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Cucurbitacins from *Bacopa monnieri* ☆

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Abstract

Four cucurbitacins, bacobitacin A–D (**1**–**4**) as well as, a known cytotoxic, cucurbitacin E (**5**) together with three known phenylethanoid glycosides, monnieraside I, III and plantioside B were isolated form the aerial part of *Bacopa monnieri*. Their structures were elucidated on the basis of extensive spectroscopic investigations (1D, 2D NMR and ESI-QTOF-MS/MS). This is the first report on the characterization of cucurbitacins in *B. monnieri*.

Keywords: *Bacopa monnieri*; Scrophulariaceae; Cucurbitacins; Bacobitacin A–D; ESI-QTOF-MS

1. Introduction

Bacopa monnieri (L.) WETTST. (Scrophulariaceae), commonly known, as ''Brahmi'' is a medicinal herb, found throughout the Indian subcontinent in wet and marshy places (Kapoor, 1990). It is used in the Ayurvedic system of medicine as a brain tonic, memory enhancer, improvement of intellect and revitaliser of sensory organs (Garai et al., 1996). Recent research has focused primarily on *Bacopa*'s cognitive enhancing effects, specifically memory, learning, and concentration and results support the traditional ayurvedic claims (Mukherjee and Dey, 1996). In addition to memory enhancing activity, it is also claimed to be useful in the treatment of cardiac, respiratory (Nadkarni, 1988) and neuropharmacological (Russo and Borrelli, 2005) disorders like insomnia, insanity, depression, psychosis, epilepsy and stress. It is reported to possess anti-inflammatory, analgesic, antipyretic, sedative (Kishore and Singh, 2005), free radical scavenging and lipid peroxidative activities (Anbarasi et al., 2005).

The major chemical constituents isolated and characterized from *B. monnieri* are dammarane type triterpenoid saponins with jujubogenin or psuedojujubogenin moieties as aglycones. The pharmacological effects of *B. monnieri* are mainly attributed to these saponins especially bacoside A and bacoside B, which are therefore, considered as bioactive marker compounds for this species (Deepak and Amit, 2004). The composition of bacoside A and bacoside B have been established very recently as a mixture of four triglycosidic and four diglycosidic saponins, respectively (bacoside A as a mixture of Bacoside A3, Bacopaside II, 3-*O*-[α-L-arabinofuranosyl-(1 → 2)-{β-D-glucopyranosyl-(1 → 3)-}-α-L-arabinopyranosyl] jujubogenin and Bacopasaponin C and bacoside B as a mixture of bacopaside N1, bacopaside N2, bacopaside-IV and bacopaside-V) (Deepak et al., 2005; Sivaramakrishna et al., 2005).

Other compounds include phenylethanoid glycosides, flavonoids and alkaloids such as brahmine and herpestine (Russo and Borrelli, 2005).

Cucurbitacins are of great interest because of the wide range of biological activity they exhibit in plants and animals. They are predominantly found in the family Cucurbitaceae but are also present in several other families of the plant kingdom. A number of compounds of this group have been investigated for their cytotoxic, hepatoprotective, antiinflammatory, cardiovascular effects (Miro, 1995) and as kairomones for diabroticite beetles (Metcalf et al., 1980, 1982). Cucurbitacin E has recently been reported to

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possess inhibitory effects on the growth of human colon, breast, lung and central nervous system cancer cell lines (Jayaprakasam et al., 2003). In continuation to our work on the isolation of novel bioactive molecules from the western Himalayan bioresources (Kumar et al., 2005, 2006a,b; Bhandari et al., 2006), here we report new cucurbitacins together with known phenylethanoid glycosides from *B. monnieri*.

2. Results and discussion

Defatted aerial parts of *B. monnieri* were extracted with 50% methanol, concentrated and kept overnight. The extract was filtered and successively fractionated with EtOAc and *n*-BuOH. The EtOAc extract was subjected to silica gel chromatography to afford cucurbitacins and phenylethanoid glycosides.

Compound **1** was obtained as white amorphous powder and displayed a molecular ion peak at *m/z* 595.2876 $[M+Na]^+$ (calcd. 595.2883) in its HRESI-QTOF-MS, corresponding to the formula $C_{32}H_{44}O_9$. The ^1H NMR data (Table 1) showed nine methyl singlets at δ 0.96, 1.08, 1.21, 1.33 (6H), 1.42, 1.47, 1.50 and 1.96 (OAc). Two olefinic protons at δ 5.71 (1H, *d*, $J = 2.2$ Hz) and 6.14 (1H, *br s*) were assigned to H-1 and H-6 and two *trans*-coupled olefinic protons of side chain were observed at δ 6.38 (1H, *d*, $J = 15.4$ Hz) and 6.93 (1H, *d*, $J = 15.4$ Hz). Two hydroxymethine signals at δ 4.51 (1H, *br s*) and 4.63 (1H, *m*) were assigned to H-7 and H-16. The decoupled ^{13}C NMR spectra of **1** (Table 1) revealed 32 carbon signals, two ascribed to acetate signals and 30 due to a tetracyclic triterpene skeleton. As evident from DEPT spectra, the ^{13}C NMR data showed nine methyls at δ 18.9, 19.3, 20.5, 21.5, 21.9, 24.6, 25.5, 27.0 and 28.7 (C-30, C-19, C-18, C-28, OAc, C-21, C-26, C-27 and C-29) and three carbonyls at δ 198.5, 214.8 and 203.7 were assignable to C-3, C-11 and C-22, respectively. Four oxygenated functions observed at δ 65.4, 70.5, 78.8, and 79.6 were ascribed to C-7, C-16, C-20 and C-25, respectively. Six olefinic signals at δ 115.3, 120.3, 124.9, 138.3, 145.3 and 150.5 revealed the position of three double bonds at C-1, C-23, C-6, C-5, C-2 and C-24 in which a double bond at C-1 was hydroxylated at C-2 position. The presence of acetate function was characterized by the presence of a singlet methyl signal at δ 1.96 (3H, *s*) in ^1H NMR and it was further confirmed by its ^{13}C NMR values at δ 21.9 and 174.4. The attachment of acetate was assigned at C-25 as its resonance shifted downfield by 6.9 ppm and beta methyl carbons showed upfield shift of 0.6–0.8 ppm. The above data indicated the presence of a cucurbitacin triterpene-type structure for compound **1** (Mata et al., 1998). The ^1H and ^{13}C NMR spectral data of **1** was very similar to that of cucurbitacin E, which was also isolated and characterized in present study, however, difference was observed due a hydroxyl substitution at C-7 position. The methyl protons of acetate showed HMBC correlation with δ 174.4 and C-25 (δ 79.6) (Fig. 2) which further confirmed the attachment of acetate at C-25. The position of hydroxyl group at C-7 was determined on the basis of comparison of ^{13}C NMR values reported earlier (Kanchanapoom et al., 2002). The β-orientation of C-7 hydroxyl group and H-8 was determined on the basis of the appearance of H-8 (δ 2.40) as a broad singlet due to a small coupling constant between H-7 and H-8 ($J < 1.0$ Hz) corresponding to the dihedral angle of approximately 90° (Kanchanapoom et al., 2002). The HMBC data also revealed the position of hydroxyl group at C-7 as the H-7 (δ 4.51) showed correlation with C-5, C-6 and C-8 and H-6 (δ 6.14) with C-5, C-7 and C-8 (Fig. 2).

The MS^2 spectra of **1** generated the fragments at *m/z* 535 $[M+Na-AcOH]^+$ and 517 $[M+Na-AcOH-H_2O]^+$ was due to the sequential loss of a 60 (AcOH) and 18 (H_2O) mass units. The fragment peaks at *m/z* 467 $[M+Na-AcOH-C_5H_7]^+$, 439 $[M+Na-AcOH-C_6H_8O]^+$ and 396 $[M+Na-AcOH-C_8H_{10}O_2]^+$ were observed due to the loss of side chain by the cleavage between C-22–C-23, C-20–C-22 and C-17–C-20. The fragments at *m/z* 203, 187 (due to retro-Diels Alder fragmentation of ring B) and 149 further confirmed the presence of hydroxyl group at C-7 position of ring B. Thus, on the basis of above spectroscopic evidences the structure of compound **1** was unambiguously assigned as 2,7β,16α,20*R*-tetrahydroxy-25-acetoxy-cucurbita-1,5,23*E*-triene-3,11,22-trione and designated as bacobitacin A (Fig. 1).

Compound **5** was isolated as a white amorphous powder. It showed a molecular ion peak at *m/z* 557.3108 $[M+H]^+$ (calcd. 557.3114) in the HRESI-QTOF-MS spectrum in positive mode which corresponded to the molecular formula $C_{32}H_{45}O_8$. The MS^2 spectrum (Scheme 1) showed the fragment ion at *m/z* 497 $[M+H-AcOH]^+$, 479 $[M+H-AcOH-H_2O]^+$ and 383 $[M+H-AcOH-C_6H_8O]^+$ due to the loss of AcOH, H_2O and side chain (C-20–C-22 cleavage), respectively. The structure of compound **5** was characterized as cucurbitacin E by comparison with its ^1H and ^{13}C NMR spectral data with those reported in literature and ESI-QTOF-MS/MS spectra (Vande and Lavie, 1983; Che et al., 1985; Seger et al., 2005).

Compound **2** was obtained as a white amorphous powder. Its positive ESI-QTOF-MS showed a sodiated molecular ion peak at *m/z* 621.3031 $[M+Na]^+$ (calcd. 621.3040) which corresponded to the molecular formula $C_{34}H_{46}O_9$. The ^1H NMR data of **2** (Table 1) showed eight methyl singlets at δ 0.93, 0.98, 1.18, 1.28 (6H), 1.37, 1.47, 1.50 and 1.94 (6H) for H-18, H-19, H-29, H-28,30, H-21, H-27, H-26 and 2× OAc. The corresponding ^{13}C NMR signals for methyls (Table 1) were observed at δ 20.1, 19.8, 27.9, 20.3, 18.3, 24.0, 26.4, 25.9 and 21.7, respectively. The peaks due to two *trans*-coupled olefinic protons were observed at δ 6.36 (1H, *d*, $J = 15.6$ Hz) and 6.96 (1H, *d*, $J = 15.6$ Hz) in ^1H NMR and their corresponding carbons at δ 120.3 and 151.9 that were assigned to the C-23 and C-24, respectively. The ^{13}C NMR spectrum showed three carbonyls at δ 198.7, 212.4 and 202.4 (C-3, C-11 and C-22), three quaternary and one methine oxygenated functions at δ 78.2 (C-20),

Table 1
^{1}H and ^{13}C NMR (300.13 and 75.46 MHz) spectral data in CD_3OD for **1**, **2**, **3** and **4**

No.	**1**		**2**		**3**		**4**	
	δC	δH *m* (J Hz)	δC	δH *m* (J Hz)	δC	δH *m* (J Hz)	δC	δH *m* (J Hz)
1	115.3	5.71 *d* (2.2)	114.8	5.82 *d* (2.1)	122.9	6.51 *d* (2.1)	123.6	6.38 *d* (2.3)
2	145.3		144.4		147.5		146.5	
3	198.5		198.7		198.7		198.7	
4	48.5		47.6		48.5		48.5	
5	138.3		138.5		138.5		138.5	
6	124.9	6.14 *br s*	120.8	5.70 *br s*	120.5	5.71 *br s*	124.5	6.14 *br s*
7	65.4	4.51 *br s*	23.6	1.37 *m*	23.9	1.42 *m*	65.9	4.63 *br s*
8	52.6	2.40 *br s*	41.6	1.97 *m*	41.8	1.96 *m*	52.7	2.43 *br s*
9	50.2		48.8		48.5		50.4	
10	36.7	3.27 *br s*	34.7	3.43 *br s*	34.7	3.56 *br s*	36.2	3.30 *br s*
11	214.8		212.4		212.4		212.7	
12	48.5	3.24 *d* (14.2)	48.8	3.12 *d* (14.4)	49.5	3.06 *d* (14.4)	48.7	3.26 *d* (14.2)
		2.61 *d* (14.2)		2.62 *d* (14.4)		2.61 *d* (14.4)		2.64 *d* (14.2)
13	50.4		51.0		51.5		51.7	
14	48.8		48.8		49.0		49.5	
15	45.2	1.88 *m*, 1.42 *m*	45.5	1.81 *m*, 1.42 *m*	45.2	1.88 *m*, 1.42 *m*	45.8	1.86 *m*, 1.44 *m*
16	70.5	4.63 *m*	78.8	4.29 *m*	71.3	4.51 *m*	71.0	4.53 *m*
17	58.6	2.30 *d* (6.9)	58.2	2.40 *d* (6.9)	58.9	2.48 *d* (6.9)	58.6	2.33 *d* (6.9)
18	20.5	0.96 *s*	20.1	0.93 *s*	20.5	0.87 *s*	20.6	0.99 *s*
19	19.3	1.08 *s*	19.8	0.98 *s*	19.3	0.96 *s*	19.3	1.11 *s*
20	78.8		78.2		78.9		78.2	
21	24.6	1.33 *s*	24.0	1.37 *s*	24.0	1.37 *s*	24.6	1.34 *s*
22	203.7		202.4		202.0		203.7	
23	120.3	6.38 *d* (15.4)	120.3	6.36 *d* (15.6)	120.5	6.38 *d* (15.6)	120.4	6.41 *d* (15.6)
24	150.5	6.93 *d* (15.4)	151.9	6.96 *d* (15.6)	150.5	6.88 *d* (15.6)	150.2	6.88 *d* (15.6)
25	79.6		79.0		79.2		79.6	
26	25.5	1.50 *s*	25.9	1.50 *s*	25.5	1.51 *s*	25.0	1.51 *s*
27	27.0	1.47 *s*	26.4	1.47 *s*	27.2	1.47 *s*	27.2	1.44 *s*
28	21.5	1.33 *s*	20.3	1.28 *s*	20.3	1.28 *s*	21.8	1.34 *s*
29	28.7	1.21 *s*	27.9	1.18 *s*	27.7	1.08 *s*	28.8	1.23 *s*
30	18.9	1.42 *s*	18.3	1.28 *s*	18.3	1.28 *s*	18.6	1.44 *s*
CH$_3$CO	21.9	1.96 *s*	21.7	1.94 *s*	21.5	1.94 *s*	21.8	1.98 *s*
CH$_3$*CO*	174.5		174.4		174.4		176.2	
CH$_3$CO			21.7	1.94 *s*				
CH$_3$*CO*			174.5					
1′ Ara (*p*)					104.1	4.61 *d* (6.7)	104.3	4.80 *d* (6.6)
2′					78.8	4.25[b]	79.2	4.28[b]
3′					72.6[a]	3.91[b]	72.7[a]	4.01[b]
4′					78.2	4.40[b]	78.8	4.51[b]
5′					65.9	3.72, 3.24[b]	64.8	3.73, 3.28[b]
1″Rham					102.5[a]	6.41 *s*	102.2[a]	6.41 *s*
2″					72.6[a]	4.77[b]	72.8[a]	4.76[b]
3″					72.4[a]	4.56[b]	72.7[a]	4.56[b]
4″					74.7[a]	4.34[b]	74.5[a]	4.35[b]
5″					69.9[a]	4.82[b]	69.7[a]	4.81[b]
6″					18.9[a]	1.65 *d* (6.0)	18.6[a]	1.65 *d* (6.1)[b]
1‴Ara (*f*)					108.9	5.21 *br s*	108.7	5.23 *br s*
2‴					93.7	4.44[b]	93.8	4.45[b]
3‴					74.7	3.78[b]	74.3	3.78[b]
4‴					84.1	4.33[b]	84.4	4.32[b]
5‴					62.0	3.65[b]	62.3	3.66[b]
1⁗Rham					102.5[a]	6.20 *s*	102.2[a]	6.21 *s*
2⁗					72.6[a]	4.49[b]	72.8[a]	4.50[b]
3⁗					72.4[a]	4.30[b]	72.7[a]	4.32[b]
4⁗					74.7[a]	4.15[b]	74.5[a]	4.11[b]
5⁗					69.9[a]	4.48[b]	69.7[a]	4.54[b]
6⁗					18.9[a]	1.66 *d* (6.1)	18.6[a]	1.66 *d* (6.1)

[a] Assignments may be interchanged.
[b] Multiplicities could not be determined due to the overlapping signals.

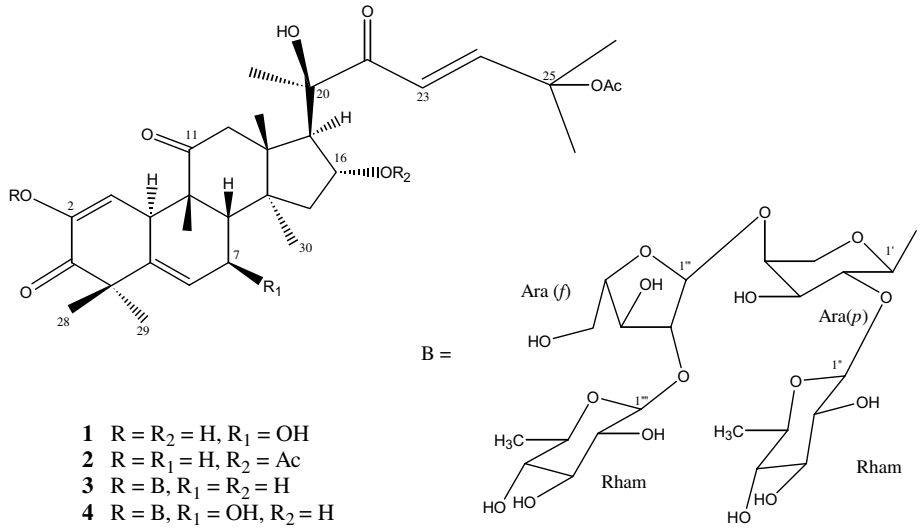

Fig. 1. Structures of compounds **1**, **2**, **3** and **4**.

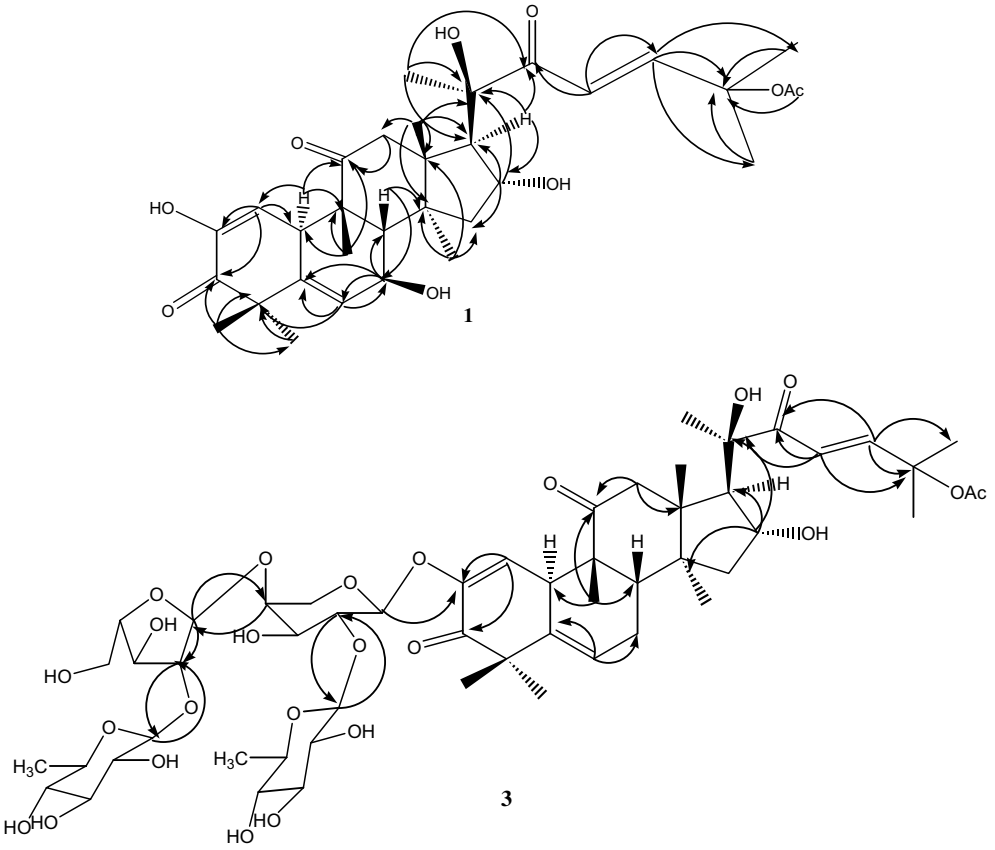

Fig. 2. The significant HMBC (→) correlations of compounds **1** and **3**.

79.0 (C-25), 144.4 (C-2) and 78.8 (C-16). The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **5** (Cucurbitacin E) but the ^{13}C NMR value of C-16 (δ 78.8) was shifted to downfield (7.5 ppm) due to acetate substitution. This attachment was further supported by HMQC, HMBC and mass spectra. In HMBC spectrum H-16 showed correlation with C-13, C-14, C-15, C-17 and C-20, which confirmed the attachment of acetate at C-16. The position of other acetate at C-25 was originally reported with cucurbitacin E. The presence of additional acetate was further confirmed by the analysis of MS^2 spectra of compound **2**. The MS^2 spectrum showed the fragment at m/z 561 $[M+Na-AcOH]^+$ and 501 $[M+Na-2\times AcOH]^+$ due to the sequential loss of two acetates. Other fragments observed were very similar to those of cucurbitacin E (**5**). On the basis of above spectral evidences the structure of

compound **2** was elucidated as 2,20*R*-dihydroxy-16α,25-diacetoxy-cucurbita-1,5,23*E*-triene-3,11,22-trione designated as bacobitacin B (Fig. 1).

Compound **3** was obtained as a white solid showing $[M+Na]^+$ peak in positive mode HRESI-QTOF-MS at *m/z* 1135.4897 (calcd. 1135.4937). On the basis of molecular mass and ^1H and ^{13}C NMR spectral data (Table 1) the molecular formula of **3** was established as $C_{54}H_{80}O_{24}$. The ^1H and ^{13}C NMR spectra were found to be identical with cucurbitacin E (**5**) except the presence of additional peaks of four sugar moieties. In ^1H NMR spectrum, signals for four anomeric protons at δ 6.41 (1H, *s*), 6.20 (1H, *s*), 5.21 (1H, *br s*) and 4.61 (1H, *d*, $J = 6.7$) were correlated with signals for four carbons at δ 102.5, 102.5, 108.9 and 104.1 by HMQC experiment and were diagnostic for the presence of four sugar residues. These anomeric signals together with other ^{13}C NMR values clearly indicated the identity of sugar residues as α-L-rhamnopyranosyl, α-L-rhamnopyranosyl, α-L-arabinofuranosyl and α-L-arabinopyranosyl, respectively. The chemical shift of C-2 and C-1 at δ 147.5 and 122.9 indicated that the four sugars were attached to C-2 of the aglycone (Seger et al., 2005). The ^1H NMR spectrum showed overlapping signals for other protons of sugar residues at δ 3.24–4.82. The linkages between sugar residues were assigned on the basis of ^{13}C NMR values and HMBC correlations. HMBC experiments established correlations between H-1′ of arabinopyranosyl and C-2 of the aglycone moiety, H-1″ of rhamnopyranosyl and C-2′ of arabinose, H-1‴ of arabifuranosyl and C-4′, H-1⁗ of rhamnopyranosyl and C-2‴ of arabinofuranosyl, suggested that a arabinopyranosyl sugar was directly attached to the C-2 of the aglycone whereas a rhamnopyranosyl (terminal) and arabifuranosyl sugars were linked to C-2′ and C-4′ of arabinopyranosyl, respectively and remaining terminal rhamnopyranosyl sugar was linked to the C-2‴ of arabinofuranosyl sugar moiety. The proposed linkages were further supported by the observation of downfield resonances of C-2′, C-4′ and C-2‴ (α-effect) and upfield resonances of adjacent carbons (β-effect). The ^1H, ^{13}C NMR and MS/MS spectral analysis of **3** indicated it to be the glycoside of cucurbitacin E (**5**). ESI-QTOF-MS/MS of **3** displayed the fragment at *m/z* 857 $[M+Na-132-146]^+$ represented glycosidic cleavage by simultaneous loss of an arabinose and a rhamnose sugar residues. Other ion at *m/z* 579 $[M+Na-2\times132-2\times146]^+$ was produced by the further simultaneous loss of an arabinose and a rhamnose sugar residues. In conclusion, **3** was assigned the structure as 2-*O*-[{α-L-rhamnopyranosyl-(1 → 2)-α-L-arabifuranosyl-(1 → 4)}-{α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl}] cucurbitacin E and designated as bacobitacin C (Fig. 1).

Compound **4** was isolated as a white amorphous powder. It showed a molecular ion peak at *m/z* 1151.4862 $[M+Na]^+$ (calcd. 1151.4886) in the HRESI-QTOF-MS spectrum in positive mode which corresponded to the molecular formula $C_{54}H_{80}O_{25}$. The ^1H and ^{13}C NMR spectra were found to be identical with bacobitacin A (**1**) except the presence of additional peaks of four sugar moieties. ^1H NMR spectrum displayed the signals for four anomeric protons at δ 6.41 (1H, *s*), 6.21 (1H, *s*), 5.23 (1H, *br s*) and 4.80 (1H, *d*, $J = 6.6$) and were correlated with signals for four carbons at δ 102.2, 102.2, 108.7 and 104.3 by HMQC experiment and were diagnostic for the presence of four sugar residues. The sugar residues were characterized as α-L-rhamnopyranosyl, α-L-rhamnopyranosyl, α-L-arabinofuranosyl and α-L-arabinopyranosyl on the basis of anomeric values and ^{13}C NMR data of sugars. The HMBC and ^{13}C NMR spectral data of **4** revealed the similar kind of glycosidic linkages as with bacobitacin C (**3**). The ^1H, ^{13}C NMR and MS/MS spectral analysis of **4** indicated it to be the glycoside of bacobitacin A (**1**). ESI-QTOF-MS/MS of **4** showed the fragment ion at *m/z* 873 $[M+Na-132-146]^+$ represents glycosidic cleavage by simultaneous loss of an arabinose and a rhamnose sugar residue. Other ion at *m/z* 595 $[M+Na-2\times132-2\times146]^+$ was produced by the further simultaneous loss of an arabinose and a rhamnose sugar residue. Other fragments were very similar to those of compound **1**. Thus, on the basis of above spectral evidences the structure of **4** was assigned as 2-*O*-[{α-L-rhamnopyranosyl-(1 → 2)-α-L-arabifuranosyl-(1 → 4)}-{α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl}]-7β,16α,20*R*-trihydroxy-25-acetoxy-cucurbita-1,15,23*E*-triene-3,11,22-trione and designated as bacobitacin D.

Other isolated compounds monnieriside II, monnieriside III and plantioside B were characterized by comparison of their ^1H and ^{13}C NMR spectral data with their reported values (Chakaravarty et al., 2002).

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Barnstead Electrothermal 9100. Optical rotation was determined on Horiba Sepa-300 Polarimeter and IR spectra were recorded on a Perkin–Elmer 1760 FT-IR spectrometer with KBr disc. Mass spectra were recorded on QTOF-Micro of Waters Micromass. NMR experiments were performed on Bruker Avance-300 spectrometer. Silica gel (60–120 mesh) for Column chromatography, TLC silica gel 60 F_{254} plates and all other chemicals used were purchased from Merck India Ltd.

3.2. Plant material

The plant material was collected in May 2004 from Institute (IHBT) farm, Palampur, Himachal Pradesh, India.

3.3. Extraction and isolation

Air-dried and powdered aerial parts (2.2 kg) were defatted with petroleum ether (b.p. 60–80 °C) in a soxhlet

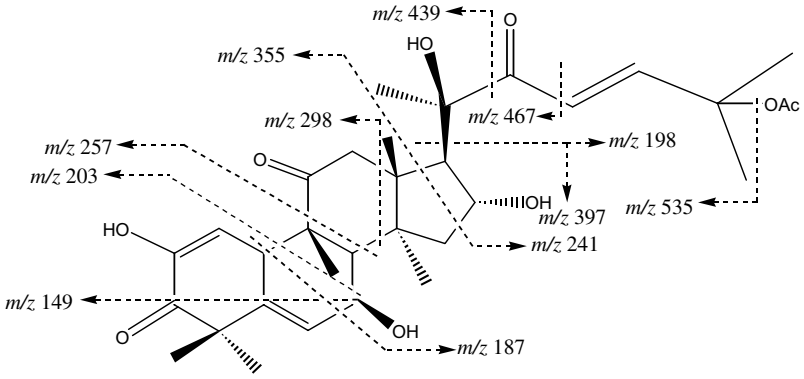

Fig. 3. ESI-MS/MS fragmentation pattern of bacobitacin A (**1**) on the basis of CID spectra.

apparatus. The defatted material was extracted with MeOH:H_2O (1:1, 3 × 2 L) in a percolator at room temperature. The combined MeOH percolations were concentrated followed by dilution with MeOH (250 mL) and kept overnight at room temperature and filtered. The precipitate formed as deep green residue was separated out. The extract was dissolved in water and partitioned, successively, with EtOAc and *n*-BuOH. The EtOAc and *n*-BuOH fractions were dried under vacuum to yield 43.3 g and 57.9 g residues respectively. The EtOAc fraction (43.3 g) was subjected to column chromatography on silica gel (60–120 mesh) using a gradient elution of hexane:ethylacetate and ethylacetate:methanol with increasing proportion of ethylacetate and methanol. A total number of 670 fractions (50 mL each) were collected from the column chromatography. Fractions 445–450, eluted with hexane:ethylacetate (7:3), on further purification by repeated column chromatography yielded a white compound **2** (20 mg). Fractions 490–496, eluted with hexane:ethylacetate (6:4), afforded compound **5** as white amorphous powder. Fractions 501–503, eluted with hexane:ethylacetate (6.5:4.5), yielded compound **1** as white amorphous powder (25 mg) on crystallization in EtOAc. Fractions 580–582 and 588–591, obtained from ethylacetate:methanol (9.5:0.5), on further purification by repeated column chromatography yielded compound **3** (19 mg) and **4** (17 mg), respectively, purified by repeated washings of ethylacetate. Fractions eluted with ethylacetate:methanol (8.5:1.5), yielded monnieriside II (25 mg) while fractions eluted with ethylacetate:methanol (6.5:3.5 and 6:4) afforded monnieriside III (20 mg) and plantioside B (23 mg), respectively.

3.4. Bacobitacin A (*1*)

White amorphous powder; $[\alpha]_D^{19}$ −33.2° ($c = 0.72$, MeOH); m.p. 150–152 °C; IR (KBr) ν_{max} cm^{-1}: 3460, 2980, 1720, 1700, 1460, 1375, 1250, 1036, 790; ^1H NMR (300.13 MHz, CD_3OD) and ^{13}C NMR (75.46 MHz, CD_3OD) see Table 1; HR-ESI-QTOF-MS *m/z*: 595.2876 $[M+Na]^+$ (calcd. for $C_{32}H_{44}O_9$, 595.2883); MS^2 *m/z*: 535 $[M+Na-AcOH]^+$, 517 $[M+Na-AcOH-H_2O]^+$, 467 $[M+H-AcOH-C_5H_7]^+$, 439 $[M+H-AcOH-C_6H_8O]^+$, 396 $[M+H-AcOH-C_8H_{10}O_2]^+$, 369, 355, 337, 298, 241, 257, 203, 198, 187, 149 (see Fig. 3).

3.5. Bacobitacin B (*2*)

White amorphous powder; $[\alpha]_D^{19}$ −42.5° ($c = 0.53$, MeOH); m.p. 135–137 °C; ^1H NMR (300.13 MHz, CD_3OD) and ^{13}C NMR (75.46 MHz, CD_3OD) see Table 1; HR-ESI-QTOF-MS *m/z*: 621.3031 $[M+Na]^+$ (calcd. for $C_{34}H_{46}O_9$, 621.3040); MS^2 *m/z*: 561 $[M+Na-AcOH]^+$, 501 $[M+Na-2\times AcOH]^+$, 543 $[M+Na-AcOH-H_2O]^+$, 525 $[M+Na-AcOH-2\times H_2O]^+$, 465 $[M+Na-2\times AcOH-2\times H_2O]^+$, 397, 315, 297, 178, 161.

3.6. Bacobitacin C (*3*)

White amorphous powder; $[\alpha]_D^{19}$ −46.7° ($c = 0.61$, MeOH); ^1H NMR (300.13 MHz, CD_3OD) and ^{13}C NMR (75.46 MHz, CD_3OD) see Table 1; HR-ESI-QTOFMS *m/z*: 1135.4897 $[M+Na]^+$ (calcd. for $C_{54}H_{80}O_{24}$, 1135.4937); MS^2 *m/z*: 857 $[M+Na-132-146]^+$, 579 $[M+Na-2\times132-2\times146]^+$.

3.7. Bacobitacin D (*4*)

White amorphous powder; $[\alpha]_D^{19}$ −44.3° ($c = 0.008$, MeOH); ^1H NMR (300.13 MHz, CD_3OD) and ^{13}C NMR (75.46 MHz, CD_3OD) see Table 1; HR-ESI-QTOFMS *m/z*: 1151.4862 $[M+Na]^+$ (calcd. for $C_{54}H_{80}O_{25}$, 1151.4886); MS^2 *m/z*: 873 $[M+Na-132-146]^+$, 595 $[M+Na-2\times132-2\times146]^+$.

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