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A CYTOTOXIC DEOXOCUCURBITACIN FROM *DESFONTAINIA SPINOSA*

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Abstract—A new cytotoxic triterpene, 11-deoxocucurbitacin I was isolated from *Desfontainia spinosa*. The structure of this compound was elucidated by extensive analysis of the high field ^{1}H NMR and ^{13}C NMR spectra and field desorption mass spectrometry. This is the first example of a cucurbitacin lacking an 11-keto functionality.

INTRODUCTION

Desfontainia spinosa Ruiz and Pav. is a small tree which grows in mountainous regions of Central and South America. Its taxonomic placement has been uncertain, having been variously located in the Solanaceae, Aquifoliaceae and Loganiaceae, though taxonomists now accept *Desfontainia* as a member of the monotypic family Desfontainiaceae [1]. In his paper Schultes [1] reports several references to the use of this plant as a narcotic and hallucinogen by certain Chilean Indian tribes. A search of the lit. has revealed no previous phytochemical work on the genus.

In a continuation of our studies on the isolation of antineoplastic constituents from higher plants, we have initiated a phytochemical study of the leaf and stem material of *D. spinosa*, collected in Costa Rica, based on the demonstrated cytotoxicity of the ethanol extract of this plant toward KB cells in culture and *in vivo* activity against P388 lymphocytic leukemia in the mouse [2].

RESULTS AND DISCUSSION

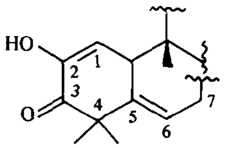
The ethanol extract showed toxicity in P388 *in vivo* and further partitioning yielded a cytotoxic fraction. This active 90% methanol fraction was passed through C-18 silica and then chromatographed on a silica gel column. Fractions were combined based on activity against P388 cells, the active fraction was separated on the chromatotron using silica gel plates and, finally, prep. TLC and crystallization from benzene afforded compound **1** as clear needles.

High resolution mass spectrometry established a molecular formula of $C_{30}H_{44}O_6$. The IR absorptions at 3350, 1680, 1645 and 1615 cm^{-1} indicated the presence of hydroxyl, conjugated carbonyl and olefinic functional groups, respectively. A partial structure (A) incorporating a diosphenol was revealed by the UV absorption at 268 nm ($\varepsilon = 6240$), which gave a bathochromic shift (312 nm, $\varepsilon = 2420$) upon addition of base. A similar shift was also observed in the UV spectrum of cucurbitacin I and suggested a tentative assignment to this group of triterpenes [3]. Examination of the ^{1}H NMR of cucurbitacin I and compound **1** revealed further similarities (Table 1).

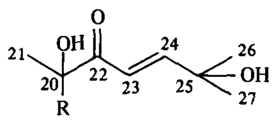
This partial structure was further supported by the olefinic proton resonance which occurred at δ6.12, $J_{\text{H-1-H-10}} = 2.3$ Hz) establishing the presence of a H-10 and a Me-9 group. The observation of ^{13}C NMR peaks at δ117.4 (C-1), 144.6 (C-2) and 199.4 (C-3) confirmed the diosphenol moiety. The ^{13}C NMR signals at δ138.7 (C-5) and 121.2 (C-6) and a ^{1}H NMR signal at δ5.67 (*t*, $J_{\text{H-6-H-7}} = 2.1$ Hz) suggested the presence of a $\Delta^{5,6}$-functional group in compound **1**.

The 470 MHz ^{1}H NMR spectrum also clearly displayed eight methyl groups (δ1.39, 1.33, 1.30, 1.24, 1.22, 1.15, 1.09 and 0.83) consistent with the basic skeleton of triterpenes. The methyl signals at δ1.39, 1.33 and 1.30 indicated attachment to carbon atoms bearing hydroxyl groups.

A typical AX pattern (doublets at δ7.07 and 6.63; $J_{AX} = 15.2$ Hz) suggested the existence of a *trans*-α,β-



Partial structure A



Partial structure B

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Table 1. ^{1}H NMR analysis for compound 1 and cucurbitacin I

	1			Cucurbitacin I		
Assignment	Chemical shift (δ)	Multiplicity	Coupling constant (J, Hz)	Chemical shift (δ)	Multiplicity	Coupling constant (J, Hz)
H-1	6.13	*d*	2.3	5.95	*d*	2.7
H-6	5.67	*t*	2.1	5.74	*brs*	
H-16	4.37	*t*	7.5	4.36	*t*	7.0
H-23	6.63	*d*	15.2	7.11	*d*	15.2
H-24	7.07	*d*	15.2	6.61	*d*	15.2

unsaturated carbonyl group. The field desorption mass spectrum showed strong peaks at m/z 113 and 387 $[\mathrm{M}-113]^+$ (Fig. 1). All these data established the presence of a typical cucurbitacin side chain (partial structure **B**). The ^{13}C NMR signals at δ203.2 (C-22), 155.3 (C-24), 119.3 (C-23), 78.6 (C-20), 71.0 (C-25), 27.6 (C-26, C-27) and 29.5 (C-21) further substantiated this conclusion.

The observation of only two carbonyl carbon peaks at δ203.2 and 199.4, ascribed to C-22 and C-3, respectively [4, 5], and the absence of a typical cyclohexanone IR absorption (> 1700 cm^{-1}) indicated the absence of the 11-keto function. Based on this evidence compound **1** is proposed to be the novel compound 11-deoxocucurbitacin I. A more complete ^{13}C NMR assignment is shown in Table 2. The chemical shifts were assigned on the basis of previously reported data [4, 5] and simple chemical shift theory.

EXPERIMENTAL

The leaves, twigs and small branches of *D. spinosa* Ruiz and Pav. were collected in 1976 in Costa Rica by Dr. Jose A. Saenz-Renauld of the Universidad de Costa Rica. The identification was confirmed by Dr. Sandra Saufferer, Economic Botany Laboratory, U.S. Department of Agriculture, Beltsville, MD 20704, where a voucher specimen supplied by Dr. Saenz-Renauld is on deposit.

Air-dried, finely ground plant material (10 kg) was percolated with 95% EtOH, yielding 1.6 kg extract. This extract was first partitioned between H_2O and $CHCl_3$, then the $CHCl_3$ solubles were partitioned between 90% MeOH and petrol to give 530 g aq. MeOH solubles. Partition fractions were tested in the P388 mouse leukemia and the 9KB cytotoxicity assays [2]. The 90% MeOH-soluble fraction was found to be active (132% T/C at 50 mg/kg in P388; ED_{50} 5.5×10^{-2} μg/ml in 9KB) and was

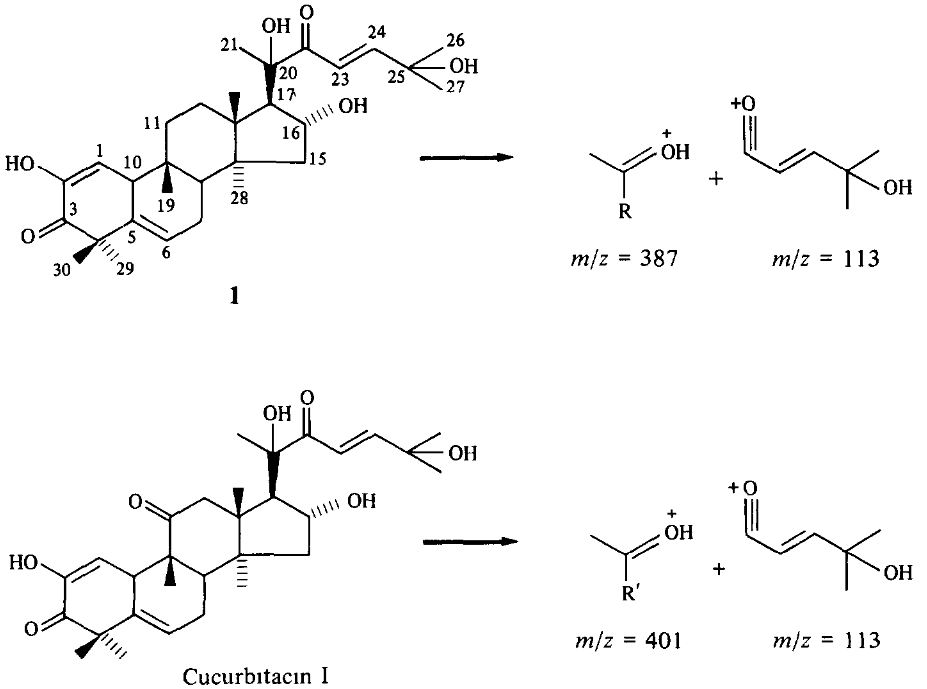

Fig. 1. Structures of 11-deoxocucurbitacin I (1) and cucurbitacin I, and mass spectral fragmentation of the side chain.

Table 2. ^{13}C NMR analysis for compound **1** and cucurbitacin I*

C No.	**1**	Cucurbitacin I†
1	117.4	115.0
2	144 6	144.6
3	199.4	198.7
4	43.3†	47.6
5	138.7	137.0
6	121.2	120.7
7	24.1	23.6
8	41.5	41.6
9	34.8	48.8
10	37.5	34.7
11	30.3§	212.9
12	31.0§	48.8
13	48.7‡	48.3
14	49.0‡	50.8
15	46.0	45.7
16	71.9	71.6
17	53.2	57.4
18	20.2‖	20.0
19	18 0‖	18.6
20	78.6	78.1
21	29.5¶	24.0
22	203.2	202.7
23	119.3	119.0
24	155.3	155.9
25	71.0	71.2
26	27.6	29.0
27	27.6	29.6
28	24.1	20 1
29	28.9¶	27.9
30	18.2‖	20.1

*In CDCl$_3$.

†In CDCl$_3$ (ref. [4]).

‡, §, ‖, ¶ Assignments with the same symbol may be interchanged.

selected for further fractionation. This fraction (530 g) was triturated with 4 l. MeOH–H$_2$O–MeCN (2:2:1), the residue separated and the soluble portion filtered through a column of C-18 phase bonded Hiflosil silica. The material (218 g) which passed through the column in the MeOH–H$_2$O–MeCN (2:2:1) solvent system was cytotoxic (ED$_{50}$ 3×10^{-1} μg/ml, 9KB). A portion of this material (104 g) was chromatographed on 2.5 kg MN 81532 silica gel. This gravity column was developed with a gradient of increasing MeOH in CHCl$_3$ and the 90 l. of eluate was collected in 60 ml fractions and pooled (fractions A–R) based on TLC. These 18 pooled fractions were tested in P388 *in vivo* and *in vitro* revealing a zone of cytotoxicity from fractions F to N. Fractions G (12 g), I (2.2 g) and K (28 g) yielded yellow ppts, respectively, upon concn. The coloured material could be removed by repeated triturations with Me$_2$CO, the washings being pooled with their respective parent fraction. These ppts were found to be semi-pure triterpene glycosides related to compound **1**.

The toxic fraction G (12 g) was further chromatographed on a dry column of silica gel, developing with 10% aq. EtOAc and 30% CH$_2$Cl$_2$ in hexane. One of the dry column fractions (100 mg) which showed two components by TLC was applied to a 2 mm silica 7749 chromatotron rotor and developed with EtOAc–CH$_2$Cl$_2$–MeOH (49:49.2). A total of 48 fractions were collected which, following examination by TLC, were pooled into five fractions. Only a partial clean-up had been obtained, so the best fractions were streaked in 10 mg aliquots, onto EM5602, 20 × 20 cm, 0.25 mm thick RP-2f glass plates which were developed 6–8 times with 10% EtOAc, 0.5% glacial HOAc in hexane Examination by short-wave UV at 254 nm showed a major band at R_f 0.45. This was scraped from the plate, eluted with Me$_2$CO, filtered and solvent evaporated to yield **1** as a white powder in an overall 0.003% yield from the plant material. This compound was cytotoxic (9PS, ED$_{50}$ 2.4×10^{-1} μg/ml; 9KB, ED$_{50}$ 1.0 $\times 10^{-2}$ μg/ml). Recrystallization from C$_6$H$_6$ gave **1** as colourless needles, mp 212–213°. The compound showed the following spectroscopic profile: UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 231 ($\varepsilon = 8500$), 268 ($\varepsilon = 6240$); IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3350 (br), 1680, 1645, 1615, 1390; Field desorption MS *m/z*: 500.319 (calc. for C$_{30}$H$_{44}$O$_6$: 500.314); ^{1}H NMR (470 MHz, CDCl$_3$): δ7.07 (1H, *d*, $J = 15.2$ Hz, H-24), 6.63 (1H, *d*, $J = 15.2$ Hz, H-23), 6.13 (1H, *d*, $J = 2.3$ Hz, H-1), 5.67 (1H, *t*, $J = 2.1$ Hz, H-6), 4.37 (1H, *t*, $J = 7.5$ Hz, H-16), 1.39 (3H, *s*, H-21), 1.33 (3H, *s*, H-26 or H-27), 1.30 (3H, *s*, H-27 or H-26), 1.24 (3H, *s*), 1.22 (3H, *s*), 1.15 (3H, *s*), 1.09 (3H, *s*), 0.83 (3H, *s*, H-28); ^{13}C NMR: see Table 2.

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