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ANTI-INFLAMMATORY TRITERPENE SAPONINS OF *PITHECELLOBIUM DULCE*: CHARACTERIZATION OF AN ECHINOCYSTIC ACID BISDESMOSIDE

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Key Word Index—*Pithecellobium dulce*; Leguminosae; triterpene glycosides; triterpene; dulcin; echinocystic acid.

Abstract—A new bisdesmodic triterpenoid saponin, dulcin was isolated from the seeds of *Pithecellobium dulce* and was identified as 3-*O*-[β-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl]-28-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl]-echinocystic acid. The known oleanolic acid saponin P_E, oleanolic acid 3-*O*-β-D-glucopyranosyl (1→2)-α-L-arabinopyranoside was also obtained. The structural features were elucidated by a combination of spectroscopic methods and some chemical transformations.

INTRODUCTION

Bhargava *et al.* [1] reported significant anti-inflammatory activity of the saponin fraction of *Pithecellobium dulce* which occurs widely throughout the greater part of India [2]. Chemical characterization of the saponin fraction, however, was yet to be established. During the study on chemical characterization of bioactive saponins from natural sources [3–11] our attention was drawn to the potential anti-inflammatory saponin of *P. dulce*. The present paper reports the isolation and characterization of a new echinocystic bisdesmoside along with identification of a known oleanolic acid glycoside P_E [12].

RESULTS AND DISCUSSION

The residue obtained on evaporation of the solvent of the methanolic extract of the defatted seeds of *P. dulce* was dissolved in water and extracted with *n*-butanol. The residue obtained after removal of the solvent afforded, on repeated chromatographic purification, two TLC homogeneous compounds which responded to positive colour tests for triterpenoid glycosides [11]. The less polar glycoside was found to be identical with saponin P_E (oleanolic acid 3-*O*-β-D-glucopyranosyl (1 → 2)-α-L-arabinopyranoside) isolated previously from *Akebia quinta* by comparison of its mp, $[\alpha]_D$ and the spectral data [12].

The more polar glycoside designated dulcin (**1**) on acid hydrolysis furnished an aglycone identified as echinocystic acid (**2**) from its physical and spectral data as well as by comparison with an authentic sample [13, 14]. The monosaccharides from the acid hydrolysate were identified as D-glucose, D-xylose and L-arabinose by PC as well as by GLC after preparation of their alditol acetates. The absolute configuration of L-arabinose was confirmed by its isolation and determination of its specific rotation. Dulcin (**1**) showed in its negative FAB mass spectrum an ion peak at *m/z* 1059 assigned to $[M-H]^-$. The other significant peaks were observed at *m/z* 927, 897, 765 and 471 ascribed to $[M-H-p]^-$, $[M-H-h]^-$, $[M-H-p-h]^-$ and $[M-H-2p-2h]^-$, respectively, where *p* and *h* denote pentose and hexose. The ^{13}C NMR spectrum displayed four anomeric carbon signals at δ 106.2, 105.1, 104.2 and 95.8. The signal at δ 95.8 suggested the presence of an ester glycosidic linkage [15] which was supported by the IR spectrum which showed an absorption at 1725 cm^{-1}. The downfield shift of +10.9 ppm for C-3 suggested a 3-*O*-glycoside linkage and thus the glycoside (**1**) was indicated to be a bisdesmoside. Treatment of **1** with methanolic KOH and subsequent purification of the product afforded the monodesmoside (**3**) which on acid hydrolysis furnished echinocystic acid (**2**) and D-glucose and L-arabinose. The negative FAB mass spectrum of **3** showed the $[M-H]^-$ peak at *m/z* 765 and other discernible peaks at *m/z* 603 and 471 assigned to $[M-H-h]^-$ and $[M-H-p-h]^-$. Thus it was evident that in compound **3** glucose was linked to arabinose which was directly attached to C-3 hydroxyl of echinocystic acid. The inter-sugar linkages of the glycone moiety of **3** were revealed by its ^{13}C NMR data upon comparison with those of methyl β-D-glucopyranoside and methyl α-L-arabinopyranoside and keeping in mind the glycosylation shift values [16, 17]. This was also confirmed by permethylation of **3** by Hakomori's method

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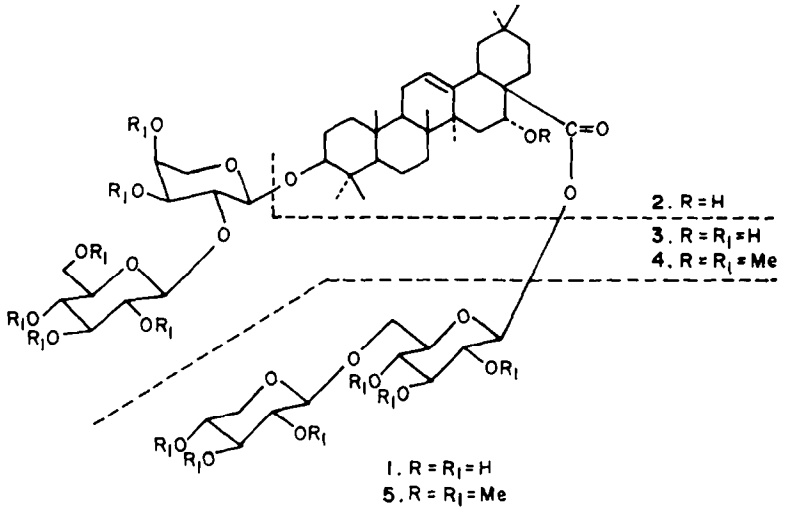

[18] followed by acid hydrolysis and identification of partially methylated sugars. The anomeric configurations were inferred from the *J* values of the respective anomeric protons in the ^{1}H NMR spectrum of the permethylate **4** (see Experimental section). Thus the structure of the prosapogenin was defined to be echinocystic acid-3-*O*-*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranoside (**3**).

The structure of dulcin (**1**) was then elucidated as follows. Permethylation of **1** by Hakomori's method yielded the permethylate (**5**) which on acid hydrolysis yielded partially methylated sugars compatible with the structure shown. The anomeric configurations of the sugar moieties were deduced from the *J* values of the respective anomeric protons of the permethylate (**5**). Thus the structure of dulcin was defined to be 3-*O*-[*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl]-28-*O*-*β*-D-xylopyranosyl (1→6)-*β*-D-glucopyranosyl]-echinocystic acid (**1**).

EXPERIMENTAL

Mps: uncorr. NMR spectra were recorded at 99.6 MHz for ^{1}H NMR and 25.1 MHz for ^{13}C NMR using TMS as int. standard. IR: KBr disc. GC analysis: 3% ECNSS-M (185 × 0.6 cm) at 190° for alditol acetates and 3% OV-225 (185 × 0.6 cm) at 195° for partially methylated alditol acetates. Negative FAB-MS were obtained on a Kratos MS-9/50 TC spectrometer in a glycerol–thioglycerol mixt. as matrix, EIMS: 70 eV.

Extraction and isolation. The air-dried powdered seed material of *P. dulce* (2.5 kg) was successively extracted with petrol (60–80°), $CHCl_3$ and MeOH. The MeOH extract was concd and partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was evapd to dryness *in vacuo* to give a residue (35 g) which was chromatographed on silica gel (750 g). Frs eluted with $CHCl_3$–MeOH (4:1) on repeated chromatographic purification yielded 2 TLC homogeneous compounds.

Saponin P_E (*oleanolic acid* 3-O-β-D-*glucopyranosyl* (1→2)-β-L-*arabinopyranoside*). The less polar fr. on crystallization from MeOH afforded needles of saponin P_E, mp 260–262° (dec.), $[\alpha]_D$ + 18.5° (MeOH: *c* 0.2). (lit. mp 263–266° (dec.) [12].

Dulcin (**1**). Powder, mp > 260° (dec.), $[\alpha]_D$ + 41.9° (pyridine; *c* 0.3); FAB-MS (negative) *m/z* (rel. int.): 1059 (100) $[M-H]^-$, 897 (18) $[M-H-glc]^-$, 927 (7) $[M-H-ara]^-$, 765 (6) $[M-H-glc-ara]^-$ and 471 (15) $[M-H-2glc-ara-xyl]^-$; ^{13}C NMR, see Table 1; IR ν_{max} cm^{-1} 3300–3500, 1725. (Found: C, 58.81; H, 7.81; $C_{52}H_{84}O_{22}$ requires: C, 58.85; H, 7.97%).

Hydrolysis of dulcin (**1**). Dulcin (**1**) was heated for 5 hr at 95° with 2 M HCl in aq. MeOH (30 ml). Usual work-up followed by CC purification and subsequent crystallization from MeOH furnished echinocystic acid (45 mg), mp 305–307°. The filtrate from the hydrolysate was worked-up [11] and analysed by PC. D-glucose, L-arabinose and D-xylose were identified using authentic samples. Conversion to respective alditol acetate [11] followed by GLC analysis confirmed the observation.

Alkaline hydrolysis of dulcin (**1**). Compound **1** (350 mg) was refluxed in aq. MeOH with 5% KOH (25 ml) for 4 hr. The mixt. was passed through Dowex 50W × 4 (4^+ form, MeOH), evapd, residue taken in *n*-BuOH, washed with H_2O, dried and crystallized from MeOH to give the prosapogenin **3** (150 mg), mp 238–40°; FAB-MS (negative) *m/z* (rel. int.) 765 (100) $[M-H]^-$, 603 (15.5) $[M-H-glc]^-$ and 471 (10) $[M-H-glc-ara]^-$. ^{13}C NMR, see Table 1. (Found: C, 64.28; H, 8.65; $C_{14}H_{66}O_{13}$ requires: C, 64.20; H, 8.67%).

Acid hydrolysis of prosapogenin (**3**). Compound **3** (30 mg) was hydrolysed as described above. The aglycone was identified as echinocystic acid and the monosaccharides were identified as D-glucose and L-arabinose in the ratio 1:1 by GLC of the alditol acetates.

Permethylation of **3**. Compound **3** (100 mg) was methylated by Hakomori's method [18] and the product was purified by CC to furnish the permethylate (**4**) as powder (60 mg); ^{1}H NMR ($CDCl_3$): δ 4.19 (1H, *d*, *J* = 6 Hz, H-1 of arabinose), 4.35 (1H, *d*, *J* = 7 Hz, H-1 of glucose). On acid hydrolysis (2 M HCl, aq. MeOH, reflux 4 hr) it yielded the

Table 1. Chemical shifts [δ_C ($\pm$0.1) of echinocystic acid (2), prosapogenin (3) and dulcin (1) in C_5D_5N

C	2	3	1	C	3	1
1	39.2	39.6	39.7	A-1	104.0*	104.2
2	27.8	28.1	28.2	A-2	80.2	80.5
3	78.0	88.9	88.6	A-3	72.5	73.0*
4	39.2	40.3	40.1	A-4	68.5	69.0
5	56.1	56.0	55.9	A-5	63.9	64.0
6	18.0	17.8	18.2	G-1	104.2*	105.1
7	33.3*	33.0*	33.1*	G-2	75.9	76.0†
8	39.4	39.0	39.2	G-3	78.0	78.2‡
9	47.0	47.4	47.4	G-4	69.8	71.0§
10	37.5	37.0	37.1	G-5	77.0	77.4‖
11	24.0	24.2	24.6	G-6	62.5	62.0
12	122.5	122.6	122.5	G'-1		95.8
13	144.6	145.0	144.9	G'-2		73.5*
14	41.5	41.2	41.1	G'-3		78.3‡
15	36.1†	36.3†	36.2†	G'-4		70.1§
16	74.2	75.0	76.1	G'-5		76.6†
17	49.7	48.5	48.7	G'-6		70.8
18	41.8	41.5	41.3	X-1		106.2
19	47.3	47.2	47.5	X-2		75.0
20	30.9	31.0	31.0	X-3		77.4‖
21	36.0†	35.5†	35.4†	X-4		70.0
22	32.4*	32.1*	32.0*	X-5		67.5
23	28.6	28.4	28.0			
24	16.5‡	16.0‡	16.1‡			
25	15.8‡	15.6‡	15.3‡			
26	17.2	17.0	17.4			
27	28.0	28.3	28.4			
28	179.6	175.0	176.2			
29	33.0	33.1	33.0			
30	24.5	24.3	24.2			

*§†‡‖ May be interchanged in each vertical column. A = arabinose, G = G' = glucose, X = xylose.

partially methylated sugars identified as 2,3,4,6-tetra-*O*-methyl-D-glucose (R_t 1.0) and 3,4-di-*O*-methyl-L-arabinose (R_t 1.35) by GLC of their alditol acetates [19, 20].

Periodate oxidation of dulcin (1). Dulcin (1) (25 mg) in MeOH was kept with a soln of sodium metaperiodate (10%, 5 ml) in the dark at room temp. for 2 days followed by work-up as usual and hydrolysis with Kiliani mixt. (4 ml) in a sealed tube. PC examination of the hydrolysate did not show the presence of any sugar.

Permethylation of dulcin (1). Dulcin (1) (50 mg) was permethylated as described earlier to yield the permethylate **5** (no hydroxyl absorption in the IR) as a powder (20 mg). ^{1}H NMR ($CDCl_3$): δ 4.06 (1H, *d*, J = 6 Hz, H-1 of arabinose), 4.30 (1H, *d*, J = 7 Hz, H-1 of glucose), 4.54 (1H, *d*, J = 6 Hz, H-1 of glucose) and 4.65 (1H, *d*, J = 6 Hz, H-1 of xylose). Hydrolysis of **5** with 2 M HCl in aq. MeOH (5 ml) at 95° for 3 hr yielded after usual work-up alditol acetates of partially methylated sugars of 2,3,4,6-tetra-*O*-methyl-D-glucose (R_t 1.00), 3,4-di-*O*-methyl-L-arabinose (R_t 1.32), 2,3,4-tri-*O*-methyl-D-glucose (R_t 2.20) and 2,3,4-tri-*O*-methyl-D-xylose (R_t 0.54) by comparison of the R_t values reported in lit. [19, 20].

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