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Abroniene, a rotenoid from the desert annual *Abronia villosa*

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Abstract

A high-throughput phytochemical investigation of *Abronia villosa* afforded a new rotenoid designated abroniine (**1**) along with the known compounds boeravinone C and lupeol. The structure of **1** was determined using NMR, MS, and optical analysis with < 400 µg of material. Compound **1** displayed moderate cytotoxicity against NCI-H460 and HL-60 human cancer cell lines with IC₅₀ values of 14 and 36 µM, respectively.

Keywords

Abronia villosa; Nyctaginaceae; rotenoid; abroniine; cytotoxicity

1. Introduction

Natural product libraries screened against biological targets continue to be valuable sources of structurally diverse compounds that provide leads and inspiration for drug discovery and development. In our continuing effort to explore minor components of plants, we have applied our miniaturized high-throughput natural product isolation, analysis, and structure elucidation methods (Eldridge et al., 2002; Hu et al., 2005) to a taxonomically diverse set of plants. Screening the resulting natural product library for cytotoxicity against human lung cancer cells revealed an active fraction from the hairy sand verbena *Abronia villosa* S. Wats. (Nyctaginaceae). From this fraction we isolated one new compound, abroniine (**1**), as well as the closely related known compound boeravinone C (**2**) and the triterpene lupeol. Compounds **1** and **2** are rotenoids, a class of compound best known for their insecticidal activity. Rotenoids are the main components of cubé resin, long used as an insecticide and fish poison (Fang and Casida, 1998, 1999). Anticancer properties of rotenoids have also been investigated (Ahmed-Belkacem et al., 2007); many of these studies have focused on the prenylated rotenoid deguelin (Kim et al., 2008; Oh et al., 2007). Very few natural products have been reported from the genus *Abronia*. *A. latifolia* has been reported to contain abronisoflavone as well as other common flavones and flavonols (Wollenweber et al., 1993).

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2. Results and discussion

Abroniene (**1**) was isolated from an organic extract of *A. villosa* along with the known compounds boeravinone C (**2**) and lupeol. Lupeol was identified by examination of the 1D and 2D NMR data and comparison of these data with those of a known sample. Compound **2** was identified by examination of the 2D NMR data and confirmed by comparison of its NMR data to those reported previously (Lami et al., 1990). The molecular formula of **1** was determined to be C₁₈H₁₆O₆ based on high-resolution electrospray ionization mass spectrometry (HRESIMS). Its ¹H NMR spectrum resembled that of **2**, but differed in the signals for the protons in the A-ring (Table 1). The proton coupling pattern of the A-ring protons, combined with COSY and HMBC signals, indicated that **1** lacks the 4-hydroxyl group found in **2**. Consistent with the lack of oxygenation at C-4, the ¹³C chemical shifts of C-4a and C-3 in **1** are each shifted 11–15 ppm downfield with respect to those in **2**.

The B/C ring junction of **1** was determined to be *trans* based on the downfield chemical shift of H-1, which has been shown to be diagnostic in the rotenoids (Unai et al., 1973). For **2**, this assignment has been further verified by NOE measurements on derivatives in which the ketone at the 12-position has been reduced to a hydroxyl (Lami et al., 1990). Unai *et al* also demonstrated a striking pattern in the optical rotation values for 12a-hydroxy rotenoids with *trans* ring junctions (Unai et al., 1973). Those with a 6aα,12aβ-configuration display large negative specific rotations, while those with a 6aβ,12aα-configuration display large positive specific rotations. The specific rotation of **1**, like that of **2**, is large and negative. Based on optical rotation as well as a likely common biosynthetic origin with **2**, we assigned the absolute configuration shown in Figure 1.

Compound **1** was tested for cytotoxicity against two human cancer cell lines: NCI-H460 (large cell lung carcinoma) and HL-60 (promyelocytic leukemia). Deguelin was also tested for comparison (Table 2). When tested over a wide concentration range in HL-60, deguelin displayed a biphasic response; its dose-response data were therefore fit to a biphasic curve, yielding two independent IC₅₀ values. The other compound/cell combinations yielded monophasic dose-response curves.

3. Experimental

3.1. General experimental procedures

NMR spectra were acquired on a Bruker Avance 600 MHz spectrometer equipped with a 5 μl CapNMR capillary microcoil probe with a 1.5 μl active volume (Magnetic Resonance Microsystems, Savoy, IL). For each compound, ¹H, gCOSY, ROESY, HSQC, and HMBC spectra were acquired; ¹³C chemical shifts (150 MHz) were obtained from the HSQC and HMBC spectra. Spectra were recorded in CD₃OD, and chemical shifts are reported with respect to the residual non-deuterated solvent signal. HRESIMS was done on a Waters LCT time-of-flight mass spectrometer with an electrospray interface and polyethylene glycol as the internal standard. The amount of each compound isolated was determined using HPLC/ELSD as previously described (Hu et al., 2005). Semipreparative HPLC was performed on a Beckman HPLC system including a Beckman 168 diode array UV detector, a Sedex Sedex 75 evaporative light scattering detector (ELSD), and an ISCO Foxy Jr. fraction collector. A splitter was used to divert 10% of the eluent to the ELSD detector, while the rest passed through a diode array UV detector and was then collected. UV λ_{max} values were taken from the diode array detector during semipreparative HPLC purification in 45–65% CH₃CN with 0.05% TFA. Optical rotation was measured on Jasco P-1010 polarimeter using a 100 μL cell with a 0.1 dm path length. Deguelin was obtained from Sigma-Aldrich.

3.2. Plant material

Aerial parts of *A. villosa* were collected in April of 2000 in the Mojave Desert of California by John Stone and Sharon Bodine of the Missouri Botanical Garden. A voucher specimen (#Stone-2833) is kept at the Missouri Botanical Garden (USA).

3.3 Extraction and isolation

Ground *A. villosa* (87 g dry weight) was extracted by shaking at room temperature with EtOH/EtOAc (1:1, v/v, 2 × 500 ml). The slurry was filtered, and the resulting solution dried to obtain 1.4 g of extract. One gram of *A. villosa* extract was then separated by silica gel flash chromatography as previously described (Eldridge et al., 2002) to generate flash fractions 2 to 5. Flash fractions 2 to 5 were further separated by reversed phase preparative HPLC to provide the *A. villosa* library. The flash fraction 2 library was prepared from a 50 mg aliquot (of 188 mg available) of flash fraction 2 using a Betasil C₁₈ column (Thermo Scientific, 21.2 × 100 mm, 5 μm) with a flow rate of 20 ml/min. The column was eluted with 60% CH₃CN for 2 min, followed by a 30 min gradient to 100% CH₃CN, then 100% CH₃CN for 8 minutes; one-minute fractions were collected from 0–40 min. Preparative HPLC fraction 7 was then further purified by semipreparative HPLC on a Synergy Hydro C₁₈ column (Phenomenex, 4.6 × 250 mm, 4 μm) eluted at 1.5 ml/min with 50% CH₃CN for 5 min, followed by a gradient to 70% CH₃CN over 20 min. Semipreparative HPLC solvents contained 0.05% (v/v) TFA. Two injections afforded 50 μg of **1** (*t*_R = 14.1 min).

To provide additional material for optical rotation measurement and biological testing, the remaining 138 mg of flash fraction 2 was subjected in two portions to preparative HPLC as above. Preparative HPLC fractions 5–8 were then pooled and purified by semipreparative HPLC as above but with a shallower gradient: 45% CH₃CN for 5 min, then a gradient to 65% CH₃CN over 20 min. Solvents again contained 0.05% (v/v) TFA. Four injections afforded 270 μg of **1** (*t*_R = 18.3 min), as well as 70 μg of **2** (*t*_R = 9.3 min). Preparative HPLC fractions 26–29 were pooled and purified by semipreparative HPLC on a Fluophase PFP column (Thermo Scientific, 7.7 × 250 mm, 5 μm) eluted at 3 ml/min with 60% CH₃CN for 5 min, followed by a gradient to 95% B over 40 min. Six injections afforded 100 μg of lupeol (*t*_R = 27.7 min).

3.4 Cytotoxicity assay

NCI-H460 and HL-60 cells were obtained from ATCC, and were grown in media (RPMI-1640 with 10% FBS for HCl-H460 or IMDM with 20% FBS for HL-60) supplemented with L-glutamine and HEPES. NCI-H460 cells were seeded into 96-well plates at 6 × 10² cells/well and allowed to adhere overnight; the media was then removed. HL-60 cells were seeded at 6 × 10⁴ cells/well in 50 μl. A stock solution of test compound in DMSO was diluted in media to generate a series of working solutions. Aliquots (100 μl for NCI-H460, or 50 μl for HL-60) of the working solutions were added to the appropriate test wells to expose cells to the final concentrations of the compounds in a total volume of 100 μl. Eight different concentrations were tested, with 2–5 wells per concentration. Camptothecin, a commercially available cytotoxic alkaloid, was used as a positive control; wells containing vehicle without compound were used as negative controls. Plates were kept for 48–72 hr in a 37°C, 5% CO₂ incubator. After incubation, viable cells were detected with the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega). Dose-response curves were generated and IC₅₀ values were determined using GraphPad Prism 5 software.

3.5 Abronione (1)

White solid; [α]_D²⁵ –633° (c 0.071, Me₂CO); UV λ_{max} 228, 296 nm; ¹H and ¹³C NMR: see Table 1; HR-ESIMS *m/z* 327.0859 [M-H][–] (calcd for C₁₈H₁₅O₆: 327.0869).

3.6 Boeravinone C (2)

$[\alpha]_D^{25} -442^\circ$ (*c* 0.024, Me₂CO); UV $\lambda_{\max}$ 218, 295 nm; ¹H and ¹³C NMR: see Table 1; ESIMS *m/z* 345 [M+H]⁺.

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Fig. 1.
Structures of compounds **1–2** and deguelin.

Table 1

NMR Spectroscopic Data (600 MHz, CD₃OD) for Abronione (1) and Boeravinone C (2)

position	abronione (1)			boeravinone C (2)		
	δ_C	δ_H (mult, J in Hz)	HMBC ^a	δ_C	δ_H (mult, J in Hz)	
1	133.2	8.34 (d, 7.9)	3, 4a, 12a	123.3	7.84 (d, 7.3)	
1a	120.6			122.3		
2	121.9	7.02 (t, 7.6)	1a, 4	121.1	6.85 (t) ^b	
3	131.7	7.26 (t, 7.5)	1, 4a	117.3	6.84 (t) ^b	
4	118.4	6.87 (d, 8.2)	2a, 4a	148.0		
4a	156.7			145.0		
6	62.7	4.36 (dd, 9.8, 4.3)	4a, 6a, 12a	62.8	4.45 ^b	
		4.43 (t, 10.6)	6a, 12a		4.45 ^b	
6a	77.6	4.61 (dd, 11.4, 4.3)	6, 12, 12a	77.9	4.67 (dd, 11.0, 4.8)	
7a	162.8			161.8		
8	91.7	6.20 (s)	7a, 9, 10, 11a, 12	91.7	6.22 (s)	
9	167.7			167.1		
10	107.5			107.4		
11	163.3			163.2		
11a	104.8			104.3		
12	196.1			nd ^c		
12a	67.6			67.3		
9-OMe	56.5	3.87 (s)	9	56.7	3.88 (s)	
10-Me	7.1	1.97 (s)	9, 10, 11	7.1	1.98 (s)	

^a HMBC correlations are from the proton(s) stated to the indicated carbon.

^b Signal partially obscured.

^c Not determined.

Table 2IC₅₀s of **1** and deguelin against human cancer cell lines (μM)

Compound	NCI-H460	HL-60
1	14	36
deguelin	12	0.0013, 19 ^a
camptothecin	0.003	0.01

^aData were fit to biphasic curve with two IC₅₀ values.