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Studies on the Constituents of the Stems of *Tinospora tuberculata* BEUMÉE. III.[1] New Diterpenoids, Borapetoside B and Borapetol B

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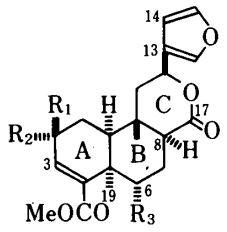
A new diterpene glucoside, borapetoside B (**1**), and its aglycone, borapetol B (**2**), were isolated from the stems of *Tinospora tuberculata* BEUMÉE as the bitter principles, and their structures were elucidated.

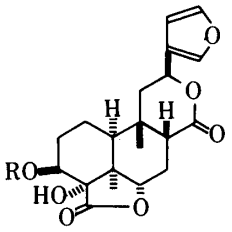
Keywords——*Tinospora tuberculata*; Menispermaceae; borapet; borapetoside B; borapetol B; bitter principle; diterpenoid; glycoside

In the preceding paper,[1,2] the structures of a phenolic glucoside, tinotuberide, two phenolic amides, *N-cis*- and *N-trans*-feruloyl tyramine, and two diterpenoids, borapetoside A (**3**) and borapetol A (**4**), isolated from the stems of *Tinospora tuberculata* BEUMÉE (syn. *T. crispa* DIERS; Thai name, borapet; Menispermaceae), were reported.

In the present work, a new diterpene glucoside, borapetoside B (**1**), and its aglycone, borapetol B (**2**) were isolated from the same source and their structures were elucidated.

Repeated chromatography of the fraction from which **3** had been isolated[1] on silicic acid columns with $CHCl_3$–MeOH–H_2O gave a new diterpene glucoside, borapetoside B (**1**), colorless needles, mp 153—154 °C, $C_{27}H_{36}O_{12}$, M^+ *m/z*: 552, $[\alpha]_D$ −15.7°, having a very bitter taste. The positive Ehrlich's test, infrared (IR) spectrum (1505, 870 cm^{-1}), mass spectrum (MS) (*m/z*: 81, 94) and ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum (Table I) of **1** showed the existence of a β-mono-substituted furan ring in **1**. On acetylation of **1** with acetic





1: R_1=H, R_2=OH, R_3=*O*-β-D-glc.pyr.
2: R_1=H, R_2=R_3=OH
5: R_1=H, R_2=OAc, R_3=*O*-β-D-glc.pyr.$(Ac)_4$
6: R_1=H, R_2=R_3=OAc
7: R_1,R_2=O, R_3=*O*-β-D-glc.pyr.
8: R_1=R_2=R_3=H

3: R=β-D-glc.pyr.
4: R=H

Chart 1

anhydride in pyridine at room temperature, a pentaacetate (**5**), colorless needles, mp 249—250 °C, $C_{37}H_{46}O_{17}$, $[\alpha]_D$ +17.0°, was obtained. Enzymatic hydrolysis of **1** with crude hesperidinase gave D-glucose, $[\alpha]_D$ +53.2°, and an aglycone, borapetol B (**2**), colorless

TABLE I. ^{1}H-NMR Data for Borapetoside B (**1**) and Related Compounds (δ)

Hydrogen	**1**[a]	**5**[b]	**2**[a]	**2**[b]	**6**[b]	**7**[a]	**4**[b]
2			4.5—4.1[d] (1H, m)	4.8—4.4[d] (1H, m)	5.58 (1H, ddd, J=3.3, 6.1, 8.2)		
3	6.34 (1H, d, J=3.5)	6.49—6.45[d] (1H, m)	6.22 (1H, d, J=3.7)	6.42 (1H, d, J=3.7)	6.43 (1H, d, J=3.3)	6.17 (1H, s)	4.02 (1H, m)
6			4.5—4.1[d] (1H, m)	4.8—4.4[d] (1H, m)	5.97 (1H, dd, J=2.0, 3.5)		5.05 (1H, dd, J=4.5, 12.0)
7				1.75 (1H, m) 2.2 (1H, m)			
8		3.30 (1H, dd, J=3.2, 12.3)		3.30 (1H, dd, J=2.5, 12.5)	3.02 (1H, dd, J=4.0, 11.5)		2.71 (1H, dd, J=5.0, 13.0)
12	5.49 (1H, dd, J=6.0, 10.0)	5.58 (1H, m)	5.49 (1H, dd, J=6.0, 10.0)	5.39 (1H, dd, J=6.0, 9.5)	5.40 (1H, dd, J=7.9, 9.2)	5.49 (1H, dd, J=7.7, 9.2)	5.69 (1H, dd, J=5.0, 12.0)
14	6.56 (1H, dd, J=0.9, 2.0)	6.49—6.45[d] (1H, m)	6.54 (1H, m)	6.42 (1H, m)	6.41 (1H, m)	6.55 (1H, m)	6.44 (1H, m)
15	7.65 (1H, dd, J=2.0, 1.6)	7.49—7.43[e] (1H, m)	7.70—7.60[e] (1H, m)	7.50—7.40[e] (1H, m)	7.43—7.46[e] (1H, m)	7.65 (1H, m)	7.43 (1H, m)
16	7.71 (1H, dd, J=1.6, 0.9)	7.49—7.43[e] (1H, m)	7.70—7.60[e] (1H, m)	7.50—7.40[e] (1H, m)	7.43—7.46[e] (1H, m)	7.72 (1H, m)	7.48 (1H, m)
$-COOCH_3$	3.69 (3H, s)	3.76 (3H, s)	3.68 (3H, s)	3.71 (3H, s)	3.78 (3H, s)	3.80 (3H, s)	
$C-CH_3$	1.45 (3H, s)	1.39 (3H, s)	1.35 (3H, s)	1.48 (3H, s)	1.40 (3H, s)	1.56 (3H, s)	1.22 (3H, s)
	0.85 (3H, s)	1.00 (3H, s)	0.85 (3H, s)	0.95 (3H, s)	1.04 (3H, s)	0.71 (3H, s)	1.14 (3H, s)
Sugar[c]	4.27 (1H, d, J=7.2)	4.14 (1H, d, J=5.1)				4.27 (1H, d, J=7.2)	
$-OCOCH_3$		2.17 (3H, s) 2.09 (3H, s) 2.06 (3H, s) 2.04 (3H, s) 2.01 (3H, s)			2.10 (3H, s) 2.06 (3H, s)		

a) Measured in DMSO-d_6 at 90 MHz. *b*) Measured in $CDCl_3$ at 90 MHz. *c*) Anomeric proton. *d,e*) Overlapped.

granules, $C_{21}H_{26}O_7$, $[\alpha]_D$ −14.1°. This aglycone (**2**) gave a diacetate (**6**), $C_{25}H_{30}O_9$, M^+ *m/z*: 474, showing no hydroxy group in the IR, on acetylation with acetic anhydride and pyridine at room temperature.

The IR absorption maxima at 1725 and 1705 cm^{-1} in **1**, and 1721 (br), 1245 and 1155 cm^{-1} in **2**, and the ^{13}C-NMR (DMSO-d_6) signals at δ 174.6 (s), 167.5 (s) and 51.6 (q) in **1**, and δ 174.8 (s), 168.0 (s) and 51.5 (q) in **2**, suggested that these compounds had a δ-lactone ring and a methoxycarbonyl group. A trisubstituted double bond was also suggested by the IR absorption at 1639 cm^{-1}, the ^{1}H-NMR (DMSO-d_6) signal at δ 6.22 (1H, d, J=3.7 Hz), and ^{13}C-NMR signals at δ 139.1 (d) and 138.5 (s) in the aglycone (**2**).

Both the ^{1}H- and ^{13}C-NMR (Tables I and II) spectra of these compounds showed the existence of two angular methyl groups (C-19 and C-20) and a δ-lactone ring between C-8 and C-12 with a β-substituted furan ring at the δ-position, resembling those of borapetol A (**4**).[1] The ^{13}C-NMR spectrum of **2** (Table II) also showed signals attributable to two tertiary

TABLE II. ^{13}C-NMR Data for Borapetoside B (**1**) and Related Compounds (δ)

Carbon	Multiplicity	**1**[a]	**5**[b]	**2**[a]	**2**[b]	**6**[b]	**7**[a]	**4**[b]
1	t	28.0[c]	25.2[c]	28.3[c]	28.7[c]	25.1	34.9	17.5[c]
2	d	62.2	66.4	62.4	64.0	66.4	197.8 s	25.5 t[c]
3	d	140.2	135.5	139.1	138.7	134.9	131.4	76.1
4	s	137.4	140.8	138.5	139.7	139.6	154.7	81.1
5	s	40.9	41.1	42.4	40.9	39.9	42.0	46.1
6	d	79.0	80.2	66.8	68.4	71.3	76.7[e]	71.4[f]
7	t	26.4[c]	25.7[c]	27.5[c]	27.3[c]	25.1	27.2	28.2
8	d	49.2	49.9	49.1	49.9	52.2	47.3	47.1[g]
9	s	36.5	37.1	36.7	37.2	37.3	36.8	35.2
10	d	40.4	40.3	40.3	40.2	41.2	40.4	47.4[g]
11	t	44.0	45.2	44.3	45.5	45.1	42.9	44.2
12	d	69.9[d]	70.2[d]	69.5	70.4	70.2	69.9[d]	70.9[f]
13	s	124.5	124.3	124.7	124.4	124.2	124.3	124.3
14	d	109.2	108.6	109.1	108.6	108.5	109.3	108.4
15	d	140.2[h]	139.7[h]	140.1[h]	139.7[h]	140.9[h]	140.5[h]	139.8[h]
16	d	143.7[h]	143.7[h]	143.7[h]	143.7[h]	143.8[h]	143.8[h]	143.9[h]
17	s	174.6	174.5	174.8	175.5	173.9	174.2	173.0
18	s	167.5	167.0	168.0	168.1	166.9	166.7	179.8
19	q	28.4	28.8	29.0	29.4	28.6	26.8	33.1
20	q	22.8	23.5	22.7	23.4	23.2	22.4	18.0
$-COOCH_3$	q	51.6	52.1	51.5	52.0	50.6	52.7	
Sugar								
1′	d	104.6	102.0				104.5	
2′	d	73.9	72.1				73.9	
3′	d	77.4[e]	72.9[e]				76.9[e]	
4′	d	69.4[d]	68.5[d]				69.4[d]	
5′	d	76.6[e]	71.6[e]				76.6[e]	
6′	t	61.0	62.1				61.0	
$-OCOCH_3$	s		170.7			170.5		
	s		170.5			169.4		
	s		170.0					
	s		169.4					
$-OCOCH_3$	q		21.0			21.1		
	q		20.7					
	q		20.5					

a) Measured in DMSO-d_6 at 22.5 MHz. *b*) Measured in $CDCl_3$ at 22.5 MHz. *c*—*h*) Assignments are interchangeable in each column.[3]

carbons (C-8 and C-10), two quaternary carbons (C-5 and C-9), three secondary carbons (C-1, C-7 and C-11), and three oxygenated methine groups (C-2, C-6 and C-12). These observations suggested that this aglycone (**2**) has a *cis* clerodane skeleton, like borapetol A (**4**).[1]

In the ^{1}H-NMR spectrum of **2**, the signals at δ 1.75 (1H, m) and 2.2 (1H, m) collapsed on irradiation at either 4.67 (H-6, 1H, m) or 3.30 (H-8, 1H, dd, J=2.5, 12.5 Hz). The signals at 4.67 and 3.30 were found to be shifted in the diacetate (**6**) to δ 5.97 (1H, dd, J=2.0, 3.5 Hz) and 3.02 (1H, dd, J=4.0, 11.5 Hz). These observations suggested a partial structure having an equatorial hydrogen and an axial hydroxyl group at C-6, a methylene at C-7, an axial hydrogen and a carbonyl group of the δ-lactone at C-8, and two angular methyl groups at C-5 and C-9. The structure of ring A was suggested by ^{1}H-NMR double resonance experiments on **6**, at δ 1.63, 2.37 (H-1, each 1H, m), 5.58 (H-2, 1H, ddd, J=3.3, 6.1, 8.2 Hz), and 6.43 (H-3, 1H, d, J=3.3 Hz). The methoxy carbonyl group at C-4, mentioned above, was deduced as the only functional group which could be located on the trisubstituted ethylene at C-3 and C-4.

Though the signal (δ 29.4) assigned to the methyl group at C-5 in the ^{13}C-NMR of **2** indicates the A/B *cis* ring juncture for the same reason as mentioned previously,[1,4] the chemical shift was observed at 3.4 ppm higher field than in floribundic acid methyl ester (**8**) (δ 32.8),[5] possessing a very similar structure. This observation suggested that the hydroxy group at C-6 is *cis* oriented with respect to the methyl group at C-5.

On reflux with acetic anhydride and anhydrous sodium acetate (conditions which cause inversion at C-8 of columbin to isocolumbin acetate[6]), **1** gave no product other than **5**. This fact indicated the B/C ring juncture to be the stable *trans* form. 2-Oxoborapetoside B (**7**), obtained from **1** by oxidation with Jones' reagent, showed a positive Cotton effect, $[\theta] = +109$ (315 nm), in the circular dichroism (CD) curve, due to the effect of the α,β-unsaturated ketone, and this indicated the absolute configuration of ring A to be as shown in the formula.

On the basis of these results, the structure of borapetol B was elucidated as methyl (2*R*,5*R*,6*S*,8*S*,9*S*,10*S*,12*S*)-15,16-epoxy-2,6-dihydroxy-cleroda-3,13(16),14-trien-17,12-olacton-18-oate (**2**).

The coupling constant (7.2 Hz) of the signal at δ 4.27 in the ^{1}H-NMR spectrum of **1**, due to the anomeric hydrogen of the glucopyranose, indicates the glycosidic linkage to have β-configuration. A remarkable glycosidation shift[7] of the signal attributable to C-6 (δ 66.8 in **2** and 79.0 in **1**) was observed, and suggested borapetoside B (**1**) to be borapetol B 6-*O*-β-D-glucopyranoside.

Borapetol B (**2**) was also found in the *n*-hexane extract, and was shown to be identical with the aglycone obtained from **1** by direct comparisons.

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. The IR spectra were measured with a Hitachi EPI-G3 or a Shimadzu IR-408 spectrometer. The ultraviolet (UV) spectra, ^{1}H-NMR, ^{13}C-NMR, MS and CD were recorded using a Hitachi 200-20 spectrometer, a JEOL FX-90Q FT-NMR spectrometer (chemical shifts are expressed in δ value (ppm), with tetramethylsilane as an internal standard), a JEOL JMS-D100 mass spectrometer, and a JASCO J20C automatic recording spectropolarimeter, respectively.

Borapetoside B (1)——The butanol extract of the crude drug (20 kg; dried stems of *Tinospora tuberculata* BEUMÉE)[1] was separated into 3 fractions by droplet counter current chromatography (DCCC) ($CHCl_3$–MeOH–H_2O, 35 : 65 : 40; upper layer as the moving phase). The second fraction was chromatographed repeatedly over silicic acid columns with $CHCl_3$–MeOH–H_2O (10 : 1 : 0.1) and $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2) as eluants, yielding borapetoside B (**1**, 6.2 g), colorless needles (from MeOH–EtOAc), mp 153—154 °C, $[\alpha]_D$ −15.7 ° (c = 1.275, MeOH). *Anal.* Calcd for $C_{27}H_{36}O_{12} \cdot 1/2H_2O$: C, 57.75; H, 6.64. Found: C, 57.58; H, 6.53. IR ν_{max}^{KBr} cm^{-1}: 3650—3225, 1725, 1705, 1505, 872. MS *m/z*: 552 (M^+), 95, 94, 81. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Borapetoside B Pentaacetate (5)——Acetylation of **1** (640 mg) with acetic anhydride in pyridine at room temperature gave borapetoside B pentaacetate (**5**, 260 mg), colorless needles (from acetone), mp 249—250 °C, $[\alpha]_D$ +17.0 ° (c = 1.84, $CHCl_3$). *Anal.* Calcd for $C_{37}H_{46}O_{17} \cdot 1/2H_2O$: C, 57.58; H, 6.14. Found: C, 57.40; H, 5.91. IR ν_{max}^{KBr} cm^{-1}: 1755, 1735, 1715, 1705, 1505, 872. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

On reflux with acetic anhydride (5 ml) and anhydrous sodium acetate (200 mg) for 5 h, **1** (100 mg) gave only **5** (52 mg), without any other product.

Enzymatic Hydrolysis of Borapetoside B (1)——A mixture of **1** (1.1 g) and crude hesperidinase (5 mg, Tanabe Co., Ltd.) in AcOH–NaOAc buffer (pH 4.5, 60 ml) was incubated at 37 °C for 3 weeks. Usual work-up yielded an aglycone, borapetol B (**2**, 0.4 g), colorless granules (from acetone–H_2O), mp 117—118 °C, $[\alpha]_D$ −14.1 ° (c = 1.165, MeOH). *Anal.* Calcd for $C_{21}H_{26}O_7 \cdot 1/2H_2O$: C, 63.15; H, 6.81. Found: C, 63.17; H, 6.83. IR ν_{max}^{KBr} cm^{-1}: 3600—3250, 1721, 1639, 1509, 1245, 1155, 872. MS *m/z*: 390 (M^+), 372, 358. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

From the aqueous layer of this reaction mixture, D-glucose (126 mg), $[\alpha]_D$ +53.2 ° (c = 2.03, H_2O), was obtained. It was identical with a standard sample of D-glucose (Wako Chem. Co., Ltd.).

Borapetol B (2)——The *n*-hexane-soluble fraction of the crude MeOH extract (crude drug, 20 kg)[1] was subjected to DCCC ($CHCl_3$–MeOH–H_2O, 65 : 35 : 20; lower layer as the moving phase) and column chromatography (silicic acid, $CHCl_3$–MeOH; 98 : 2) successively, yielding two fractions. The more polar fraction gave colorless granules (from acetone–H_2O, 0.706 g), which were identified as borapetol B (**2**) by IR, ^{1}H- and ^{13}C-NMR comparisons, and mixed melting point determination.

Borapetol B Diacetate (6)——On acetylation with acetic anhydride (5 ml) in pyridine (5 ml) at room temperature,

2 (200 mg) gave borapetol B diacetate (**6**, 180 mg), colorless granules (from acetone–H_2O), mp 118—119 °C, $[\alpha]_D$ +34.0° (c=0.67, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1730, 1635, 1500, 872. MS m/z: 474 (M^+), 94, 81. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

2-Oxoborapetoside B (7)——Oxidation of **1** (1.4 g) with Jones' reagent (1.5 ml) for 2 min at 0 °C afforded 2-oxoborapetoside B (**7**, 358 mg), white powder (from MeOH–H_2O), mp 125—129 °C, $[\alpha]_D$ −27.7° (c=0.505, MeOH). CD (c=0.505, MeOH) $[\theta]^{22}$ (nm): 0 (322), +109 (315) (positive maximum), 0 (307). *Anal.* Calcd for $C_{27}H_{34}O_{12}\cdot 3/2H_2O$: C, 56.15; H, 6.46. Found: C, 56.15; H, 6.41. IR ν_{max}^{KBr} cm^{-1}: 3700—3200, 1725, 1675, 872. UV λ_{max}^{MeOH} nm (ε): 213 (6998), 244 (3572). MS m/z: 550 (M^+), 372, 371. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

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