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 from the Twigs of *Podocarpus fleuryi*

A new 5(6→7)abeo-sterol with rarely reported [6-5-6-5]-fused rings, 3β,5β,6-trihydroxy-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.

$^1$H 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: C_{29}H_{48}O, MW: 412; \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 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); \(^{13}\)C-NMR (CDCl\(_3\), 125 MHz) \(\delta\) 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).

**\(\beta\)-Sitosterol linoleate (3)**
Yellowish oil, MF:C_{47}H_{80}O_{2}, MW: 676; \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 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); \(^{13}\)C-NMR (CDCl\(_3\), 125 MHz): \(\delta\) 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').
4β-Hydroxyl-4(20→5),10(18→9)abeo-pimar-15(16)-ene (4)

Colorless oil, MF: C_{20}H_{34}O, 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: C_{20}H_{28}O₃, 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: C_{20}H_{28}O₃, 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, CH₂OH), 56.0 (q, C-8), 55.9 (q, C-9).

p-Hydroxybenzyl alcohol (9)
Colorless needle, 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 needles, MF: C₁₃H₁₀O₄, 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: C₁₃H₁₄O₆, MW:290; ¹H-NMR (500 MHz, CD₄COCD₃): 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 (CD₄OCD₃, 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 \textbf{1}. 
Original spectra of compound 1.

$^1$HNMR:
$^{13}$CNMR:
H-H COSY:
HSQC:
HMBC:
ROESY Spectrum
HRESIMS:

Qualitative Analysis Report

Data Filename: NZE:5.d
Sample Type: Sample
Instrument Name: Instrument 1
Acq Method: SIM

IRM Calibration Status: Success

User Spectra

Fragmentor Voltage: 135
Collision Energy: 0
Ionization Mode: ESI

Peak List

| MW/Z  | Abund | Formula       | Ion  |
|-------|-------|---------------|------|
| 163.1231 | 1    | 2H3O+        |      |
| 413.3773 | 1    | C2H3O2      |      |
| 471.3803 | 1    | C2H5O2      | (M+H)+ |
| 472.3837 | 1    | C2H5O2      | (M+H)+ |
| 487.3548 | 1    | C2H5O2      | (M+H)+ |
| 919.7119 | 1    | C2H5O2      |      |
| 920.7754 | 1    | C2H5O2      |      |
| 921.7784 | 1    | C2H5O2      |      |

Formula Calculator Element Limits

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

Formula Calculator Results

| Formula | Calculated Mass | Calculated Mz | Mz | Diff. (mDa) | Diff. (ppm) | DBE |
|---------|----------------|---------------|----|-------------|-------------|-----|
| C2H5O2  | 418.3917       | 471.3803      | 471.3803 | 0.6         | 1.4         | 4000 |

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