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DAMMARANE-TYPE TRITERPENOID SAPONINS FROM *BACOPA MONNIERA*

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Key Word Index—*Bacopa monniera*; Scrophulariaceae; bacopasaponins A, B and C; pseudojujubogenin; triterpenoid saponins.

Abstract—Three new dammarane-type triterpenoid saponins, bacopasaponins A, B and C, of biological interest have been isolated from the reputed Indian medicinal plant *Bacopa monniera* and identified as 3-*O*-α-L-arabinopyranosyl-20-*O*-α-L-arabinopyranosyl-jujubogenin, 3-*O*-[α-L-arabinofuranosyl (1→2)α-L-arabinopyranosyl]pseudojujubogenin and 3-*O*-[β-D-glucopyranosyl (1→3){α-L-arabinofuranosyl (1→2)}α-L-arabinopyranosyl]pseudojujubogenin by spectroscopic methods and some chemical transformations. The hitherto undetermined configurations at C-20 and C-22 of pseudojujubogenin were elucidated by phase-sensitive ROESY, and ^1H and ^{13}C signals of the saponins were assigned by DEPT, ^1H–^1H COSY, HSQC and HMBC techniques.

INTRODUCTION

Bacopa monniera Wettst, popularly known as 'Brahmi', is held in high repute as a nervine tonic, cardio tonic and diuretic in Indian traditional medicine [1]. The alcoholic extract was reported to improve the performance of rats in various learning experiments as manifested by better acquisition, consolidation and retention of newly acquired behavioural responses [2–4]. Saponins, which are present as a complex mixture, are the main constituents of the alcoholic extract and as such these are believed to be the active constituents of this fraction. In earlier chemical investigations [5, 6] attempts were made for isolation and characterization of the saponins from this herb. The isolation has been reported of three aglycones ebelin lactone (**1**) [7], bacogenin A_1 (**2**) [8] and partially characterized bacogenin A_3 (**3**) [9], which are artefacts, and two genuine sapogenins, jujubogenin [10] and pseudojujubogenin [11]. However, the structure of pseudojujubogenin was not completely elucidated, the configurations at C-20 and C-22 remaining undetermined. The structural elucidation has been described of two new dammarane-type triterpenoid saponins, bacoside A_1 [12] and bacoside A_3 [13], both of which are jujubogenin glycosides. In continuation of our chemical investigation on the pharmaceutically important naturally occurring saponins [14–16] our attention was drawn to the potential saponins of the plant occurring as a very complex mixture. This paper reports the isolation and structural elucidation of three new saponins of biological interest from the plant.

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RESULTS AND DISCUSSION

The *n*-butanol soluble fraction of the methanol extract of the leaves of *B. monniera* was partially purified by chromatography on silica gel (see Experimental). Further purification, using a combination of silica gel column chromatography and preparative TLC on silica gel G followed by crystallization, yielded three pure saponins designated as bacopasaponins A–C (**4**–**6**), each giving a positive Liebermann–Burchard (L.B.) test for triterpenoids and the Molisch test for sugars.

Acid hydrolysis of bacopasaponin A (**4**) yielded a major aglycone characterized as ebelin lactone (**1**) and a monosaccharide constituent identified as L-arabinose by its isolation and by a study of its properties (see Experimental). It is known that **1** is an artefact derived from the genuine sapogenin, jujubogenin, by acid catalysed transformation during hydrolysis [10] and as such jujubogenin was supposed to be the aglycone of **4**. The positive-ion FAB mass spectrum of **4** showed peaks at *m/z* 759, 737, 587 and 460 attributable to $[M + Na]^+$, $[M + H]^+$, $[M + H - \text{arabinose}]^+$ and $[M + Na - 2 \times \text{arabinose} + H]^+$. The saponin showed a cationized ion at *m/z* 775 assignable to $[M + K]^+$ as the base peak in the positive-ion FAB mass spectrum in a glycerol–thioglycerol matrix containing KCl [16, 17]. The negative-ion FAB mass spectrum of the compound

exhibited the $[M-H]^-$ peak at m/z 735. Thus, acid hydrolysis and FAB-MS results disclosed that saponin **4** is a jujubogenin bisdesmoside, arabinose being attached to two positions of the aglycone. The pyranose form of both the arabinose units, as well as their points of attachment at C-3 and C-20 hydroxyls, were ascertained from the ^{13}C chemical shifts of jujubogenin [11] and of bacopasapcnin A, taking into consideration the glycosylation shift values [18, 19]. The ^1H and ^{13}C NMR assignments (Tables 1 and 2) were made with the help of DEPT, ^1H–^1H COSY, HSQC and HMBC experiments. Thus, the structure of bacopasaponin A was defined as 3-*O*-α-L-arabinopyranosyl-20-*O*-α-L-arabinopyranosyl-jujubogenin (**4**).

Acid hydrolysis of bacopasaponin B (**5**) afforded a major aglycone which was characterized as bacogenin A_1 (**2**) [7, 8] and the sugar component was identified as L-arabinose. Compound **2** is known to be an artefact derived from the genuine sapogenin, pseudojujubogenin, by acid catalysed rearrangement during hydrolysis. As previously mentioned the configurations at C-20 and C-22 of pseudojujubogenin were yet to be determined. The positive-ion FAB-mass spectrum of **5** showed the $[M+H]^+$ peak at m/z 737 as the base peak. The other discernible peaks were observed at m/z 605, 473 and 455 ascribable to $[M+H-\text{arabinosyl}]^+$, $[M+H-2\times\text{arabinosyl}]^+$ and $[M+H-\text{arabinosyl-arabinose}]^+$, respectively. The sequential loss of two arabinosyls, as well as arabinosyl arabinose, indicated the presence of a diarabinoside chain attached to the aglycone at a single point instead of the two arabinose units being attached to two different positions as in **4**. The linkage of the disaccharide chain at C-3 of the aglycone as well as the inter-sugar connection could be inferred from the ^{13}C NMR spectrum of **5** (Table 2), taking into consideration the glycosylation shift rules [18, 19]. The NMR techniques of DEPT, ^1H–^1H COSY, HSQC and HMBC were successfully used for unambiguous assignments of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) and determination of the ring sizes of the sugar units. The configurations at C-20 and C-22 of the aglycone, pseudojujubogenin were determined by phase-sensitive rotating frame overhauser enhancement spectroscopy (ROESY). The ROEs observed in phase-sensitive ROESY are shown in Fig. 1, which suggest 20(*S*) and 22(*R*) configurations. Thus, the structure of bacopasaponin B was elucidated as 3-*O*-[α-L-arabinofuranoxyl $(1\rightarrow2)$α-L-arabinopyranosyl]pseudojujubogenin (**5**).

Bacopasaponin C (**6**) on acid hydrolysis yielded bacogenin A_1 as the major sapogenin, which is an artefact formed from pusedojujubogenin during acid hydrolysis. The sugar constituents were identified as L-arabinose and D-glucose. The positive-ion FAB-mass spectra displayed the base peak at m/z 921 assignable to $[M+Na]^+$ and other discernible peaks at m/z 899, 767, 737, 473 and 455 ascribable to $[M+H]^+$, $[M+H-\text{arabinosyl}]^+$, $[M+H-\text{glucosyl}]^+$, $[M+H-2\times\text{arabinosyl-glucosyl}]^+$ and $[M+H-\text{arabinosyl-glucosyl-arabinose}]^+$, respectively. The negative-ion FAB-mass spectrum exhibits significant peaks at m/z 897, 765, 735 and 603 attributable to $[M-H]^-$, $[M-H-\text{arabinosyl}]^-$, $[M-H-\text{glucosyl}]^-$ and $[M-H-\text{arabinosyl-glucosyl}]^-$, respectively. The hydrolysis and FAB-mass spectrometry results indicated bacopasaponin C to be pseudojujubogenin trisaccharide, the sugar moiety containing a terminal arabinose and a terminal glucose. The attachment of the sugar moiety at C-3 were determined by NMR spectroscopy. The ^1H and ^{13}C NMR signals were assigned unambiguously (Tables 1 and 2) with the help of DEPT, ^1H–^1H COSY and HSQC techniques. The assigned ^{13}C data were in good agreement with the glycosylation shift rules. The ROESY technique was employed to define the configurations at C-20 and C-22 of pseudojujubogenin. The structure of bacopasaponin C was thus elucidated as 3-*O*-[β-D-glucopyranosyl $(1\rightarrow3)$]{α-L-arabinofuranosyl $(1\rightarrow2)$}α-L-arabinofuranosyl]pseudojujubogenin (**6**).

Dammarane-type triterpenoid saponins are major constituents of several reputed herb drugs. Ginseng, the widely known plant drug, has been used for centuries as an expensive traditional medicine, and ginseng saponins are mainly protopanaxadiol and protopanaxatriol glycosides [20]. Jujubogenin glycosides have been isolated from several reputed medicinal plants (Rhamnaceae and Scrophulariaceae) [21]. It appears that pseudosapogenin glycosides have been reported so far from this Indian herb drug *B. monniera*. We are pursuing our studies to isolate the other saponin constituents of the plant to provide a basis for discussion of their biological activity in relation to their chemical structures.

EXPERIMENTAL

The plant material was collected from 24-Parganas, West Bengal, and was identified in the Indian Botanic Garden, Howrah. A voucher specimen is deposited in the herbarium of the Indian Institute of Chemical Biology. Mps: uncorr. IR: KBr discs; ^1H NMR; 399.65 MHz and ^{13}C NMR: 100.40 MHz in pyridine-d_5. FAB-MS were obtained on a VG-ZAB-SE mass spectrometer using glycerol–thioglycerol as matrix with salt or without salt for positive-ion spectra and nitrobenzyl alcohol for negative-ion spectra. Cs^+ was used as a bombarding particle operating at 5 kV accelerating voltage, equipped 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) $CHCl_3$–MeOH–H_2O (35:13:2). 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 acetate.

The air dried powdered leaves (1.4 kg) of *B. monniera* collected from places near to Calcutta were successively extracted in a percolator with petrol (60–80°), $CHCl_3$ and MeOH. The MeOH extract was concd

Table 1. ^{1}H NMR chemical shifts of bacopasaponins A (**4**), B (**5**) and C (**6**) in pyridine-d_5*

4	**5**	**6**
1 α 0.75 (*ddd*, *J* = 3.5, 13.4, 13.4 Hz)	1 α 0.76 (ddd, J = 3.9, 13.5, 13.5 Hz)	1 α 0.71 (*ddd*, *J* = 3.4, 13.6, 13.6 Hz)
β 1.50 (*ddd*, *J* = 2.7, 2.7, 13.4 Hz)	β 1.50 (*ddd*, *J* = 2.0, 3.9, 13.5 Hz)	β 1.48 (*m*)
2 α 1.80 (*m*)	2 α 1.82 (*m*)	2 α 1.82 (*m*)
β 2.10 (*dddd*, *J* = 2.7, 3.5, 4.4, 13.9 Hz)	β 2.10 (*dddd*, *J* = 3.9, 3.9, 4.4, 13.7 Hz)	2.10 (*dddd*, *J* = 3.4, 3.4, 4.1, 13.7 Hz)
3.24 (*dd*, *J* = 4.4, 11.7 Hz)	3.24 (*dd*, *J* = 4.4, 11.7 Hz)	3 3.19 (*dd*, *J* = 4.1, 11.7 Hz)
4 —	4 —	4 —
5 0.62 (*dd*, *J* = 1.7, 11.2 Hz)	5 0.65 (*dd*, *J* = 1.7, 11.7 Hz)	5 0.65 (*brd*, *J* = 11.5 Hz)
6 α 1.23 (*m*)	6 α 1.32 (*m*)	6 α 1.27 (*m*)
β 1.36 (*m*)	β 1.45 (*m*)	β 1.42 (*m*)
7 α 1.40 (*m*)	7 α 1.47 (*m*)	7 α 1.47 (*m*)
β 1.26 (*m*)	β 1.35 (*m*)	β 1.32 (*m*)
8 —	8 —	8 —
9 0.80 (*dd*, *J* = 2.4, 12.4 Hz)	9 0.85 (*dd*, *J* = 2.5, 12.7 Hz)	9 0.80 (*dd*, *J* = 2.0, 12.6 Hz)
10 —	10 —	10 —
11 α 1.82 (*m*)	11 α 1.53 (*m*)	11 α 1.47 (*m*)
β 1.17 (*m*)	β 1.33 (*m*)	β 1.28 (*m*)
12 α 1.67 (*dddd*, *J* = 3.8, 13.2, 13.2, 13.2 Hz)	12 α 1.75 (*m*)	12 α 1.70 (*m*)
β 1.77 (*m*)	β 1.90 (*m*)	β 1.86 (*m*)
13 2.95 (*dddd*, *J* = 1.4, 4.4, 5.4, 13.2 Hz)	13 2.82 (*dddd*, *J* = 1.4, 4.4, 5.4, 13.2 Hz)	13 2.78 (*ddd*, *J* = 4.6, 5.4, 12.7 Hz)
14 —	14 —	14 —
15 α 2.32 (*d*, *J* = 8.5 Hz)	15 α 2.43 (*d*, *J* = 8.3 Hz)	15 α 2.38 (*d*, *J* = 8.3 Hz)
β 1.33 (*d*, *J* = 8.5 Hz)	β 1.33 (*dd*, *J* = 1.2, 8.3 Hz)	β 1.46 (*brd*, *J* = 8.3 Hz)
16 —	16 —	16 —
17 1.31 (*d*, *J* = 5.4 Hz)	17 1.63 (*dd*, *J* = 1.3, 7.8 Hz)	17 1.58 (*brd*, *J* = 7.8 Hz)
18 0.96 (*s*)	18 1.05 (*s*)	18 1.02 (*s*)
19 0.66 (*s*)	19 0.72 (*s*)	19 0.65 (*s*)
20 —	20 —	20 —
21 1.35 (*s*)	21 1.36 (*s*)	21 1.33 (*s*)
22 α 1.97 (*dd*, *J* = 1.7, 13.9 Hz)	22 2.58 (*ddd*, *J* = 2.2, 2.2, 10.5 Hz)	22 2.60 (*ddd*, *J* = 2.2, 2.2, 10.2 Hz)
β 1.39 (*dd*, *J* = 11.0, 13.9 Hz)	23 α 3.85 (*dd*, *J* = 2.2, 10.7 Hz)	23 α 3.79 (*dd*, *J* = 2.2, 10.7 Hz)
23 5.27 (*ddd*, *J* = 1.7, 7.3, 11.0 Hz)	β 4.68 (*dd*, *J* = 2.2, 10.7 Hz)	β 4.63 (*dd*, *J* = 2.2, 10.7 Hz)
24 5.43 (*dsept*, *J* = 7.3, 1.3 Hz)	24 5.82 (*dsept*, *J* = 10.7, 1.2 Hz)	24 5.77 (*dsept*, *J* = 10.2, 1.2 Hz)
25 —	25 —	25 —
26 1.62 (*d*, *J* = 1.3 Hz)	26 1.68 (*d*, *J* = 1.2 Hz)	26 1.64 (*d*, *J* = 1.2 Hz)
27 1.75 (*d*, *J* = 1.3 Hz)	27 1.60 (*d*, *J* = 1.2 Hz)	27 1.58 (*d*, *J* = 1.2 Hz)
28 1.17 (*s*)	28 1.22 (*s*)	28 1.21 (*s*)
29 0.86 (*s*)	29 0.96 (*s*)	29 1.00 (*s*)
30 α 4.17 (*d*, *J* = 7.5 Hz)	30 α 4.24 (*d*, *J* = 7.6 Hz)	30 α 4.21 (*d*, *J* = 7.6 Hz)
β 4.04 (*dd*, *J* = 1.4, 7.5 Hz)	β 4.16 (*d*, *J* = 7.6 Hz)	β 4.14 (*d*, *J* = 7.6 Hz)
3-*O*-Ara	3-*O*-Ara	3-*O*-Ara
1 4.70 (*d*, *J* = 7.0 Hz)	1 4.89 (*d*, *J* = 6.3 Hz)	1 4.76 (*d*, *J* = 6.8 Hz)
2 4.33 (*dd*, *J* = 7.0, 8.8 Hz)	2 4.40 (*dd*, *J* = 6.3, 8.0 Hz)	2 4.43 (*dd*, *J* = 6.8, 8.8 Hz)
3 4.09 (*dd*, *J* = 2.2, 8.8 Hz)	3 4.18 (*dd*, *J* = 6.6, 8.0 Hz)	3 4.18 (*dd*, *J* = 2.7, 8.8 Hz)
4 4.25 (*m*)	4 4.26 (*m*)	4 4.47 (*ddd*, *J* = 2.7, 2.7, 2.7 Hz)
5 a 4.23 (*m*)	5 a 4.28 (*m*)	5 a 4.16 (*dd*, *J* = 2.7, 10.0 Hz)
b 3.75 (*dd*, *J* = 2.5, 12.7 Hz)	b 3.77 (*dd*, *J* = 3.4, 12.7 Hz)	b 3.73 (*dd*, *J* = 2.7, 10.0 Hz)
20-*O*-Ara	Ara(f)	Ara(f)
1 4.78 (*d*, *J* = 6.6 Hz)	1 6.10 (*d*, *J* = 1.2 Hz)	1 6.00 (*d*, *J* = 2.7 Hz)
2 4.29 (*dd*, *J* = 6.6, 8.6 Hz)	2 4.98 (*dd*, *J* = 1.2, 2.7 Hz)	2 4.93 (*dd*, *J* = 2.7, 5.1 Hz)
3 4.08 (*dd*, *J* = 2.4, 8.6 Hz)	3 4.81 (*dd*, *J* = 2.7, 4.6 Hz)	3 4.77 (*dd*, *J* = 5.4, 7.3 Hz)
4 4.23 (*m*)	4 4.89 (*m*)	4 4.71 (*ddd*, *J* = 3.4, 3.7, 7.3 Hz)
5 a 4.14 (*dd*, *J* = 3.0, 12.2 Hz)	5 a 4.26 (*m*)	5 a 4.25 (*dd*, *J* = 3.4, 12.0 Hz)
b 3.65 (*dd*, *J* = 2.0, 12.2 Hz)	b 4.17 (*m*)	b 4.16 (*dd*, *J* = 3.7, 12.0 Hz)
		Glc
		1 5.04 (*d*, *J* = 7.8 Hz)
		2 3.89 (*dd*, *J* = 7.8, 9.0 Hz)
		3 4.14 (*dd*, *J* = 9.0, 9.0 Hz)
		4 4.04 (*dd*, *J* = 9.0, 9.5 Hz)
		5 3.87 (*ddd*, *J* = 2.4, 5.6, 9.5 Hz)
		6 a 4.40 (*dd*, *J* = 2.4, 11.7 Hz)
		b 4.17 (*dd*, *J* = 5.6, 11.7 Hz)

*Assignments aided by ^{1}H–^{1}H COSY, HSQC and HMBC experiments.
Ara: arabinose; Glc: glucose; f: furanose.

Table 2. ^{13}C NMR chemical shifts of bacopasaponins A (**4**), B (**5**) and C (**6**) in pyridine-d_5*

Carbon	4	5	6	4		5		6	
1	38.3	38.8	38.7	3-*O*-Ara		3-*O*-Ara		3-*O*-Ara	
2	26.7	26.7	26.6	1	107.3	1	105.8	1	105.3
3	88.6	88.9	88.6	2	72.9	2	76.3	2	76.7
4	39.7	39.6	39.7	3	74.6	3	73.4	3	83.1
5	56.2	56.2	56.1	4	69.4	4	68.5	4	68.2
6	18.3	18.3	18.2	5	66.6	5	65.6	5	65.4
7	36.0	36.1	35.9	20-*O*-Ara		Ara(f)		Ara(f)	
8	37.5	37.5	37.4	1	98.8	1	109.9	1	110.0
9	53.0	53.1	52.9	2	73.1	2	81.2	2	83.5
10	37.3	37.3	37.1	3	75.1	3	78.8	3	77.7
11	21.8	21.8	21.6	4	69.3	4	88.1	4	84.7
12	28.4	28.6	28.4	5	66.6	5	62.8	5	61.9
13	36.1	37.2	37.0					Glc	
14	53.7	53.5	53.3					1	104.6
15	37.4	37.0	36.8					2	74.9
16	110.2	110.3	110.2					3	77.6
17	55.2	51.4	51.1					4	71.2
18	18.8	18.9	18.7					5	78.1
19	16.3	16.3	16.2					6	62.2
20	75.8	71.9	71.7						
21	25.1	27.2	26.8						
22	41.6	46.3	45.9						
23	68.8	66.1	66.0						
24	127.4	124.2	124.0						
25	133.8	132.9	132.9						
26	25.7	26.1	25.9						
27	18.3	18.5	18.3						
28	28.1	28.1	27.7						
29	16.7	16.7	16.4						
30	65.9	65.9	65.8						

*Assignments aided by HSQC and HMBC experiments.

and partitioned between H_2O and *n*-BuOH. The *n*-BuOH layer was washed with H_2O, and then removed under red. pres. The residue (52 g) thus obtained was dissolved in a minimum amount of MeOH, adsorbed on silica gel, dried and extracted successively with $CHCl_3$, EtOAc, Me_2CO and $CHCl_3$–MeOH (4:1). The last 3 frs were separately subjected to CC on silica gel ($CHCl_3$–MeOH, 24:1), and similar frs so obtained were further purified by prep. TLC (solvent system B) followed by crystallization. Thus, **4** (85 mg), **5** (58 mg) and **6** (140 mg) were obtained.

Bacopasaponin A (**4**). Crystallized from EtOAc–

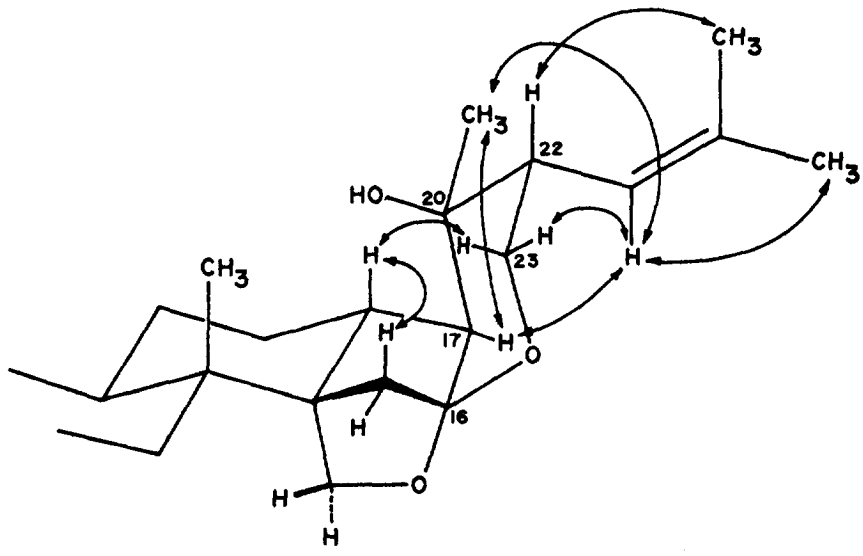
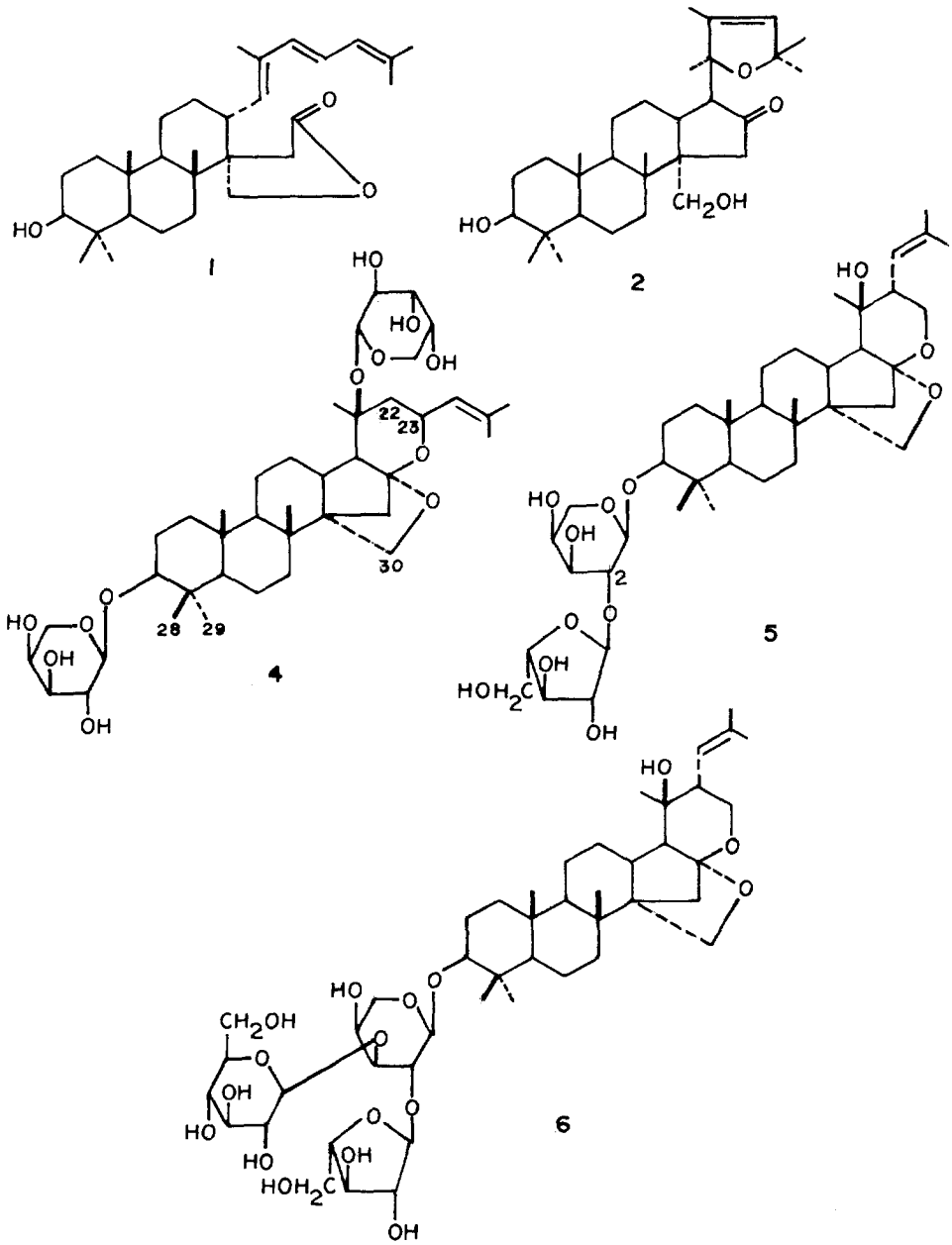


Fig. 1. ROEs observed in phase-sensitive ROESY.



MeOH as micro needles, mp 256° $[\alpha]_D$ −90° (MeOH, *c* 1.3); IR ν_{max}^{KBr} cm^{-1}: 3300–3600 (hydroxyl), 1452, 1390, 1310, 1290, 1215, 1140, 1078, 1052, 930; ^1H and ^{13}C NMR (Tables 1 and 2); FAB-MS (positive) *m/z* (rel. int.): 759 (40), 737 (38), 587 (100), 460 (35), KCl added 775 [M + K] (100), 759 (23), 737 (8), 587 (60); FAB-MS (negative) *m/z* 735 $[M-H]^-$ (100) (Found: C, 65.28; H, 8.70; $C_{40}H_{64}O_{12}$ requires C, 65.19; H, 8.75%).

Hydrolysis of **4**. Compound **4** (100 mg) was hydrolysed with 2M HCl in aq. MeOH (25 ml) on a water bath for 5 hr. Usual work-up followed by CC purification on silica gel furnished **1**, mp 173° (lit. [12] 172°). 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 tested for carbohydrates by PC using an authentic sample. Only one spot corresponding to L-arabinose was detected. That the arabinose was the L-enantiomer was confirmed by its isolation by prep. PC and comparison of its specific rotation with that of L-arabinose. The other portion of the conc. filtrate was reduced with $NaBH_4$ and worked up as usual. The residue was acetylated with Ac_2O–pyridine (1:1), worked up and subjected to GLC analysis using the column mentioned above. Only one peak corresponding to arabinitol acetate was detected using an authentic sample.

Bacopasaponin B (**5**). Crystallized from EtOAc–

MeOH as microneedles, mp 283° $[\alpha]_D$ −65.4° (MeOH, *c* 1.3); IR_{max}^{KBr} cm^{-1}: 3200–3600 (hydroxyl), 1452, 1390, 1305, 1290, 1262, 1122, 1000, 860, 790; ^1H and ^{13}C NMR (Tables 1 and 2); FAB-MS (positive) *m/z*: 737 (100), 605 (10), 473 (24), 455 (34); FAB-MS (negative) *m/z* 735 (100) (Found: C, 65.10; H, 8.68; $C_{40}H_{64}O_{12}$ requires C, 65.19; H, 8.75%).

Hydrolysis of **5**. Compound **5** (100 mg) was hydrolysed with 2M HCl in aq. MeOH (25 ml) on a water bath for 6 hr and worked up in the usual way. The purified major aglycone was found to be identical with **2**, mp 241–242° (lit. [7] mp 242°). Its ^1H NMR and mass spectral data were comparable to those of an authentic sample.

The filtrate from the hydrolysate was worked up as described for **4**, and the carbohydrate constituent was identified as L-arabinose by PC and GLC.

Bacopasaponin C **6**. Crystallized from EtOAc–MeOH as micro needles, mp 222° $[\alpha]_D$ −47.5° (MeOH, *c* 1.2); IR_{max}^{KBr} cm^{-1}: 3200–3600 (hydroxyl) 1450, 1390, 1290, 1260, 1140 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); FAB-MS (positive) *m/z*: 921 (100), 899 (9), 767 (6), 737 (13), 473 (57), 455 (76), FAB-MS (negative) *m/z*: 897 (100), 765 (9), 735 (19) (Found: C, 60.42; H, 8.20; $C_{46}H_{74}O_{17}$ requires C, 60.34; H, 8.29).

Hydrolysis of **6**. Compound **6** (150 mg) was hydrolysed with 2 M HCl in aq. MeOH in the usual way. The major aglycone obtained was found to be identical with **2**.

The filtrate from the hydrolysate was worked up in the usual way and the sugar constituents were identified as D-glucose and L-arabinose by PC and GLC.

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