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

NAOMICHI FUKUDA,[a] MICHIKO YONEMITSU,[a] TAKEATSU KIMURA,*,[a]
SHIZUKO HACHIYAMA,[b,2a)] KAZUMOTO MIYAHARA,[b,2b)]
and TOSHIO KAWASAKI[b,2b)]

*Daiichi College of Pharmaceutical Sciences,[a] 22–1, Tamagawa-cho, Minami-ku,
Fukuoka 815, Japan and Faculty of Pharmaceutical Sciences,
Kyushu University,[b] 3–1–1, Maidashi,
Higashi-ku, Fukuoka 812, Japan*

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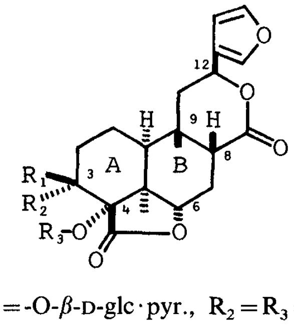
A new diterpene glucoside, borapetoside A (**1**), and its aglycone, borapetol A (**2**), were isolated from the stems of *Tinospora tuberculata* BEUMÉE as the bitter principles, and their structures were elucidated.

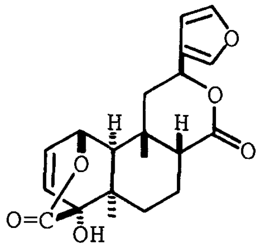
Keywords——*Tinospora tuberculata*; Menispermaceae; borapet; borapetoside A; borapetol A; bitter principle; diterpenoid; glycoside; X-ray analysis

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

In this paper, we report two new diterpenoid bitter principles, borapetoside A (**1**) and borapetol A (**2**), newly isolated from the same source. The butanol fraction, described in the preceding paper,[1] was separated into 3 fractions by droplet counter current chromatography (DCCC), and tinotuberide was isolated from the third fraction. Repeated chromatography of the second fraction on silicic acid columns with $CHCl_3$–MeOH–H_2O, gave a new diterpene glucoside, borapetoside A (**1**), colorless needles, mp 172—173 °C, $C_{26}H_{34}O_{12}$, M^+ *m*/*z* 538, $[\alpha]_D$ +2.60 °, having a very bitter taste.



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



7

Chart 1

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

Hydrogen	**1**[a]	**4**[b]	**2**[c]	**2**[b]	**6**[b]	**7**[c]
1						5.30 (1H, dd, J=2.0, 5.2)
3	4.04 (1H, m)	3.87 (1H, m)	4.02 (1H, m)	4.02 (1H, m)	5.10 (1H, m)	6.2—6.5 (2H, 2-H and 3-H, m)
6	5.02 (1H, dd, J=6.6, 9.0)	4.67 (1H, dd, J=6.0, 12.0)	5.04 (1H, dd, J=4.8, 12.0)	5.05 (1H, dd, J=4.5, 12.0)	4.84 (1H, dd, J=4.4, 11.4)	
8	2.78 (1H, dd, J=9.0, 9.0)	2.77 (1H, dd, J=6.0, 13.2)	2.72 (1H, dd, J=5.4, 12.7)	2.71 (1H, dd, J=5.0, 13.0)	2.77 (1H, dd, J=6.0, 12.0)	2.40 (1H, dd, J=3.5, 13.5)
12	5.90 (1H, dd, J=6.0, 12.0)	5.67 (1H, dd, J=6.0, 12.0)	5.70 (1H, dd, J=6.0, 12.0)	5.69 (1H, dd, J=5.0, 12.0)	5.71 (1H, dd, J=5.2, 11.6)	5.56 (1H, dd, J=4.4, 12.4)
14	6.55 (1H, dd, J=0.9, 1.8)	6.42 (1H, dd, J=0.9, 1.8)	6.44 (1H, m)	6.44 (1H, m)	6.44 (1H, dd, J=0.9, 1.7)	6.6 (1H, m)
15	7.51 (1H, dd, J=1.8, 1.8)	7.41 (1H, dd, J=1.8, 1.8)	7.42 (1H, m)	7.43 (1H, m)	7.44 (1H, dd, J=1.6, 1.7)	7.4 (1H, m)
16	7.64 (1H, dd, J=0.9, 1.8)	7.46 (1H, dd, J=0.9, 1.8)	7.48 (1H, m)	7.48 (1H, m)	7.50 (1H, dd, J=0.9, 1.6)	7.7 (1H, m)
CH_3	1.23 1.12	1.19 1.14	1.22 1.14	1.22 1.14	1.25 1.14	1.11 0.86
Sugar	4.33[d] (1H, d, J=7.8)					
OH			3.50[e] (2H)	3.30[e] (2H)	3.52[e] (1H)	
CH_3CO		1.99, 2.02, 2.09, 2.11			2.09	

a) Measured in CD_3OD at 100 MHz. *b*) Measured in $CDCl_3$ at 90 MHz. *c*) Measured in DMSO-d_6 at 90 MHz. *d*) Anomeric proton. *e*) Confirmed by addition of D_2O.

Positive Ehrlich's test, infrared spectra (IR; 1505, 870 cm^{-1}), mass spectra (MS; m/z 81, 94) and ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra (Table I) of **1** and **2** showed the existence of a β-mono-substituted furan ring in **1**.

On acetylation of **1** with acetic anhydride in pyridine at room temperature, a tetraacetate (**4**), colorless needles, $C_{34}H_{42}O_{16}$, $[\alpha]_D$ −9.20 °, was obtained. As this tetraacetate (**4**) still possessed a free hydroxy group (IR; 3655, 3537 cm^{-1}), it was refluxed with acetic anhydride in pyridine, and a pentaacetate (**5**), colorless needles, $C_{36}H_{44}O_{17}$, $[\alpha]_D$ 0.0 °, was obtained.

On enzymatic hydrolysis of **1** with crude hesperidinase, D-glucose, $[\alpha]_D$ +49.1 °, and an aglycone, borapetol A (**2**), colorless needles, $C_{20}H_{24}O_7 \cdot CH_3OH$, $[\alpha]_D$ +28.1 °, were obtained. This aglycone gave a monoacetate (**6**), $C_{22}H_{26}O_8$, M^+ m/z 418, showing IR absorption at 3500 cm^{-1} (OH), on acetylation at room temperature. Thus, **1** was deduced to have four hydroxy groups on a glucoside moiety in addition to a tertiary hydroxy group.

IR absorption maxima at 1762 and 1703 cm^{-1} in **1**, and 1769 and 1721 cm^{-1} in **2**, and ^{13}C-NMR signals [δ 172.3 (s) an 178.1 (s) in **2**], suggested that these compounds had both a γ- and a δ-lactone ring. The tertiary hydroxy group was found to be located at the α-position of

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

Carbon	Multiplicity	**1**$^{a)}$	**2**$^{a)}$	**2**$^{b)}$	**6**$^{b)}$	**4**$^{b)}$	**5**$^{b)}$	**7**$^{a)}$
1	t	17.4$^{f)}$	17.5$^{f)}$	17.5$^{f)}$	17.4$^{f)}$	17.3$^{f)}$	16.9$^{f)}$	73.0 d
2	t	24.6$^{f)}$	24.7$^{f)}$	25.5$^{f)}$	25.4$^{f)}$	25.3$^{f)}$	25.4$^{f)}$	130.0 d
3	d	75.6$^{d)}$	74.3	76.1	75.8	81.0	78.5	135.5 d
4	s	79.5	80.5	81.1	75.6	80.0	87.9	80.2
5	s	45.2	45.4	46.1	46.1	45.8	46.9	36.7
6	d	69.8$^{c)}$	69.5$^{c)}$	71.4$^{c)}$	72.7$^{c)}$	70.5	70.6	25.6 t
7	t	27.2$^{f)}$	28.1$^{f)}$	28.2$^{f)}$	25.5$^{f)}$	27.3$^{f)}$	28.4$^{f)}$	16.9
8	d	46.3$^{e)}$	46.5$^{e)}$	47.1$^{e)}$	47.0$^{e)}$	47.0$^{e)}$	46.3$^{e)}$	43.2$^{e)}$
9	s	34.3	34.4	35.2	35.1	35.0	35.3	34.5
10	d	46.1$^{e)}$	46.3$^{e)}$	47.4$^{e)}$	47.0$^{e)}$	46.8$^{e)}$	46.1$^{e)}$	45.0$^{e)}$
11	t	42.9	43.0	44.2	44.0	44.2	44.0	39.9
12	d	69.4$^{c)}$	69.4$^{c)}$	70.9$^{c)}$	70.9$^{c)}$	70.5	70.6	70.0
13	s	124.2	124.3	124.3	124.2	124.0	124.4	124.9
14	d	108.7	108.7	108.4	108.4	108.2	108.3	108.9
15	d	140.3	140.3	139.8	139.8	139.5	139.6	140.0
16	d	143.5	143.5	143.9	143.8	143.6	143.8	143.4
17	s	172.6	172.3	173.0	172.9	172.4	172.1	173.1
18	s	177.6	178.1	179.8	177.6	177.8	171.4	174.1
19	q	32.6	32.4	33.1	32.9	32.9	33.2	27.0
20	q	17.9	17.5	18.0	18.5	18.5	18.5	23.6
Sugar								
1′	d	102.4				101.8	101.9	
2′	d	74.1				71.5	71.8	
3′	d	76.7$^{d)}$				75.2$^{d)}$	74.5$^{d)}$	
4′	d	73.1				68.4	68.6	
5′	d	75.8$^{d)}$				72.2$^{d)}$	72.3$^{d)}$	
6′	t	60.7				61.8	61.8	
$\mathrm{CH_3\underline{C}O}$	s				161.9	169.2	168.7	
						169.5	169.4	
						169.7	169.7	
						170.2	169.9	
							170.4	
$\mathrm{\underline{C}H_3CO}$	q				21.2	20.5	20.5	
						20.7	20.6	
							20.9	
							21.7	

a) Measured in DMSO-d_6 at 22.5 MHz. *b*) Measured in $\mathrm{CDCl_3}$ at 22.5 MHz. *c*—*f*) Assignments are interchangeable in each column.

one of these carbonyl groups, since the signals at δ 80.0 (s) and 177.8 (s) in the ^{13}C-NMR spectrum of **4** shifted to δ 87.9 (s) and 171.4 (s) upon vigorous acetylation.

The ^{1}H- and ^{13}C-NMR spectra (Tables I and II) both showed the other lactone ring to be very similar to that of columbin (**7**),[3] which possesses a δ-lactone ring between C-8 and C-17 with a β-substituted furan ring at the δ-position.

In addition to the signals assigned to the two carbonyl carbons and the four olefinic carbons of the furan ring, the ^{13}C-NMR spectrum of **2** (Table II) showed signals attributable to two angular methyl groups (C-19 and C-20), two tertiary carbons (C-8 and C-10), two quaternary carbons (C-5 and C-9), four secondary carbons (C-1, C-2, C-7 and C-11), a quaternary oxygen-bearing carbon (C-4), and three oxygenated methine groups (C-3, C-6 and C-12). The signals at δ 69.4 and 69.5 (in DMSO-d_6) in **2** showed no significant difference from the corresponding signals in **1**, **4** and **5**, and were assigned to C-6 and C-12. Comparison of these observations with the NMR spectra of columbin (**7**) led us to conclude that the aglycone

(**2**) was a diterpenoid having a clerodane skeleton.

In the ^{1}H-NMR spectrum of **1**, the double doublet signal at δ 5.90 (J=6.0, 12.0 Hz), also found at δ 5.56 (J=4.4, 12.4 Hz) in **7**, was observed as a sharp doublet on irradiation at either δ 2.05 (m) or 2.31 (m), and was assigned as an axial hydrogen at C-12 adjacent to a lactone oxygen, a furan ring and a methylene (C-11). The broad singlet at δ 4.02 in the ^{1}H-NMR spectrum of **2** was shifted downfield on formation of the acetate (**6**) (δ 5.10, m). The corresponding signal at δ 3.87 in the ^{1}H-NMR spectrum of **4** was sharpened on irradiation at either δ 1.86 or 2.23, and was assigned to an equatorial hydrogen at C-3 adjacent to a methylene group.

The double doublet signal at δ 4.67 (H-6, J=6.0, 12.0 Hz) in the spectrum of **4** was observed as a sharp doublet on irradiation at either δ 1.86 or 2.23. The double doublet signal of **2** at δ 2.72 (J=5.4, 12.7 Hz) was found to be very similar to the signal of columbin (**7**) [δ 2.40 (1H, dd, J=3.5, 13.5 Hz)] which was assigned to the axial hydrogen at C-8. The signals of **1** at δ 2.24 (1H, m) and 2.41 (1H, m) collapsed on irradiation at either δ 2.78 (H-8, dd, J=9.0, 9.0 Hz) or 5.02 (H-6, dd, J=6.6, 9.0 Hz), which correspond to the signals of **2** at δ 2.72 and 5.04 and those of **4** at δ 2.77 and 4.67. These observations suggested a partial structure having an axial hydrogen and the oxygen of the γ-lactone at C-6, a methylene at C-7, an axial hydrogen and the carbonyl group of the δ-lactone at C-8, and two angular methyl groups at C-5 and C-9. The γ-lactone ring was also suggested to include C-4, C-5 and C-6, because there

TABLE III. Fractional Atomic Coordinates ($\times 10^4$) and Anisotropic Thermal Parameters ($\times 10^3$)

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(1)	8599 (4)	5049 (7)	2406 (6)	36 (3)	25 (3)	38 (3)	−1 (3)	10 (2)	1 (3)
C(2)	9671 (4)	4673 (8)	1802 (5)	33 (3)	32 (3)	34 (3)	−4 (3)	10 (2)	2 (3)
C(3)	9759 (4)	2854 (7)	1398 (5)	26 (3)	34 (3)	31 (3)	−2 (3)	6 (2)	−2 (3)
C(4)	9630 (4)	1741 (7)	2664 (5)	31 (3)	29 (3)	32 (3)	4 (3)	6 (2)	2 (3)
C(5)	8689 (4)	2182 (7)	3562 (5)	21 (2)	29 (3)	27 (3)	−1 (2)	4 (2)	−1 (2)
C(6)	7799 (4)	1366 (7)	2658 (5)	34 (3)	29 (3)	32 (3)	−7 (3)	6 (2)	−4 (3)
C(7)	6758 (4)	1277 (8)	3354 (6)	31 (3)	39 (4)	35 (3)	−11 (3)	6 (2)	−6 (3)
C(8)	6466 (4)	3166 (8)	3528 (5)	23 (2)	44 (3)	29 (3)	−1 (3)	2 (2)	2 (3)
C(9)	7299 (4)	4332 (7)	4297 (5)	24 (3)	31 (3)	34 (3)	1 (2)	6 (2)	−2 (3)
C(10)	8440 (4)	4069 (7)	3745 (5)	19 (2)	29 (3)	30 (3)	−2 (2)	6 (2)	−1 (2)
C(11)	7286 (4)	4101 (8)	5901 (5)	27 (3)	44 (4)	29 (3)	−1 (3)	5 (2)	−4 (3)
C(12)	6374 (4)	3020 (8)	6352 (5)	27 (3)	45 (3)	28 (3)	−1 (3)	0 (2)	0 (3)
C(13)	6113 (4)	3302 (9)	7832 (5)	34 (3)	51 (4)	28 (3)	0 (3)	3 (2)	1 (3)
C(14)	5339 (5)	2404 (11)	8551 (6)	45 (4)	97 (6)	29 (3)	−16 (4)	6 (3)	−4 (4)
C(15)	5391 (5)	2964 (12)	9838 (6)	37 (3)	106 (7)	39 (3)	−6 (4)	9 (3)	8 (4)
C(16)	6606 (6)	4339 (11)	8753 (6)	70 (5)	84 (6)	33 (3)	−23 (5)	15 (3)	−6 (4)
C(17)	5393 (4)	3344 (8)	4163 (5)	27 (3)	52 (4)	31 (3)	−6 (3)	3 (2)	2 (3)
C(18)	9304 (5)	−52 (8)	2196 (6)	57 (4)	30 (3)	37 (3)	7 (3)	20 (3)	2 (3)
C(19)	8880 (4)	1238 (8)	4951 (6)	33 (3)	39 (3)	35 (3)	9 (3)	6 (2)	8 (3)
C(20)	6943 (4)	6162 (8)	4010 (6)	37 (3)	33 (3)	57 (4)	8 (3)	18 (3)	1 (3)
O(3)	8981 (3)	2403 (5)	321 (3)	35 (2)	39 (2)	29 (2)	−8 (2)	7 (2)	−4 (2)
O(4)	10614 (3)	1707 (6)	3472 (4)	29 (2)	60 (3)	43 (2)	10 (2)	2 (2)	10 (2)
O(6)	8240 (3)	−258 (5)	2271 (4)	52 (2)	25 (2)	49 (2)	−9 (2)	21 (2)	−8 (2)
O(12)	5380 (3)	3400 (6)	5531 (4)	22 (2)	63 (3)	31 (2)	−4 (2)	4 (1)	0 (2)
O(15)	6159 (4)	4184 (8)	10013 (4)	78 (3)	105 (5)	32 (2)	−11 (4)	12 (2)	−16 (3)
O(17)	4574 (3)	3484 (7)	3472 (4)	20 (2)	99 (4)	41 (2)	−5 (2)	−4 (2)	7 (3)
O(18)	9866 (4)	−1153 (6)	1819 (5)	79 (3)	32 (2)	66 (3)	14 (3)	36 (3)	−1 (2)
C(M)	2551 (5)	4060 (13)	1089 (8)	47 (4)	98 (7)	85 (5)	−6 (5)	4 (4)	45 (6)
O(M)	2440 (3)	3260 (8)	2390 (5)	34 (2)	90 (4)	91 (4)	−6 (4)	−7 (2)	32 (4)

The anisotropic thermal parameters are expressed in the form: $\exp[-2\pi(U_{11}h^2a^{*2}+\cdots+2U_{23}klb^*c^*)]$. The standard deviation for the last digit is given in parentheses.

was no other available position in the rest of the deduced skeleton.

Blunt and Stothers[4] reported that the resonance of the angular methyl carbon at C-10 in A/B-*cis* steroids is more than 10 ppm to lower field as compared with that in the corresponding A/B-*trans* compounds. The same tendency is also observed in related diterpenes such as haplolciliatic acid[5] and sonderianin[6] (A/B-*trans*) (δ 20.7 and 19.7, respectively), and columbin (7) and floribundic acid methyl ester[7] (A/B-*cis*) (δ 27.0 and 32.8

TABLE IV. Bond Lengths and Their Standard Deviations (Å)

C(1)–C(2)	1.532 (8)	C(9)–C(10)	1.577 (8)
C(1)–C(10)	1.533 (8)	C(9)–C(11)	1.562 (9)
C(2)–C(3)	1.508 (8)	C(9)–C(20)	1.546 (9)
C(3)–C(4)	1.527 (8)	C(11)–C(12)	1.521 (9)
C(3)–O(3)	1.435 (7)	C(12)–C(13)	1.503 (9)
C(4)–C(5)	1.550 (8)	C(12)–O(12)	1.474 (8)
C(4)–C(18)	1.549 (9)	C(13)–C(14)	1.422 (11)
C(4)–O(4)	1.427 (8)	C(13)–C(16)	1.342 (11)
C(5)–C(6)	1.529 (8)	C(14)–C(15)	1.320 (13)
C(5)–C(10)	1.549 (8)	C(15)–O(15)	1.377 (12)
C(5)–C(19)	1.544 (9)	C(16)–O(15)	1.376 (11)
C(6)–C(7)	1.509 (9)	C(17)–O(12)	1.324 (8)
C(6)–O(6)	1.467 (7)	C(17)–O(17)	1.202 (9)
C(7)–C(8)	1.562 (9)	C(18)–O(6)	1.357 (7)
C(8)–C(9)	1.559 (9)	C(18)–O(18)	1.198 (8)
C(8)–C(17)	1.523 (9)	C(M)–O(M)	1.423 (12)

The standard deviation for the last digit is given in parentheses.

TABLE V. Bond Angles and Their Standard Deviations (°)

C(2)–C(1)–C(10)	112.5 (5)	C(8)–C(9)–C(20)	107.3 (5)
C(1)–C(2)–C(3)	111.5 (5)	C(10)–C(9)–C(11)	112.5 (5)
C(2)–C(3)–C(4)	109.7 (5)	C(10)–C(9)–C(20)	109.0 (5)
C(2)–C(3)–O(3)	111.9 (5)	C(11)–C(9)–C(20)	105.6 (5)
C(4)–C(3)–O(3)	109.6 (5)	C(1)–C(10)–C(5)	111.1 (5)
C(3)–C(4)–C(5)	115.9 (5)	C(1)–C(10)–C(9)	112.6 (5)
C(3)–C(4)–C(18)	109.9 (5)	C(5)–C(10)–C(9)	111.3 (5)
C(3)–C(4)–O(4)	108.6 (5)	C(9)–C(11)–C(12)	113.7 (5)
C(5)–C(4)–C(18)	100.0 (5)	C(11)–C(12)–C(13)	113.4 (6)
C(5)–C(4)–O(4)	111.5 (5)	C(11)–C(12)–O(12)	111.3 (5)
C(18)–C(4)–O(4)	110.6 (5)	C(13)–C(12)–O(12)	104.8 (5)
C(4)–C(5)–C(6)	98.3 (4)	C(12)–C(13)–C(14)	125.6 (7)
C(4)–C(5)–C(10)	116.8 (5)	C(12)–C(13)–C(16)	127.3 (7)
C(4)–C(5)–C(19)	106.9 (5)	C(14)–C(13)–C(6)	107.0 (7)
C(6)–C(5)–C(10)	109.4 (5)	C(13)–C(14)–C(15)	106.9 (8)
C(6)–C(5)–C(19)	111.2 (5)	C(14)–C(15)–O(15)	110.7 (8)
C(10)–C(5)–C(19)	113.3 (5)	C(13)–C(16)–O(15)	109.6 (7)
C(5)–C(6)–C(7)	113.2 (5)	C(8)–C(17)–O(12)	117.9 (6)
C(5)–C(6)–O(6)	104.2 (4)	C(8)–C(17)–O(17)	122.6 (6)
C(7)–C(6)–O(6)	115.1 (5)	O(12)–C(17)–O(17)	119.5 (6)
C(6)–C(7)–C(8)	102.6 (5)	C(4)–C(18)–O(6)	110.1 (5)
C(7)–C(8)–C(9)	118.1 (5)	C(4)–C(8)–O(18)	127.8 (6)
C(7)–C(8)–C(17)	110.7 (5)	O(6)–C(18)–O(18)	122.1 (6)
C(9)–C(8)–C(17)	109.9 (5)	C(6)–O(6)–C(18)	107.3 (4)
C(8)–C(9)–C(10)	111.2 (5)	C(12)–O(12)–C(17)	118.1 (5)
C(8)–C(9)–C(11)	111.0 (5)	C(15)–O(15)–C(16)	105.9 (7)

The standard deviation for the last digit is given in parentheses.

respectively). The signal of the angular methyl group in **2** was found at δ 32.4, and hence the A/B-ring junction was deduced to be *cis*.

3-Oxoborapetol A (**3**), obtained from **2** by oxidation with Jones' reagent, showed a positive Cotton effect in the circular dichroism (CD) curve, due to the effect of the axial 4-hydroxy group, and this indicated the absolute configuration of ring A to be as shown in the formula.

Unequivocal proof for the whole structure, including the relative stereochemistry of rings B and C was obtained by single crystal X-ray analysis of **2** using the direct method. The positional and thermal parameters with their standard deviations are listed in Table III. The bond lengths and bond angles are given in Tables IV and V. An ORTEP drawing[8] is shown in Chart 2. The absolute structure of borapetol A was thus determined as (5*S*,8*R*,10*S*)-3*S*,4*R*-4*R*-dihydroxy-15,16-epoxy-cleroda-13(16),14-diene-17,12*S*: 18,6*S*-diolactone (**2**).

The coupling constant (7.8 Hz) of the signal at δ 4.33 in the ^{1}H-NMR spectrum of **1**, due to the anomeric hydrogen,indicates the glycosidic linkage to have β-configuration. Accordingly, borapetoside A (**1**) was deduced to be borapetol A 3-*O*-β-D-glucopyranoside.

Borapetol A(**2**) was also found in the *n*-hexane extract. It was separated by successive chromatographies, and shown to be identical with the aglycone obtained from **1** by mixed melting point determination and IR, ^{1}H-NMR and ^{13}C-NMR comparisons.

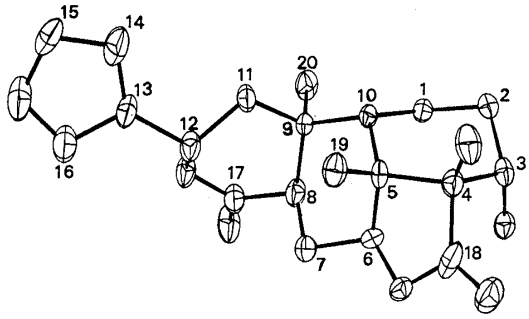


Chart 2. An ORTEP Drawing of Borapetol A (2)

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured with a Hitachi EPI-G3 or a Shimadzu IR-408 spectrometer. UV spectra, ^{1}H-NMR, ^{13}C-NMR, MS and CD were recorded using a Hitachi 200-20 spectrometer, a JEOL PS-100 NMR spectrometer, a JEOL FX-90Q FT-NMR spectrometer (chemical shifts are expressed in δ values (ppm), with tetramethylsilane as an internal standard), a JEOL JMS-D100 mass spectrometer, and a JASCO J20C automatic recording spectropolarimeter.

Borapetoside A (1)——The butanol extract of the crude drug (20 kg; dried stems of *Tinospora tuberculata* BEUMÉE)[1] was separated into 3 fractions by 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 A (**1**, 15.2 g), colorless needles (from MeOH–H_2O), mp 172—173 °C, $[\alpha]_D$ +2.60 ° (c = 1.0, MeOH). *Anal.* Calcd for $C_{26}H_{34}O_{12} \cdot H_2O$: C, 56.11; H, 6.52. Found: C, 56.24; H, 6.79. IR ν_{max}^{KBr} cm^{-1}: 3580—3170, 1762, 1703, 1505, 870. MS *m/z*: 538 (M^+), 376, 359, 341, 109, 95, 94, 81. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Borapetoside A Tetraacetate (4)——Acetylation of **1** (1.0 g) with acetic anhydride in pyridine at room temperature gave borapetoside A tetraacetate (**4**; 740 mg), colorless needles (from *n*-hexane–EtOAc), mp 235—237 °C, $[\alpha]_D$ −9.20 ° (c = 1.00, $CHCl_3$). *Anal.* Calcd for $C_{34}H_{42}O_{16} \cdot 1/2H_2O$: C, 57.06; H, 6.06. Found: C, 57.23; H, 6.05. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3655, 3537, 1800—1730, 1600, 1265—1190, 877. MS *m/z*: 359, 341, 109, 95, 94, 81. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Borapetoside A Pentaacetate (5)——Reflux of **4** (300 mg) with acetic anhydride in pyridine for 2 h gave borapetoside A pentaacetate (**5**: 198 mg), colorless needles (from acetone), mp 263—265 °C, $[\alpha]_D$ 0 ° (c = 0.825, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1790, 1750, 1735, 1270—1205, 879. MS *m/z*: 748 (M^+; $C_{36}H_{44}O_{17}$), 109, 95, 94, 81. ^{1}H-NMR ($CDCl_3$) δ: 1.13 (3H, s), 1.20 (3H, s), 2.00, 2.01, 2.03, 2.08, 2.11 (each 3H, s). ^{13}C-NMR: Table II.

Enzymatic Hydrolysis of Borapetoside A (1)——A mixture of **1** (1.0 g) and crude hesperidinase (10 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 A (**2**: 220 mg), colorless needles (from MeOH), mp 137—138 °C, $[\alpha]_D$ +28.1 ° (c = 1.46, pyridine). *Anal.* Calcd for $C_{20}H_{24}O_7 \cdot CH_3OH$: C, 61.75; H, 6.91. Found: C, 61.90; H, 7.23. IR ν_{max}^{KBr} cm^{-1}: 3515, 3405, 1769, 1721, 1508, 870. MS m/z: 376 (M^+), 95, 94, 81. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

From the aqueous layer of this reaction mixture, D-glucose (187 mg), $[\alpha]_D$ +49.1 ° (c = 2.34, H_2O), was obtained. It was identical with commercial D-glucose (Wako Chem. Co., Ltd.).

Borapetol A (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 less polar fraction gave colorless needles (from MeOH–H_2O), which were identified as borapetol A (**2**) by IR, ^{1}H- and ^{13}C-NMR, and mixed melting point.

Borapetol A Monoacetate (6)——On acetylation with acetic anhydride (5 ml) in pyridine (5 ml) at room temperature, **2** (100 mg) gave borapetol A monoacetate (**6**, 55 mg), colorless needles (from *n*-hexane–EtOAc), mp 147 °C, $[\alpha]_D$ +26.5 ° (c = 0.945, $CHCl_3$) was obtained. IR ν_{max}^{KBr} cm^{-1}: 3500, 1775, 1735, 1720, 1245. MS m/z: 418 (M^+, $C_{22}H_{26}O_8$), 95, 94, 81. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

3-Oxoborapetol A (3)——Oxidation of **2** (60 mg) with Jones' reagent (0.3 ml) for 30 min at 0 °C, afforded 3-oxoborapetol (**3**, 41 mg), colorless needles (from MeOH–$CHCl_3$), mp 230—235 °C. IR ν_{max}^{KBr} cm^{-1}: 3500—3300, 1790, 1750—1675, 875. MS m/z: 374 (M^+), 95, 94, 81. CD (c = 0.315, MeOH) $[\theta]^{22}$ (nm): 0 (348), +2377 (302.5), +23.8 (254).

X-Ray Analysis of Borapetol A (2)——$C_{20}H_{24}O_7 \cdot CH_3OH$ (M_r 408.46), monoclinic, space group: $P2_1$, $a = 12.610$ (4), $b = 7.974$ (2), $c = 9.676$ (2) Å, $V = 969.4$ (5) Å^3, $D_{obs} = 1.37$ g/cm^3 (in aq. KI solution) $D_{calcd} = 1.40$ g/cm^3, $Z = 2$. The determination of lattice constants and diffracted intensities was carried out on a SYNTAX $P\bar{1}$ automated four-circle diffractometer using a crystal with approximate dimensions of 0.2 × 0.3 × 0.6 mm. Intensity data were collected by the ω-scan method (bisecting mode) with graphite-monochromated Mo K_α radiation ($\lambda = 0.71069$ Å) within the limit $\omega \leq 25.0°$ to give 1583 unique reflections. The intensities were corrected for the usual Lorenz and polarization factors.

Solution of the phase problem was performed *via* a multi solution weighted tangent formula approach[8] for 223 reflections ($E \geq 1.50$). From the subsequent E-map the sites of 24 plausible non-hydrogen atoms were located. Positions of remaining C and O atoms were derived from successive D-Fourier syntheses. The structure along with one molecule of methanol was refined by the block-diagonal least-squares method with anisotropic thermal parameters ($R = 0.095$). Apart from the hydrogens of hydroxy groups and methanol, H atoms were included in the final refinement at calculated positions assuming a C–H bond length of 1.08 Å and with an isotropic thermal parameter ($B = 3.5$). The hydrogen parameters were not refined. The final R-factor value of 0.049 was attained for the 1539 observed reflections ($I \geq 2.3\sigma$). The atomic scattering factors were taken from International Tables for X-ray Crystallography.[9]

All calculations were performed on a FACOM M-190 compouter at the Computer Center, Kyushu University, using UNICS II[10] and MULTAN[11] programs.

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