Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 110, No. 4, August 1998, pp. 409–414.

Novel triterpenoidal saponin from the seeds of *Pithecellobium dulce*

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MS received 2 March 1998; revised 25 May 1998

Abstract. A novel triterpenoid saponin was isolated from the seeds of *Pithecellobium dulce*, which has been identified as 3-O[β-D-glucopyranosyl (1→2) -α-*L*-arabinopyranosyl] 28-O [α-*L*-rhamnopyranosyl(1→4) β-D-glucopyranosyl (1→4) β-D-glucopyranosyl] echinocystic acid by spectral analysis and chemical degradation methods.

Keywords. *Pithecellobium dulce benth.; mimosaceae;* triterpene saponin; bisdesmoside; 3-O[-β-D-glucopyranosyl (1→2) α-L-arabinopyranosyl] 28-O [α-L-rhamnopyranosy (1→4) β-D-glucopyranosyl (1→4) β-D-glucopyranosyl] echinocystic acid.

1. Introduction

Pithecellobium dulce benth. (mimosaceae) is indigeneous to India. It has been reported to possess significant medicinal value[1]. Earlier workers have reported triterpenoid saponin, flavon and several flavonoidal glycoside in it. A new triterpenoid saponin has been isolated from its seeds.

2. Results and discussion

The defatted seeds of *P. Dulce* were extracted with hot aqueous *n*-butanol. The extract was concentrated and the residue was subjected to various column chromatography examinations, with solvents of increasing polarity and finally the compound was found homogeneous on preparative TLC, compound I, analysed for $C_{59}H_{96}O_{27}$ M^+ 1236 m.p. 210–213°, which responded to all colour reactions of triterpenoid[2] and which also proved positive to the Molisch test, indicating I to be a triterpenoid glycoside.

Saponin I on acid hydrolysis furnished echinocystic acid II[3], L-rhamnose, L-arabinose and D-glucose (Co Pc), in the ratio of 1:1:3 (GLC).

The ion peak at m/z 1235 in FABMS of saponin I assigned for $[M-H]^-$. The important fragmentation peaks were obtained at 1103, $[M-H-p]^-$ 1072$[M-H-h]^-$, 940$[M-H-p-h]^-$ 464[M–H–3h]. In the ^{13}CNMR spectrum of I, five anomeric proton signals at δ 104·8 (Arab.) 105·5 (glucose$_1$), 96·2 (glucose$_2$) 104·6 (glucose$_3$) and 102·2 (rhamnose) were obtained. The signal at 96·2 assigned for the presence of an ester glycosidic linkage[4]. By comparing the ^{13}C NMR spectra of I and II, the downfield shift of + 11 ppm for C-3, indicated a 3-O glycosidic linkage, thereby confirming the bisdesmosidic nature of glycoside I. The oligosaccharide moiety (composed of 2 glucose

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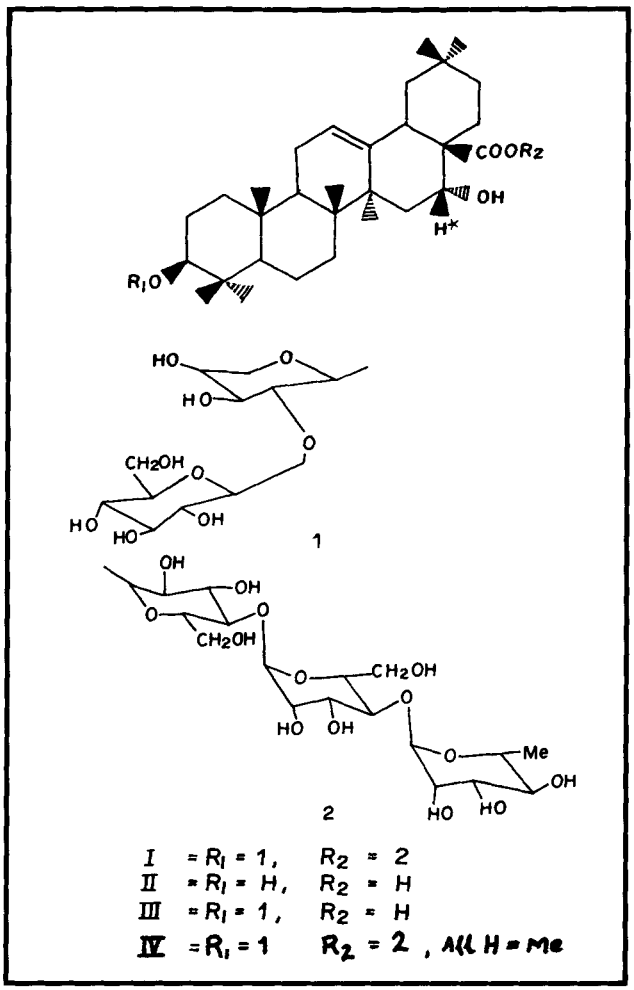

Figure 1.

and a rhamnose molecule) was bound by a β-ester glycosidic linkage to the C-28 carboxyl group of the aglycone. It was found β-glycosidic, through C-1 of one glucose to aglycone, again β-glycosidic, through C-4 of one glucose to C-1 of another glucose and α glycosidic through C-4 of second glucose and C-1 of rhamnose [5,6].

This was further confirmed by the methanolysis and reduction of the methylated product IV. IV is methylated product of I, in which all the hydroxyl groups are replaced by methoxy group (no hydroxyl absorption in the IR spectrum of IV). IV was obtained by the exhaustive methylation of saponin I by the Kuhn method. Methanolysis of IV gave, 16 mono-O-methyl echinocystic acid, methyl 2, 3, 4 trio-methyl rhamnopyranoside, methyl 3, 4 di-O-methyl arabinopyranoside, methyl 2, 3, 4, 6, tetra-O-methyl glucopyranoside and 2, 3, 6 tri-O-methyl glucopyranoside. IV on reduction with $LiAlH_4$ yielded sorbitol derivative and a product VI, which on methanolysis yielded 16 mono-O-methyl-olean-12-ene-3, 28, diol and methyl 2, 3, 4 tri-O-methyl arabinopyranoside and methyl 2, 3, 4, 6-tetra-O-methyl glucopyranoside.

Saponin I on treatment with methanolic KOH, yielded monodesmoside III, analysed for $C_{41}H_{66}O_{13}M^+$ 766[7]. The negative FABMS of III indicated the $[M-H]^-$ peak at 765. Another two important peaks were obtained at m/z 603 $[M-H-h]^-$ and 471 $[M-H-p-h]^-$, indicating that in III the D-glucose was linked to L-arabinose, which was directly attached to C-3 hydroxyl of echinocystic II. This was further confirmed by per-methylation of III by the Hokomories method[8], followed by acid hydrolysis and by identifying the partially methylated sugars and the methylated product IIIa. In ^1H NMR of IIIa the coupling constant suggested that the glycosidic linkage of L-arabinose was α and that of D glucose was β (see § 3). Thus, the prosapogenin III has been identified as echinocystic acid 3-O-β-D glucopyranosyl $(1\rightarrow2)$ α-L arabinopyranoside. The acid hydrolysis of III provided echinocystic acid II and D glucose and L rhamnose.

On the basis of the above deliberations the saponin I was identified as 3-O-[β-D-glucopyranosyl $(1\rightarrow2)$ α-L arabinopyranosyl] 28-O [α-L-rhamnopyranosyl $(1\rightarrow4)$ β-D glucopyranosyl $(1\rightarrow4)$ β-D glucopyranosyl echinocystic acid.

3. Experimental

3.1 *Plant materials*

Seeds of *P. dulce* were collected locally from the University campus in August 96. Voucher Specimen (No. XXXII S) has been deposited in the Department of Chemistry, Dr H S Gour University, Sagar).

3.2 *Extraction and isolation*

Air dried and powdered seeds of *P. dulce* (2 Kg) were extracted with hot petroleum ether. The extract was concentrated under reduced pressure and the residue was subjected to successive extraction with chloroform, acetone and methanol. The methanol extract was concentrated and extracted with aqueous *n*-butanol in a separating funnel. The *n*- butanol layer was separated and evaporated to give a crude compound (25 g), which was subjected to silica-gel column chromatography. It was then subjected to preparative TLC when it was found to be homogeneous.

SAPONIN I - Amorphous, powder, analysed for $C_{59}H_{96}O_{27}$, m.p. 210–212° M^+ 1236, ν max 3300, 3500 cm^{-1} (–OH group), 1730 cm^{-1} (–COO– group), FABMS m/z 1235 $[M-H]^-$, 1103 $[M-H-p]^-$, 1072 $[M-H-h]^-$, 940 $[M-H-h-p]^-$, 464 [M–H–3h]. (*p* denotes pentose ring and *h* denotes hexose ring).

3.3 *Acid hydrolysis of saponin I*

65 mg of Saponin I refluxed with 10 ml of 2% H_2SO_4 for 6 h. After purification and recrystallization from methanol, echinocytic acid precipitated out $C_{30}H_{48}O_4$ M^+ 472, m.p. 226–228°, ^{13}C NMR (see table 1). Sugar moieties (in the hydrolysate) were identified as D-glucose, L-arabinose and L-rhamnose by Co Pc and GLC.

3.4 *Alkaline hydrolysis of saponin I*

Compound I (250 mg) was refluxed with 5% methanolic KOHC (15 ml) for 5 h. After evaporation the residue was obtained by adding butanol. It was filtered and washed and identified as prosapogenin III, analysed for $C_{41}H_{66}O_{13}$, m.p. 240–242°, M^+ 766 EIMS, negative FABMS m/z 765 $[M-H]^-$, 603 $[M-H-h]^-$ and 471 $[M-H-p-h]^-$ ^{13}C NMR (see tables 1 and 2).

Table 1. ^{13}C NMR (C_5D_5N) data for I, II and III.

Position	I	II*	III*
1.	40·2	39·2	39·8
2.	28·6	28·2	28·7
3.	89·2	78·0	89·3
4.	40·5	40·1	40·5
5.	56·2	56·1	56·3
6.	18·5	18·0	17·9
7.	33·6	32·8	33·1
8.	39·8	39·4	39·0
9.	50·2	47·2	47·5
10.	37·5	35·6	37·2
11.	24·8	24·2	24·8
12.	122·2	122·5	122·8
13.	145·1	144·6	145·4
14.	40·8	42·5	41·6
15.	36·5	36·1	36·4
16.	76·6	74·2	75·0
17.	49·2	49·7	48·8
18.	40·5	41·8	41·5
19.	47·8	47·3	47·5
20.	31·2	30·9	31·2
21.	35·6	36·0	35·0
22.	32·5	32·4	32·2
23.	28·2	28·6	28·8
24.	16·4	16·5	16·2
25.	15·5	15·8	15·6
26.	17·5	17·2	17·0
27.	28·8	28·2	28·4
28.	176·6	179·5	175·5
29.	33·4	33·2	33·6
30.	24·4	24·2	24·3

*Signals were assigned by comparison with reported data [5,9].

Table 2. ^{13}C NMR data of sugar moieties of I and III in (C_5D_5N).

C-3 Sugar	I	III	C-28 Sugar	I
Arabinose	104·8	103·8	Rhamnose	102·2
$Glucose_1$	105·5	105·1	$Glucose_2$	96·2
			$Glucose_3$	104·6

3.5 *Exhaustive methylation of saponin I and methanolysis of methylated product*

Kuhn method - Saponin I (5mg) dissolved in 3 ml DMF was methylated with 1 ml MeI mixed with 30 mg Ag_2O at room temperature for 3 days. The filtrate of the above reaction mixture was again mixed with MeI (2 ml) and Ag_2O (70 mg) and methylated in the same manner as above. A filtrate of the second reaction mixture when mixed with water gave a precipitate, which was dissolved by adding KCN (solid). The solution was

extracted with chloroform. After removal of the solvent, the methylated product IV was obtained (2 mg). 1 mg of IV was subjected to acid hydrolysis with 10% HCl (dry) in 1 ml MeOH for 6 h. The reaction mixture was treated with Ag_2CO_3, filtered and evaporated, the residue obtained was 16 mono-O-methyl echinocystic acid, identified by comparison of it's methyl ester with a known sample [10] and a sugar part, composed of four types of methylated sugars. Methyl 2, 3, 4 tri-O-methyl rhamnopyranoside, methyl 2, 3, 4, 6 tetra-O-methyl glucopyranoside, methyl 2, 3, 6 tri-O-methyl glucopyranoside and methyl 3, 4 di-O methyl arabino pyranoside (Co TLC and Co GLC). The various parameters for GLC are the following:

Linear temperature programming	:	55°C–230°C at a rate of 2·5°C per min.
Detector	:	FID
Carrier gas	:	N_2
Pressure in carrier gas	:	1·20 ml/cm^{-2}
Injection temperature	:	170°C
Detector temperature	:	230°C

3.6 *Reduction of IV with $LiAlH_4$*

1 mg of IV in dry THF (0.5 ml) containing a suspension of $LiAlH_4$ (1 mg) refluxed for $2\frac{1}{2}$ h. After cooling, the reaction mixture was treated with water and then acidified with HCl to dissolve the ppt. The solution was concentrated and extracted with $CHCl_3$. After removal of the solvent sorbitol derivative (Co TLC) product V was obtained. Acid hydrolysis of V furnished methyl 2, 3, 4 tri-O-methyl arabinopyranoside, methyl 2, 3, 4, 6 tetra-O-methyl glucopyranoside (CoTLC and CoGLC) and 16-mono-O- methyl olean-12-ene-3, 28, diol, MS m/z (rel. int.): 472 (M^+, 24), 457 (M^+–Me8), 454 (M^+–H_2O, 4), 264 (88), 233 (80), 207 (24), 201 (100), identified by comparision (Co TLC and MS) with an authentic sample prepared from methyl 16-mono-O methyl echinocystate [10].

3.7 *Permethylation of III*

Compound III (50 mg) was methylated by Hakamori's method [8] and after purification by column chromatography methylated product IIIa was obtained ^{1}H NMR ($CDCl_3$); δ 4·20 (1H, d, J = 6 Hz, H-1 of Ara.), 4·38 (1H, d, J = 7 Hz, H-1 of glucose) on acid hydrolysis (methanolic HCl / refluxed for 4 h) it yielded the partially methylated sugars identified as 2, 3, 4, 6-tetra-O-methyl-D glucose and 3, 4 di-O-methyl-L-arabinose by GLC [11,12].

Acknowledgement

Thanks are due to the Head, Regional Sophisticated Instrumentation Centre (Central Drug Research Institute, Lucknow) for recording various spectra and Head, Department of Chemistry, Dr H S Gour University, for providing facilities.

References

1. Krishnamurthy A (ed.) *The Wealth of India. Raw Materials* 1969 8th edn (New Delhi: CSIR Publication) 140
   Kuhn R. Angew Chem. **67** 32
2. Mohato S B, Roy S K, Sahu N P and Sen S 1991 *Tetrahedron* **47** 5215

3. Arihara S, Nigam S K, Suzaki Y, Tanaka M and Yoshikawa K 1997 *J. Nat. Prod.* **60** 1268
4. Kundu A P, Mohato S B and Nandy A K 1992 *Tetrahedron* **48** 2483
5. Seo S, Shimaoka A, Tori K and Tomita Y 1974 *Tetrahedron Lett.* 4227
6. Sakamoto I, Tanakao O and Yamasaki K 1977 *Chem. Pharm. Bull.* **25** 3437
7. Mahoto S B and Sahu N P 1994 *Phytochemistry* **37** 1425
8. Hakomori S 1964 *J. Biochem. (Tokyo)*
9. Seo S, Tomita Y, Tori K and Yoshimura Y 1978 *J. Am. Chem. Soc.* **100** 3331
10. Akoi T and Suga T 1978 *Phytochemistry* **17** 771
11. Jensson P E, Kenne L, Liedgren H, Lindberg B and Lonngren J 1976 *Chem. Commun. Univ. Stockholm* **8** 23
12. Lonngren J and Pilotti A 1971 *Acta Chem. Scand.* **25** 1144