Pergamon

0031-9422(95)00220-0

Phytochemistry, Vol. 40, No. 3, pp. 923-925, 1995
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0031-9422/95 $9.50 + 0.00

WITHAJARDIN E, A WITHANOLIDE FROM *DEPREA ORINOCENSIS*

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(*Received* 6 *December* 1994)

Key Word Index — *Deprea orinocensis*; Solanaceae; withanolides; steroids; 2D NMR.

Abstract—A new withanolide belonging to the withajardins series was isolated from *Deprea orinocensis* and identified by 2D NMR spectroscopic data. The compound has an unusual pattern of substitution in the A-ring.

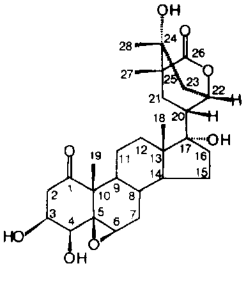
INTRODUCTION

Recently, we have reported the isolation and structural elucidation of a new type of withanolides, the withajardins [1], characterized by having a bicyclic side-chain on C-17 that possesses a homocyclic six-membered ring and a six-membered lactone ring. A similar moiety, but with a homocyclic five-membered ring instead, is also presents in the acnistins [2, 3]. We describe here the isolation and structural determination of withajardin E (**1**), a 14-deoxywithajardin.

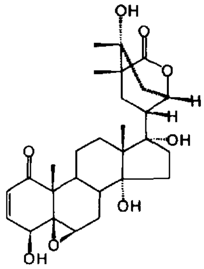
RESULTS AND DISCUSSION

Column chromatography followed by preparative TLC of an extract of *Deprea orinocensis* (H & B) Rafinesque, gave the known withajardins A, B, C and D a new more polar substance, withajardin E (**1**) ($C_{28}H_{40}O_8$ by HRMS). This compound has a structure close to that of withajardin B (**2**) [1], since their ^1H and ^{13}C NMR spectra were essentially identical in the region associated with the bicyclic moiety on C-17 in the steroidal nucleus. The ^1H NMR spectrum of **1** (Table 1) displayed two singlet signals at $\delta 1.00$ and 0.99 for the 27-Me and 28-Me, respectively, and a broad proton singlet at $\delta 4.52$ for H-22. On the other hand, chemical shifts for the C-20 to C-28 carbon atoms are also similar in the ^{13}CNMR spectra of both **1** and **2** (Table 2).

A broad singlet signal at $\delta 3.14$ ($W_{1/2} = 6.0$ Hz) in the ^1H NMR spectrum of **1**, besides the presence in the ^{13}C NMR spectrum of signals corresponds to a quaternary carbon atom and to a methine group, both bearing an oxygen atom, at $\delta 63.7$ and 57.2, respectively, confirmed the existence of the same 5,6-β-epoxy functionality as that found in withajardin B (**2**) (Tables 1 and 2).



Withajardin E (**1**)



Withajardin B (**2**)

The main observed difference in the ^1H and ^{13}C NMR spectra of **1**, compared with those of **2**, was lack of the typical signals for the 2-ene-1-one system. The singlet at $\delta 209.5$ in the ^{13}C NMR spectrum of **1** was assigned to an isolated ketone group [4]. Furthermore, the ^1H NMR spectrum of **1** displayed four signals at $\delta 5.39$ (*d*, $J = 2.9$ Hz), 5.20 (*d*, $J = 2.7$ Hz), 5.11 (*s*) and 4.94 (*s*) interchangeable with deuterium oxide, and so assignable to hydroxy groups.

A homonuclear ^1H-^1H COSY experiment indicated the presence in the molecule of three important spin systems: H-3 ($\delta 3.88$) showed COSY connectivity with the H-2_{ax} ($\delta 2.47$), H-2_{eq} ($\delta 2.64$) and H-4 ($\delta 3.12$) protons and also with the 4-OH hydroxy group; the H-4 methine proton displayed strong cross-peaks with the H-3 proton and with the 4-OH hydroxy group at $\delta 5.20$. These data indicated the presence in **1** of a $-CH_2CH(OH)CH(OH)-$ partial fragment.

Assignments of all the functional groups were achieved by HMQC and HMBC experiments. In the HMQC spectrum, the carbon atom at $\delta 42.5$ (C-2) was coupled with the geminal protons at $\delta 2.47$ (H-2_{ax}) and $\delta 2.64$ (H-2_{eq}), while the carbon atom at $\delta 67.5$ (C-3) showed interaction with the proton at $\delta 3.88$. The carbon atom at $\delta 68.8$ (C-4) interacts with the proton at $\delta 3.12$. On the

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Table 1. ^{1}H NMR spectra of withajardins E (1) and B (2)*

Proton	E (1)	B (2)†
2_{ax}	2.47 *dd* (8.1, 15.5)	
		6.15 *d* (9.8)
2_{eq}	2.64 *dd* (3.3, 15.5)	
3	3.88 *dd* (3.7, 7.7)	7.07 *dd* (6.3, 9.8)
4	3.12 *d* (2.9)	3.55 *dd* (6.3, 4.3)
6	3.14 *brs* (6.0)	3.24 *brs* (4.5)
7a	2.13 *m*	1.98 *dt* (4.7, 17.0)
7b	1.23 *m*	1.16 *d* (13.6)
8	1.29 *m*	1.67 *m*
9	1.52 *m*	1.40 *m*
11a	1.80 *m*	1.90 *m*
11b	1.24 *m*	1.54 *m*
12a	‡	1.80 *m*
12b	‡	1.61 *m*
14	1.56 *m*	
15a	1.62 *m*	1.76 *m*
15b	1.17 *m*	1.45 *m*
16a	1.98 *m*	2.16 *m*
16b	1.58 *m*	‡
18	0.62 *s*	0.79 *s*
19	1.10 *s*	1.30 *s*
20	2.21 *d* (11.8)	2.28 *m*
21_{ax}	2.10 *m*	2.28 *m*
21_{eq}	1.36 *dd* (11.8, 13.7)	1.50 *m*
22	4.52 *brs* (6.0)	4.62 *brs* (6.0)
23_{ax}	2.39 *d* (15.0)	2.42 *d* (14.6)
23_{eq}	1.84 *dd* (3.0, 15.0)	1.82 *m*
27	1.00 *s*	0.97 *s*
28	0.99 *s*	0.99 *s*
3-OH	5.39 *d* (2.9)	
4-OH	5.20 *d* (2.7)	5.58 *d* (4.2)
14-OH		5.39 *s*
17-OH	4.94 *s*	6.24 *s*
24-OH	5.11 *s*	4.90 *s*

*Taken in DMSO-d_6; scalar coupling determined by HOMO 2D*J*.

‡From ref. [1].

†Could not be assigned because of overlap.

Table 2. ^{13}C NMR of withajardins E (1) and B (2)*

C	E (1)	B (2)†
1	209.5 *s*	201.6 *s*
2	42.5 *t*	131.3 *d*
3	67.6 *d*	145.0 *d*
4	68.8 *d*	68.6 *d*
5	63.7 *s*	63.0 *s*
6	57.2 *d*	59.2 *d*
7	31.2 *t*	24.9 *t*
8	29.5 *d*	33.3 *d*
9	41.8 *d*	36.8 *d*
10	49.6 *s*	47.1 *s*
11	20.3 *t*	19.3 *t*
12	30.8 *t*	25.2 *t*
13	47.0 *s*	50.0 *s*
14	48.9 *d*	85.1 *s*
15	23.3 *t*	23.1 *t*
16	34.7 *t*	35.9 *t*
17	82.5 *s*	84.2 *s*
18	15.1 *q*	18.5 *q*
19	14.5 *q*	16.1 *q*
20	41.1 *d*	42.0 *d*
21	26.0 *t*	26.0 *t*
22	76.4 *d*	76.5 *d*
23	40.1 *t*	40.8 *t*
24	69.6 *s*	69.6 *s*
25	47.5 *s*	47.3 *s*
26	177.2 *s*	177.2 *s*
27	14.3 *q*	14.5 *q*
28	27.2 *q*	27.7 *q*

*Taken in DMSO-d_6. Multiplicities were determined by DEPT.

†From ref. [1].

other hand, the methyl groups at δ0.62 (18-Me), 0.99 (28-Me), 1.00 (27-Me) and 1.10 (19-Me), had one-bond interactions with the carbon atoms at δ15.1, 27.2, 14.3 and 14.5, respectively. Finally, a correlation was observed between H-22 (δ4.52), the 'fingerprint' of the withanolides [4, 5] and the carbon atom at δ76.4.

In the HMBC experiment (Table 3) the carbonyl carbon atom at δ209.5 (C-1) has a long-range coupling with the H-2_{ax} proton (two-bond correlation) and with the 19-Me (three-bond correlation), while the H-2_{eq} proton (δ2.64) displayed a two-bond correlation with the C-3 carbon atom and a three-bond correlation with the C-4 one. Similarly, the epoxy-bearing C-5 quaternary carbon atom (δ63.7) showed three long-range correlations: two three-bond connectivities with 4-OH and 19-Me, respectively, and a two-bond connectivity with the H-4 proton.

The analysis of the connectivities in this experiment, for the bicyclic side-chain on C-17 (Table 3) revealed the same correlations as in the case of withajardin B (2). These data confirmed the existence in **1** of the same type of bicyclic side-chain on C-17 as that found in withajardin B, the presence of a 3,4 dihydroxy-1-ketone grouping in the A-ring and a 5,6-epoxy group in the B-ring. This pattern for the A- and B-rings is unusual in the withanolide family of natural products and to the best of our knowledge it has been previously reported [6] only for physangulide, isolated from *Physalis angulata*.

Finally, when compared with that of withajardin B, the ^{13}C NMR spectrum of **1** showed upfield shifts for the C-8, C-13 and C-15 carbon atom signals and downfield shifts for the C-7, C-9 and C-12 carbon atoms (Table 2). This behaviour can be interpreted as being due to the absence of the hydroxy group on C-14, which was confirmed in the HMBC experiment, since the doublet signal at δ48.9 (C-14) showed a three-bond correlation with 18-Me.

All the above data are in accordance with withajardin E (**1**) having the structure 14-deoxy-2,3-dihydro-3β-hydroxy-withajardin B. The relative 3β,4β-stereochemistry assigned to the hydroxy groups in the A-ring is in accordance with the observed low value for the coupling constant $J_{2,3} = 2.9$ Hz and the observed Δ value (+ 0.51) for the 19-Me when the ^{1}H NMR spectrum was taken in pyridine-d_5.

Table 3. Long-range correlations (HMBC experiment of withajardins E and **B**)

C	E (**1**)	B (**2**)*
1	2, 19-Me	2, 3, 19-Me
2	*	
3	2_{eq}, 4-OH	4, 5
4	2_{eq}	2, 3, 4-OH
5	4, 4-OH, 19-Me	4, 4-OH, 19-Me
6	*	
7	6	6
8	6	6
9	19-Me	7, 19-Me
10	2_{eq}, 19-Me	2, 4, 19-Me
12	18-Me	18-Me
13	18-Me	14-OH, 18-Me
14	18-Me	18-Me
15	*	
16	17-OH	20
17	18-Me, 21_{ax}	17-OH, 21_{ax}
20	17-OH, 21_{ax}	17-OH
21	27-Me	27-Me
23	24-OH	28-Me
24	21_{ax}, 22, 23_{ax}, 24-OH, 28-Me	23_{ax}, 24-OH, 28-Me
25	21_{ax}, 27-Me	21_{ax}, 23_{ax}, 24-OH, 27-Me
26	21_{ax}	21_{ax}, 22
28	24-OH	24-OH

*From ref. [1].

†Correlations were not observed.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were recorded on Brucker AMX400 and WP200SY spectrometers, respectively, running at 100.6 MHz for ^{13}C and 400 MHz for ^{1}H. IR spectra were performed on a Perkin–Elmer 1600 (FTIR) spectrometer. HRMS were run on a VG-Micromass ZAB at 70 eV. LRMS were run on a Hewlett-Pakard, model 5995.

Extraction and isolation. The plant material was collected and processed as previously reported [1].

Withajardin E (**1**). Amorphous powder (15 mg); HRMS: $[M - H_2O]$ *m/z*: 486.26191 (calc. for $C_{28}H_{38}O_7$, 486.26175); IR ν_{max} cm^{-1}: 3450, 3100, 1720, 1700, 1420, 1350, 1220; ^{1}H and ^{13}C NMR: see Tables 1 and 2; EIMS *m/z* (rel. int.): 486 $[M - H_2O]^+$ (1), 450 $[M - 3 \times H_2O]$ (1), 238 (50), 125 (15), 55 (100).

Acknowledgements — F. E. is indebted to COLCIENCIAS (Colombia) for a Fellowship. J.G.L. and A.G.G thanks the Areces Foundation (Spain) for financial support.

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