Cytotoxic Cucurbitacin Constituents from *Sloanea zuliaensis*

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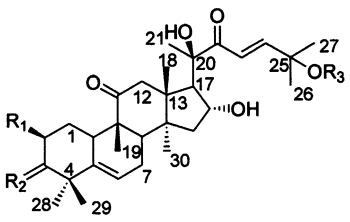
A new cucurbitacin D analogue, 2-deoxycucurbitacin D (**1**), as well as cucurbitacin D (**2**) and 25-acetylcucurbitacin F (**3**) were isolated from *Sloanea zuliaensis*. Compound **1** was found only in the young leaves of the plant and not in the mature leaves, and its structure was established using 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,[1] 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**)[2] and 25-acetylcucurbitacin F (**3**).[3] 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_1 = H, R_2 = O, R_3 = H
2 R_1 = OH, R_2 = O, R_3 = H
3 R_1 = OH, R_2 = αOH, R_3 = Ac

Compound **1** gave a molecular ion peak at *m*/*z* 499.3022 $[M - 1]^+$ in its HRCIMS, corresponding to the formula $C_{30}H_{44}O_6$. The ^1H and ^{13}C NMR data of **1** showed eight methyl singlets (δ_H/δ_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 δ_H 5.75 (δ_C 119.1, C-6), two *trans*-coupled olefinic protons at δ_H 6.68 and 7.13 (J = 15.2 Hz; δ_C 119.6, 155.6; C-23, C-24), three carbonyls at δ_C 213.6, 213.1, 202.9 (C-3, C-11, C-22), and three oxygenated functions δ_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,[4] which showed a similarity to that of cucurbitacin D (**2**) isolated from the same plant material,[2] 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 $^1H-^1H$ 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[2] and 25-acetylcucurbitacin F,[3,5] respectively. TLC profiles of extracts from young and mature leaves indicated the absence of **1** in mature leaves.

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

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

Table 1 shows the GI_{50} (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.[6]

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 $CDCl_3$ at 300 MHz for ^1H and 75 MHz for ^{13}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_{254s}) 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.[7] 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.[8] The activity was retained in the MeOH fraction (1.2 g), which was subjected to flash chromatography on Si gel using $CHCl_3$/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 $CHCl_3$ 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; $[\alpha]^{25}_D$ +51.0° (*c* 0.03, MeOH); IR (KBr) ν_{max} 3410, 2950, 1710, 1700, 1465, 1380 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 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-12α), 2.73 (1H, d, J = 14.8 Hz, H-12*β*), 2.65 (1H, m, H-10), 2.64 (1H, d, J = 6.9 Hz, H-17), 2.45 (1H, m, H-2α), 2.40 (1H, m, H-2*β*, H-7α), 1.90 (1H, br d, J = 6.1 Hz, H-8), 1.87 (1H, m, H-1α), 1.85 (1H, m, H-7*β*), 1.83 (1H, dd, J = 13.3, 6.9 Hz, H-15*β*), 1.50 (1H, m, H-1*β*), 1.42 (3H, s, Me-21), 1.40 (1H, d, J = 13.3 Hz, H-15α), 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); ^{13}C NMR (75 MHz, $CDCl_3$) δ 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 $[M - 1]^+$ (20), 498 (33), 482 (12), 439 (2), 388 (6), 369 (6), 326 (4), 189 (6), 112 (30), 96 (100); HRCIMS *m*/*z* 499.30225 $[M - 1]^+$ (calcd for $C_{30}H_{43}O_6$, 499.30596).

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