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Bacopasaponins E and F: two jujubogenin bisdesmosides from *Bacopa monniera*

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Abstract

Two new dammarane-type jujubogenin bisdesmosides, bacopasaponins E and F of biological interest have been isolated from the reputed Indian medicinal plant *Bacopa monniera* and defined as 3-*O*-[β-D-glucopyranosyl(1 → 3){α-L-arabinofuranosyl(1 → 2)}α-L-arabinopyranosyl]-20-*O*-(α-L-arabinopyranosyl) jujubogenin and 3-*O*-[β-D-glucopyranosyl(1 → 3){α-L-arabinofuranosyl(1 → 2)}β-D-glucopyranosyl]-20-*O*-α-L-arabinopyranosyl) jujubogenin respectively by spectroscopic methods and some chemical transformations.

Keywords: *Bacopa monniera*; Scrophulariaceae; Bacopasaponin E; Bacopasaponin F; Jujubogenin; Ttriterpenoid saponin

1. Introduction

Bacopa monniera Wettst. (Scrophulariaceae) is widely used as a nervine tonic, cardio tonic and diuretic in Indian traditional medicine (Chopra, Chopra & Verma, 1969). The activities of the herb are believed to be associated with the polar extractive, which contains mainly saponins as a complex mixture. Attempts have been made to isolate and define the different constituents of the saponin fraction and structure elucidation of six new dammarane-type triterpenoid saponins, bacoside A_1 (Jain & Kulshrestha, 1993), bacoside A_3 (Rastogi, Pal & Kulshrestha, 1994) and bacopasaponins A, B, C and D (Garai, Mahato, Ohtani & Yamasaki, 1996a; Garai, Mahato, Ohtani & Yamasaki, 1996b) has been reported thus far. In view of growing interest on the herb because of its beneficial effects, we pursued our studies for complete characterisation of the saponin constituents. This paper reports isolation and structure elucidation of two new bisdesmosidic jujubogenin glycosides.

2. Results and discussion

The *n*-butanol soluble fraction of a methanol extract of the leaves of *B. monniera* was dissolved in minimum volume of methanol, adsorbed on silica gel and fractionated by successive elution with chloroform, ethyl acetate, acetone and 20% methanol in chloroform. The methanol-chloroform extract on purification by column chromatography and preparative TLC on silica gel followed by solvent treatment and crystallization furnished two new saponins designated bacopasaponins E (**1**) and F (**2**) which gave positive froth test for saponins, Liebermann-Burchard test for triterpenes and Molisch test for sugars.

Acid hydrolysis of **1** liberated a mixture of aglycones of which the major one was identified as ebelin lactone (**3**) (Kulshrestha & Rastogi, 1973). The sugar constituents were identified by PC and GC as D-glucose and L-arabinose. The compound **3** is known to be an artefact derived from the genuine sapogenin, jujubogenin (**4**) by acid catalysed rearrangement during hydrolysis (Kawai, Akiyama, Ogihara & Shibata, 1974) and as such the genuine aglycone of saponin **1** was assumed to be **4** which was ascertained by ^{1}H and ^{13}C NMR data of the intact saponin **1**.

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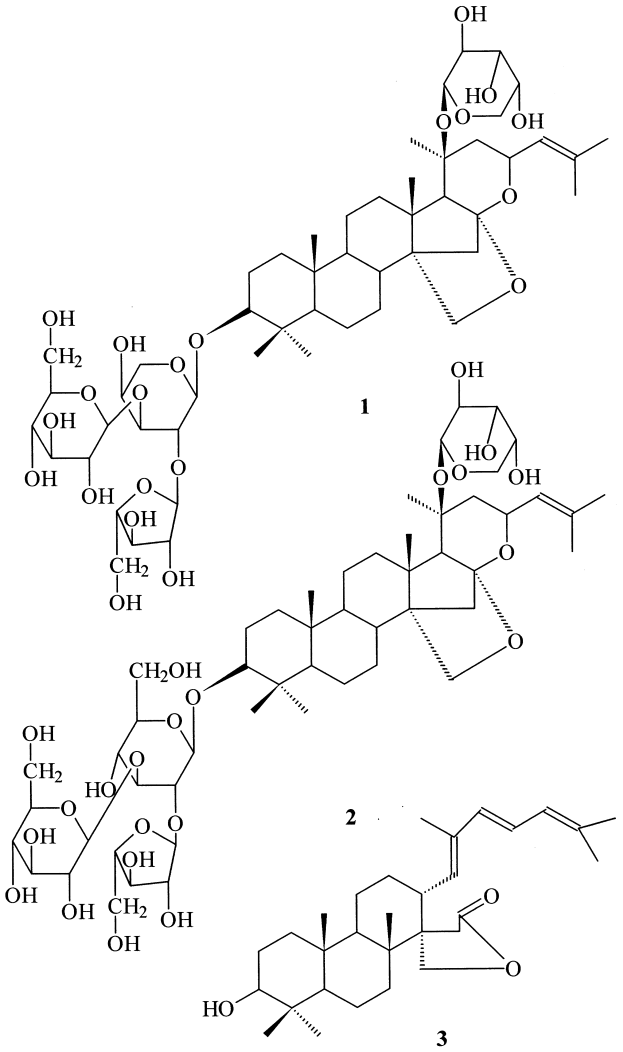
The positive-ion FAB mass spectrum of saponin **1** displayed significant peaks at *m/z* 1031, 881, 749, 587, 586 and 454 assigned to $[M+H]^+$, $[M+H\text{-arabinose}]^+$, $[M+H\text{-arabinose-arabinosyl}]^+$, $[M+H\text{-arabinose-arabinosyl-glucosyl}]^+$, $[M+H\text{-arabinose-arabinosyl-glucosyl-H}]^+$ and $[M+H\text{-arabinose-2} \times \text{arabinosyl-glucosyl-H}]^+$ respectively. The acid hydrolysis and FAB-MS results indicated that saponin **1** is a jujubogenin bisdesmoside, one arabinose unit and a trisaccharide unit consisting of two arabinose and a glucose being attached to two positions of the aglycone **4**. The attachments of the sugar moieties at C-3 and C-20 of the aglycone, the pyranose form of the glucose unit, pyranose forms of the two arabinose units and furanose form of one arabinose unit, as well as intersugar linkages, were determined by ^{13}C NMR chemical shift values (Kasai, Okihara, Asakawa, Mizutani & Tanaka, 1979; Tori, Seo, Yoshimura, Arita & Tomita, 1978). The ^{13}C NMR assignments (Table 1) were made with the help of DEPT and by comparison with the ^{13}C values of bacopasaponins A, B and C (Garai et al., 1996a). Thus the structure of bacopasaponin E was elucidated as 3-*O*-[β-D-glucopyranosyl(1 → 3){α-L-arabinofuranosyl(1 → 2)}α-L-arabinopyranosyl]-20-*O*-(α-L-arabinopyranosyl) jujubogenin (**1**).

Acid hydrolysis of bacopasaponin F (**2**) also furnished ebelin lactone (**3**) as the major aglycone and as such the genuine aglycone of saponin **2** was also assumed to be jujubogenin (**4**). The ^1H and ^{13}C NMR data of the intact saponin **2** also supported this presumption. The sugar constituents liberated by the acid hydrolysis were identified as D-glucose and L-arabinose by PC and GC.

The positive-ion FAB-MS of bacopasaponin F exhibited discernible peaks at *m/z* 1061, 911, 779, 617 and 454 attributed to $[M+H]^+$, $[M+H\text{-arabinose}]^+$ $[M+H\text{-arabinose-arabinosyl}]^+$, $[M+H\text{-arabinose-arabinosyl-glucosyl}]^+$ and $[M+H\text{-arabibnose-arabinosyl-2} \times \text{glucosyl}]^+$. Thus the acid hydrolysis and FAB-MS results suggested that saponin **2** is a bisdesmoside, one arabinose unit and a trisaccharide unit containing two glucose and one arabinose being attached to two positions of the aglycone **4**. Taking into consideration the ^{13}C NMR data of saponin **2** and glycosylation shift values (Kasai et al., 1979; Tori et al., 1978), the attachments of the sugar moieties at C-3 and C-20 of the aglycone **4**, the pyranose form of two glucose units, pyranose form of one and furanose form of another arabinose unit were determined. Assignments of the ^{13}C NMR data of saponin **2** were made with the help of DEPT and by comparison with the ^{13}C values of bacopasaponins A, B and C (Garai et al., 1996a). Consequently the structure of bacopa saponin F was defined as 3-*O*-[β-D-glucopyranosyl(1 → 3){α-L-arabinofuranosyl(1 → 2)}β-D-glucopyranosyl]-20-*O*-(α-L-arabinopyranosyl) jujubogenin (**2**).



It may be mentioned that three saponins isolated from *Zizyphus joazeiro* (Higuchi et al., 1984) contain the same chain of sugars at the C-3 position of jujubogenin.

It is noteworthy that dammarane-type triterpenoid saponins are major constituents of several reputed herb drugs including ginseng which is one of the most widely known plant drug used for centuries as an expensive traditional medicine (Shibata, 1986). Jujubogenin glycosides have been isolated from a number of reputed medicinal plants (e.g. Rhamnaceae and Scrophulariaceae) (Lins Brando, Dubois, Teixera & Wagner, 1992; Mahato & Garai, 1998). However, it appears that in addition to jujubogenin glycosides pseudojujubogenin glycosides have been reported so far only from this Indian herb drug *B. monniera* (Garai et al., 1996a, 1996b). We are pursuing our studies to isolate the other saponin constituents of the plant to provide a basis for discussion of their biological activities in relation to their chemical structure.

Table 1
^{13}C NMR chemical shifts of bacopasaponins E(**1**) and F(**2**) in pyridine-d_5[a]

Carbon	1	2
1	38.6	38.6
2	26.7	26.6
3	88.5	88.6
4	39.7	39.6
5	56.0	56.0
6	18.1	18.2
7	35.8	35.9
8	37.0	37.0
9	52.8	52.8
10	37.3	37.3
11	21.5	21.6
12	28.2	28.3
13	35.8	35.9
14	53.6	53.7
15	37.2	37.3
16	110.1	110.1
17	55.0	55.0
18	18.6	18.6
19	16.4	16.5
20	75.5	75.6
21	25.0	25.1
22	41.3	41.4
23	68.7	68.7
24	127.3	127.4
25	133.7	133.7
26	25.6	25.6
27	18.2	18.3
28	27.6	27.7
29	16.2	16.2
30	65.8	65.9
3-*O*-Ara (p) **1**; 3-*O*-Glc **2**		
1	105.5	105.0
2	77.0	79.1
3	83.5	89.0
4	68.4	70.2
5	65.8	78.4
6		62.6
Ara(f)		
1	110.1	109.8
2	83.8	83.8
3	77.7	77.7
4	84.6	84.7
5	61.9	62.0
Glc		
1	104.8	104.6
2	75.0	75.4
3	77.9	77.6
4	71.3	71.5
5	78.3	78.5
6	62.3	62.3
20-*O*-Ara(p) **1**; 20-*O*-Ara(p) **2**		
1	98.6	98.7
2	72.9	73.0
3	75.1	75.0
4	69.2	69.3
5	66.6	66.7

[a] Glc (p) = glucopyranose; Ara(p) = arabinopyranose; Ara(f) = arabinofuranose.

3. 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 Institute, Mps: uncorr, IR: KBr discs; ^1H and ^{13}C NMR: 300 MHz in pyridine-d_5. FAB-MS (positive ion) were obtained on VG-ZAB-SE mass-spectrometer 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, 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. HPLC:Waters with 515 pump, 680 gradient controller and 2487 dual absorbance detector.

The air dried powdered leaves of the plant (2 kg) were successively extracted in a percolator with petrol (b.p. 60–80°C), $CHCl_3$ 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 80 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 fr was purified by CC on silica gel using $CHCl_3$-MeOH (20:1) as mobile phase and prep. TLC (solvent system B) followed by prep. HPLC on a μBondapak C18 column (10 μm) (7.8 × 300 mm) using solvent system, methanol-water (65:35), flow rate 2 ml/min, detection at 210 nm and injection of 60 μl containing approximately 3 mg of the material.

3.1. Bacopasaponin E (*1*)

Amorphous powder (MeOH–ether), mp 266–270°C (dec), $[\alpha]_D$ −27.72° (MeOH, *c* 0.44). IR ν_{max}^{KBr} cm^{-1}: 3320–3600 (hydroxyl), 1635, 1575, 1420, 1380, 1298, 1260, 1214,1025, ^1H NMR (pyridine-d_5): 0.64 (3H, s, 19-Me), 0.94 (3H, s, 29-Me), 1.03 (3H, s, 18-Me), 1.25 (3 H, s, 28-Me), 1.48 (3H, s, 21-Me), 1.68 (3H, s, 26-Me), 1.80 (3H, s, 27-Me), 4.75 (1H, d, J = 7 Hz, 1-H of arabinose unit), 4.84 (1H, d, J = 7 Hz, 1-H of arabinbose unit), 5.05 (1H, d, J = 8 Hz, 1-H of glucose unit), 6.05 (1H, brs, 1-H of arabinofuranose unit), 5.33 (1H, t-like, 23-H), 5.48 (1H, m, 24-H). FAB-MS (positive) *m/z* (rel.int.): 1031 (100), 881 (25), 749(23), 587 (28), 586 (31), 454 (74).

3.2. Hydrolysis of **1**

Saponin **1** (60 mg) was hydrolysed with 2 M HCl in aq. MeOH (16 ml) on a water bath for 5 h and worked up in the usual way. The purified major aglycone was identified as ebelin lactone (**3**) (Garai et al., 1996a) by comparison with an authentic sample. The filtrate from the hydrolysate was neutralised with Ag_2CO_3, filtered and a portion of the filtrate was concd under reduced 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 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.

3.3. Bacopasaponin F (**2**)

Amorphous powder (MeOH–ether), mp 260-264°C (dec.), $[\alpha]_D$ −37.72° (MeOH, *c* 0.44), IR ν_{max}^{KBr} 3310-3610, 1640, 1575, 1420, 1386, 1300, 1260, 1215, 1030. ^{1}H NMR (pyridine-d_5): 0.61 (3H, s, 19-Me), 0.93 (3H, s, 29-Me), 1.03 (3H, s, 18-Me), 1.25 (3H, s, 28-Me), 1.48 (3H, s, 21-Me), 1.69 (3H, s, 26-Me), 1.80 (3H, s, 27-Me), 4.51 (1H, d, J = 8 Hz, 1-H of glucose unit), 4.81 (1H, d, J = 6.5 Hz, 1-H of arabinose unit), 4.85 (1H, d, J = 6.8 Hz, 1-H of arabinose unit), 5.17 (1H, d, J = 8 Hz, 1-H of glucose unit) 6.22 (1H, brs, 1-H of arabinofuranose unit), 5.31 (1H, t-like, 23-H), 5.47 (1H, m, 24-H), FAB-MS (positive) m/z (rel. int.): 1061 (100), 911 (36), 779 (40), 617 (40), 616 (26), 454 (65).

3.4. Hydrolysis of **2**

Compound **2** (55 mg) was hydrolysed with 2 M-HCl in aq. MeOH in the usual way. The major aglycone obtained was found to be identical with ebelin lactone **3** (Garai et al., 1996a).

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

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