SUPPLEMENTARY MATERIAL

A New 5(6→7)abeo-sterol from the Twigs of *Podocarpus fleuryi*

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A new 5(6→7)abeo-sterol with rarely reported [6-5-6-5]-fused rings, 3 β ,5 β ,6-trihydroxyl-*B*-norsitostane (**1**), along with ten known compounds were isolated from the twigs of *Podocarpus fleuryi*. Their structures were elucidated by means of extensive spectroscopic analysis. Compound **1** was assessed for their cytotoxicity against five human tumour cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW-480), and the result showed that it had no activity.

Keywords: *Podocarpus fleuryi*; Podocarpaceae; abeo-sterol

Supporting information

Spectroscopic data of the known compounds **2-11**.

Figure S1. Selected HMBC and H-H correlations of **1**.

Figure S2. Selected ROESY correlations of **1**.

Original spectra of compound **1**.

^1H NMR Spectrum (500 MHz, CDCl_3)

^{13}C NMR Spectrum (125 MHz, CDCl_3)

COSY Spectrum

HSQC Spectrum

HMBC Spectrum

ROESY Spectrum

HRESIMS

IR

Spectroscopic data of the known compounds 2-11.

Sitostenone (2)

Colorless needles, MF: C₂₉H₄₈O, MW: 412; ¹H-NMR (500 MHz, CDCl₃): δ 0.73 (3H, s, H-18), 0.83 (3H, d, *J* = 6.8 Hz, H-27), 0.85 (3H, d, *J* = 6.8 Hz, H-26), 0.87 (3H, t, *J* = 7.0 Hz, H-29), 0.94 (3H, d, *J* = 6.5 Hz, H-21), 1.20 (3H, s, H-19), 5.74 (1H, s, H-4);

¹³C-NMR (CDCl₃, 125 MHz) δ

35.6 (t, C-1), 32.1 (t, C-2), 199.7 (s, C-3), 123.7 (d, C-4), 171.7 (s, C-5), 33.8 (t, C-6), 33.0 (t, C-7), 35.7 (d, C-8), 53.8 (d, C-9), 38.6 (s, C-10), 21.0 (t, C-11), 39.6 (t, C-12), 42.4 (s, C-13), 55.9 (d, C-14), 24.2 (t, C-15), 28.2 (t, C-16), 56.0 (d, C-17), 12.0 (q, C-18), 17.4 (q, C-19), 36.1 (d, C-20), 18.7 (q, C-21), 34.0 (t, C-22), 26.7 (t, C-23), 45.8 (d, C-24), 29.2 (d, C-25), 19.8 (q, C-26), 19.0 (q, C-27), 23.1 (t, C-28), 12.0 (q, C-29).

β-Sitosterol linoleate (3)

Yellowish oil, MF: C₄₇H₈₀O₂, MW: 676; ¹H-NMR (500 MHz, CDCl₃): δ 0.70 (3H, s, H-18), 0.82 (3H, d, *J* = 7.2 Hz, H-27), 0.85 (3H, d, *J* = 7.2 Hz, H-26), 0.86 (3H, t, *J* = 7.0 Hz, H-29), 0.89 (3H, t, *J* = 6.8 Hz, H-18'), 0.90 (3H, d, *J* = 6.8 Hz, H-21), 1.04 (3H, s, H-19), 2.27 (2H, t, *J* = 8.0 Hz, H-2'), 2.78 (2H, t, *J* = 7.0 Hz, H-11'), 4.61 (1H, m, H-3), 5.32 (4H, m, H-9', 10', 12', 13'), 5.36 (1H, m, H-6); ¹³C-NMR (CDCl₃, 125 MHz): δ 37.0 (t, C-1), 27.8 (t, C-2), 73.7 (t, C-3), 38.2 (t, C-4), 139.7 (s, C-5), 122.6 (d, C-6), 31.9 (t, C-7), 32.0 (d, C-8), 50.0 (d, C-9), 36.6 (s, C-10), 21.0 (t, C-11), 39.7 (t, C-12), 42.3 (s, C-13), 56.7 (d, C-14), 24.3 (t, C-15), 28.3 (t, C-16), 56.1 (d, C-17), 11.9 (q, C-18), 19.3 (q, C-19), 36.2 (d, C-20), 19.1 (q, C-21), 34.0 (t, C-22), 26.1 (t, C-23), 45.8 (d, C-24), 29.1 (d, C-25), 18.8 (q, C-26), 19.8 (q, C-27), 23.1 (t, C-28), 12.0 (q, C-29), 173.3 (s, C-1'), 36.2 (t, C-2'), 25.1 (t, C-3'), 29.2 (t, C-4'), 29.3 (t, C-5'), 31.9 (t, C-6'), 29.7 (t, C-7'), 27.2 (t, C-8'), 128.1 (d, C-9'), 130.0 (d, C-10'), 25.6 (t, C-11'), 129.8 (d, C-12'), 128.0 (d, C-13'), 22.7 (t, C-14'), 29.1 (t, C-15'), 31.9 (t, C-16'), 22.7 (t, C-17'), 14.1 (q, C-18').

4 β -Hydroxyl-4(20 $\rightarrow$ 5),10(18 $\rightarrow$ 9)abeo-pimar-15(16)-ene (4)

Colorless oil, MF: C₂₀H₃₄O, MW:290; ¹H-NMR (500 MHz, CDCl₃): δ 0.77 (3H, s, H-20), 0.97 (3H, s, H-17), 1.05 (3H, s, H-19), 1.31 (3H, s, H-18), 4.85 (1H, dd, J = 10.5, 1.0 Hz, H-16a), 4.91 (1H, dd, J = 17.5, 1.0 Hz, H-16b), 5.82 (1H, dd, J = 17.5, 10.5 Hz, H-15); ¹³C-NMR (CDCl₃, 125 MHz): δ 20.5 (t, C-1), 23.4 (t, C-2), 36.9 (t, C-3), 76.0 (s, C-4), 42.7 (s, C-5), 32.4 (t, C-6), 25.7 (t, C-7), 42.5 (d, C-8), 36.9 (s, C-9), 51.3 (d, C-10), 35.8 (t, C-11), 32.1 (t, C-12), 36.3 (s, C-13), 39.3 (t, C-14), 151.4 (d, C-15), 108.6 (t, C-16), 23.2 (q, C-17), 23.4 (q, C-18), 15.3 (q, C-19), 12.4 (q, C-20).

Inumakoic acid (5)

Colorless oil, MF: C₂₀H₂₈O₃, MW:316; ¹H-NMR (500 MHz, CDCl₃): δ 1.10 (3H, s, H-20), 1.32 (3H, d, J = 7.5 Hz, H-16), 1.34 (3H, s, H-18), 1.35 (3H, d, J = 7.5 Hz, H-17), 1.50 (1H, d, J = 12.8 Hz, H-5), 1.99 (1H, m, H-2), 2.04 (1H, m, H-3), 2.21 (1H, m, H-1), 2.21 (1H, m, H-6), 2.96 (1H, d, J = 5.0 Hz, H-7), 3.29 (1H, m, H-15), 6.54 (1H, d, J = 8.6 Hz, H-13), 7.00 (1H, d, J = 8.6 Hz, H-12); ¹³C-NMR (CDCl₃, 125 MHz): δ 37.2 (t, C-1), 20.4 (t, C-2), 40.1 (t, C-3), 43.8 (s, C-4), 52.1 (d, C-5), 21.1 (t, C-6), 30.0 (t, C-7), 134.3 (s, C-8), 141.0 (s, C-9), 38.5 (s, C-10), 152.1 (s, C-11), 124.2 (d, C-12), 114.6 (d, C-13), 130.9 (s, C-14), 27.3 (d, C-15), 20.3 (q, C-16), 20.4 (q, C-17), 28.6 (q, C-18), 183.9 (s, C-19), 23.2 (q, C-20).

Lambertic acid (6)

Colorless oil, MF: C₂₀H₂₈O₃, MW:316; ¹H NMR (500 MHz, CDCl₃): δ 1.23 (3H, d, J = 7.0 Hz, H-17), 1.24 (3H, s, H-20), 1.25 (3H, d, J = 7.0 Hz, H-16), 1.27 (3H, s, H-19), 1.55 (1H, m, H-1a), 1.57 (2H, m, H-6), 2.01 (2H, m, H-3), 2.04 (2H, m, H-2), 2.20 (1H, m, H-1b), 2.25 (1H, dd, J = 12.9 Hz, 2.5 Hz, H-5), 2.84 (2H, m, H-7), 3.12 (1H, s, H-15), 6.65 (1H, m, H-11), 6.86 (1H, s, H-14); ¹³C-NMR (CDCl₃, 125 MHz): δ 38.3 (t, C-1), 21.1 (t, C-2), 38.3 (t, C-3), 43.8 (s, C-4), 52.8 (d, C-5), 19.9 (t, C-6), 31.3 (t, C-7), 126.7 (s, C-8), 146.5 (s, C-9), 37.5 (s, C-10), 111.9 (d, C-11), 150.9 (s,

C-12), 131.9 (s, C-13), 127.5 (d, C-14), 26.8 (d, C-15), 22.7 (q, CH₃-16), 22.5 (q, CH₃-17), 28.8 (q, CH₃-18), 182.2 (s, C-19), 23.2 (q, C-20).

Secoisolariciresinol-9,9'-acetonide (7)

Colorless oil, MF: C₂₃H₃₀O₆, MW: 402; ¹H-NMR (500 MHz, CDCl₃): δ 1.38 (6H, s, 2× CH₃), 1.59 (1H, m, H-8, 8'), 2.63 (2H, dd, *J* = 14.8 Hz, 7.9 Hz, H-7b, 7'b), 2.65 (2H, dd, *J* = 14.8 Hz, 8.2 Hz, H-7a, 7'a), 3.39 (2H, dd, *J* = 12.5 Hz, 4.5 Hz, H-9a, 9'a), 3.80 (3H, 2 -OCH₃), 3.81 (2H, dd, *J* = 12.5, 2.5 Hz, H-9b, 9'b), 6.50-6.80 (6 ArH); ¹³C-NMR (CDCl₃, 125 MHz): δ 132.4 (t, C-1, 1'), 111.4 (s, C-2, 2'), 146.4 (s, C-3, 3'), 143.7 (d, C-4, 4'), 113.9 (d, C-5, 5'), 121.9 (s, C-6, 6'), 37.2 (t, C-7, 7'), 43.0 (d, C-8, 8'), 61.9 (t, C-9, 9'), 55.7 (q, 2 OCH₃), 24.8 (q, 2 CH₃), 101.3 (s, O-C-O).

3,4-Dimethoxybenzyl alcohol (8)

Yellow oil, MF: C₉H₁₂O₃, MW: 168; ¹H-NMR (500 MHz, CDCl₃): δ 3.89 (6H, s, H-8, H-9), 4.64 (2H, s, H-7), 6.87 (3H, m, H-2, H-5, H-6); ¹³C-NMR (CDCl₃, 125 MHz): δ 133.6 (s, C-1), 110.5 (d, C-2), 149.2 (s, C-3), 148.6 (s, C-4), 111.1 (d, C-5), 119.4 (d, C-6), 65.3 (t, C-7), 56.0 (q, C-8), 55.9 (q, C-9).

p-Hydroxybenzyl alcohol (9)

Colorless neddle, MF: C₇H₈O₂, MW: 124; ¹H-NMR (500 MHz, CD₃COCD₃): δ 4.56 (2H, m, -CH₂OH), 6.80 (2H, m, H-2, H-6), 7.20 (2H, m, H-3, H-5); ¹³C-NMR (CD₃OCD₃, 125 MHz): δ 157.0 (s, C-1), 116.0 (d, C-2, C-6), 130.2 (d, C-3, C-5), 133.0 (s, C-4), 65.9 (t, CH₂OH).

Chrysophanol (10)

Yellow neddles, MF: C₁₅H₁₀O₄, MW: 254; ¹H-NMR (500 MHz, CDCl₃): δ 2.49 (3H, q, 3-CH₃), 7.13 (1H, m, H-2), 7.29 (1H, d, *J* = 8.9 Hz, 1.5 Hz, H-7), 7.68 (1H, d, *J* = 8.8 Hz, 7.9 Hz, H-6), 7.69 (1H, m, H-4), 7.84 (1H, d, *J* = 8.0 Hz, 1.5 Hz, H-5); ¹³C-NMR (CDCl₃, 125 MHz): δ 162.8 (s, C-1), 124.4 (d, C-2), 149.4 (s, C-3), 121.4 (d, C-4), 124.6 (d, C-5), 137.0 (d, C-6), 120.0 (d, C-7), 162.5 (s, C-8), 192.6 (s, C-9),

182.1 (s, C-10), 133.7 (s, C-11), 115.9 (s, C-12), 113.8 (s, C-13), 133.3 (s, C-14), 22.3 (q, 3-CH₃).

(-)-Epicatechin (11)

White amorphous powder, MF: C₁₅H₁₄O₆, MW:290; ¹H-NMR (500 MHz, CD₃COCD₃): δ 2.79 (1H, dd, *J* = 16.0 Hz, 3.8 Hz, H-4), 2.80 (1H, dd, *J* = 16.6 Hz, 4.8 Hz, H-4), 4.26 (1H, s, H-3), 4.89 (1H, m, H-2), 6.01 (1H, t, *J* = 2.8 Hz, H-8), 6.10 (1H, d, *J* = 2.8 Hz, H-6), 6.83 (1H, s, 5'-OH), 6.84 (1H, m, H-6'), 7.01 (1H, s, H-2'); ¹³C-NMR (CD₃OCD₃, 125 MHz): δ 77.9 (d, C-2), 65.7 (d, C-3), 27.6 (t, C-4), 155.9 (s, C-5), 95.2 (d, C-6), 156.1 (s, C-7), 94.6 (d, C-8), 155.6 (s, C-9), 98.61 (s, C-10), 130.7 (s, C-1'), 113.9 (d, C-2'), 143.8 (d, C-3'), 144.0 (s, C-4'), 118.1 (s, C-5'), 114.5 (d, C-6').

Figure S1. Selected HMBC and H-H correlations of 1.

Figure S2. Selected ROESY correlations of **1**.

Original spectra of compound **1**.

¹H NMR:

[illegible]

2D COSY NMR spectrum of compound 1. The x and y axes both range from 0.5 to 4.5 ppm. The 1D ^1H NMR spectrum is projected along the top and left axes. Significant cross-peaks are observed between 0.8-1.5 ppm and 1.5-2.0 ppm, and between 3.5-4.0 ppm and 4.0-4.5 ppm.

[illegible]

1D ¹H NMR Spectrum (Top):

Chemical Shift (ppm)	Integration
~1.0	1.00
~1.5	1.00
~2.0	1.00
~2.5	1.00
~3.0	1.00
~3.5	1.00
~4.0	1.00
~4.5	1.00
~5.0	1.00
~5.5	1.00
~6.0	1.00
~6.5	1.00
~7.0	1.00

1D ¹³C NMR Spectrum (Left):

Chemical Shift (ppm)
~10
~15
~20
~25
~30
~35
~40
~45
~50
~55
~60
~65
~70

2D HSQC Spectrum Data (Approximate):

¹ H (ppm)	¹³ C (ppm)
1.0	10
1.5	15
2.0	20
2.5	25
3.0	30
3.5	35
4.0	40
4.5	45
5.0	50
5.5	55
6.0	60
6.5	65
7.0	70

ROESY Spectrum

HRESIMS:

Qualitative Analysis Report

Data Filename	NZE-5.d	Sample Name	NZE-5
Sample Type	Sample	Position	P1-C7
Instrument Name	Instrument 1	User Name	
Acq Method	SIBU.m	Acquired Time	4/17/2015 11:27:06 AM
IRM Calibration Status	Success	DA Method	Default.m
Comment			

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra

Peak List

m/z	z	Abund	Formula	Ion
163.1231	1	23024.64		
413.3773	1	29795.7		
471.3803	1	97383.55	C29 H52 O3	(M+Na)+
472.3837	1	31602.42	C29 H52 O3	(M+Na)+
487.3548	1	33275.45		
919.7719	1	105999.09		
920.7754	1	68924.18		
921.7784	1	22089.46		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	30
S	0	5

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C29 H52 O3	448.3917	471.3809	471.3803	0.6	1.4	4.0000

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IR: