Published in final edited form as:
Phytochemistry. 2014 February ; 98: 232–235. doi:10.1016/j.phytochem.2013.11.005.

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 C_{28} 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 C_{28} 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

^{1}H, ^{13}C(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).

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 CH_2Cl_2 partition phase of the CH_2Cl_2–MeOH (1:1) extract of *J. caulescens* var. *bipinnatifida* (see Experimental section). The molecular formula of the major component **1** was determined to be $C_{28}H_{38}O_7$ 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 ^{13}C NMR (APT) and HSQC spectra for **1** (Table 1) displayed 28 carbon signals differentiated as five CH_3, seven CH_2, 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 $C_{28}H_{36}$. 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 $\delta_{H\text{-}27}$ 1.77 and $\delta_{H\text{-}28}$ 1.89 (each 3H, s), one oxygenated methine group at $\delta_{H\text{-}23}$ 4.82 (1H, s); and $\delta_{C\text{-}26}$ 175.2 (C), $\delta_{C\text{-}24}$ 157.3 (C), $\delta_{C\text{-}25}$ 123.8 (C), $\delta_{C\text{-}23}$ 82.4 (CH), $\delta_{C\text{-}28}$ 12.0 (CH_3), and $\delta_{C\text{-}27}$ 8.4 (CH_3)], 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 [$\delta_{H\text{-}22}$ 3.95 (1H, dd, J = 11.1, 1.9 Hz), $\delta_{H\text{-}21}$ 1.00 (3H, d, J = 6.7 Hz); $\delta_{C\text{-}22}$ 68.8 (CH), $\delta_{C\text{-}12}$ 98.6 (C), $\delta_{C\text{-}17}$ 80.5 (C), and $\delta_{C\text{-}21}$ 10.2 (CH_3)], as well as a 5β,6β-epoxy group [$\delta_{H\text{-}6}$ 3.12 (1H, brs), $\delta_{C\text{-}5}$ 64.1(C) and $\delta_{C\text{-}6}$ 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 ^{13}C NMR and HSQC of **1** showed signals for an isolated keto (δ_C 213.2) and two extra methylenes [δ_C 34.2 (CH_2) 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 (CH_2) 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)H_2-C(3)H_2-C(4)H_2-; the chemical shift values of H_2-2 [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 H_2- 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), ^{1}H-^{1}H COSY fragment of-C(21)H_2-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 ^{1}H, 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 × 10 mm, 5 μm).

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 CH_2Cl_2-MeOH (50:50, 2.0 L) at room temperature. After removing the solvents under vacuum, the extract (50 g) was suspended in 300 mL H_2O, followed by successive partitions with *n*-hexane, dichloromethane and *n*-butanol (3 × 300 mL). The resulting CH_2Cl_2 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 CH_2Cl_2:EtOAc (10:1, 6:1, and 4:1) with decreasing amounts of CH_2Cl_2 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 CH_3CN (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 CH_3CN (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 CH_2Cl_2:acetone (10:1, 5:1, and 3:1) with decreasing amounts of CH_2Cl_2 to afford two fractions. The first fraction (150 mg) was subjected to CombiFlash CC (24 g silica gel), eluted with CH_2Cl_2: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 CH_2Cl_2:EtOAc (3:1) to afford jaborosalactone 39 (**4**, 7 mg).

3.3.1. 2,3-dihydrotrechonolide A (1)—Cubic colorless crystals; $[\alpha]^{25}_{D}$-2.2 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) 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 $C_{28}H_{38}O_7Na$, 509.2515); ^1H NMR and ^{13}C 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 mm^3) obtained from CH_2Cl_2-CH_3CN (1:1) using Cu Kα radiation (λ = 1.54178 Å) on a Bruker APEX2 diffractometer equipped with a Bruker MicroStar microfocus rotating anode X-ray source and Helios multilayer optics. Crystal data for **1**: $C_{28}H_{38}O_7$ (formula weight 486.58), Orthorhombic, space group $P2_12_12_1$, 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) $Å^3$, D_c = 1.320 Mg/m^3, 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 [$I > 2\sigma(I)$], 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; $[\alpha]^{25}_{D}$ -0.8 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) 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 $C_{28}H_{38}O_8Na$, 525.2464); ^1H NMR and ^{13}C NMR, see Tables 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Mr. Luis González for the collection of the plant material. This fieldwork was supported by the ICBG Project “Bioactive Agents from Dryland Biodiversity in Latin America” grant 5 UO1 TW 00316-10 from the National Institutes of Health (NIH) Fogarty International Center (FIC) to B.N.T., whereas the chemical work was supported by KU Grant 2506014-910/099 to B.N.T. The authors are grateful to National Science Foundation (NSF) MRI grant CHE-0923449, which was used to purchase a Bruker APEX2 X-ray diffractometer and the software. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH/FIC and NSF.

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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.

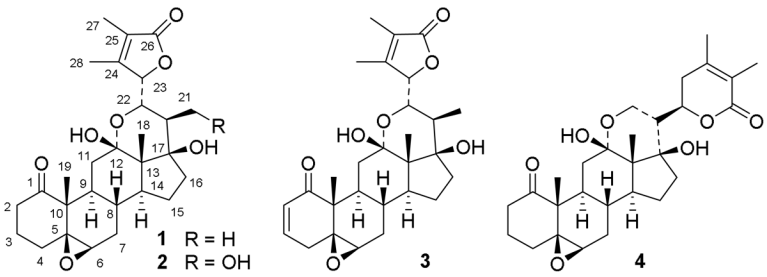

Fig. 1.
Withanolides **1-4** isolated from *Jaborosa caulescens* var. *bipinnatifida*

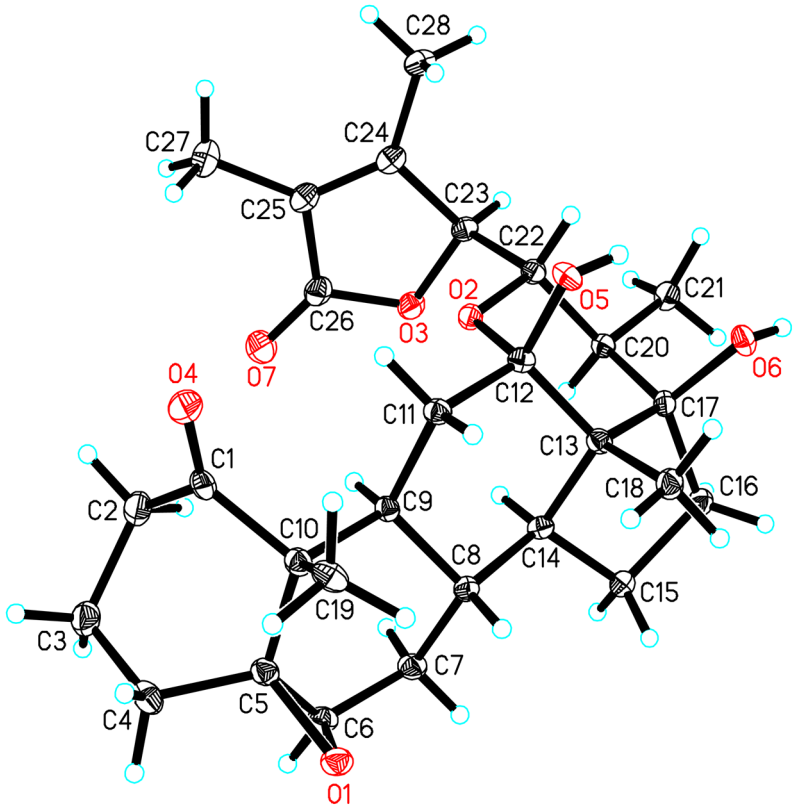

Fig. 2.
X-ray ORTEP drawing of 2,3-dihydrotrechonolide A (**1**)

Table 1

^{1}H (400 MHz) and ^{13}C (125 MHz) NMR data of withanolides **1** and **2** in $CDCl_3$

	1			**2**		
Pos.	**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	β2.64 ddd (14.5, 9.6, 6.2)	34.4	CH_2	β2.64 ddd (14.5, 9.6, 6.2)
			α2.22 ddd (14.5, 8.7, 5.3)			α 2.22 ddd (14.5, 8.7, 5.3)
3	17.7	CH_2	1.91 m, 1.88 m	17.8	CH_2	1.91 m, 1.88 m
4	29.8	CH_2	β1.95 m, α 1.23 m	29.9	CH_2	β1.95 m, α 1.24 m
5	64.1	C		64.1	C	
6	60.2	CH	3.12 brs	60.2	CH	3.13 s
7	30.8	CH_2	β2.16 ddd (14.5, 3.6, 2.7)	30.7	CH_2	β2.15 ddd (14.5, 3.6, 2.7)
			α 1.38 ddd (14.5, 11.0, 1.4)			α 1.36 ddd (14.5, 11.0, 1.4)
8	28.8	CH	1.46 qd (11.0, 3.6)	28.9	CH	1.45 qd (11.0, 3.6)
9	40.7	CH	1.18 td (11.0, 4.5)	40.6	CH	1.18 td (11.0, 4.5)
10	52.6	C		51.9	C	
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

	1			2		
Pos.	**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