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A 15β-HYDROXYWITHANOLIDE FROM *DATURA FEROX*

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Key Word Index—*Datura ferox*; Solanaceae; withanolide; 15β-hydroxynicandrin B.

Abstract—The leaves of *Datura ferox* yielded a new withanolide, 5α,12α,15β-trihydroxy-6α,7α-epoxy-1-oxo-22*R*-witha-2,24-dienolide (15β-hydroxynicandrin B), in addition to nicandrin B, withanicandrin, withastramonolide and withametelin E previously reported from *Datura* species. The structure and stereochemistry of 15β-hydroxynicandrin B was established on the basis of spectroscopic evidence and molecular modelling.

INTRODUCTION

Steroidal lactones (C-28) (withanolides) based on the ergostane framework, have been isolated from various genera of the Solanaceae family [1]. To date, six *Datura* species have been reported to contain withanolides, namely *D. quercifolia* [2], *D. stramonium* [2], *D. metel* [1, 3–5], *D. tatura* [6], *D. fastuosa* [7, 8] and *D. ferox* [2].

From leaves of *D. ferox* collected in Argentina we have isolated a new withanolide, 15β-hydroxynicandrin B (**1**). The plant also contained, in addition to nicandrin B (withaferoxolide) (**2**) and withanicandrin (**3**) previously isolated from its leaves [2], withastramonolide (**4**) and withametelin E (**5**) which have been reported from *D. stramonium* [2] and *D. metel* [4], respectively.

RESULTS AND DISCUSSION

15β-Hydroxynicandrin B (**1**), $C_{28}O_7H_{38}$ showed in its FAB-mass spectrum (*m*-nitrobenzylalcohol, KCl) a quasi-molecular ion $[M + K]^+$ at *m/z* 525. The ^{1}H NMR spectum (Table 1) had two olefinic protons, a double double doublet at δ6.61 and a double doublet at δ5.87 corresponding to H-3 and H-2, respectively. The signal at δ3.05 was assigned to H-6 by analogy with known 5α-hydroxy-6α,7α-epoxywithanolides [2]. An AB system at δ2.61 was observed in the five withanolides isolated from this plant, it was assigned to the methylene hydrogens at position C-4 based on the spin–spin couplings and ^{1}H–^{1}H correlations with H-2 and H-3 (COSY 45 and DQF COSY).

The presence of two methyl singlets at δ1.89 and 1.94 indicated a typical α,β-unsaturated δ-lactone side-chain bearing methyl groups at positions C-24 and C-25. At the high-field end of the spectrum three signals appeared for Me-18 (δ1.06), Me-21 (δ1.10) and Me-19 (δ1.20). The broad signal at δ3.96 ($W_{1/2}$ 6.0 Hz) was characteristic of a 12α-hydroxywithanolide [2].

The main difference observed in the ^{1}H NMR spectrum of **1** when compared with the related compounds **2**–**5**, was a downfield shift (*ca* 0.35 ppm) of the signal for H-7, which appeared at δ3.72, and the presence of an additional signal at δ4.45 assigned to H-15.

The ^{13}C NMR spectrum of **1** (Table 2), complemented by DEPT spectra, was in agreement with the proposed structure. The spectral data were closely related to those of nicandrin B (**2**) for rings A–C and the side-chain [2, 9], the main differences being in the signals for carbons C-8, C-14, C-15, C-16 and C-18. The signal at δ69.8 was assigned to C-15, bearing the hydroxyl group.

The presence of a hydroxy group at position 15β, was established by NOESY experiments and molecular modelling considerations using the PM3 semi-empirical method (Table 3). The NOESY spectrum showed a strong correlation between the signal at δ4.45 (assigned to H-15) and H-7. Molecular modelling calculations indicated that the H-15/H-7 distance was below 3.0 Å for both the 15α and 15β isomers of **1** confirming the presence of the substituent at position C-15. However no NOE was observed between H-15 and Me-18, thus, H-15 must be in the α-orientation.

To our knowledge, the only other 15β-hydroxywithanolide reported is the allylic alcohol nicaphysalin C, recently isolated from *Nicandra physaloides* [10].

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra were measured at 200.13 and 50.32 MHz respectively in $CDCl_3$ with

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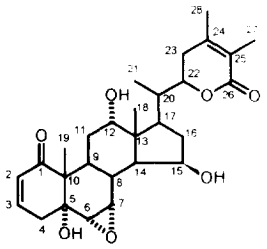
Table 1. ^{1}H NMR spectral data for 15β-hydroxynicandrin B (**1**) in $CDCl_3$

H	δ	H	δ
2	5.87 *dd* (2.9, 10.1)	16α	2.32 *ddd* (14.1, 13.3, 5.5)
3	6.61 *ddd* (2.5, 5.0, 10.1)	16β	1.41 *ddd* (14.1, 9.0, 1.7)
4α	2.52 *dd* (18.0, 5.0)	17	1.92 *ddd* (13.3, 9.0, 1.7)
4β	2.70 *ddd* (18.0, 2.9, 2.5)	18	1.06 *s*
6	3.05 *d* (3.8)	19	1.20 *s*
7	3.72 *dd* (3.8, 1.7)	20	2.12 *m*
8	2.21 *dt* (1.7, 10.8)	21	1.10 *d* (6.5)
9	2.10 *dt* (2.7, 10.8)	22	4.35 *dt* (13.5, 3.5)
11α	2.85 *dt* (13.0, 2.7)	23α	1.99 *dd* (18.3, 3.5)
11β	1.61 *ddd* (13.0, 10.8, 3.5)	23β	2.51 *dd* (18.3, 13.5)
12	3.96 *brs* ($W_{1/2}$ 6.0)	27	1.89 *s*
14	1.87 *dd* (10.8, 5.5)	28	1.94 *s*
15	4.45 *dt* (1.7, 5.5)		

Chemical shifts in δ from TMS. Coupling constants (in parentheses) in Hz.

Assignments are based on phase-sensitive DQF COSY spectrum and coupling constants.



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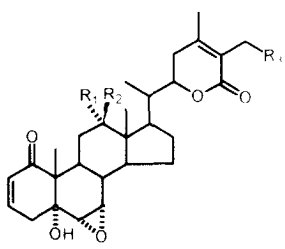

2 R_1=OH, R_2=R_3=H

3 R_1, R_2=O, R_3=H

4 R_1=R_3=OH, R_2=H

5 R_2=R_3=OH, R_1=H

Table 2. ^{13}C NMR spectral data of **1** in $CDCl_3$

C	δ	C	δ
1	203.6	15	69.8
2	128.9	16	40.7
3	140.0	17	43.4
4	36.8	18	14.7
5	73.4	19	15.0
6	56.2	20	38.8
7	56.7	21	12.1
8	32.6	22	78.4
9	28.9	23	29.9
10	50.7	24	149.4
11	29.9	25	122.0
12	73.1	26	167.3
13	46.7	27	12.5
14	48.4	28	20.5

TMS as int. standard. Multiplicity determinations (DEPT) and 2D NMR spectra (DQF COSY, NOESY) were obtained using standard Bruker software. FAB-MS were determined on a VG ZAB-BEQQ mass spectrometer. PM3 calculations were performed with AMPAC 4.5 (Semichem, Shawnee, KS).

Plant material and isolation procedure. Whole *Datura ferox* plants were collected in San Antonio de Litín, Córdoba province, Argentina. A voucher specimen is deposited at the Museo Botánico, Universidad Nacional de Córdoba [CORD]. Dried and pulverized leaves (987 g) were extracted with $CHCl_3$–Me_2CO–MeOH at room temp. The resulting soln was filtered and evapd to dryness. The residue was partitioned with hexane–MeOH–H_2O (10:3:1), the hexane layer was discarded and the methanolic layer concd and extracted exhaustively with $CHCl_3$. The organic layer was evapd to dryness. The residue (3.15 g) was chromatographed on silica gel. Elution with EtOAc–hexane–*iso*-PrOH (30:3:2) afforded four frs containing withanolides which were further purified by flash chromatography using EtOAc–hexane–*iso*-PrOH mixts of increasing polarity. Early frs of eluate (EtOAc–hexane–*iso*-PrOH 100:10:1) yielded withanicandrin (**3**) (10.6 mg), crystals from EtOAc–hexane, mp 264–266° (lit. [2] 267°). Continued elution with EtOAc–hexane–*iso*-PrOH (50:5:1) furnished a mixt. of two withanolides which were purified by prep. reverse-phase TLC, MeOH–H_2O (7:3) yielding withametelin (**5**) (1.0 mg), crystals from EtOAc–hexane, mp 259–260° (lit. [4] 260–262°) and 5α,12α,15β-trihydroxy-6α,7α-epoxy-1-oxo-22*R*-witha-2,24-dienolide (15β-hydroxynicandrin B) (**1**) (1.5 mg), crystals from EtOAc–hexane, mp 266–267°; ^{1}H and ^{13}C NMR: Tables 1 and 2; FAB-MS (*m*-nitrobenzyalcohol, KCl) *m/z* (rel. int.): 525 $[M + K]^+$ (100), 487 $[M + 1]^+$ (17), 485 $[M - 1]^+$ (15), 469 (10), 451 (12). Further elution with EtOAc–hexane–*iso*-PrOH (50:5:2) afforded withastramonolide (**4**) (16 mg), crystals from EtOAc–hexane, mp 271–273° (lit. [2] 269–270°) and nicandrin B (withaferoxolide) (**2**) (34 mg), crystals from

Table 3. Correlations displayed by 15β-hydroxynicandrin B (**1**) in the NOESY spectrum*

H	NOESY correlations	H–H distance (Å)†
7	H-15	2.8
8	H-18, H-19	< 2.4
11β	H-18, H-19	< 2.2
12	H-18	2.4
20	H-18	2.4

*Interactions between vicinal and geminal hydrogens are not included.

†Calculated distances for the most stable conformer of **1** (AMPAC 4.5, PM3).

EtOAc–hexane, mp 271–272° (lit. [2] 278°, [9] 246–248°). ^{1}H NMR spectra for the four known withanolides (**2**–**5**) were in agreement with the reported data [2, 4, 9].

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