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BACOPASAPONIN D—A PSEUDOJUJUBOGENIN GLYCOSIDE FROM *BACOPA MONNIERA*

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Key Word Index—*Bacopa monniera*; Scrophulariaceae; bacopasaponin D; pseudojujubogenin; triterpenoid saponin.

Abstract—A new dammarane-type pseudojujubogenin glycoside, bacopasaponin D, has been isolated from the reputed Indian medicinal plant *Bacopa monniera* and defined as 3-*O*-[α-L-arabinofuranosyl(I$\rightarrow$2)β-D-glucopyranosyl]pseudojujubogenin by spectroscopic methods and some chemical transformations. The ^{13}C signals of the saponin were assigned by DEPT, ^{1}H–^{1}H COSY and HSQC techniques. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Bacopa monniera Wettst (Scrophulariaceae) is reputed to be a nervine tonic, cardio tonic and diuretic in Indian traditional medicine [1]. The activities are believed to be associated with the polar fraction, which contains mainly saponins as a complex mixture. The isolation and structural elucidation of two new jujubogenin glycosides, bacoside A_1 [2] and bacoside A_3 [3], have recently been reported. In our previous communication [4] we reported the structural eludication of three new dammarane-type triterpenoid saponins, bacopasaponins A, B and C. This paper reports the isolation and structural elucidation of a new pseudojujubogenin glycoside from the plant.

RESULTS AND DISCUSSION

The *n*-butanol soluble fraction of a methanol extract of the leaves of *B. monniera* was fractionated by adsorption on silica gel followed by successive elution with chloroform, ethylacetate, acetone and 20% methanol in chloroform. The last three extracts were separately purified by a combination of silica gel column chromatography, preparative TLC on silica gel G followed by solvent treatment and crystallization. Thus, besides bacopasaponins A, B and C, a new saponin designated bacopasaponin D (**1**) was isolated, which gave a positive Liebermann–Burchard test for triterpenoids and Molisch test for sugars.

Acid hydrolysis of **1** furnished a major aglycone characterized as bacogenin A_1 (**2**), and the sugar components were identified as D-glucose and L-arabinose. Compound **2** is known to be an artefact derived from the genuine sapogenin, pseudojujubogenin, by acid catalysed rearrangement during hydrolysis [5], and jujubogenin yields the artefact ebelin lactone on similar acid treatment [6]. The mechanisms for the formations of bacogenin A_1 and ebelin lactone from pseudojujubogenin and jujubogenin, respectively, have been proposed [5, 6].

It may also be argued that in pseudojujubogenin an initial cyclization followed by cleavage of the 16-ketal group may lead to the formation of bacogenin A_1 (**2**). In jujubogenin the 20-hydroxyl group and the isobutenyl side chain are in a geometrically unfavourable disposition to cyclize to a tetrahydrofuran ring. However, dehydration and subsequent retro-Diels–Alder collapse of the dihydropyran ring yields ebelin lactone.

The positive-ion FAB mass spectrum of saponin **1** diaplayed significant peaks at *m/z* 789, 767, 635, 473, 455 and 437 attributable to $[M+Na]^+$, $[M+H]^+$, $[M+H-\text{arabinosyl}]^+$, $[M+H-\text{arabinosyl-glucosyl}]^+$ and $[M+H-\text{arabinosyl-glucosyl-}H_2O]^+$, respectively. The hydrolysis and FAB mass spectral results disclosed bacopasaponin D to be a pseudojujubogenin disaccharide, the sugar moiety containing a terminal arabinose linked to a glucose, which is attached to the aglycone. The attachment of the sugar moiety at C-3 of the aglycone, the pyranose form of the glucose unit and furanose form of the arabinose, as well as linkage of the arabinose to C-2 of the glucose, were determined by ^{13}C NMR chemical shifts values [7, 8]. The ^{13}C NMR assignments (Table 1) were made with the help of DEPT, ^{1}H–^{1}H COSY and HSQC experiments. The sequence of sugar units as well as their ring sizes were ascertained by permethylation followed by hydrolysis and identification of the resulting partially methylated sugars. Thus, 3, 4, 6-tri-*O*-methyl-D-glucose

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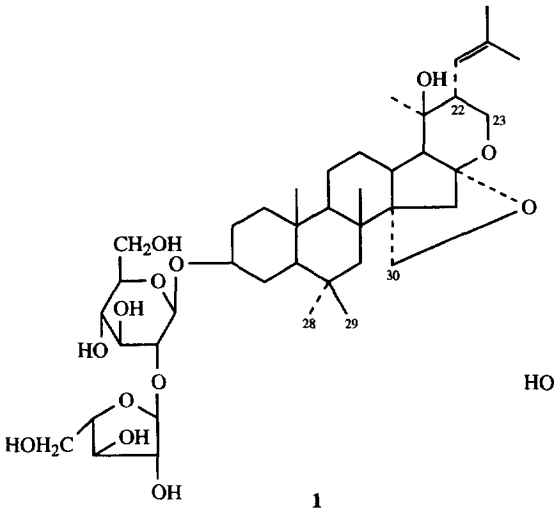

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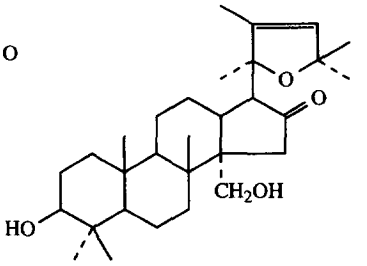

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and 2,3,5-tri-*O*-methyl-L-arabinose were identified by GC analysis of their alditol acetates [9]. Consequently, the structure of bacopasaponin D was elucidated as 3-*O*-[α-L-arabinofuranosyl (1→2) β-D-glucopyranosyl] pseudojujubogenin (**1**).

Table 1. ^{13}C NMR chemical shifts of bacopasaponin D (**1**) in pyridine-d_5*

C	1		1
		Glc(p)	
1	38.9	1	105.9
2	26.8	2	78.3
3	89.1	3	89.1
4	39.7	4	71.9
5	56.2	5	78.2
6	18.3	6	61.8
		Ara(f)	
7	36.1	1	109.7
8	37.6	2	80.9
9	53.0	3	78.8
10	37.2	4	88.5
11	21.8	5	62.9
12	28.7		
13	37.2		
14	53.6		
15	37.0		
16	110.4		
17	51.4		
18	18.9		
19	16.3		
20	71.9		
21	27.2		
22	46.3		
23	66.2		
24	124.2		
25	132.9		
26	26.1		
27	18.5		
28	28.1		
29	16.9		
30	63.0		

*Assignments aided by DEPT and HSQC experiments.

Glc(p) = glucopyranose; Ara(f) = arabinofuranose.

It is noteworthy that dammarane-type triterpenoid saponins are major constituents of a number of reputed herb drugs including ginseng. Although jujubogenin glucosides have been isolated from several reputed medicinal plants (e.g. Rhamnaceae and Scrophulariaceae) [10], it appears that pseudojujubogenin glycosides are so far reported only from this Indian herb drug *B. monniera*. We are pursuing our studies on the isolation of other saponin constituents of the plant to provide a basis for discussion of their biological activity in relation to their chemical structures.

EXPERIMENTAL

Plant material was collected from 24-Parganas, West Bengal, and identified in the Indian Botanic Garden, Howrah. A voucher specimen is deposited in the herbarium of the Institute. Mps: uncorr. IR: KBr discs; ^1H NMR: 399.65 MHz and ^{13}C NMR: 100.40 MHz in pyridine-d_5. FAB-MS (positive ion) were obtained on a VG-ZAB-SE mass spectrometry using glycerol–thioglycerol as matrix. Cs^+ was used as bombarding particle operating at 5 kV accelerating voltage with a 20 kV conversion dynode. TLC: silica gel G (BDH) plates using the solvent systems (A) $CHCl_3$–pyridine–H_2O (80:19:1) and (B) $CHCl_3$–EtOAc–MeOH–H_2O (75:10:14:1). The spots on the TLC plates were visualized by spraying L.B. reagent. PC: Whatman No. 1 with solvent system *n*-BuOH–pyridine–H_2O (6:4:3); a satd soln of aniline oxalate in H_2O was used as staining agent. GC: ECNSS-M, 3% on Gas-Chrom Q at 190° for alditol acetates and OV-225 on Gas-Chrom Q at 195° for partially methylated alditol acetates.

The air-dried powdered leaves (1.5 kg) of *B. monniera* collected in the neighbourhood of Calcutta were successively extracted in a percolator with petrol (60–80°), $CHCl_3$ and MeOH. The MeOH extract was concd and partitioned between H_2O and *n*-BuOH. The *n*-

BuOH layer was washed with H_2O, and distilled under red. pres. The residue (57 g) was dissolved in a minimum vol. of MeOH, adsorbed on silica gel, dried and eluted successively with $CHCl_3$, EtOAc, Me_2CO and $CHCl_3$–MeOH (4:1). The last 3 frs were separated purified by CC on silica gel using $CHCl_3$–MeOH (24:1) as mobile phase and prep. TLC (solvent system B) followed by crystallization. Thus, bacopasaponin D (35 mg) was obtained besides bacopasaponins A, B and C.

Bacopasaponin D (**1**). Crystallized from MeOH as micro-needles. mp 250°, $[\alpha]_D$ −42° (MeOH, *c* 0.14), IR ν_{max}^{KBr} cm^{-1}: 3300–3600 (hydroxyl), 1637, 1574, 1419, 1382, 1297, 1258, 1214, 1026. ^1H NMR (pyridine-d_5): δ 0.71 (3H, *s*, 19-Me), 1.01 (3H, *s*, 29-Me), 1.05 (3H, *s*, 18-Me), 1.27 (3H, *s*, 28-Me), 1.37 (3H, *s*, 21-Me), 1.61 (3H, *d*, J = 1.2 Hz, 27-CH_3), 1.69 (3H, *d*, J = 1.2 Hz, 26-Me), 2.59 (1H, *ddd*, J = 2.2, 2.2, 10.5 Hz, 22-H), 2.81 (1H, *dddd*, J = 1.4, 4.4, 5.4, 13.2 Hz, 13-H), 3.30 (1H, *dd*, J = 4.4, 11.7 Hz, 3-H), 3.85 (1H, *dd*, J = 2.2, 10.7 Hz, 23-αH), 4.68 (1H, *dd*, J = 2.2, 10.7 Hz, 23-βH). 4.95 (1H, *d*, J = 8 Hz, 1-H of glucose unit), 6.31 (1H, *d*, J = 1.2 Hz, 1-H of arabinose unit), 5.82 (1H, *dsept*, J = 10.7, 1.2 Hz, 24-H); FAB-MS (positive) *m/z* (rel. int.): 789(10), 767(100), 635(5), 473(5), 455(20), 437(6) (found: C, 64.23; H, 8.61%; $C_{41}H_{66}O_{13}$ requires C, 64.20; H, 8.67%).

Hydrolysis of **1**. Compound **1** (50 mg) was hydrolysed with 2 M HCl in Aq. MeOH (15 ml) on a water bath for 5 hr and worked up in the usual way. The purified major aglycone was identified as bacogenin A_1 (**2**), mp 241–242° (lit. [11] mp 242°). Its ^1H NMR and MS data were comparable to those of an authentic sample. The filtrate from the hydrolysate was neutralized with Ag_2CO_3, filtered, and a portion of the filtrate was concd under red. pres. and examined for carbohydrates by PC using authentic samples. Two spots were detected corresponding to L-arabinose and D-glucose. That the arabinose was the L-enantiomer was ascertained by its isolation by prep. PC and comparison of its specific rotation with that of L-arabinose. The other portion of the filtrate was reduced with $NaBH_4$, worked up as usual, the residue acetylated with Ac_2O–pyridine (1:1) and then subjected to GC analysis using the column mentioned above. Two peaks corresponding to glucitol and arabinitol acetates were detected using authentic samples.

Permethylation of **1** *and hydrolysis of the product.* Compound **1** (100 mg) was permethylated by the method of ref. [12] with NaH (150 mg) in DMSO (15 ml) and CH_3I (6 ml) under N_2 atmosphere in the usual way. Work up as usual yielded a gummy residue which was purified by CC over silica gel with petrol–EtOAc (2:3) as eluent to yield the permethylate as a powder. No OH band was observed in the IR spectrum. The permethylated product was hydrolysed by heating under reflux with 2 M HCl in aq. MeOH (10 ml) for 5 hr, worked up as usual and the filtrate neutralized with Ag_2CO_3 and filtered. The material isolated after usual work up was reduced with $NaBH_4$, acetylated with Ac_2O–pyridine (1:1) and then subjected to GC analysis on the column mentioned above. The peaks corresponding to 3,4,6-tri-*O*-methyl-D-glucitol triacetate and 2,3,5-tri-*O*-methyl-L-arabinitol diacetate were identified by comparison of the R_f values with those of authentic samples.

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