group in **7** instead of the terminal arabinosyl group in **6**. The location of the xylosyl group in **7** must be linked to the C-2 position of arabinose (Ara-1) from the observation of a long-range correlation between the signal of H-1 (δ 4.98) of the xylose and the signal of C-2 (δ 80.8) of the arabinose. Therefore, compound **7** was formulated as oleanolic acid 3-*O*-β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside.

Compound **4** showed the ion peak at *m/z* 897 $[M-H]^-$, 16 mass units more than that of **2**. A ^{13}C NMR spectral comparison of **4** with **2** showed that **4** differed structurally from **2** only in its D ring, though the sugar unit was also affixed to the C-3 position. Acid hydrolysis of **4** afforded echinocystic acid (**9**)[5], D-glucose and L-arabinose. The presence of a hydroxyl group at C-16 was deduced from the downfield chemical shifts of C-15 (+7.8 ppm), C-16 (+40.9 ppm) and C-17 (+2.1 ppm) compared with those of **2**. The NOE between H-16 (δ 5.24) and Hα-22 (δ 2.42) indicated an α-configuration of the C-16-OH. The ^1H and ^{13}C NMR shifts of the sugar moiety at C-3 were in good agreement with those of **2**. The structure of **4** was therefore determined as echinocystic acid 3-*O*-α-L-arabinopyranosyl (1 → 2)-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside.

Compound **1** showed an ion peak at *m/z* 765 $[M-H]^-$, 132 mass units less than that of **4** and gave L-arabinose, D-glucose and **9** on acid hydrolysis. In the ^{13}C NMR spectrum of **1**, two glycosylated carbon signals were observed at δ 89.1 (C-3) and 70.0 (C-6 of Glc), indicating a vicinose (Ara $\xrightarrow{6}$ Glc) to be attached at C-3-OH. Therefore, compound **1** was deduced to



be echinocystic acid 3-*O*-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside.

Compound **5** showed an ion peak at *m/z* 897 $[M-H]^-$, 16 mass units more than that of **2** and gave L-arabinose, D-glucose, D-xylose and **9** on acid hydrolysis. The carbon signals due to the sugar moieties of **5** were superimposable on those of **3**. Thus, compound **5** was determined to be echinocystic acid 3-*O*-β-D-xylopyranosyl-(1 → 2)-α-L - arabinopyranosyl-(1 → 6)-β-D-glucopyranoside.

Pithedulosides A and E were identified as compounds **1** and **5**, respectively, obtained from the acid hydrolysate of saponin GS-C, isolated previously from *Gleditsia japonica* by Konoshima *et al.* [5, 6]. As far as we know, pithedulosides A and E are described here for the first time from a natural source.

EXPERIMENTAL

Mps (uncorr.) were measured with a Yanagimoto micromelting point apparatus. Optical rotations were recorded on a Jasco DIP-140 digital polarimeter, and NMR spectra were recorded on Jeol JNM-GX-400 and Varian UNITY 600 spectrometers in pyridine-d_5 soln using TMS as int. standard. NMR experiments included ^1H–^1H COSY, ^{13}C–^1H COSY, HMBC, TOCSY and ROESY. Coupling constants (*J* values) are given in Hz. The FAB-MS (Xe gun, 10 kV, triethylene glycol as matrix) was measured on a Jeol JMS-PX303 mass spectrometer. For CC, Kieselgel 60 (230–400 mesh, Merck), and for TLC, silica gel 60F-254 (Merck) were used. HPLC was carried out with a Waters ALC/GPC 244 instrument.

Plant material. The legume of *P. dulce* Benth was collected in August 1995, and the mesocarp was removed to obtain the seeds.

Extraction and isolation of compounds **1**–**7**. Powdered seed (5.0 kg) of *P. dulce* was percolated with EtOH and the percolate was freed of petrol and Et_2O soluble constituents. The residue was dissolved in the minimum amount of EtOH and saponin pptd by addition of a large excess of Et_2O. The Et_2O was decanted and the saponin filtered over silica gel to yield straw coloured powder (150 g). Half of it (75 g) was subjected to CC on silica gel, eluting with CH_2Cl_2–MeOH–H_2O (25:6:0.1, 25:8:0.1, 25:10:0.1, 13:7:2, 6:4:1) and then with MeOH to give 6 frs (1–6) in order of elution. Fr. 2 (4.0 g) was subjected to CC on silica gel, eluting with CH_2Cl_2–MeOH–EtOAc–H_2O (2:2:4:1, lower phase) to give 3 frs (2-1–2-3). Fr. 2-2 was further purified by HPLC (YMC ODS, 33% CH_3CN) to furnish **1**, (45 mg). Fr. 3 (6.0 g) was subjected to CC on silica gel, eluting with CH_2Cl_2–MeOH–H_2O (25:8:0.1) to give 4 frs (3-1–3-4), and fr. 3-3 was purified by HPLC (YMC ODS, 34% CH_3CN) to furnish **2** (45 mg) and **3** (50 mg). Fr. 4 (9.5 g) was sepd by CC over silica gel, eluting first with CH_2Cl_2–MeOH–H_2O (25:8:0.1) and then with CH_2Cl_2–MeOH–EtOAc–H_2O (2:2:4:1, lower phase) to give 5 frs (frs 4 and 5-1–5-5). Frs 4, 5-3 and 5-4 were further purified by repeated HPLC (YMC ODS, 33–35% CH_3CN) to yield **4** (200 mg) and **5** (80 mg) from frs 4 and 5-3, and **6** (100 mg) and **7** (80 mg) from frs 4 and 5-4, respectively.

Pithedulosside A (**1**). Needles, mp 216–218°; $[\alpha]_D$ −8.2° (MeOH; *c* 4.6). IR ν_{max}^{film} cm^{-1}: 3450, 1690, 1090, 1045. FAB-MS *m/z*: 765 $[M-H]^-$, 633 $[M-Ara-H]^-$. (Found: C, 62.60; H, 8.92, $C_{41}H_{66}O_{13}.H_2O$ requires: C, 62.73, H, 8.73.) ^1H NMR (400 MHz): δ 0.86 (3H, *s*, H-25), 0.98 (3H, *s*, H-24), 1.02 (3H, *s*, H-26), 1.08 (3H, *s*, H-29), 1.19 (3H, *s*, H-30), 1.25 (3H, *s*, H-23), 1.83 (3H, *s*, H-27), 3.31 (1H, *dd*, *J* = 11.7, 4.4 Hz, H-3), 3.62 (1H, *dd*, *J* = 12.4, 5.0 Hz, H-18), 5.25 (1H, *m*, H-16), 5.61 (1H, *m*, H-12), 4.86 (1H, *d*, *J* = 7.3 Hz, H-1 of Glc), 4.94 (1H, *d*, *J* = 6.6 Hz, H-1 of Ara). ^{13}C NMR: Tables 1 and 2.

Pithedulosside B (**2**). Needles, mp 220–222°; $[\alpha]_D$ +10.5° (pyridine; *c* 5.7). IR ν_{max}^{film} cm^{-1}: 3450, 1690, 1080, 1040. Negative FAB-MS *m/z*: 881 $[M-H]^-$, 749 $[M-Ara-H]^-$. Positive FAB-MS *m/z*: 921 $[M+K]^+$. (Found: C, 62.60; H, 8.92, $C_{46}H_{74}O_{16}.6H_2O$ requires: C, 56.97, H, 8.74.) ^1H NMR (400 MHz): δ 0.84 (3H, *s*, H-25), 0.95 (3H, *s*, H-24), 0.99 (6H, *s*, H-26, H-29), 1.01 (3H, *s*, H-30), 1.32 (3H, *s*, H-27), 1.34 (3H, *s*, H-23), 3.27 (1H, *dd*, *J* = 13.0, 4.5 Hz, H-18), 3.48 (1H, *dd*, *J* = 11.7, 4.4 Hz, H-3), 5.44 (1H, *m*, H-12), 4.89 (1H, *d*, *J* = 8.1 Hz, H-1 of Glc), 5.03 (1H, *d*,

Table 1. ^{13}C NMR data for aglycone moieties of compounds **1**–**7** (in pyridine-d_5, δ values)

C	1, 4, 5	2, 3, 6, 7
1	38.8	38.9
2	26.8	26.9
3	89.1	88.9
4	39.6	39.7
5	55.9	55.8
6	18.6	18.7
7	33.5	33.3
8	40.0	39.7
9	47.4	48.1
10	37.1	37.2
11	23.9	23.9
12	122.5	122.7
13	145.1	144.9
14	42.2	42.2
15	36.2	28.4
16	74.8	23.9
17	48.9	46.8
18	41.5	42.1
19	47.2	46.5
20	31.1	31.1
21	36.3	34.4
22	33.0	33.3
23	28.3	28.4
24	17.1	17.2
25	15.7	15.6
26	17.5	17.5
27	27.3	26.4
28	180.1	180.3
29	33.5	33.4
30	24.8	23.9

J = 6.6 Hz, H-1 of Ara-2), 5.09 (1H, *d*, J = 5.4 Hz, H-1 of Ara-1). ^{13}C NMR: Tables 1 and 2.

Pitheduloside C (**3**). Needles, mp 182–184°; $[\alpha]_D$ +3.6° (pyridine; *c* 3.7). IR ν_{max}^{film} cm^{-1}: 3450, 1690, 1050, 1030. Negative FAB-MS *m/z*: 881 $[M-H]^-$, 749 $[M-Ara-H]^-$. Positive FAB-MS *m/z*: 921 $[M+K]^+$. (Found: C, 56.47; H, 8.36, $C_{46}H_{74}O_{16}.5H_2O$ requires: C, 56.77, H, 8.70.) ^1H NMR (400 MHz, pyridine-d_5): δ 0.84 (3H, *s*, H-25), 0.98 (3H, *s*, H-24), 0.99 (6H, *s*, H-26, H-29), 1.00 (3H, *s*, H-30), 1.33 (6H, *s*, H-23, H-27), 3.27 (1H, *dd*, J = 13.0, 4.5 Hz, H-18), 3.53 (1H, *dd*, J = 11.7, 4.4 Hz, H-3), 5.43 (1H, *m*, H-12), 4.91 (1H, *d*, J = 7.3 Hz, H-1 of Glc), 4.99 (1H, *d*, J = 6.6 Hz, H-1 of Xyl), 5.15 (1H, *d*, J = 5.1 Hz, H-1 of Ara). ^{13}C NMR: Tables 1 and 2.

Pitheduloside D (**4**). Needles, mp 186–188°, $[\alpha]_D$ -9.8° (pyridine; *c* 6.3). IR ν_{max}^{film} cm^{-1}: 3400, 1690, 1075, 1025. FAB-MS *m/z*: 897 $[M-H]^-$, 765 $[M-Ara-H]^-$, 633 $[M-2Ara-H]^-$, 471 $[M-Glc-2Ara-H]^-$. (Found: C, 56.79; H, 8.51, $C_{46}H_{74}O_{16}.5H_2O$ requires: C, 55.86, H, 8.56.) ^1H NMR (600 MHz): δ 0.87 (3H, *s*, H-25), 0.99 (3H, *s*, H-24), 1.02 (3H, *s*, H-26), 1.05 (3H, *s*, H-29), 1.17 (3H, *s*, H-30), 1.33 (3H, *s*, H-23), 1.75 (1H, *br d*, J = 12.6 Hz, H_α-15), 1.87 (3H, *s*, H-27), 2.26 (1H, *dt*, J = 13.4, 4.3 Hz, H_β-22), 2.37 (1H, *br d*, J = 12.6 Hz, H_β-15), 2.42 (1H, *br d*, J = 13.4 Hz, H_α-22), 2.51 (1H, *dt*, J = 12.6, 4.3 Hz, H_α-21), 2.81 (1H, *t*, J = 13.4 Hz, H_α-19), 3.48 (1H, *dd*, J = 11.2, 4.0 Hz, H-3), 3.61 (1H, *dd*, J = 13.4, 5.0 Hz, H-18), 5.24 (1H, *m*, H-16), 5.61 (1H, *m*, H-12), 4.91 (1H, *d*, J = 7.8 Hz, H-1 of Glc), 5.06 (1H, *d*, J = 6.8 Hz, H-1″ of Ara), 5.10 (1H, *d*, J = 5.1 Hz, H-1 of Ara). ^{13}C NMR: Tables 1 and 2.

Pitheduloside E (**5**). Needles, mp 227–229°, $[\alpha]_D$ -8.6° (MeOH; *c* 4.5). IR ν_{max}^{film} cm^{-1}: 3450, 1690, 1040, 1030. FAB-MS *m/z*: 897 $[M-H]^-$. (Found: C, 58.95; H, 8.36, $C_{46}H_{74}O_{17}.2H_2O$ requires: C, 59.08, H, 8.41.) ^1H NMR (400 MHz): δ 0.88 (3H, *s*, H-25), 0.99 (3H, *s*, H-24), 1.02 (3H, *s*, H-26), 1.05 (3H, *s*, H-29), 1.18 (3H, *s*, H-30), 1.32 (3H, *s*, H_3-23), 1.89 (3H, *s*, H-27), 3.60 (1H, *dd*, J = 11.7, 4.4 Hz, H-18), 3.52 (1H, *dd*, J = 12.4, 5.0 Hz, H-3), 5.25 (1H, *m*, H-16), 5.60 (1H, *m*, H-12), 4.91 (1H, *d*, J = 7.3 Hz, H-1 of Glc), 4.99 (1H, *d*, J = 6.5 Hz, H-1 of Xyl), 5.15 (1H, *d*, J = 5.2 Hz, H-1 of Ara). ^{13}C NMR: Tables 1 and 2.

Pitheduloside F (**6**). Needles, mp 226–228°; $[\alpha]_D$ +6.1° (pyridine; *c* 3.6). IR ν_{max}^{film} cm^{-1}: 3400, 1670, 1080, 1050. Negative FAB-MS *m/z*: 1043 $[M-H]^-$, 911 $[M-Ara-H]^-$, 881 $[M-Glc-H]^-$, 779 $[M-2Ara-H]^-$. (Found: C, 56.09; H, 8.22, $C_{52}H_{84}O_{21}.7H_2O$ requires: C, 53.32, H, 8.43.) ^1H NMR (400 MHz, pyridine-d_5): δ 0.84 (3H, *s*, H-25), 0.94 (3H, *s*, H-24), 0.98 (3H, *s*, H-26), 1.00 (3H, *s*, H-29), 1.11 (3H, *s*, H-30), 1.31 (6H, *s*, H-23, H-27), 3.28 (1H, *dd*, J = 13.0, 4.5 Hz, H-18), 3.40 (1H, *dd*, J = 11.7, 4.4 Hz, H-3), 5.44 (1H, *m*, H-12), 4.90 (1H, *d*, J = 7.3 Hz, H-1 of Glc-1), 5.07 (1H, *d*, J = 7.1 Hz, H-1 of Ara-2), 5.11 (1H, *d*, J = 5.1 Hz, H-1 of Ara-1), 5.42 (1H, *d*, J = 7.6 Hz, H-1 of Glc-2). ^{13}C NMR: Tables 1 and 2.

Pitheduloside G (**7**). Needles, mp 193–195°, $[\alpha]_D$ +7.5° (pyridine; *c* 1.4). IR ν_{max}^{film} cm^{-1}: 3400, 1670, 1080, 1050. Negative FAB-MS *m/z*: 1044 $[M-H]^-$. (Found: C, 49.95; H, 8.36, $C_{52}H_{84}O_{21}.5H_2O$ requires: C, 55.01, H, 8.35.) ^1H NMR (600 MHz, pyridine-d_5): δ 0.84 (3H, *s*, H-25), 0.93 (3H, *s*, H-24), 0.98 (3H, *s*, H-26), 0.99 (3H, *s*, H-29), 1.10 (3H, *s*, H-30), 1.28 (3H, *s*, H-27), 1.31 (3H, *s*, H_3-23), 3.28 (1H, *dd*, J = 13.0, 4.5 Hz, H-18), 3.45 (1H, *dd*, J = 11.7, 4.4 Hz, H-3), 5.42 (1H, *m*, H-12), 4.90 (1H, *d*, J = 7.3 Hz, H-1 of Glc-1), 4.98 (1H, *d*, J = 7.1 Hz, H-1 of Xyl), 5.14 (1H, *d*, J = 5.1 Hz, H-1 of Ara), 5.40 (1H, *d*, J = 7.6 Hz, H-1 of Glc-2). ^{13}C NMR: Tables 1 and 2.

Acid hydrolysis of pitheduloside B (**2**). A soln of **2** (30 mg) in 5% H_2SO_4–dioxane (1:1) was heated at 100° for 2 hr. The reaction mixt. was diluted with H_2O and extracted with $CHCl_3$. The organic layer was concd to dryness and yielded **8** (15 mg): needles, mp 310–312°, $[\alpha]_D$ +72.8° ($CHCl_3$; *c* 1.5). IR ν_{max}^{film} cm^{-1}: 3450, 1690, 1080, 1040. EI-MS *m/z*: 456 $[M]^+$. The aq. layer was neutralized with Amberlite IRA-45 and evapd *in vacuo* to dryness. The sugar was determined by using RI detection (Waters 410) and chiral detection (Shodex OR-1) in HPLC (Shodex RSpak DC-613, 75% CH_3CN, 1 ml min^{-1}, 70°) in comparison with authentic sugars (10 mM each of L-Ara and D-Glc). The sugar part gave two peaks showing positive

Table 2. ^{13}C NMR data for sugar moieties of compounds **1–7** (in pyridine-d_5, δ values)

C	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
3-*O*-Glc-1			H-1		H-1		H-1		H-1		H-1		H-1	
	H-1													
1	107.0	4.86 *d* (7.3)	106.9	4.89 *d* (8.1)	106.8	4.91 *d* (7.3)	106.9	4.91 *d* (7.8)	106.9	4.91 *d* (7.3)	105.1	4.90 *d* (7.3)	105.1	4.90 *d* (7.3)
2	75.6		75.7		75.7		75.7		75.7		83.0		83.1	
3	78.6		78.4		78.4		78.3		78.4		78.0		78.1	
4	72.0		72.1		72.3		72.1		72.3		71.8		72.0	
5	76.7		76.1		76.3		76.0		76.2		75.8		76.0	
6	70.0		69.7		69.6		69.7		69.6		69.3		69.5	
Ara-1 (1 → 6)														
1	105.3	4.94 *d* (6.6)	102.6	5.09 *d* (5.4)	102.4	5.15 *d* (5.1)	102.5	5.10 *d* (5.1)	102.4	5.15 *d* (5.2)	102.5	5.11 *d* (5.1)	102.5	5.14 *d* (5.1)
2	72.3		79.7		80.6		79.7		80.5		79.7		80.8	
3	74.4		72.9		72.7		72.9		72.6		72.9		72.7	
4	69.1		67.8		67.3		67.8		67.3		67.7		67.4	
5	66.4		64.7		64.4		64.7		64.3		64.6		64.6	
Ara-2 or Xyl (1 → 2)														
1			105.7	5.03 *d* (6.6)	106.4	4.99 *d* (6.6)	105.7	5.06 *d* (6.8)	106.3	4.99 *d* (6.5)	105.7	5.07 *d* (7.1)	106.4	4.98 *d* (7.1)
2			73.0		75.5		72.9		75.4		72.9		75.6	
3			74.4		77.9		74.4		77.9		74.4		77.9	
4			69.0		70.9		69.1		70.9		69.0		70.9	
5			66.8		67.6		66.8		67.5		66.7		67.6	
Glc-2 (1 → 2)														
1											106.0	5.42 *d* (7.6)	106.0	5.40 *d* (7.6)
2											77.2		77.2	
3											78.0		78.1	
4											71.7		71.7	
5											78.4		78.4	
6											62.7		62.7	

optical rotation at 6.20 min (L-Ara, 6.18 min) and 7.38 min (D-Glc, 7.36 min).

Acid hydrolysis of pithedulosides D (**4**). Acid hydrolysis of **4** (40 mg) was carried out in the same way as described for **2** to yield **9** (15 mg): needles, mp 225–227°, $[\alpha]_D$ +39.8° (EtOH; *c* 1.5). IR ν_{max}^{film} cm^{-1}: 3400, 1680, 1080, 1050. EI-MS *m/z*: 472 $[M]^+$. The aq. layer was analysed in the same way as described for **2** to identify L-Ara and D-Glc.

Acid hydrolysis of pithedulosides C (**3**), *F* (**6**) *and G* (**7**). Acid hydrolysis of **3**, **6**, and **7** (each 5 mg) was performed in the same way as described for **2** and the product was analysed by TLC (CH_2Cl_2–MeOH, 20:1) and HPLC (C_8, 60% CH_3CN) with authentic oleanolic acid as a standard. The aq. layer was treated as described for **2**. **3**, and **7**: D-Xyl (5.75 min), L-Ara and D-Glc; **6**: L-Ara and D-Glc.

Acid hydrolysis of pithedulosides A (**1**) *and E* (**5**). Acid hydrolysis of **1** and **5** (each 5 mg) was carried out in the same way as described for **3**, **6** and **7** to give **9** and **1**: L-Ara and D-Glc; **5**: D-Xyl, L-Ara and D-Glc.

REFERENCES

1. Misra, G. and Nigam, S. K., *Quarterly Journal of Crude Drug Research*, 1975, **16**, 158.
2. Nigam, S. K. and Mitra, C. R., *Planta Medica*, 1970, **18**, 44.
3. Sahu, N. P. and Mahato, S. B., *Phytochemistry*, 1994, **37**, 1425.
4. Kasai, R., Okihara, M., Asakawa, J., Mizutani, K. and Tanaka, O., *Tetrahedron*, 1979, **35**, 1427.
5. Konoshima, T. and Sawada, T., *Chemical and Pharmaceutical Bulletin*, 1982, **30**, 2747.
6. Konoshima, T., Fukusha, H., Inui, H., Sato, K. and Sawada, T., *Phytochemistry*, 1981, **20**, 139.