Bacopasides III—V: Three New Triterpenoid Glycosides from *Bacopa monniera*

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Received October 1, 2002; accepted November 18, 2002

Three new saponins, designated as bacopasides III, IV and V have been isolated from *Bacopa monniera* WETTST. and their structures have been elucidated as 3-*O*-α-L-arabinofuranosyl (1→2)-β-D-glucopyranosyl jujubogenin (1), 3-*O*-β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl jujubogenin (2) and 3-*O*-β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl pseudojujubogenin (3) mainly on the basis of two dimensional (2D) NMR and other spectral analyses.

Key words *Bacopa monniera*; Scrophulariaceae; saponin; jujubogenin; pseudojujubogenin; 2D NMR

In continuation of our studies[1,2] on the bioactive glycosidic fraction of *Bacopa monniera*, a herb having confirmed nervine tonic activity,[3—5] we undertook a reinvestigation of the *n*-butanol soluble fraction of the methanol extract of the plant. Earlier workers reported six saponins, *viz.* bacopasaponins A—F from the same fraction.[6—8] During the present investigation, we have extensively used HPLC for isolation of the minor constituents and finally have been able to isolate three new triterpenoid glycosides, designated as bacopaside III (**1**), bacopaside IV (**2**) and bacopaside V (**3**). We report herein the isolation and structure determination of these three saponins.

Results and Discussion

The *n*-butanol soluble fraction of the methanol extract of the plant was dissolved in minimum volume of methanol, adsorbed on silica gel and fractionated by successive extraction with hot solvents, such as chloroform, ethyl acetate, acetone and methanol–chloroform (1 : 4) in a Soxhlet apparatus. The acetone extract was subjected to column chromatography (CC) over silica gel and the combined saponin-containing fractions was then subjected to prep. HPLC. The minor peaks were collected to give the saponins **1**—**3**. All the compounds gave positive froth test for saponins and Libermann–Burchard test for triterpenes.

The sugar constituents present in the saponins were identified as glucose and arabinose by paper chromatography of their acid hydrolysates. Gas chromatography (GC) of the alditol acetates derived from the hydrolysates showed that glucose and arabinose were present in 1 : 1 ratio in all the saponins (**1**—**3**). The absolute configuration of the sugars were determined to be D and L respectively by the specific rotations of the sugars isolated by prep. paper chromatography of the acid hydrolysates of the saponins.

High-resolution positive ion FAB mass spectrum (FAB-MS) of all the three saponins (**1**—**3**) showed $[M+H]^+$ at *m*/*z* 767.4601 corresponding to the molecular formula $C_{41}H_{66}O_{13}$. ^{13}C-NMR spectrum (Table 1) of each of the saponins (**1**—**3**) displayed signals for 41 carbons, of which 30 were assigned to the aglycone moiety and the remaining 11 must be due to one glucose and one arabinose units. A comparison of the ^{13}C chemical shifts of the three saponins revealed that the compounds differ not only with respect to the linkages of the sugar units, but also with respect to their aglycone moiety. While the ^{13}C chemical shifts of the aglycone moiety of **1** and **2** were found to be very close to those of jujubogenin 3-*O*-α-L-arabinofuranosyl-(1→2)-[β-D-glucopyranosyl(1→3)]-α-L-arabinopyranoside (**4**),[9] those of **3** were found to be almost identical with ^{13}C chemical shifts of the aglycone part of pseudojujubogenin-3-*O*-glycosides, *viz.* bacopasides I (**5**).[1,2] It was, therefore, evident that bacopasides III and IV (**1**, **2**) are jujubogenin-3-*O*-glycosides and bacopaside V (**3**) must be pseudojujubogenin-3-*O*-glycoside.

Having thus identified the aglycone moiety of all the saponins **1**—**3**, attention was given to the sugar units and their linkages in the compounds. It has already been established that each of the saponins contain one unit each of D-

Table 1. ^{13}C-NMR Chemical Shifts[a)] of Bacopasides III (**1**), IV (**2**) and V (**3**) in Pyridine-d_5

Carbon	1	2	3	Carbon	1	2	3
1	38.7	38.7	38.7	24	127.2	127.2	124.2
2	26.8	26.8	26.8	25	134.1	134.1	132.9
3	89.0	88.5	88.6	26	25.6	25.6	26.1
4	39.6	39.8	39.8	27	18.3	18.4	18.5
5	56.1	56.1	56.2	28	28.4	28.0	28.0
6	18.3	18.4	18.3	29	16.9	16.8	16.8
7	36.0	36.1	36.1	30	65.8	65.8	65.9
8	37.5	37.6	37.6	3-*O*-Glc (**1**), 3-*O*-Ara(p) (**2**, **3**)			
9	53.0	53.0	53.01	1′	105.9	107.4	107.4
10	37.2	37.3	37.3	2′	78.2	72.0	71.9
11	21.8	21.8	21.8	3′	78.1	84.2	84.2
12	28.5	28.6	28.6	4′	71.8	69.3	69.3
13	37.1	37.1	37.1	5′	78.2	67.1	67.0
14	53.8	53.8	53.5	6′	62.9	—	—
15	36.9	36.9	36.9	Ara(f) (**1**), Glc (**2**, **3**)			
16	110.6	110.6	110.3	1″	109.6	106.5	106.4
17	54.0	54.0	51.3	2″	80.8	75.8	75.8
18	18.9	18.9	18.9	3″	78.7	78.4	78.4
19	16.3	16.3	16.4	4″	88.5	71.6	71.6
20	68.6	68.5	71.9	5″	62.8	78.7	78.7
21	30.1	30.1	27.2	6″	—	62.7	62.7
22	45.5	45.5	46.3				
23	68.5	68.6	66.1				

a) Chemical shifts were assigned on the basis of 2D NMR, *viz.* ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC spectral analyses. Glc=glucopyranose; Ara(p)=arabinopyranose; Ara(f)=arabinofuranose.

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glucose and L-arabinose. ^{13}C chemical shifts (Table 1) of the carbons of the sugar units as well as the ^{1}H chemical shifts and the splitting pattern (Table 2) of the anomeric proton signals of these units present in each of the compounds clearly revealed[1)] that the glucose unit exists in the pyranose form in all the three compounds, whereas the arabinose unit exists in

Table 2. ^{1}H-NMR Chemical Shifts[a)] of Bacopasides III (**1**), IV (**2**) and V (**3**) in Pyridine-d_5

Proton	**1**	**2**	**3**
H_3-18	1.05 (s)	1.08 (s)	1.08 (s)
H_3-19	0.68 (s)	0.74 (s)	0.75 (s)
H_3-21	1.39 (s)	1.38 (s)	1.39 (s)
H_3-26	1.69 (s)	1.70 (s)	1.69 (s)
H_3-27	1.67 (s)	1.67 (s)	1.62 (s)
H_3-28	1.29 (s)	1.30 (s)	1.30 (s)
H_3-29	1.03 (s)	0.98 (s)	0.98 (s)
H-3	3.29 (dd, *J*=11.4, 4.2)	3.33 (dd , *J*=11.6, 4.3)	3.34 (dd, *J*=11.5, 4.3)
H-13	2.82 (m)	2.83 (m)	2.86 (m)
H-23/H_2-23	5.20 (dd, *J*=9.3, 9.3)	5.20 (dd , *J*=9.4, 9.4)	3.89 (d, *J*= 10.2), 3.75[b)]
H-24	5.53 (d, *J*=7.8)	5.54 (d, *J*=7.6)	5.86 (d, *J*=10.2)
H-1′	4.97 (d, *J*=7.8)	4.76 (d, *J*=7.3)	4.77 (d, *J*=7.2)
H-2′	4.05 (dd, *J*=7.8, 8.0)	4.60 (dd, *J*=7.3, 7.8)	4.61 (dd, *J*=7.2, 8.0)
H-3′	4.27[b)]	4.24[b)]	4.25[b)]
H-4′	4.14 (dd, *J*=9.0, 9.0)	4.45 (br s)	4.46 (br s)
H-5′	3.96 (m)	3.75 (d, *J*=11.9), 4.24[b)]	3.75 (d, *J*=12.0), 4.24[b)]
H_2-6′	4.38 (dd, *J*=11.2, 5.1), 4.59 (d, *J*=11.2)	—	—
H-1″	6.38 (br s)	5.41 d (*J*=7.6)	5.41 (d, *J*=7.8)
H-2″	5.11 (br s)	4.05 dd (*J*=7.6, 8.0)	4.05 (dd, *J*=7.8, 8.0)
H-3″	4.90 (br s)	4.24[b)]	4.25[b)]
H-4″	4.99[b)]	4.26[b)]	4.24[b)]
H-5″	4.20,[b)] 4.30[b)]	4.01 (m)	4.01 (m)
H_2-6″	—	4.40 (dd, *J*=12.5, 4.5), 4.57 (d, *J*=12.5)	4.40 (dd, *J*=11.4, 4.6), 4.57 (d, *J*=11.4)

a) Chemical shifts were assigned on the basis of 2D NMR, *viz.* ^{1}H–^{1}H COSY, HMQC and HMBC spectral analyses. Figures in the parentheses are the coupling constants in Hz. *b*) Signals were overlapped by other signal(s) and therefore their multiplicities could not be determined.

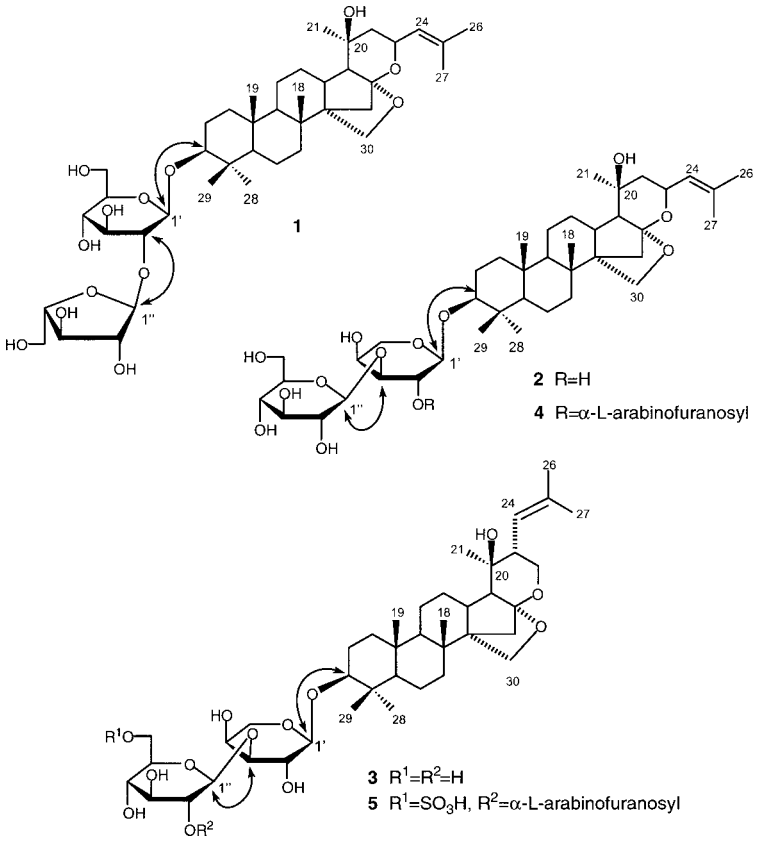

Fig. 1. Bacopasides from *B. monniera* and Their HMBC Correlations Depicted in Arrow

the furanose form in **1** and in the pyranose form in **2** and **3**. The linkages between the sugar units of the disaccharide moiety of saponin **1** was determined to be arabinofuranosyl-(1→2)-glucopyranose, and those of **2** and **3** were ascertained to be glucopyranosyl-(1→3)-arabinopyranose from the analyses of their heteronuclear multiple bond correlation (HMBC) spectra (Fig. 1). The HMBC data also revealed that the disaccharide moiety was attached to C-3 of the respective aglycone in all the three saponins.

On the basis of the above evidence, bacopasides III—V can be represented as 3-*O*-*α*-L-arabinofuranosyl(1→2)-*β*-D-glucopyranosyl jujubogenin (**1**), and 3-*O*-*β*-D-glucopyranosyl(1→3)-*α*-L-arabinopyranosyl jujubogenin (**2**) and 3-*O*-*β*-D-glucopyranosyl(1→3)-*α*-L-arabinopyranosyl pseudojujubogenin (**3**) respectively.

Literature survey showed that a saponin, designated as rugoside-A, having the same chemical structure as that of bacopaside IV (**2**) was reported[10] from *Zizyphus rugosa*. However, the reported mp (224—227 °C) was found to be ~50° less than that of **2**. No other physical data, *viz.* $[\alpha]_D$, FAB-MS, ^{1}H-NMR or ^{13}C-NMR of the compound was provided except few ^{1}H-NMR data of the permethylated derivative of the compound. On scrutiny it was found that the signal for the anomeric proton of the arabinopyranose unit has been reported to be obtained as a broad singlet. This reported multiplicity of the anomeric proton can only be explained on the basis of a furanose structure but not a pyranose structure for the arabinose unit. We, therefore, feel that bacopaside IV and rugoside-A are not the same compound and rugoside-A may have an arabinofuranose unit instead of an arabinopyranose as suggested.[10]

Experimental

Melting points were measured in open capillaries and are uncorrected. 1D and 2D NMR spectra were recorded using a JEOL JNM ALPHA 500 and ALPHA 600 MHz spectrometers in pyridine-d_5. Chemical shifts are expressed as δ values using tetramethylsilane (TMS) as internal standard. High-resolution FAB-MS were measured on a JEOL HX-110 mass spectrometer using nitrobenzyl alcohol (NBA) as the matrix (with/without KCl/NaCl). GC was done on ECNSS-M, 3% on Gas-Chrom Q column at 190 °C for alditol acetates which were identified by co-injection of the authentic samples. HPLC was done on a C_{18} column (Senshu PAK, 8 mm i.d.×250 mm) with H_2O–MeOH (30 : 70, 25 : 75) as the mobile phase and UV absorbance detector (217 nm). Solvent system employed for paper chromatography (PC) was EtOAc–pyridine–H_2O (2 : 1 : 2).

Extraction and Isolation The air-dried and powdered aerial parts of *Bacopa monniera* (1.5 kg) were successively extracted with petroleum ether (bp 60—80 °C) and MeOH in a percolator at room temp. The methanol extract was concentrated and partitioned between H_2O and *n*-BuOH. The *n*-BuOH layer was evaporated under reduced pressure to get a deep brown residue (80 g). A portion (30 g) was dissolved in minimum volume of MeOH, adsorbed on silica gel, dried and successively extracted with $CHCl_3$, EtOAc, Me_2CO and $CHCl_3$–MeOH (4 : 1) in a Soxhlet apparatus. The Me_2CO extract (6 g) was subjected to chromatography over a column of silica gel (35 cm×5 cm) using MeOH–$CHCl_3$ (1 : 19, 1 : 9). Initial eluate with MeOH–$CHCl_3$ (1 : 19) gave mainly bacopasaponin A and B.[6] Further elution with MeOH–$CHCl_3$ (1 : 9) furnished a gummy residue (1.8 g). A portion (0.4 g) was then subjected to prep. HPLC to yield bacopasides III (**1**, 41 mg), IV (**2**, 62 mg) and V (**3**, 25 mg).

Bacopaside III (**1**): Recrystallized from MeOH–H_2O in micro needles, mp 232—234 °C (dec.), $[\alpha]_D^{23}$ −44.8° (*c*=0.52, MeOH.). High-resolution (HR)-FAB-MS (positive) *m/z*: 767.4601 $[M+H]^+$, 789.4406 $[M+Na]^+$, 805.4140 $[M+K]^+$. Calcd for $C_{41}H_{67}O_{13}$: 767.4563, for $C_{41}H_{66}O_{13}Na$: 789.4383 and for $C_{41}H_{66}O_{13}K$: 805.4122. ^{13}C-NMR: Table 1. ^{1}H-NMR: Table 2.

Bacopaside IV (**2**): Recrystallised from MeOH as fine needles, mp 272—274 °C (dec.), $[\alpha]_D^{23}$ −5.2° (*c*=0.50, MeOH). HR-FAB-MS (positive) *m/z*: 767.4601 $[M+H]^+$, 789.4406 $[M+Na]^+$. Calcd for $C_{41}H_{67}O_{13}$: 767.4563 and for $C_{41}H_{66}O_{13}Na$: 789.4383. ^{13}C-NMR: Table 1. ^{1}H-NMR: Table 2.

Bacopaside V (**3**): Recrystallised from MeOH as fine needles, mp 274—276 °C (dec.), $[\alpha]_D^{23}$ −24.9° (*c*=0.38, MeOH). HR-FAB-MS (positive) *m/z*: 767.4601 $[M+H]^+$, 789.4425 $[M+Na]^+$, 805.4156 $[M+K]^+$. Calcd for $C_{41}H_{67}O_{13}$: 767.4563, for $C_{41}H_{66}O_{13}Na$: 789.4383 and for $C_{41}H_{66}O_{13}K$: 805.4122. ^{13}C-NMR: Table 1. ^{1}H-NMR: Table 2.

Hydrolysis of the Saponins (1—3) Compounds **1**, **2** and **3** (15 mg each) were hydrolysed separately with 2 N HCl in aq. MeOH (10 ml) on a steam bath for 6 h. The reaction mixtures were cooled, diluted with water (10 ml) and extracted with $CHCl_3$. The aqueous parts were neutralized with Ag_2CO_3 and filtered. The filtrates were concentrated under reduced pressure and examined for sugars by PC. Two spots were detected corresponding to arabinose (*Rf* 0.234) and glucose (*Rf* 0.185) in all three cases.

Small portions of the filtrate concentrates were converted to alditol acetates by the usual procedure and the products were subjected to GC analysis. Two peaks corresponding to glucitol acetate and arabinitol acetate in 1 : 1 ratio in all the three cases were observed.

The sugar components of the remaining portion of the filtrate concentrates were resolved by prep. PC and proved to be L-arabinose and D-glucose by their specific rotations in all the three cases.

Acknowledgements One of the authors (S. G.) is indebted to CSIR, New Delhi for providing financial help in the form of RA.

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