

## Cytotoxic Cucurbitacin Constituents from *Sloanea zuliaensis*

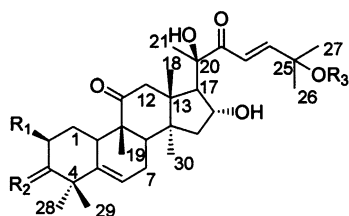
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A new cucurbitacin D analogue, 2-deoxycucurbitacin D (**1**), as well as cucurbitacin D (**2**) and 25-acetylcucurbitacin F (**3**) were isolated from *Sloanea zuliaensis*. Compound **1** was found only in the young leaves of the plant and not in the mature leaves, and its structure was established using spectroscopic means. Compounds **1–3** demonstrated potent cytotoxic activity against breast (MCF-7), lung (H-460), and central nervous system (SF-268) human cancer cell lines.

Within the framework of an International Cooperative Biodiversity Groups (ICBG) project based in Panama, aimed at discovering *inter alia* novel potential antitumor agents,<sup>1</sup> total methanolic/EtOAc extracts of young and mature leaves of *Sloanea zuliaensis* Pittier (Elaeocarpaceae) showed cytotoxic activity against the MCF-7, H-460, and SF-268 human cancer cell lines (Table 1). Neither phytochemical nor biological reports on *S. zuliaensis* were found in the literature. Bioassay-guided fractionation of the total extract of young leaves of *S. zuliaensis*, using MCF-7, H-460, and SF-268 human cancer cell lines, resulted in the isolation of 2-deoxycucurbitacin D (**1**) along with cucurbitacin D (**2**)<sup>2</sup> and 25-acetylcucurbitacin F (**3**).<sup>3</sup> However, **2** and **3** were isolated only from the mature leaves. The structure determination of the new natural product **1** and the cytotoxic activity of compounds **1–3** are discussed herein.



- 1** R<sub>1</sub> = H, R<sub>2</sub> = O, R<sub>3</sub> = H  
**2** R<sub>1</sub> = OH, R<sub>2</sub> = O, R<sub>3</sub> = H  
**3** R<sub>1</sub> = OH, R<sub>2</sub> = αOH, R<sub>3</sub> = Ac

Compound **1** gave a molecular ion peak at  $m/z$  499.3022  $[M - 1]^+$  in its HRCIMS, corresponding to the formula C<sub>30</sub>H<sub>44</sub>O<sub>6</sub>. The <sup>1</sup>H and <sup>13</sup>C NMR data of **1** showed eight methyl singlets ( $\delta_H/\delta_C$  0.95/19.9; 1.15/19.6; 1.24/28.5; 1.26/22.8; 1.34/19.0; 1.38 (6H)/29.2, 29.5; 1.42/23.9), an olefinic proton at  $\delta_H$  5.75 ( $\delta_C$  119.1, C-6), two *trans*-coupled olefinic protons at  $\delta_H$  6.68 and 7.13 ( $J = 15.2$  Hz;  $\delta_C$  119.6, 155.6; C-23, C-24), three carbonyls at  $\delta_C$  213.6, 213.1, 202.9 (C-3, C-11, C-22), and three oxygenated functions  $\delta_C$  71.5, 71.2, 78.1 (C-16, C-25, C-20). The above data indicated the presence of a cucurbitacin triterpene-type structure,<sup>4</sup> which

**Table 1.** Cytotoxic Activity of Plant Extracts and Compounds **1–3** from *S. zuliaensis*

compound/extract	GI <sub>50</sub> (μg/mL)		
	MCF-7	H-460	SF-268
<i>S. zuliaensis</i> young leaves MeOH/EtOAc extract	1.50	1.00	1.00
<i>S. zuliaensis</i> mature leaves MeOH/EtOAc extract	1.50	1.10	2.10
2-deoxycucurbitacin D ( <b>1</b> )	0.041	0.032	0.210
cucurbitacin D ( <b>2</b> )	0.020	0.013	0.021
25-acetylcucurbitacin F ( <b>3</b> )	0.110	0.065	0.087
adriamycin	$8.0 \times 10^{-7}$	$3.0 \times 10^{-7}$	$8.5 \times 10^{-7}$

showed a similarity to that of cucurbitacin D (**2**) isolated from the same plant material,<sup>2</sup> except for the absence of one oxygenated function. HMBC cross-peak connectivities showed correlations of H-2/C-3, C-1; H-17/C-16, C-13; H-16/C-13, C-20, C-14, and the <sup>1</sup>H–<sup>1</sup>H COSY NMR showed correlations of H-2/H-1α, H-1β; H-10/H-1α, H-1β; H-16/H-17, H-15β. On the basis of the above spectroscopic data, the structure of **1** was assigned as 2-deoxycucurbitacin D (**1**), a new natural product. The spectroscopic data of compounds **2** and **3** were identical to those of the previously known cucurbitacin D<sup>2</sup> and 25-acetylcucurbitacin F,<sup>3,5</sup> respectively. TLC profiles of extracts from young and mature leaves indicated the absence of **1** in mature leaves.

Table 1 shows the GI<sub>50</sub> (the concentration required to inhibit 50% of cell growth) values of compounds **1–3** against MCF-7, H-460, and SF-268 human cancer cell lines. Compounds **1–3** showed potent activity. Compounds **2** and **3** have been reported to be active against different human tumor cell lines.<sup>6</sup>

### Experimental Section

**General Experimental Procedures.** Melting points were uncorrected. Optical rotations were measured with an Autopol III (Rudolph Research Analytical Co.) polarimeter. IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. NMR spectra were recorded using a Bruker Avance 300 spectrometer in CDCl<sub>3</sub> at 300 MHz for <sup>1</sup>H and 75 MHz for <sup>13</sup>C NMR. Mass spectra were obtained on a Kratos MS50TC mass spectrometer. Silica gel [Merck, Kieselgel 60 (0.063–0.200 and 0.015–0.040 mm)] was used for column chromatography. Silica gel plates (Merck, Kieselgel 60 F<sub>254s</sub>) were used for TLC.

**Cytotoxicity Bioassays.** The cytotoxicity bioassay was performed against breast (MCF-7), lung (H-460), and central nervous system (SF-268) human cancer cell lines according to

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the method of Monks et al.<sup>7</sup> During the isolation process, the activity of all fractions was monitored using all three cell lines.

**Plant Material.** Fresh young and mature leaves of *S. zuliaensis* were collected from Monumento Natural Barro Colorado, Barro Colorado, Panama (N 9°14' 2", W 79°39'30") in December 2001. A voucher specimen (50976) is deposited in the Herbarium of the University of Panama (PMA).

**Extraction and Isolation.** Fresh young leaves (500 g) were extracted and subjected to solvent partitioning as described before.<sup>8</sup> The activity was retained in the MeOH fraction (1.2 g), which was subjected to flash chromatography on Si gel using CHCl<sub>3</sub>/MeOH mixtures in order of increasing polarity (0 to 15% MeOH), yielding three secondary fractions (SM1–3). Fraction SM1 was chromatographed on a Si gel Lobar column, which on elution with 2% MeOH in CHCl<sub>3</sub> yielded pure **1** (4 mg, 0.000008%), **2** (2 mg, 0.000004%), and **3** (30 mg, 0.00006%). The fresh mature leaves (770 g) were subjected to the same isolation procedures as for the young leaves described above, which afforded **2** (5 mg, 0.000006%) and **3** (16.3 mg, 0.000021%).

**2-Deoxycucurbitacin D (1):** colorless crystals; mp 153–155 °C;  $[\alpha]_D^{25} +51.0^\circ$  (c 0.03, MeOH); IR (KBr)  $\nu_{\max}$  3410, 2950, 1710, 1700, 1465, 1380 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.13 (1H, d, *J* = 15.2 Hz, H-24), 6.68 (1H, d, *J* = 15.2 Hz, H-23), 5.75 (1H, dd, *J* = 5.4, 1.4 Hz, H-6), 4.42 (1H, t, *J* = 6.9 Hz, H-16), 3.25 (1H, d, *J* = 14.8 Hz, H-12 $\alpha$ ), 2.73 (1H, d, *J* = 14.8 Hz, H-12 $\beta$ ), 2.65 (1H, m, H-10), 2.64 (1H, d, *J* = 6.9 Hz, H-17), 2.45 (1H, m, H-2 $\alpha$ ), 2.40 (1H, m, H-2 $\beta$ , H-7 $\alpha$ ), 1.90 (1H, br d, *J* = 6.1 Hz, H-8), 1.87 (1H, m, H-1 $\alpha$ ), 1.85 (1H, m, H-7 $\beta$ ), 1.83 (1H, dd, *J* = 13.3, 6.9 Hz, H-15 $\beta$ ), 1.50 (1H, m, H-1 $\beta$ ), 1.42 (3H, s, Me-21), 1.40 (1H, d, *J* = 13.3 Hz, H-15 $\alpha$ ), 1.38 (6H, s, Me-26, -27), 1.34 (3H, s, Me-30), 1.26 (3H, s, Me-29), 1.24 (3H, s, Me-28), 1.15 (3H, s, Me-19), 0.95 (3H, s, Me-18); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  213.6 (s, C-3), 213.1 (s, C-11), 202.9 (s, C-22), 155.6 (d, C-24), 140.8 (s, C-5), 119.6 (d, C-23), 119.1 (d, C-6), 78.1 (s, C-20), 71.5 (d, C-16), 71.2 (s, C-25), 57.5 (d, C-17), 51.0 (s, C-13), 50.9 (s, C-4), 49.0 (s, C-9), 48.7 (s, C-14), 48.3 (t, C-12), 45.5 (t, C-15), 42.4 (d, C-8), 38.0 (t, C-2), 36.0 (d, C-10), 29.5 (q, C-26), 29.2 (q, C-27), 28.5 (q, C-28), 24.6 (t, C-1, -7), 23.9 (q, C-21), 22.8 (q, C-29), 19.9 (q, C-18), 19.6 (q, C-19), 19.0

(q, C-30); CIMS *m/z* 499 [M – 1]<sup>+</sup> (20), 498 (33), 482 (12), 439 (2), 388 (6), 369 (6), 326 (4), 189 (6), 112 (30), 96 (100); HRCIMS *m/z* 499.30225 [M – 1]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>43</sub>O<sub>6</sub>, 499.30596).

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