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New picrotoxane terpenoids from *Picrodendron baccatum*

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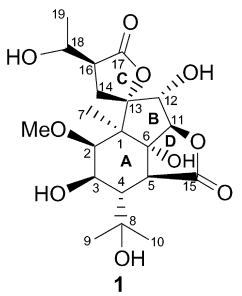
Abstract—Two new picrotoxane norditerpenoid lactones, picrodendrins X (**1**) and Y (**2**), and four new picrotoxane sesquiterpenoid lactones, picrodendrins Z (**3**), α (**4**), β (**5**) and picrodendrioside A (**6**) were isolated from the bark and leaves of *Picrodendron baccatum*. Their structures were determined by spectral analysis and X-ray crystallographic analysis.

1. Introduction

Picrodendron baccatum Klug et Urban (Euphorbiaceae) is used as an insecticide in folk medicine in Dominica.[1] In a previous paper, we reported the isolation and characterization of 22 picrotoxane terpenoids, picrodendrins, from the plant;[2] some of them showed potent noncompetitive blocking activity on rat brain $GABA_A$ receptors and against housefly's.[3a] Further investigation on the bark and leaves of the plant led to the purification of two new picrotoxane norditerpenoid lactones named picrodendrins X (**1**) and Y (**2**), along with four picrotoxane seaquiterpenoid lactones designated as picrodendrioside A (**6**) and picrodendrins Z (**3**), α (**4**) and β (**5**). In the present paper, we report the isolation and structure elucidation of these new compounds.

2. Results and discussion

Picrodendrin X (**1**), an amorphous solid, $[\alpha]_D=-13.5°$ (pyridine), has a molecular formula of $C_{20}H_{30}O_{10}$ as determined from its high resolution FABMS (*m/z* 431.1907, $[M+H]^+$). The ^{13}C NMR spectrum (Table 1) showed twenty signals for five methyls, a methylene, eight methines including three oxygen bearing ones, four quaternary carbons, and two γ-lactones (δ 177.5 and 179.0). The presence of γ-lactone moieties was also supported by the IR spectrum which showed an absorption at 1755 cm^{-1}. The ^1H NMR spectrum of **1** (Table 1) displayed signals for a methoxyl (δ 3.67, s, 3H), a secondary methyl (δ 1.64, d, 3H), and three tertiary methyl singlets at δ 1.51, 1.68 and 1.76. Furthermore, absence of any absorption in the UV region for conjugated chromophore, coupled with the degree of unsaturation (us=6) suggested that **1** possesses a tetracyclic terpenoid lactone frame work. Interpretation of $^1H-^1H$ COSY indicated the presence of three independent spin systems: H-2 to H-5, H-11/H-12, and H-14/H-16/H-18/H-19. The HMBC correlations between H-9 (δ 1.68), H-10 (δ 1.76) and the methine carbon at C-4 (δ 50.8) suggested the location of a hydroxyisopropyl group at C-4. The location of one of the γ-lactone rings between C-11 and C-15 was evident from the HMBC correlation between the γ-lactone carbon (C-15, δ 179.0) and the methine protons at H-4 (δ 3.04), H-5 (δ 3.45) and H-11 (δ 5.29). The location of the second γ-lactone ring was deduced from the observed three bond correlation of C-17 (δ 177.5) with H-14 and H-18. This was further confirmed by comparing the ^{13}C chemical shifts of **1** with those of picrodendrin W (**7**) isolated previously from the same source.[2g] The orientation of the protons and the relative stereochemistry were supported by a phase sensitive NOESY experiment (Fig. 1). Key nOe interactions between H-2/H-3, H-2/H-7, H-2/H-14α, H-3/H-9, H-5/H-10, H-5/H-11, H-9/H-10 and H-12/H-14β suggested that H-2, H-3, H-5, H-11, the methyl group at C-1 and the hydroxyisopropyl group at C-4 are oriented on the same face of the molecule. The ring system is supposed to be formed by the fusion of a cyclohexane (A), a cyclopentane (B) and two γ-lactones (C and D). Ring A is *cis*-connected with ring B and D that are also *cis*-connected each other. Thus on the basis of extensive 2D NMR spectral analysis and comparison of the spectral data with those of **7**, it was concluded that picrodendrin X possesses the relative stereostructure as shown in **1**.



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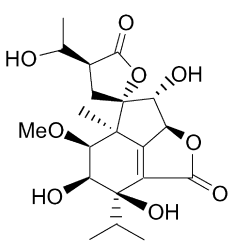

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Keywords: *Picrodendron baccatum*; picrodendrin X; picrodendrin Y; picrodendrin Z; picrodendrin α; picrodendrin β; picrodendrioside A; picrotoxane.

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Table 1. NMR spectral data for compounds **1**, **2** and **7** in pyridine-d_5

Position	**1**			**7**		**2**		
	δ_H (J in Hz)[a]	δ_C[b]	HMBC[c]	δ_H (J in Hz)[a]	δ_C[b]	δ_H (J in Hz)[a]	δ_C[b]	HMBC[c]
1		55.5	H-2, 5, 7, 14α, 14β		49.5		54.4	H-2, 5, 7, 14α, 14β
2	3.91 shs	86.2	H-7, 2-OMe	3.87 d (1.8)	87.2	4.28 s	84.9	H-3, 4, 7, 12
3	4.77 brd (8.1)	70.2	H-2, 4, 5	4.56 brs	72.1	4.09 dd (11.0, 1.1)	68.4	H-2, 4, 5
4	3.04 dd (8.1, 1.8)	50.8	H-2, 5, 9, 10		74.6	3.13 dd (11.0, 15.0)	48.2	H-2, 5, 9, 10
5	3.45 d (1.8)	51.3	H-4		132.4	3.35 d (15.0)	45.4	H-4, 11
6		82.4	H-2, 4, 5, 7		168.4		77.8	H-2, 4, 5, 7, 12
7 (Me)	1.51 s	14.9		1.38 s	17.1	1.24 s	6.0	
8		73.3	H-4, 5, 9, 10	2.64 sep (6.7)	35.3		83.8	H-4, 5, 9, 10
9 (Me)	1.68 s	28.0	H-10	1.24 d (6.7)	18.4	1.64 s	29.3	H-4, 10
10 (Me)	1.76 s	28.9	H-9	1.20 d (6.7)	18.6	1.56 s	20.3	H-4, 9
11	5.29 dd (5.5, 1.1)	96.3		5.58 d (8.0)	84.8	4.74 d (6.4)	74.0	H-5
12	4.97 d (5.5)	83.5	H-14β	4.15 d (8.0)	82.0	4.68 s	80.5	H-14α, 14β
13		95.4	H-7, 14α, 14β		96.4		93.8	H-7, 14α, 14β, 16
14α	2.82 dd (13.6, 11.4)	27.5		2.68 dd (14.7, 11.6)	28.5	2.58 dd (13.7, 11.0)	28.8	H-16
14β	2.68 dd (13.6, 8.4)			2.73 dd (14.7, 7.9)		2.68 dd (13.7, 5.5)		
15		179.0	H-4, 5, 11		171.3		173.3	H-4, 5
16	3.26 ddd (11.4, 8.4, 5.1)	47.4	H-14α, 14β, 19	3.27 ddd (11.6, 7.9, 5.5)	47.0	2.99 ddd (11.0, 5.5, 3.3)	46.0	H-14α, 14β, 19
17		177.5	H-14α, 14β, 16, 18		177.0		176.2	H-14α, 14β, 16
18	4.62 qui (6.2)	67.9	H-14β, 19	4.60 qui (6.1)	67.8	4.28 s	68.4	H-14α, 14β, 16, 19
19 (Me)	1.64 d (6.2)	20.7		1.58 d (6.1)	20.8	1.61 d (6.4)	21.2	H-16
2-OMe	3.67 s	60.3	H-2	3.66 s	61.0			

[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.
[c] HMBC data are expressed as protons exhibiting $^{2-3}J_{CH}$ coupling to the carbons as indicated.

Picrodendrin Y (**2**), $[\alpha]_D$=+119.6° (pyridine), was obtained as prisms. Its molecular formula was determined as $C_{19}H_{26}O_9$ by its high-resolution FABMS (*m/z* 399.1653, $[M+H]^+$) in combination with the ^{13}C NMR spectra. Its IR spectrum showed absorption bands corresponding to a hydroxyl (3447 cm^{-1}) and two lactone (1772 and 1733 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed signals due to a secondary methyl (δ 1.61, d, 3H) and three tertiary methyl (δ 1.24, 1.64, 1.56, s, each 3H) groups. Analysis of the ^1H–^1H COSY spectrum revealed the existence of two independent spin systems (H-2 to H-5 and H-14/H-16/H-18/H-19). Comparison of ^1H and ^{13}C NMR spectral data (Table 1) with those of **1** suggested that both posses spiro-γ-lactone ring but they differ in the conformation of the cyclohexane ring with axial protons (H-3, 4 and 5) or equatorial ones. The coupling constants observed between H-3 and H-4 (J_{HH}=11.0 Hz) and those of H-4 and H-5 (J_{HH}=15.0 Hz) indicated the transdiaxial relationships of H-3, H-4 and H-5. The $^3J_{CH}$ observed between the γ-lactone carbonyl carbon (C-15) and H-4 in the HMBC spectrum (Table 1) revealed the location a γ-lactone. Furthermore, the HMBC correlations of H-9 (δ 1.64) and H-10 (δ 1.56) with C-4 (δ 48.2) suggested the location of these methyl groups at C-8. On the other hand, no spin coupling was observed between H-11 and H-12 of **2** in the ^1H NMR, though such coupling had been usually observed in the picrodendrins by us earlier.[2] HMBC spectra also revealed long range correlations between H-12 and C-2 in agreement with the presence of another furan ring with an ether bond from C-2→C-12. The evidences suggested that **2**

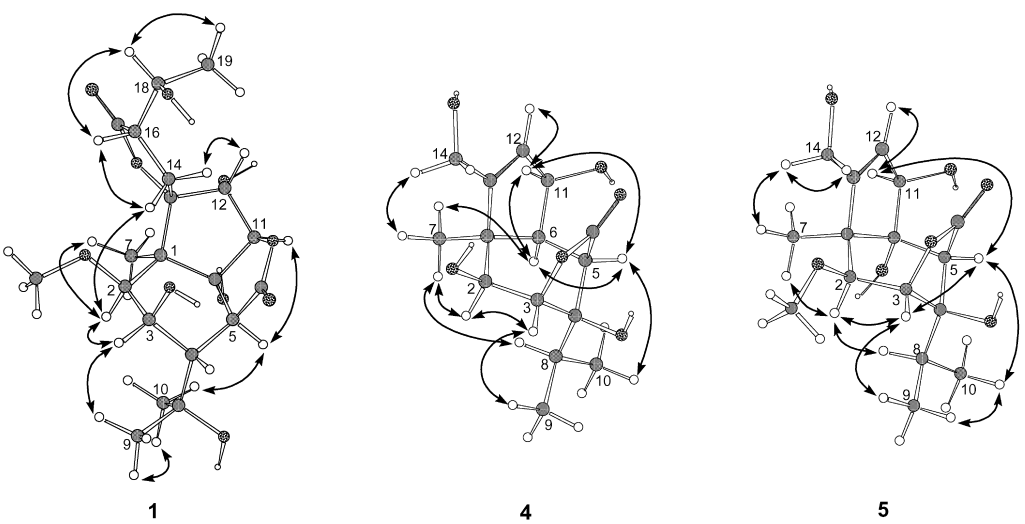
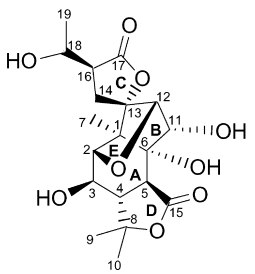


Figure 1. Key nOe Correlations of **1**,**4** and **5**.

might have a new carbon skeleton. In order to ascertain the full structure using X-ray crystallographic analysis, we subjected **2** to recrystallization from methanol which furnished suitable prisms. Figure 2 shows a perspective ORTEP drawing of **2**. It reveals that the ring system is formed by the fusion of a cyclohexane (A), a cyclopentane (B), two γ-lactones (C and D) and a tetrahydro furan ring (E), with ring C spiro-connected at C-13. Ring A assumes a chair form due to fusion of five-membered rings B and E. Twist rings B and E are *cis*-connected with ring A with a mirror symmetry to each other. This conformation explains the upfield shift of C-7 (δ 6.0) in the ^{13}C NMR spectrum due to steric compression of H-2 to C-7 and H-14α. The minimum energy conformation of **2** calculated by Macro-Model (ver. 5) showed that the dihedral angle of H11–C11–C12–H12 was 74.9°, with J_{calcd}=0.25 Hz. This also explains the absence of spin coupling between H-11 and H-12 in the ^{1}H NMR spectrum. Thus the relative stereochemistry of picrodendrin Y was confirmed as **2**.

Picrodendrin β (**5**), $[\alpha]_D$=+91.3° (pyridine), was obtained as an amorphous solid. Its molecular formula was determined as $C_{16}H_{24}O_7$ by HRMS (*m/z* 382.1622, $[M]^+$). The IR spectrum displayed absorption bands corresponding to hydroxyl (3423 cm^{-1}) and a γ-lactone (1774 cm^{-1}). The ^{1}H NMR spectrum (Table 2) showed signals due to an isopropyl (δ 1.20, 1.45, each 3H, d and δ 2.92, 1H, sep), a tertiary methyl (δ 1.67, s, 3H) and a methoxyl (δ 3.39, s, 3H) groups. The ^{13}C NMR spectrum (Table 2) showed the signals of a γ-lactone carbonyl carbon (δ 176.9) and a pair of olefinic carbons (δ 123.9 and 159.1). The ^{1}H–^{1}H COSY spectrum contained four independent spin systems (H-2/H-5, H-9/H-10, H-11/H12, and H-14α/H-14β) as well as a W-type long range coupling correlation was observed between H-3 and H-5. The HMBC spectrum showed correlations of the carbon resonance at δ 82.0 (C-4) to protons at δ 1.20 (H-9) and δ 1.45 (H-10), which suggested that the isopropyl group was located at C-4. Further HMBC correlations between the γ-lactone carbonyl carbon (C-15, δ 176.9) and two methine protons (H-3, δ 5.05 and H-5, δ 3.14), as also of H-5 (δ 3.14) to C-1, 4, and 6 (δ 54.5, 82.0, 79.6 respectively) revealed that the γ-lactone ring extended from C-3 to C-5. The correlations of 2H-14 (δ 4.72 and 5.06) with C-12 (δ 123.9) and C-13 (δ 159.1) indicated that the hydroxymethyl group was located at C-13. The stereochemistry of **5** was determined by a phase-sensitivity NOESY experiment (Fig. 1). Key nOe correlations between H-2/H-3, H-2/H-7, H-2/H-8, H-3/H-5, H-3/H-9, H-5/H-10, H-5/H-11, H-9/H-10 and H-7/H-14α indicated that H-2, H-3, H-5, H-11, the methyl group at C-1 and the isopropyl group at C-4 were all on the same face of the molecule. From the foregoing evidences it was established that picrodendrin β has the relative stereochemistry as depicted in **5**.



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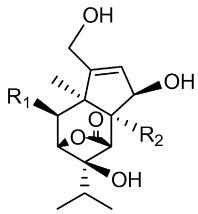
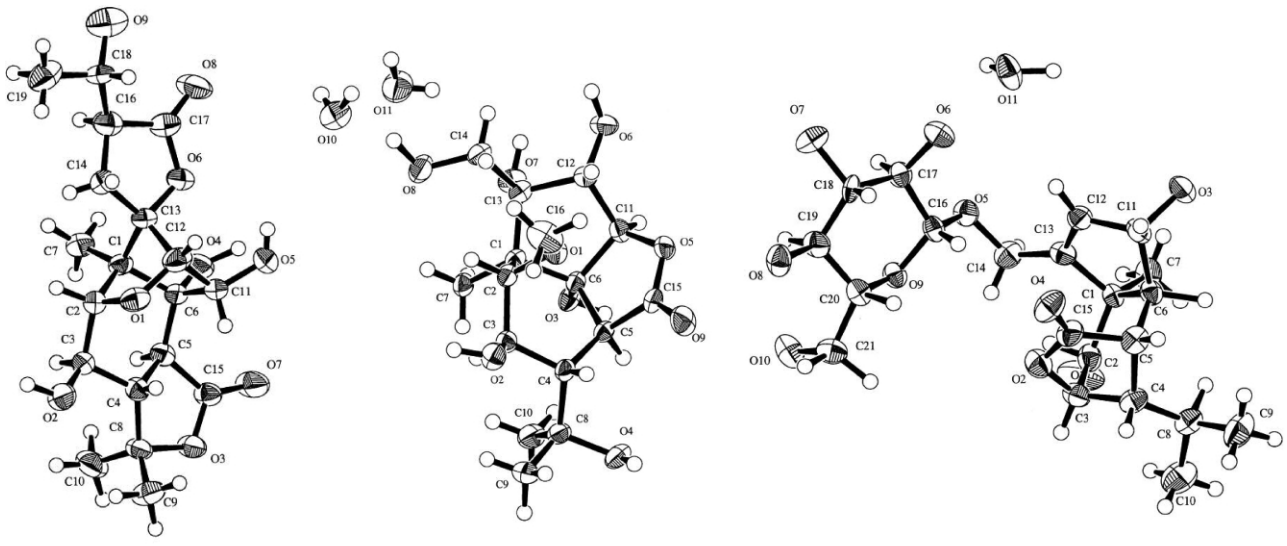


	R_1	R_2
4:	OH	H
5:	OMe	OH

Picrodendrin α (**4**), $[\alpha]_D$=+98.7° (pyridine), was obtained as colorless prisms, IR ν_{max} 3327 (OH) and 1761 (5-ring lactone) cm^{-1}. The molecular formula $C_{15}H_{22}O_6$ was determined from its high resolution FABMS (*m/z* 297.1349, $[M-H]^+$ as well as from the ^{13}C NMR spectra. The ^{1}H NMR spectrum of **4** displayed signals due to an isopropyl (δ 1.50, 1.21, d, each 3H, and δ 3.00, sep, 1H) and a tertiary methyl (δ 2.06, s, 3H) groups besides these for a trisubstituted double bond (δ 6.40, d, *J*=2.2 Hz). The ^{13}C NMR spectrum (Table 2) showed signals for a γ-lactone carbonyl carbon at δ 178.3 and a pair of olefinic carbons (δ 126.0 and 156.3). Comparison of the NMR spectral data of **4** with those of **5** indicated close resemblance between them. The most significant differences observed were the



2 **3** **6**

Figure 2. The ORTEP Drawing of **2**, **3** and **6**.

Table 2. NMR spectral data for compounds **4** and **5** in pyridine-d_5

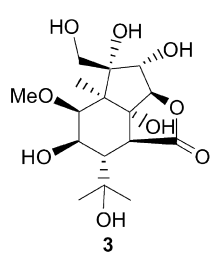
Position	**4**			**5**		
	δ_H (J in Hz)[a]	δ_C[b]	HMBC[c]	δ_H (J in Hz)[a]	δ_C[b]	HMBC[c]
1		50.7	H-2, 3, 5, 6, 7, 11, 12, 14α, 14β		54.5	H-2, 3, 5, 7, 11, 12
2	4.96 d (3.7)	69.4	H-3, 7	3.78 s	86.0	H-3, 7, 2-OMe
3	5.09 dd (3.7, 1.1)	87.4	H-2, 5	5.05 s	77.1	
4		81.0	H-2, 3, 5, 6, 8, 9, 10		82.0	H-3, 5, 9, 10
5	3.04 dd (4.4, 1.1)	52.4	H-6, 11	3.14 d (1.1)	59.3	H-3
6	2.93 d (4.4)	56.3	H-2, 5, 7, 12		79.6	H-5, 7, 12
7 (Me)	2.06 s	27.8	H-6	1.67 s	25.1	H-2
8	3.00 sep (6.6)	29.2	H-9, 10	2.92 sep (6.6)	30.9	H-4, 9, 10
9 (Me)	1.50 d (6.6)	17.8	H-8, 10	1.20 d (6.6)	15.4	H-4, 8, 10
10 (Me)	1.21 d (6.6)	17.0	H-8, 9	1.45 d (6.6)	18.3	H-8, 9
11	5.46 d (2.2)	78.8	H-5, 6, 11, 12	5.05 s	83.9	H-5
12	6.40 d (2.2)	126.0	H-6, 11, 14α, 14β	6.58 d (2.2)	123.9	H-14α, 14β
13		156.3	H-6, 7, 11, 12, 14α, 14β		159.1	H-2, 7, 14α, 14β
14α	4.63 d (15.0)	59.6	H-12	4.72 brd (15.4)	61.4	
14β	4.79 d (15.0)			5.06 d (15.4)		
15		178.3	H-3, 5, 6		176.9	H-3, 5
2-OMe				3.39 s	58.2	H-2

[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.
[c] HMBC data are expressed as protons exhibiting $^{2-3}J_{CH}$ coupling to the carbons as indicated.

disappearance of the signal for the methoxyl group and the upfield shift of C-2 (δ 69.4) in **4**, indicating that a hydroxyl group was located at C-2. Moreover, a new signal was observed due to a methine proton at H-6 (δ 2.93, d, 1H) coupled (J_{HH}=4.4 Hz) to the signal for H-5. The stereochemistry of **4** was established from a phase sensitivity NOESY experiment (Fig. 1). The most significant nOe correlations observed were between H-2/H-3, H-2/H-7, H-3/H-9, H-5/H-10, H-7/H-6, H-5/H-6, H-5/H-11 and H-6/H-11, indicating that H-2, H-3, H-5, H-6, the methyl group at C-1, and the isopropyl group at C-4 were all on the same face of the molecule. Thus, the relative stereochemistry of picrodendrin α was established as **4**.

Picrodendrin Z (**3**), $[\alpha]_D$=−7.8° (pyridine), was obtained as prisms and deduced to have the molecular formula $C_{16}H_{26}O_9$ as determined from its high resolution FABMS (*m/z* 363.1655, $[M+H]^+$) in conjunction with the NMR data. Its IR spectrum showed bands at 3553 and 3474 (OH), and 1735 (5-ring lactone) cm^{-1}. The ^1H spectrum of **3** (Table 3) displayed signals for a methoxyl (δ 3.75, s, 3H) and three tertiary methyl (δ 1.70, 1.76, 1.80, s, each 3H) groups. The ^{13}C NMR spectrum (Table 3) showed 16 signals for three methyls, a hydroxy methylene, six methines including four oxymethines, and five quaternary carbons including three oxysubstituted ones (δ 73.3, 83.5, 84.1) and a γ-lactone (δ 179.4). The above evidences coupled with the absence of any absorption in the UV region and the calculated degree of unsaturaion (us=4) suggested **3** to be a tricyclic sesquiterpenoid lactone. Comparison of ^1H and ^{13}C NMR signals with those of **1** revealed that most of the signals were similar except those of the spiro-γ-lactone ring present in **1**. However, the appearance of signals at δ 4.19 and 4.23 (each d, J_{HH}=11.4 Hz) assigned to 2H-14 suggested that in **3** the spirolactone ring of **2** was replaced with a hydroxy and a hydroxymethylene groups. Finally, the relative stereochemistry of picrodendrin Z was established unambiguously as **3** by X-ray crystallographic analysis (Fig. 2).

Picrodendrioside A (**6**), $[\alpha]_D$=+136.3° (pyridine), was obtained as prisms. Its molecular formula was determined as $C_{21}H_{30}O_{10}$ by its high resolution FABMS (*m/z* 465.1737, $[M+Na]^+$) in combination with the ^{13}C NMR spectra. The IR spectrum showed absorption bands at 3400, 1776 and 1722 cm^{-1} corresponding to hydroxyl, γ-lactone and ketonic groups, respectively. Presence of a γ-lactone carbonyl supported by the ^{13}C NMR signal at δ 176.9. The other functional groups revealed by the ^1H and ^{13}C NMR spectra of **6** were a tertiary methyl group (δ 1.78, s, 3H), an isopropyl group (δ 0.81, 0.86, d, each 3H, and δ 1.56, sep, 1H), an olefinic bond (δ 130.0 and 147.2) and a ketone (δ 207.2). The ^1H and ^{13}C NMR spectral data of **6** were basically similar to those of **4**. The most significant difference observed was the presence of a ketone, located at C-2 in place of a hydroxyl group, and a glucose (^1H: δ 4.93, 1H, d and ^{13}C: δ104.0, 75.3, 78.5, 71.5, 78.5 and 62.5) moiety. Fragment ion peaks *m/z* 263 $[M-C_6H_{11}O_6]^+$ and *m/z* 179 $[M-C_{15}H_{19}O_4]^+$ were observed in the FABMS supporting the presence of a glucose residue. These evidences suggested that **6** is a sesquiterpenoid glucoside. The coupling constant (J=7.8 Hz) of the anomeric proton (H-1′, δ 4.93) in the ^1H NMR spectrum suggested that the glucose was present in β- configuration.



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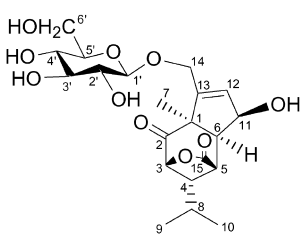

6

Hydrolysis of **6** with β-glucosidase afforded D-glucose with $[\alpha]_D$=+37.8° and the aglycone **6a**. The attachment of the glucose moiety to C-14 of the sesquiterpene was deduced

Table 3. NMR spectral data for compounds **3** and **6** in pyridine-d_5

Position	**3**			**6**		
	δ_H (J in Hz)[a]	δ_C[b]	HMBC[c]	δ_H (J in Hz)[a]	δ_C[b]	HMBC[c]
1		58.4	H-2, 5, 7, 11, 14α, 14β		60.5	H-3, 5, 6, 7, 11, 12
2	4.09 s	86.5	H-7, 2-OMe		207.2	H-3, 4, 7
3	4.84 brd (8.1)	70.3	H-2, 4, 5	4.76 d (5.4)	84.7	H-4, 5
4	3.07 dd (8.1, 1.9)	51.2	H-2, 3, 5, 9, 10	2.42 qui (5.4)	53.2	H-3, 8, 9, 10
5	3.31 d (1.9)	50.3	H-4	3.03 t (4.4)	43.7	H-3, 4, 6, 11
6		83.5	H-2, 4, 5, 7, 11	2.82 d (4.9)	54.1	H-4, 5, 7, 12
7 (Me)	1.80 s	16.2	H-2	1.78 s	25.4	H-6
8		73.3	H-3, 4, 5, 9, 10	1.56 sep (6.4)	25.0	H-4, 9, 10
9 (Me)	1.76 s	29.0	H-4, 10	0.81 d (6.4)	19.3	H-4, 8, 10
10 (Me)	1.70 s	27.9	H-4, 9	0.86 d (6.4)	20.6	H-4, 8, 9
11	5.27 d (4.0)	99.1	H-12	5.42 brs	77.8	H-6, 12
12	4.97 d (4.0)	80.7	H-11, 14α, 14β	6.59 d (1.5)	130.0	H-6, 14α, 14β
13		84.1	H-14α, 14β		147.2	H-6, 7, 11, 12, 14α, 14β
14α	4.19 d (11.4)	65.3	H-12	4.43 dt (15.1, 1.5)	65.3	H-1′
14β	4.23 d (11.4)			4.80 dd (15.1, 1.5)		
15		179.4	H-4, 5, 11		176.9	H-3, 5, 6
2-Ome	3.75 s	59.9	H-2			
1′				4.93 d (7.8)	104.0	H-14α, 14β, 2′
2′				4.05 t (8.3)	75.3	
3′				4.27 t (6.4)	78.5	H-2′
4′				4.28 dd (18.1, 11.7)	71.5	H-3′
5′				3.93 sep (2.4)	78.5	H-4′
6′a				4.38 dd (11.7, 4.9)	62.5	
6′b				4.49 dd (11.7, 2.4)		

[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.
[c] HMBC data are expressed as protons exhibiting $^{2-3}J_{CH}$ coupling to the carbons as indicated.

from the long-range correlation shown between H-14 (δ 4.43, 4.80, d, each 1H) of the aglycone and anomeric carbon (δ 104.0) of glucose. In addition, a ^{13}C NMR spectral comparison of **6a** with **6** revealed a glycosylation shift (+6.5) in the resonance of C-14. Finally the structure and stereochemistry of picrodendrioside A was established unambiguously as **6** from X-ray crystallographic studies. Figure 2 shows a perspective ORTEP drawing of the crystal. To the best of our knowledge **6** represents the first sesquiterpene-glucoside reported from the picrotoxane terpenoid groups.

3. Experimental

3.1. General procedures

Melting points are uncorrected. IR spectra were recorded as KBr pellets on a JASCO 7300 FTIR spectrometer. UV spectra were recorded on a Hitachi 340 spectrophotometer in MeOH. Optical rotations were determined on a JASCO DIP-4 digital polarimeter. Low-resolution EIMS were measured on a JEOL D-300 mass spectrometer. HREIMS and FABMS were measured on a JEOL DX-303 mass spectrometer. ^1H, ^{13}C and 2D NMR spectra were recorded on a JEOL α-500 spectrometer (^1H at 500 MHz, ^{13}C at 125 MHz) and a JEOL EX-400 spectrometer (^1H at 400 MHz, ^{13}C at 100 MHz), using tetramethylsilane as internal standard.

Isolation. Dried bark (8.0 kg) of *P. baccatum* collected in Indonesia in September 1986 was extracted with MeOH (49 L). The residue remaining (1.9 kg) after removal of the solvent was extracted successively with $CHCl_3$, AcOEt and *n*-BuOH. The *n*-BuOH fraction (302 g) was chromatographed on reversed phase highly porous polymer resin Diaion HP-20 (2 kg, Mitsubishi Kasei) and eluted with following gradient, H_2O, H_2O–MeOH (8:2, 6:4, 4:6, and 2:8) and MeOH. The H_2O–MeOH (8:2) eluent was further purified by silica gel C.C. [solvent system CH_2Cl_2–MeOH (9:1)] and medium pressure LC [silica gel D-60 (Fuji Silysia) with the solvent system $CHCl_3$–MeOH (10:1)] and HPLC [Capcell pack C18 SG120 with the solvent system H_2O–MeOH (2:1)] to afford picrodendrin X (**1**, 5 mg). The H_2O eluent was chromatographed on a silica gel column and eluted with CH_2Cl_2–MeOH (9:1), which affords picrodendrin Z (**3**, 41 mg). And the CH_2Cl_2–MeOH (9:1) eluent was further purified by medium pressure LC [the solvent system benzene–AcOEt (4:1)] to afford picrodendrin Y (**2**, 158 mg).

Dried leaves (10 kg) of *P. baccatum* were extracted with MeOH (115 L). The residue remaining (3.6 kg) after removal of the solvent was extracted successively with $CHCl_3$ and *n*-BuOH. The *n*-BuOH fraction (385 g) was chromatographed on Diaion HP-20 C.C. and eluted with following, H_2O and H_2O–MeOH (9:1). The H_2O eluent was further purified by silica gel C.C. [the solvent system $CHCl_3$–MeOH (7:3) and $CHCl_3$–MeOH (9:1)] to afford picrodendrin α (**4**, 31 mg). The H_2O–MeOH (9:1) eluent was further purified by silica gel C.C. [the solvent system $CHCl_3$–MeOH (95:5) and benzene–AcOEt (4:1)] and by HPLC [ODS with the solvent system H_2O–MeOH (2:1)] to afford picrodendrin β (**5**, 63 mg). On the other hand, the same H_2O–MeOH (9:1) eluent was also purified by silica gel C.C. [the solvent system $CHCl_3$–MeOH (7:3)

and AcOEt–acetone (1:1)] and by ODS C.C [the solvent system H_2O–MeOH (7:3)] to afford picrodendrioside A (**6**, 14 mg).

3.1.1. Picrodendrin X (1). Amorphous solid, mp 234–236°C (MeOH), $[\alpha]_D^{25}=-13.5°$ (c=0.52, pyridine). IR ν_{max} (KBr) cm^{-1}: 3428, 1755, 1170, 1106, 1069 and 1020. EIMS m/z (rel. int.): 336 ($[M-49]^+$, 1), 308 (2), 232 (3), 205 (10), 177 (14), 149 (33), 132 (20), 115 (25), 91 (20), 77 (20), 65 (9), 59 (100), 42 (70), 30 (29). HRFABMS m/z: 431.1907 $[M+H]^+$ (calcd for $C_{20}H_{31}O_{10}$, 431.1917). ^1H NMR and ^{13}C NMR: see Table 1.

3.1.2. Picrodendrin Y (2). Colorless prisms, mp 293–294°C (MeOH), $[\alpha]_D^{24}=+119.6°$ (c=1.00, pyridine). IR ν_{max} (KBr) cm^{-1}: 3447, 1772, 1733, 1286, 1170, 1108, 1075 and 1018. EIMS m/z (rel. int.): 336 ($[M-62]^+$, 2), 318 (3), 309 (4), 292 (6), 265 (6), 247 (4), 211 (8), 193 (14), 167 (27), 153 (20), 127 (54), 109 (27), 95 (52), 85 (46), 67 (25), 55 (58), 43 (100), 32 (13). HRFABMS m/z: 399.1653 $[M+H]^+$ (calcd for $C_{19}H_{27}O_9$, 399.1655). ^1H NMR and ^{13}C NMR: see Table 1.

Crystal data for **2**. Crystals of **2**, crystallized from methanol, belong to the orthorhombic space group $P2_12_12_1$. Lattice constants and intensity data were measured on AFC7R diffractometer equipped with a device for graphite-monochromated Cu Kα radiation. Crystal data: $C_{19}H_{26}O_9$, a=14.317 (1), b=16.136 (2), c=7.701 (2) Å, Z=4, F.W.=398.41, D_c=1.487 g/cm^3, μ(Cu Kα)=10.06 cm^{-1}. A total of 1571 independent reflections with $I>3.00\sigma(I_o)$ were used for structure analysis. The structure was determined by a direct method (SAPI91)[4] and refined by full-matrix least squares (DIRDIF92).[5] The final refinement cycle gave R=0.052 (Rw=0.071). The final Fourier difference synthesis showed a maximum and minimum of +0.58 and −0.29 e$^-$/Å^3, respectively.

3.1.3. Picrodendrin Z (3). Colorless prisms, mp 236°C (MeOH), $[\alpha]_D^{25}=-7.8°$ (c=1.10, pyridine). IR ν_{max} (KBr) cm^{-1}: 3553, 3474, 3384, 1735, 1368, 1109, 1070, 1025 and 1010. EIMS m/z (rel. int.): 327 ($[M-35]^+$, 1), 295 (5), 223 (5), 206 (8), 177 (11), 161 (13), 149 (19), 121 (20), 108 (35), 95 (25), 69 (22), 59 (92), 43 (100), 32 (78). HRFABMS m/z: 363.1655 $[M+H]^+$ (calcd for $C_{16}H_{27}O_9$, 363.1655). ^1H NMR and ^{13}C NMR: see Table 3.

Crystal data for **3**. Crystals of **3**, crystallized from methanol, belong to the orthorhombic space group $P2_12_12_1$. Lattice constants and intensity data were measured on a Rigaku AFC7R diffractometer equipped with a device for graphite-monochromated Cu Kα radiation. Crystal data: $C_{18}H_{26}O_9$, a=9.165 (2), b=22.235 (2), c=8.997 (2) Å, Z=4, F.W.=389.40, D_c=1.400 g/cm^3, μ(Cu Kα)=9.57 cm^{-1}. A total of 1613 independent reflections with $I>3.00\sigma(I)$ and $2\theta<120.09$ were used for structure analysis. The structure was determined by a direct method (MITHRIL84)[6] and refined by full-matrix least squares (DIRDIF94).[7] The final refinement cycle gave R=0.036 (Rw=0.058). The final Fourier difference synthesis showed a maximum and minimum of +0.22 and −0.40 e$^-$/Å^3, respectively.

3.1.4. Picrodendrin α (4). Colorless prisms, mp 132°C (MeOH), $[\alpha]_D^{24}=+98.7°$ (c=1.39, pyridine). IR ν_{max} (KBr) cm^{-1}: 3327, 1761, 1356, 1099, 1030 and 999. EIMS m/z (rel. int.): 280 ($[M-H_2O]^+$, 5), 257 (8), 239 (9), 219 (37), 193 (51), 175 (13), 163 (17), 149 (22), 121 (26), 109 (43), 95 (19), 79 (18), 71 (43), 55 (24), 43 (100). HRFABMS m/z: 297.1349 $[M-H]^+$ (calcd for $C_{15}H_{21}O_6$, 297.1338). ^1H NMR and ^{13}C NMR: see Table 2.

3.1.5. Picrodendrin β (5). Colorless solid, $[\alpha]_D^{24}=+91.3°$ (c=1.13, pyridine). IR ν_{max} (KBr) cm^{-1}: 3423, 1774, 1655 and 1080. EIMS m/z (rel. int.): 328 ($[M]^+$, 12), 310 (2), 267 (9), 249 (11), 235 (9), 221 (9), 207 (8), 189 (11), 161 (11), 137 (19), 113 (34), 95 (15), 84 (100), 77 (12), 71 (33), 56 (60), 43 (50). HRMS m/z 328.1622 $[M]^+$ (calcd for $C_{16}H_{24}O_7$, 328.1515). ^1H NMR and ^{13}C NMR: see Table 2.

3.1.6. Picrodendrioside A (6). Colorless prisms, mp 212°C (MeOH), $[\alpha]_D^{24}=+136.3°$ (c=0.98, pyridine). IR ν_{max} (KBr) cm^{-1}: 3400, 2972, 2931, 1776, 1722, 1344, 1074 and 1024. EIMS m/z (rel. int.): 263 ($[M-C_6H_{11}O_6]^+$, 100), 246 (5), 237 (16), 218 (106), 203 (29), 175 (31), 147 (20), 121 (22), 109 (39), 97 (18), 91 (25), 85 (21), 73 (48), 60 (32), 44 (45). FABMS m/z 481 $[M+K]^+$, 465 $[M+Na]^+$, 460 $[M+NH_4]^+$, 443 $[M+H]^+$, 263 $[M-C_6H_{11}O_6]^+$, 179 $[M-C_{15}H_{19}O_4]^+$. HRFABMS m/z: 465.1737 $[M+Na]^+$ (calcd for $C_{21}H_{30}O_{10}Na$, 465.1737). ^1H NMR and ^{13}C NMR, see Table 3.

3.2. Enzymatic hydrolysis of picrodendrioside A (6)

A solution of **6** (5 mg) in an acetate buffer (pH=5.6, 1 mL) was treated with β-glucosidase (Sigma, No. G-0395, from almond, ca. 50 mg) at 37°C overnight. The reaction mixture was extracted with AcOEt, and the AcOEt extract was evaporated under reduced pressure to afford aglycon (**6a**, 5 mg). The aqueous phase was passed through a silica gel column (SiO_2 2 g, $CHCl_3$–MeOH–H_2O (14:6:1), lower phase) to afford D-glucose ($[\alpha]_D^{25}=+37.8°$, c=0.09, 24 h after dissolution in H_2O).

3.2.1. Aglycone 6a. Colorless prisms, mp 169–170°C (MeOH), $[\alpha]_D^{24}=+256.8°$ (c=0.37, pyridine). IR ν_{max} (KBr) cm^{-1}: 3298, 2920, 1797, 1718, 1302, 1111 and 1018. EIMS m/z (rel. int.): 262 ($[M-H_2O]^+$, 49), 237 (4), 219 (133), 191 (7), 175 (18), 167 (8), 149 (24), 121 (11), 109 (55), 97 (43), 83 (34), 71 (71), 57 (100), 43 (72). FABMS m/z 303 $[M+Na]^+$, 298 $[M+NH_4]^+$, 279 $[M-H]^+$, 263 $[M-OH]^+$. ^1H NMR (pyridine-d_5) δ: 0.83 (3H, d, J=6.4 Hz, H-9), 0.87 (3H, d, J=6.4 Hz, H-10), 1.61 (1H, sep, J=6.4 Hz, H-8), 1.88 (3H, s, H-7), 2.45 (1H, qui, J=5.5 Hz, H-4), 2.86 (1H, d, J=4.8 Hz, H-6), 3.06 (1H, t, J=4.6 Hz, H-5), 4.43 (1H, d, J=15.9 Hz, H-14α), 4.55 (1H, d, J=15.9 Hz, H-14β), 4.78 (1H, d, J=5.5 Hz, H-3), 5.48 (1H, shs, H-11) and 6.50 (1H, d, J=1.8 Hz, H-12). ^{13}C NMR (pyridine-d_5) δ: 19.3 (C-9), 20.6 (C-10), 25.0 (C-8), 25.6 (C-7), 43.7 (C-5), 53.3 (C-4), 54.4 (C-6), 58.8 (C-14), 60.3 (C-1), 77.8 (C-11), 84.8 (C-3), 127.8 (C-12), 152.5 (C-13), 177.0 (C-15) and 207.0 (C-2).

Crystal data for **6**. Crystals of **6**, crystallized from methanol, belong to the orthorhombic space group $P2_12_12_1$. Lattice constants and intensity data were measured on a Rigaku AFC7R diffractometer equipped with a device for

graphite-monochromated Cu Kα radiation. Crystal data: $C_{21}H_{30}O_{10}$, a=8.884 (1), b=31.318 (3), c=7.982 (1) Å, Z=4, F.W.=442.46, D_c=1.323 g/cm^3, μ(Cu Kα)=8.94 cm^{-1}. A total of 1951 independent reflections with $I>3.00\sigma(I_o)$ and $2\theta<120.04$ were used for structure analysis. The structure was determined by a direct method (SAPI91)[4] and refined by full-matrix least squares (DIRDIF92).[5] The final refinement cycle gave R=0.069 (Rw=0.100). The final Fourier difference synthesis showed a maximum and minimum of +0.43 and −0.44 e$^-$/Å^3, respectively.

Crystallographic data (excluding structure factors) for the structures, picrodendrioside A (**6**), picrodendrin Y (**2**) and Z (**3**), in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 212069-212071, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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