

A New Antifeedant Withanolide from *Jaborosa lanigera*

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Z. Naturforsch. **2007**, *62b*, 573–576; received December 16, 2005

A new trechonolide-type withanolide, jaborosolactol, possessing a hemiketal ring system and a 5-membered lactol, has been isolated from the aerial parts of *Jaborosa lanigera*. The structure was elucidated based on spectroscopic and spectrometric methods (1D and 2D NMR, MS). The feeding deterrent activity of the compound against the Mediterranean fruit fly *Ceratitis capitata* was examined.

Key words: *Jaborosa*, Withanolides, Jaborosolactol

Introduction

The withanolides are a group of natural C₂₈-steroidal lactones built on an intact or arranged ergostane framework that occurs mainly in plants of certain genera of *Solanaceae*. The first member of this group of compounds, withaferin A, was isolated from the well-known Indian medicinal plant, *Withania somnifera* [1] and its structure was fully elucidated by Lavie and coworkers in 1965 [2]. The withanolides exhibit a variety of biological activities as antifeedant, immunosuppressive and cancer chemoprevention activity [3].

Jaborosa Miers is a South American genus belonging to the *Solanaceae* family that comprises about 23 species, 11 of which are almost exclusively distributed in Argentina [4]. Previous studies on populations of *J. laciniata*, *J. magellanica*, *J. leucothrica* and

J. sativa gave a group of withanolides with a γ -lactone ring in the side chain known as trechonolide-type. The first unsaturated- γ -lactone side-chain withanolide known as trechonolide A (**1**) [5] was isolated from *J. laciniata*, and its 24,25-epoxy analogue, jaborosolactone U (**2**) [6] was found in *J. sativa*. The epoxy- γ -lactol-side-chain found in the trechonolide isolated from *J. lanigera* (**3**) has no precedent among the withanolides (Fig. 1).

Results and Discussion

Jaborosolactol (**3**) was isolated as a major component from the aerial part of *J. lanigera*. The HREIMS showed a molecular ion corresponding to the formula C₂₈H₃₈O₇, whereas the EIMS showed peaks at $m/z = 297$ (60%) corresponding to the cleavage between C-20 and C-17, distinctive for this type of structure.

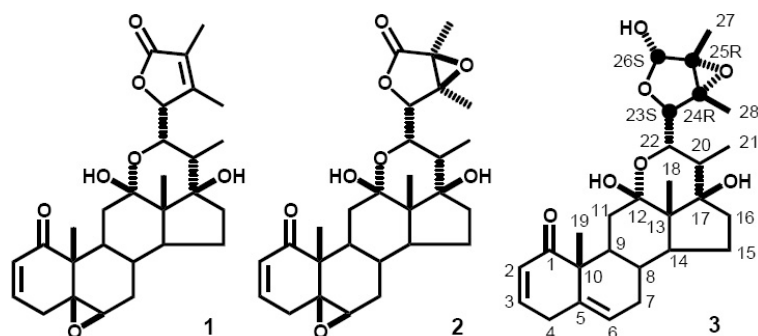


Fig. 1. Structures of compounds 1–3.

Table 1. ^1H and ^{13}C NMR spectral data of compound **3** in CDCl_3^a .

C	$^1\text{H}(\delta)$	$^{13}\text{C}(\delta)$	C	$^1\text{H}(\delta)$	$^{13}\text{C}(\delta)$	C	$^1\text{H}(\delta)$	$^{13}\text{C}(\delta)$
1	–	203.3	11	α : 2.28 β : 1.86	37.1	20	2.10	35.5
2	5.90	127.5	12	–	99.5	21	1.02	10.4
3	6.74	145.1	13	–	47.7	22	4.20	73.0
4	α : 2.86 β : 3.26	33.3	14	2.00	47.3	23	4.29	80.4
5	–	136.0	15	α : 1.66 β : 1.52	22.6	24	–	68.2
6	5.59	124.1	16	α : 1.66 β : 1.82	33.5	25	–	65.2
7	α : 1.51 β : 2.07	25.2	17	–	80.2	26	5.11	98.4
8	1.55	32.3	18	1.07	12.2	27	1.52	10.35
9	1.90	40.6	19	1.22	18.9	28	1.55	13.8
10	–	49.6						

^a Chemical shifts (δ) downfield from TMS, 500.13 and 125.77 MHz; ^{13}C NMR signals were assigned according to the H-C correlations obtained from HETCOR.

The assignment was done on the basis of ^1H COSY-45 and ^{13}C - ^1H HETCOR experiments. In the low field end of the ^1H NMR spectrum, the compound exhibited signals at $\delta = 5.90$, 6.74 and 5.59, which were assigned to H-2, H-3 and H-6, respectively, of a 1-oxo-2,5-dienewithanolide [7]. The ^1H and ^{13}C NMR data for **3** closely resembled those of jaborosalactone **U** (**2**), isolated from *J. sativa* [6] for rings C and D, and the side chain. However, the absence of a non-conjugated lactone carbonyl and the presence of two quaternary carbons at $\delta = 68.2$ and 65.2, and a methine at $\delta = 98.37$ in the ^{13}C NMR spectrum (Table 1) assigned to C-24, C-25 and C-26, respectively, suggested the presence of a 24,25-epoxy- γ -lactol side-chain. The C-27 and C-28 methyl hydrogens at 1.53 and 1.55 ppm and the presence of a signal at 5.11 ppm assigned to H-26 were in agreement with the proposed structure.

The stereochemistry at positions C-24, C-25 and C-26 of jaborosalactol (**3**) was assigned based on spectroscopic and molecular modeling considerations. Two orientations are possible for the epoxide, either 24*R*, 25*R* or 24*S*, 25*S*, which, combined with the two possible configurations at C-23 (23*R* or 23*S*) led to four possible stereochemical arrangements for the lactol side-chain. Further, three rotamers around the C-22-C-23 bond are possible in each case. In addition, we have to consider the stereochemistry of C-26 (26*R* or 26*S*). Thus, the twenty-four structures were generated and their geometry optimized by molecular modeling using the AM1 semiempirical method.

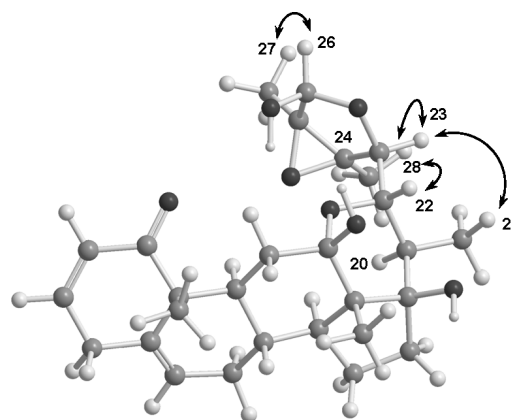


Fig. 2. Stereoscopic view of the predominant conformer of jaborosalactol (**3**) as predicted by AM1 calculations and NMR data (ROEs).

ROESY experiments carried out on jaborosalactol (**3**) showed that at short mixing times (0.7 sec) extremely weak or no ROE cross-peaks were observed between protons more than 3.0 Å apart. Under these conditions, strong peaks were observed for the pairs H-26/H-27, H-23/H-22, H-23/H-21, H-23/H-28, H-22/H-21, and H-22/H-28. An extremely weak ROE cross-peak was barely detected for the pair H-20/H-23 and for H-26/H-23. The small coupling constant observed between H-22/H-23 allowed us to discard those structures where H-22 and H-23 have an *anti* arrangement. The carbon chemical shift of C-23 indicates a 23*S* configuration at this position [8]. We considered only conformers corresponding to the non *anti* configurations and discarded those having H-23/H-21 and H-22/H-28 distances larger than 3.1 Å for which almost no ROEs should be observed. Thus, we only had to consider two possible structures, 23*S*,24*R*,26*S* and 23*S*,24*R*,26*S*. Table 2 summarizes the relative energy of the two remaining structures and relevant geometrical data. Considering the interproton distances in Table 2, only the conformer 23*S*,24*R*,26*S* (indicated as b) is compatible with the observed NOEs (Figs. 1, 2). These results imply the same absolute configuration at position 23 as in trechonolide A (**1**) [5, 8] and the opposite orientation of the 24,25-epoxide as in jaborosalactone **U** (**2**). Based on our results, we also conclude that the stereochemistry of C-26 in **3** is *S*.

Feeding deterrent activity of jaborosalactol (**3**) was studied against the Mediterranean fruit fly *Ceratitis capitata* W. (Diptera: Tephritidae). The bioassays were performed incorporating the products into Terán larval diet [9] in concentrations of 500 ppm. Daily obser-

Table 2. Geometrical data from AM1 calculations for the possible configurations of **3**^a.

Configuration	Conformer	Relative energy ^b (kcal/mol)	H-22-C-C-H-23 (degrees)	D23-28 ^c (Å)	D23-21 ^c (Å)	D23-20 ^c (Å)	D22-28 ^c (Å)	D26-27 ^c (Å)
23S,24S,26S	a	2.77	161.3	2.97	2.59	2.45	3.27	3.25
23S,24R,26S	b	0.97	56.8	2.50	2.22	3.19	2.50	2.65
observed ^d	–	–	–	< 3.0	≪ 3.0	~ 3.0	≪ 3.0	< 3.0

^a Distances larger than 4.0 (Å) are not listed; ^b relative energies are given within each set of rotamers around the C-22-C-23 bond; ^c distances between hydrogens at the specific positions. When more than one hydrogen is present, the shortest distance is indicated; ^d NOESY spectrum with mixing time 0.7 sec showed measurable NOEs only between pairs of hydrogens separated by less than 2.9–3.0 Å.

vations were made in order to the record the number of puparia and adults. The DT 50 % (time needed to reach the adult stage of 50 % of the surviving individuals) was evaluated. The DT 50 % obtained were 20.10 (19.82–20.43) for jaborosolactol and 18.99 (18.71–19.26) for the control. The results have shown that jaborosolactol produced a significant delay in development of neonate larvae.

Experimental Section

General experimental procedures

¹H and ¹³C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer at 200.13 and 50.32 MHz, respectively, and on a Bruker AM-500 at 500.13 and 125.77 MHz, respectively. Multiplicity determinations (DEPT-135) and 2D spectra (COSY-45, HETCOR and ROESY) were obtained using standard Bruker software. Chemical shifts are given in δ downfield from TMS as internal standard. EIMS were collected on a VG Trio-2 mass spectrometer at 70 eV by direct inlet; HREIMS (70 eV) were measured on a VG ZAB-BEQQ mass spectrometer. IR and UV spectra were measured on a Nicolet Magna 550 FT IR and a Hewlett-Packard 8451A spectrophotometer, respectively. AM1 calculations were performed with the MOPAC module in Chem3D 8.0 (Cambridge Soft). The melting point was taken on a Fisher-Johns apparatus and is uncorrected. Vacuum liquid chromatography (VLC) and column flash chromatography were carried out on Kieselgel 60-G (Merck) and Kieselgel S 0.040–0.063 mm, respectively. TLC analysis was performed on silica gel 60 F254 (0.2 mm thick).

Plant material

Whole *Jaborosa lanigera* plants were collected in February 2001 in the Refugio de Mulas Muertas, La Rioja Province, Argentina. A Voucher specimen is deposited at the Museo Botánico, Universidad Nacional de Córdoba under No. CORD 258.

Extraction and isolation

Leaves and stems (782 g) of *J. lanigera* were extracted as previously described [7]. The residue obtained after evap-

oration of the combined extracts was initially fractionated by vacuum liquid chromatography using hexane-EtOAc mixtures of increasing polarity (100:0–0:100) as eluant. The fraction eluted with hexane-EtOAc 40:60 was further fractionated by flash chromatography yielding jaborosolactol (**3**) (165 mg).

Bioassays

Groups of 10 newly hatched larvae (1–2 d old) were reared on an artificial diet into which the test compounds had been incorporated. The artificial diet was prepared according to the method of Terán [9]. Test compound **3** was dissolved in ethanol to obtain a final concentration of 500 ppm. Control larvae were exposed to an artificial diet to which ethanol had been added. The puparia and adult number were daily registered. Four replicates of each treatment were assayed.

Jaborosolactol (**3**)

White solid. – M.p. 260–262 °C. – UV/vis (MeOH): λ_{\max} (log ϵ) = 215 nm (3.19) – IR (dry film): ν = 3429, 2959, 2938, 1719, 1662, 1265, 1027 cm^{-1} . – ¹H NMR (500.13 MHz, CDCl₃, assignments based on ¹H–¹H-COSY): δ = 1.02 (d, J = 6.6 Hz, 1H, 21-H), 1.07 (s, 3H, 18-H), 1.22 (s, 3H, 19-H), 1.51 (m, 1H, 7 α -H), 1.52 (s, 3H, 27-H), 1.55 (m, 1H, 8-H), 1.55 (s, 3H, 28-H), 1.86 (m, 1H, 11 β -H), 1.90 (m, 1H, 9-H), 2.07 (m, 1H, 7 β -H), 2.10 (m, 1H, H-20), 2.28 (m, 1H, 11 α -H), 2.86 (dd, J = 21.2, 4.7 Hz, 1H, 4 α -H), 3.26 (ddd, J = 21.2, 2.6, 1.8 Hz, 1H, 4 β -H), 4.20 (dd, J = 12.4, 1.0 Hz, 1H, 22-H), 4.29 (d, J = 1.0 Hz, 1H, 23-H), 4.40 (d, J = 12.4 Hz, 1H, 26-OH), 5.11 (d, J = 12.4 Hz, 1H, 26-H), 5.59 (d, J = 5.8 Hz, 1H, 6-H), 5.90 (dd, J = 10.2, 1.8 Hz, 1H, 2-H), 6.74 (ddd, J = 10.2, 4.7, 2.6 Hz, 1H, 3-H). – ¹³C NMR (125.77 MHz) see Table 1. – MS (EI, 70 eV): m/z (%) = 486 [M]⁺ (1), 468 [M⁺–18] (6), 450 (1), 298 (21), 297 (60), 283 (18), 280 (3), 265 (11), 168 (4), 107 (38), 97 (47), 55 (100). – HREIMS: m/z = 486.2620 [M]⁺ (C₂₈H₃₆O₆, requires 486.2618).

Acknowledgements

We thank Dr Silvina Bado, Facultad de Agronomía, Universidad de Buenos Aires for investigation of the deterrent activity. Financial support by CONICET (Argentina) and Universidad de Buenos Aires is gratefully acknowledged.

- [1] P. A. Kurup, *Current Sci.* **1956**, 25, 57.
- [2] D. Lavie, E. Glotter, Y. Shvo, *J. Chem. Soc.* **1965**, 30, 1774.
- [3] A. S. Anjaneyulu, D. S. Rao, P. W. Lequesne, *Nat. Prod. Chem.* **1998**, 20, 135.
- [4] A. T. Hunziker, G. Barboza, *Flora Fanerogámica Argentina* **1998**, 54, 1.
- [5] D. Lavie, R. Bessalle, M. J. Pestchanker, H. E. Gottlieb, F. Frolow, O. S. Giordano, *Phytochemistry* **1987**, 26, 1791.
- [6] G. M. Bonetto, R. R. Gil, J. C. Oberti, A. S. Veleiro, G. Burton, *J. Nat. Prod.* **1995**, 58, 705.
- [7] A. M. Cirigliano, A. S. Veleiro, G. M. Bonetto, J. C. Oberti, G. Burton, *J. Nat. Prod.* **1996**, 59, 717.
- [8] V. E. Nicotra, N. S. Ramacciotti, R. R. Gil, J. C. Oberti, G. E. Feresin, C. A. Guerrero, R. F. Baggio, M. T. Garland, G. Burton, *J. Nat. Prod.* **2006**, 69, 783.
- [9] H. R. Terán, *Rev. Agron.* **1977**, 14, 17.