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Withanolides from *Jaborosa caulescens* var. *bipinnatifida*

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Abstract

Two new withanolides 2,3-dihydrotrechonolide A (**1**) and 2,3-dihydro-21-hydroxytrechonolide A (**2**) were isolated along with two known withanolides trechonolide A (**3**) and jaborosalactone 39 (**4**) from *Jaborosa caulescens* var. *bipinnatifida* (Solanaceae). The structures of **1-2** were elucidated through 2D NMR and other spectroscopic techniques. In addition, the structure of withanolide **1** was confirmed by X-ray crystallographic analysis.

Keywords

2, 3-dihydrotrechonolide A; 2, 3-dihydro-21-hydroxytrechonolide A; Withanolide; *Jaborosa caulescens* var. *bipinnatifida* ; Solanaceae

1. Introduction

Withanolides are a group of modified, highly-oxygenated C₂₈ ergostane-type steroids, present primarily in several genera of the Solanaceae which include *Acnistus*, *Datura*, *Dunalia*, *Jaborosa*, *Physalis*, *Vassobia* and *Withania*. In recent years, these C₂₈ steroids have attracted attention due to their structural diversity as well as their biological activities including promising antitumor, insecticidal and phytotoxic capacities (Chen et al., 2011; Misico et al., 2011; Zhang et al., 2012a). Recently we reported the isolation and characterization of a series of withanolides with structural variations in the steroidal nucleus and the side chain from *Datura wrightii* (Zhang et al., 2013), *Physalis longifolia* (Zhang et al., 2011; Zhang et al., 2012b), *Vassobia brevifolia* (Samadi et al., 2010), and *Withania somnifera* (Tong et al., 2011). There are a total of 23 *Jaborosa* species which are mostly

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Supporting Information

¹H, ¹³C(APT), and 2D NMR spectra of withanolides **1** and **2** are available. Crystallographic data for the structure of **1** as reported in this paper were deposited with the Cambridge Crystallographic Data Centre, under reference number CCDC 943258. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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distributed throughout South America and contain abundant and diverse withanolides (Misico et al., 2011). In continuing our withanolide research, we examined the above-ground biomass of the Chilean perennial shrub *Jaborosa caulescens* var. *bipinnatifida* Gillies & Hook. Herein we report the isolation and structure elucidation of two new withanolides (**1** and **2**) as well as two known withanolides (**3** and **4**) from the species.

2. Results and discussion

Compounds **1-4** were isolated from the CH₂Cl₂ partition phase of the CH₂Cl₂-MeOH (1:1) extract of *J. caulescens* var. *bipinnatifida* (see Experimental section). The molecular formula of the major component **1** was determined to be C₂₈H₃₈O₇ by HRESIMS and NMR experiments, equating to ten double-bond equivalents. The IR absorptions of **1** revealed the presence of hydroxyl (3328 cm⁻¹), ester (1748 cm⁻¹) and keto (1712 cm⁻¹) groups. The ¹H NMR spectrum (Table 1) showed the presence of five methyl groups at δ_H 0.97 (3H, s), 1.00 (3H, d, *J* = 6.7 Hz), 1.07 (3H, s), 1.77 (3H, s), and 1.89 (3H, s); three protons attached to oxygenated carbons at δ_H 3.12 (1H, brs), 3.95 (1H, dd, *J* = 11.1, 1.9 Hz), 4.82 (1H, s). The ¹³C NMR (APT) and HSQC spectra for **1** (Table 1) displayed 28 carbon signals differentiated as five CH₃, seven CH₂, seven CH (including three oxygenated at δ_C 60.2, 68.8, and 82.4), and nine C (including a keto carbonyl at δ_C 213.2, an ester carbonyl at δ_C 175.2, two olefinic at δ_C 157.3 and 123.8, an hemiketal or ketal at δ_C 98.6, as well as two oxygenated at δ_C 80.5 and 64.1) groups, corresponding to C₂₈H₃₆. The remaining two hydrogen atoms were therefore assigned as two OH groups, indicating that seven rings must be present in the structure.

The NMR data of **1** closely resembled those of trechonolide A (**3**) (Figure 1), a seven-ringed withanolide first reported in *Jaborosa laciniata* (syn: *Trechonaetes laciniata*) (Lavie et al., 1987). Compounds **1** and **3** were found to contain identical α,β-unsaturated γ-lactone units in the side chain [two vinylic methyl groups at δ_{H-27} 1.77 and δ_{H-28} 1.89 (each 3H, s), one oxygenated methine group at δ_{H-23} 4.82 (1H, s); and δ_{C-26} 175.2 (C), δ_{C-24} 157.3 (C), δ_{C-25} 123.8 (C), δ_{C-23} 82.4 (CH), δ_{C-28} 12.0 (CH₃), and δ_{C-27} 8.4 (CH₃)], a hemiketal bridge formed by the 22-hydroxyl group and a ketone at C-12 to result in a six-membered ring with a β-oriented hydroxyl at C-12 [δ_{H-22} 3.95 (1H, dd, *J* = 11.1, 1.9 Hz), δ_{H-21} 1.00 (3H, d, *J* = 6.7 Hz); δ_{C-22} 68.8 (CH), δ_{C-12} 98.6 (C), δ_{C-17} 80.5 (C), and δ_{C-21} 10.2 (CH₃)], as well as a 5β,6β-epoxy group [δ_{H-6} 3.12 (1H, brs), δ_{C-5} 64.1 (C) and δ_{C-6} 60.2 (CH)].

The main differences between **1** and **3** were observed within the A ring moiety of the steroid nucleus. The ¹H NMR of compound **1** revealed the absence of the olefinic protons pertaining to the conjugated 1-oxo-2-ene moiety observed in **3**. Instead, the ¹³C NMR and HSQC of **1** showed signals for an isolated keto (δ_C 213.2) and two extra methylenes [δ_C 34.2 (CH₂) corresponding to δ_H 2.64 (1H, ddd, *J* = 14.5, 9.6, 6.2 Hz) and 2.22 (1H, ddd, *J* = 14.5, 8.7, 5.3 Hz); and δ_C 17.7 (CH₂) corresponding to δ_H 1.91 (1H, m) and 1.88 (1H, m)] groups. These observations suggested compound **1** was 2,3-dihydrotrechonolide A. This was further supported by ¹H-¹H COSY fragment of -C(2)H₂-C(3)H₂-C(4)H₂-; the chemical shift values of H₂-2 [δ_H 2.64 (1H, ddd, *J* = 14.5, 9.6, 6.2 Hz) and 2.22 (1H, ddd, *J* = 14.5, 8.7, 5.3 Hz)]; the HMBC correlations between H₂-2, H-3 (δ_H 1.88, 1H, m) and C-1 (δ_C 213.2); as well as the superimposable NMR signals for the moieties of rings A and B observed in both **1** and jaborosalactone **39** (**4**), reported from the same species (Nicotra et al., 2007).

Finally, the structure of **1** was confirmed through a single-crystal X-ray diffraction experiment (Figure 2). Thus, withanolide **1** was determined as 2,3-dihydrotrechonolide A. The full assignments of NMR data of **1** (Table 1) were obtained by 2D-NMR including ¹H-¹H COSY, multiplicity edited-HSQC, HMBC and ROESY experiments.

The molecular formula of compound **2** was determined to be $C_{28}H_{38}O_8$ by HRESIMS and NMR experiments. Its IR absorptions and NMR data (Table 1) resemble those of **1**. The main differences between **2** and **1** were observed in the C-21 NMR signals. The absent C-21 methyl signal along with the appearance of a pair of double doublets at δ_H 4.14 (1H, dd, $J = 12.4, 1.4$ Hz) and 3.93 (1H, dd, $J = 12.4, 3.9$ Hz) in compound **2** suggested that **2** was a 21-hydroxy derivative of **1**. This observation was supported by the resonance signals of C-21 (CH_2 , δ_C 59.9, δ_H 4.14 and 3.93), 1H - 1H COSY fragment of-C(21)H₂-C(20)H-C(22)H-CH(23)- [H_2 -21 δ_H 4.14 (1H, dd, $J = 12.4, 1.4$ Hz) and 3.93 (1H, dd, $J = 12.4, 3.9$ Hz); H-20 δ_H 2.19 (1H, ddd, $J = 11.3, 3.9, 1.4$ Hz); H-22 δ_H 4.69 (1H, dd, $J = 11.3, 1.9$ Hz); and H-23 δ_H 5.08 (1H, s)]. It was confirmed by HMBC correlations between H_2 -21 (δ_H 4.14 and 3.93) and C-20 (δ_C 59.9), C-22 (δ_C 64.7), and C-17 (δ_C 83.0), as well as the superimposable NMR signals for the side chain of **1** and jaborosalactone 41, reported from the same species (Nicotra et al., 2007). Thus, withanolide **2** was determined as 2,3-dihydro-21-hydroxytrechonolide A.

Withanolides **1-4** share a distinct structural moiety, a hemiketal bridge, that has only been reported in withanolides from the Solanaceous genera *Deprea* (Su et al., 2003) and *Jaborosa* (Misico et al., 2011). This structural moiety is formed by the interaction of 22-hydroxyl (or 21-hydroxyl) and C-12 ketone groups, resulting in a six-membered ring composed of C-12, 13, 17, 20, 22 (or 21) and an oxygen atom. In addition, compound **4** contains a commonly encountered δ -lactone in the side chain, whereas compounds **1-3** possess a less prevalent γ -lactone structural moiety that is confined to the genera *Deprea*, *Jaborosa*, and *Physalis*. In conclusion, compounds **1** and **2** are two new additions to this limited structural sub-type of withanolides.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Rudolph RS Autopol IV automatic polarimeter. IR data were obtained with a Thermo Nicolet Avatar 380 FT-IR spectrometer. NMR spectra were recorded with a Bruker AV-400 or AV-500 instrument with a cryoprobe for 1H , APT ^{13}C , COSY, HSQC, HMBC, and ROESY. Chemical shift values are given in δ (ppm) using the peak signals of the solvent $CDCl_3$ (δ_H 7.26 and δ_C 77.2) as references and coupling constants were reported in Hz. HRESIMS data were collected with a LCT Premier time of flight mass spectrometer (Waters Corp., Milford, MA). Column chromatography was performed on CombiFlash columns (Teledyne Isco, Lincoln, NE). Normal-phase silica gel G TLC plates (w/UV 254) and reversed-phase C_{18} TLC plates (w/UV 254) (Sorbent Technologies, Atlanta, GA) were used for fraction and compound detection. The spots were visualized using UV light at 254 nm and spraying with 10% EtOH-sulfuric acid reagent. Semi-preparative HPLC was performed on an Agilent 1200 unit equipped with a DAD detector, utilizing a Lichrospher RP-18 column (250 \times 10 mm, 5 μ m).

3.2 Plant material

Above-ground biomass of *J. caulescens* var. *bipinnatifida* was collected by Luis González in the Cordillera de Santa Ana, in the vicinity of La Serena (latitude: 29°48'3 S; longitude: 70°2'7 W), Coquimbo (IV region), Chile on Jan. 15, 1995. The plant was authenticated by Gloria Montenegro of Pontificia Universidad Católica de Chile (P. U. C.). A voucher specimen, 0561, was deposited in the Herbarium of the P. U. C. Intellectual Property Agreements were signed by the collaborating institutions and deposited at the National Institutes of Health.

3.3 Extraction and isolation

The collected biomass was air dried, ground to a coarse powder (475 g) and stored in an air-tight dark container until processing time. It was extracted three times with CH₂Cl₂-MeOH (50:50, 2.0 L) at room temperature. After removing the solvents under vacuum, the extract (50 g) was suspended in 300 mL H₂O, followed by successive partitions with *n*-hexane, dichloromethane and *n*-butanol (3 × 300 mL). The resulting CH₂Cl₂ fraction (7.5 g) collected was applied to a CombiFlash column (220 g silica gel) and eluted subsequently with a mixture of hexane-acetone (95:5, 90:10, 80:20, 67:33, 50:50, 33:67), in order of increasing concentrations of acetone.

The 20% acetone fraction (750 mg) was subjected to CombiFlash CC (40 g silica gel), eluted with CH₂Cl₂:EtOAc (10:1, 6:1, and 4:1) with decreasing amounts of CH₂Cl₂ to afford two fractions. Fraction 1 (120 mg) was subjected to CombiFlash CC (24 g silica gel), eluted with hexane:EtOAc (1:1) to afford a fraction of the major compound. This fraction was subjected to semi-HPLC, eluted by isocratic CH₃CN (38%) to afford 2,3-dihydrotrechonolide A (**1**, 42 mg). Fraction 2 (140 mg) was subjected to CombiFlash CC (24 g silica gel), eluted with hexane:EtOAc (1:1) to afford a withanolide-containing fraction. This fraction was subjected to semi-HPLC, eluted by isocratic CH₃CN (41%) to afford trechonolide A (**3**, 15 mg).

The 33% acetone fraction (890 mg) was subjected to CombiFlash CC (80 g silica gel), eluted with CH₂Cl₂:acetone (10:1, 5:1, and 3:1) with decreasing amounts of CH₂Cl₂ to afford two fractions. The first fraction (150 mg) was subjected to CombiFlash CC (24 g silica gel), eluted with CH₂Cl₂:EtOAc (4:1) to afford 2,3-dihydro-21-hydroxytrechonolide A (**2**, 5 mg). The second fraction (200 mg) was subjected to a CombiFlash CC (24 g silica gel), eluted with CH₂Cl₂:EtOAc (3:1) to afford jaborosalactone 39 (**4**, 7 mg).

3.3.1. 2,3-dihydrotrechonolide A (1)—Cubic colorless crystals; $[\alpha]_D^{25}$ -2.2 (*c* 0.1, CHCl₃); UV (CHCl₃) ν_{\max} (log ϵ) 225 (4.25) nm; IR (neat) ν_{\max} 3328 (br), 2943, 2833, 1748, 1712, 1446, 1018 cm⁻¹; HRESIMS *m/z* 509.2500 [M+Na]⁺ (calcd for C₂₈H₃₈O₇Na, 509.2515); ¹H NMR and ¹³C NMR, see Tables 1.

3.3.2 Single-Crystal X-ray structure determination of 2,3-dihydrotrechonolide A (1)—Crystal analysis was performed with a colorless cubic crystal (dimensions 0.20 × 0.19 × 0.12 mm³) obtained from CH₂Cl₂-CH₃CN (1:1) using Cu K α radiation (λ = 1.54178 Å) on a Bruker APEX2 diffractometer equipped with a Bruker MicroStar microfocussing rotating anode X-ray source and Helios multilayer optics. Crystal data for **1**: C₂₈H₃₈O₇ (formula weight 486.58), Orthorhombic, space group *P*2₁2₁2₁, T = 100(2) K, crystal cell parameters *a* = 11.1455(3) Å, *b* = 11.9845(4) Å, *c* = 18.3310(5) Å, *V* = 2448.53(12) Å³, *D_c* = 1.320 Mg/m³, *Z* = 4, F(000) = 1048, absorption coefficient μ = 0.763 mm⁻¹. A total of 17017 reflections were collected in the range 4.41 < θ < 69.80°, with 4487 independent reflections [*R*_(int) = 0.0188], completeness to θ = 66° was 99.8%. Multi-scan absorption correction applied; full-matrix least-squares refinement on *F*², the number of data/restraints/parameters were 4487/0/469; goodness-of-fit on *F*² = 1.041; final *R* indices [*I* > 2 σ (*I*)], *R*₁ = 0.0248, ωR^2 = 0.0650; *R* indices (all data), *R*¹ = 0.0248, ωR^2 = 0.0650; largest difference peak and hole, 0.239 and -0.159 e/Å⁻³.

3.3.3. 2,3-dihydro-21-hydroxytrechonolide A (2)—Solid; $[\alpha]_D^{25}$ -0.8 (*c* 0.1, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 225 (3.88) nm; IR (neat) ν_{\max} 3323 (br), 2943, 2832, 1737, 1708, 1449, 1021 cm⁻¹; HRESIMS *m/z* 525.2484 [M+Na]⁺ (calcd for C₂₈H₃₈O₈Na, 525.2464); ¹H NMR and ¹³C NMR, see Tables 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Four withanolides **1-4** were isolated from *Jaborosa caulescens* var. *bipinnatifida* (Solanaceae).
- 2,3-dihydrotrechonolide A (**1**) and 2,3-dihydro-21-hydroxytrechonolide A (**2**) are new and uncommon.
- The structure of withanolide **1** was confirmed by X-ray crystallographic analysis.

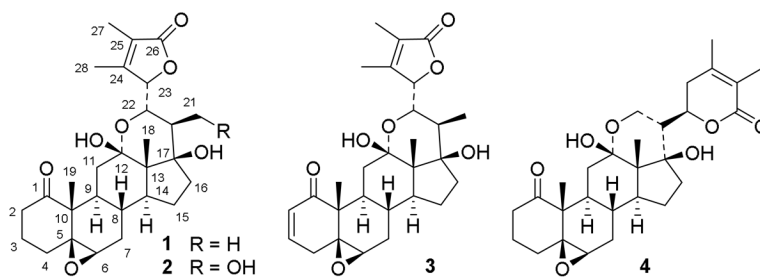


Fig. 1.
Withanolides **1-4** isolated from *Jaborosa caulescens* var. *bipinnatifida*

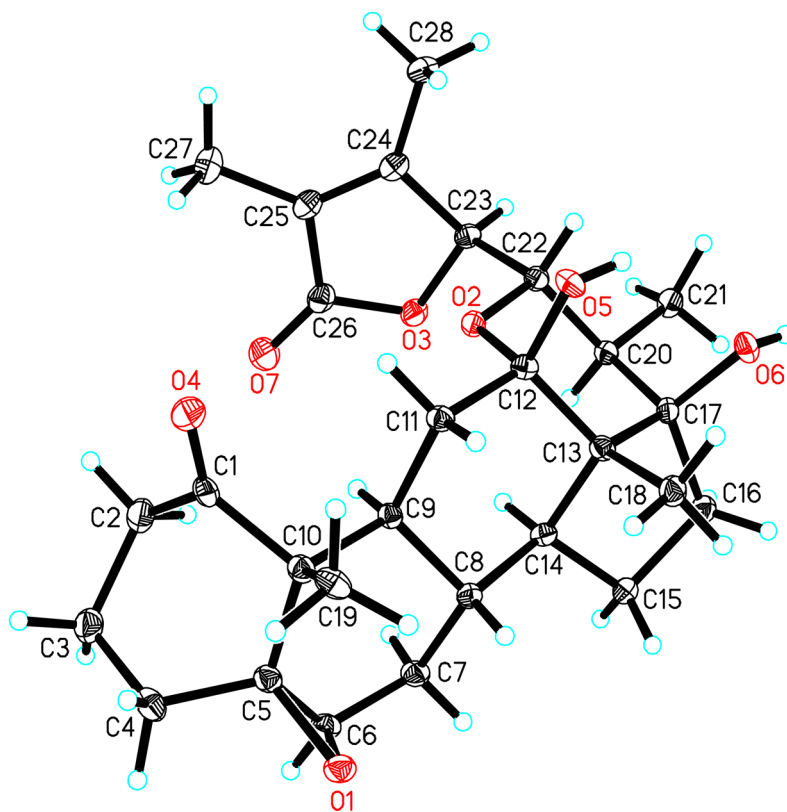


Fig. 2.
X-ray ORTEP drawing of 2,3-dihydrotrechonolide A (**1**)

Table 1

¹H (400 MHz) and ¹³C (125 MHz) NMR data of withanolides **1** and **2** in CDCl₃

Pos.	1			2		
	δ_c	Mult.	δ_H (J in Hz)	δ_c	Mult.	δ_H (J in Hz)
1	213.2	C		213.2	C	
2	34.2	CH ₂	β 2.64 ddd (14.5, 9.6, 6.2)	34.4	CH ₂	β 2.64 ddd (14.5, 9.6, 6.2)
3	17.7	CH ₂	α 2.22 ddd (14.5, 8.7, 5.3)	17.8	CH ₂	α 2.22 ddd (14.5, 8.7, 5.3)
4	29.8	CH ₂	1.91 m, 1.88 m	29.9	CH ₂	1.91 m, 1.88 m
5	64.1	C	β 1.95 m, α 1.23 m	64.1	C	β 1.95 m, α 1.24 m
6	60.2	CH	3.12 brs	60.2	CH	3.13 s
7	30.8	CH ₂	β 2.16 ddd (14.5, 3.6, 2.7)	30.7	CH ₂	β 2.15 ddd (14.5, 3.6, 2.7)
8	28.8	CH	α 1.38 ddd (14.5, 11.0, 1.4)	28.9	CH	α 1.36 ddd (14.5, 11.0, 1.4)
9	40.7	CH	1.46 qd (11.0, 3.6)	40.6	CH	1.45 qd (11.0, 3.6)
10	52.6	C	1.18 td (11.0, 4.5)	51.9	C	1.18 td (11.0, 4.5)
11	35.2	CH ₂	1.55 m, 1.19 m	35.4	CH ₂	1.55 m, 1.24 m
12	98.6	C		98.5	C	
13	47.4	C		47.6	C	
14	46.4	CH	1.88 m	46.5	CH	1.80 m
15	22.8	CH ₂	1.95 m, 1.44 m	22.6	CH ₂	1.65 m, 1.46 m
16	34.4	CH ₂	1.86 m, 1.60 m	34.9	CH ₂	2.00 m, 1.73 m
17	80.5	C		83.0	C	
18	11.9	CH ₃	0.97 s	11.2	CH ₃	0.96 s
19	12.6	CH ₃	1.07 s	12.8	CH ₃	1.08 s
20	35.3	CH	2.30 (11.1, 6.7)	41.0	CH	2.19 ddd (11.3, 3.9, 1.4)
21	10.2	CH ₃	1.00 d (6.7)	59.9	CH ₂	4.14 dd (12.4, 1.4) 3.93 dd (12.4, 3.9)
22	68.8	CH	3.95 dd (11.1, 1.9)	64.7	CH	4.60 dd (11.3, 1.9)
23	82.4	CH	4.82 s	82.3	CH	5.08 s

Pos.	1			2		
	δ_C	Mult.	δ_H (J in Hz)	δ_C	Mult.	δ_H (J in Hz)
24	157.3	C		157.5	C	
25	123.8	C		123.7	C	
26	175.2	C		175.3	C	
27	8.4	CH ₃	1.77 s	8.4	CH ₃	1.78 s
28	12.0	CH ₃	1.89 s	12.1	CH ₃	1.93 s