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Withajardins, Withanolides with a New Type of Skeleton Structure of Withajardins A, B, C and D Absolute Configuration of Withajardin C

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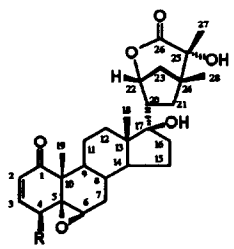
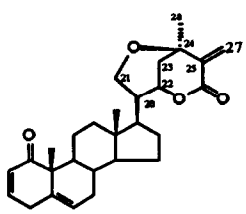
ABSTRACT : Withajardins A, B, C and D are withanolides with a new skeleton isolated from the leaves of *Deprea orinocensis*. Their structure and the absolute configuration of withajardin C were established by spectral analysis, including 2D-NMR and X-ray diffraction. The ^{13}C NMR assignments of withajardins and acnistins were compared and biogenetic pathways to these compounds were formulated.

The withanolides are a group of steroid lactones which have been isolated from several genera of Solanaceae[1,2] and recently have also been found in a soft coral [3]. Several of these substances have displayed various types of biological activity, such as cytotoxic[4], anticancer[5], immunosuppresive[6], anti-inflammatory[7] and hepatoprotective [8] properties.

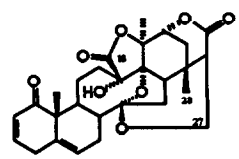
The witanolides are characterized chemically by a lactone chain at C-17 and different oxidations, mainly in the A, B and E rings[2] hower biogenetic transformations can produce very modified compounds such as the physalins[9], trechonolide[10], prejaborol[11], withamelins[12] and acnistins[13] (Fig 1). In the course of a biological screening we studied the species *Dunalia solanaceae*[14], finding acnistins A and E in the former and now withajardins A ,B ,C and D with a new skeleton related to that of the acnistins are reported from the leaves of *Deprea orinocensis*.



R=H , Acnistin A (5)
R=OH , Acnistin E (6)



Withametelin

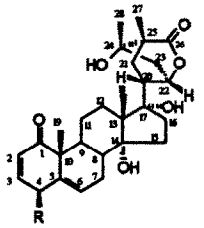


Physalin B

Figure 1. Withanolides derived-skeleton

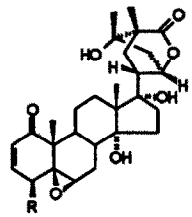
The structures of acnistins A and E have recently been studied[14] by 2D-NMR, starting the analysis of the HMBC spectrum at the lactone carbonyl, easily identified by its shifts at 178.92 and 179.09 ppm, respectively; in this way, sequentially following the two and three-bond correlations, the chemical shifts of the bicyclic side chain could be unambiguously assigned as could the rest of the steroid system. Furthermore, the structure of acnistin D was confirmed by X-ray diffraction analysis, enabling its absolute configuration to be established. The application of these bidimensional techniques to acnistin E (with a structure earlier determined by X-ray diffraction[13]) and to 3-methoxy-acnistin E provided conclusive proof of the validity of the assignments.

The chemical shifts of carbon atoms of the three acnistins were then compared with those found for withajardins A-D (Fig. 2) and from these data it would appear to be indicated that a homocyclic five membered side chain ring was present as in tha acnistins. Nonetheless, some discrepancies were observed for the shifts of the atoms C-20, C-22, C-24, C-25, C-27 and C-28, in particular (Table I). HMBC[15] and HMQC[16] experiments on the four withajardins enabled their structures to be established conclusively and an X-ray diffraction analysis of the withajardin C established its absolute configuration. It was thus ascertained that the withajardins have a new carbon skeleton with a homocyclic six membered side chain ring.



R = OH Withajardin A (1)

R = OAc Withajardin D (2)



R = OH Withajardin B (3)

R = OAc Withajardin C (4)

Figure 2. Structure of Withajardins A-D

Table I . ^{13}C NMR of Withajardins A-D (1-4) and Acnistins A (5) and E (6)

	Withajardins				Acnistins	
Carbon	A (1)[a]	D (2)[b]	B (3)[a]	C (4)[b]	A (5)[b]	E (6)[b]
1	203.23 s	202.39 s	201.64 s	200.61 s	202.36 s	202.37 s
2	127.40 d	130.83 d	131.28 d	133.73 d	129.87 d	133.08 d
3	146.15 d	140.00 d	145.06 d	139.70 d	143.56 d	141.31 d
4	67.38 d	70.20 d	68.59 d	71.32 d	31.00 t	70.27 d
5	138.09 s	134.33 s	63.01 s	60.91 s	62.14 s	64.02 s
6	128.75 d	133.16 d	59.22 d	60.73 d	62.89 d	61.69 d
7	24.38 t	25.01 t	24.91 t	25.75 t	32.76 t	30.92 t
8	35.77 d	36.15 d	33.32 d	35.98 d	30.74 d	30.72 d
9	35.86 d	36.32 d	36.81 d	37.41 d	43.85 d	43.14 d
10	48.78 s	49.36 s	47.07 s	47.62 s	46.94 s	47.85 s
11	21.17 t	21.51 t	19.33 t	20.87 t	22.69 t	21.52 t
12	26.13 t	26.41 t	25.24 t	25.31 t	33.26 t	32.97 t
13	49.95 s	50.67 s	49.95 s	50.60 s	48.54 s	46.80 s
14	85.21 s	86.80 s	85.09 s	86.90 s	50.42 d	50.71 d
15	32.15 t	33.70 t	32.11 t	33.52 t	23.50 t	23.96 t
16	35.73 t	35.90 t	35.93 t	35.92 t	36.92 t	36.69 t

Table I. (Cont.) ^{13}C NMR of Withajardins A-D (1-4) and Acnistins A (5) and E (6)

	Withajardins				Acnistins	
Carbon	A (1)[a]	D (2)[b]	B (3)[a]	C (4)[b]	A (5)[b]	E (6)[b]
17	84.26 s	85.09 s	84.18 s	85.09 s	85.43 s	85.07 s
18	18.92 q	19.91 q	18.51 q	19.60 q	14.41 q	14.00 q
19	21.85 q	21.36 q	16.10 q	15.98 q	14.20 q	16.57 q
20	41.85 d	41.25 d	41.96 d	41.25 d	51.33 d	51.36 d
21	26.00 t	27.12 t	26.00 t	27.09 t	37.09 t	37.13 t
22	76.54 d	77.00 t	76.46 d	77.10 d	84.05 d	84.03 d
23	40.93 t	41.67 t	40.83 t	41.67 t	41.20 t	41.22 t
24	69.53 s	70.27 s	69.55 s	70.30 s	45.30 s	45.35 s
25	47.28 s	47.80 s	47.27 s	47.91 s	76.63 s	76.70 s
26	177.21 s	178.29 s	177.15 s	178.80 s	179.09 s	178.92 s
27	14.47 q	14.44 s	14.46 q	14.52 q	25.55 q	25.56 q
28	27.67 q	27.49 s	27.68 q	27.57 q	19.92 q	19.89 q
MeCOO		170.20 s		170.10 s		
MeCOO		21.61 q		19.91 s		

[a] : Recorded at 100.0 MHz, DMSO-d6. [b]: Recorded at 100.0 MHz, $CDCl_3$. Multiplicities were determined by DEPT experiments.

Results and Discussion

Withajardin C (4), $C_{30}H_{40}O_9$ (Table II) has an α,β-unsaturated carbonyl in which the olefin protons form part of an AMX system as can be deduced from the ^1H-^1H COSY spectrum since a doublet is seen at 7.00 ppm (J=6.0Hz and 9.8 Hz), a doublet at 6.25 ppm (J = 9.8 Hz) and another one at 4.26 ppm (J = 6.0 Hz); the presence of an acetate and an epoxide were deduced from the singlet at d 2.06 (3H) and d 3.24 (1H) (Table III).

Table II. Mass, IR Spectra and mps. of Withajardins 1-4

Compound	Formula	Highest Mass ions observed[a]	IR, cm^{-1}	m.p., °C
Withajardin A (1)	$C_{28}H_{38}O_7$	450.2574 450.2568 (M^+-H_2O)	3300,1725,1660	225, descomp.
Withajardin B (3)	$C_{28}H_{38}O_8$	487 (M^+-Me)	3400,1730,1665	228, descomp.
Withajardin C (4)	$C_{30}H_{40}O_9$	545.2747 545.2769 (M^++ H^+)	3450.1740,1725,1660	268, descomp.
Withajardin D (2)	$C_{30}H_{40}O_8$	450 (M^+-AcOH-H_2O)	3350,1735,1725,1660	182, descomp.

a : Observed / Calculated for specific formula. I.E., 70 eV, except for withajardin C, 15 eV

Table III . ^{1}H NMR of Withajardins A-D (1-4)

H	Withajardin A(1)[a]	Withajardin D(2)[b]	Withajardin B(3)[a]	Withajardin C(4)[b]
2	5.81 (d, 4.3)	6.01 (d, 10.0)	6.15 (d, 9.8)	6.25 (d ,9.8)
3	6.81 (dd, 4.6, 10.0)	6.69 (dd, 4.7, 10.0)	7.07 (dd, 6.3, 9.8)	7.00 (dd, 6.0, 9.8)
4	4.55 (t, 4.5)	5.76 (d, 4.7)	3.55 (dd, 4.3, 6.1)	4.62 (d, 6.0)
6	6.82 (d, 4.2)	6.09 (d, 2.8)	3.24 (brs, 4.5)	3.30 (brs, 5.0)
7-ax	2.09 (dd, 3.8, 18.6)	1.95 (m)	1.98 (dt, 4.7, 17.0)	2.5 (d, 11.8)
7-eq	1.21 (d,14.29		1.16 (d, 13.6)	1.25 (d, 13.7)
8	1.75 (m)	1.82 (m)	1.67 (m)	1.80 (m)
9	1.50 (m)	1.42 (m)	1.40 (m)	1.57 (m)
11-ax		1.89 (m)	1.90 (m)	1.90 (m)
11-eq	1.56 (dd, 4.7, 10.2)		1.54 (m)	1.64 (dd, 4.5, 11.0)
12-ax	1.81 (m)	1.78 (m)	1.80 (m)	1.90 (m)
12-eq	1.60 (m)	1.61 (m)	1.61 (m)	1.62 (m)
15-β	1.78 (m)	1.75 (m)	1.76 (m)	1.74 (m)
15-α	1.47 (dd, 4.2, 11.0)	1.46 (m)	1.45 (m)	1.47 (dd, 4.3, 13.0)
16-β	1.99 (dq, 4.2, 11.5, 16.3)	1.89 (m)	2.15 (m)	2.15 (m)
16-α	1.77 (m)	2.05 (m)		1.82 (m)
18	0.84 (s)	0.89 (s)	0.79 (s)	0.86 (s)
19	1.33 (s)	1.39 (s)	1.30 (s)	1.41 (s)
20	2.24 (m)	2.25 (m)	2.28 (m)	2.25 (m)
21-ax	2.16 (d, 13.4)	2.18 (m)	2.28 (m)	2.18 (m)
21-eq	1.40 (dd, 1.9, 11.6)	1.45 (m)	1.50 (m)	1.47 (d, 12.2)
22	4.62 (brs, 6.0)	4.61 (brs, 6.0)	4.62 (brs, 6.0)	4.62 (d,1.7)
23-ax	2.42 (d, 14.4)	2.46 (d, 15.0)	2.42 (d, 14.6)	2.44 (d,13.7)
23-eq	1.85 (m)	1.85 (m)	1.82 (m)	1.87 (m)
27	0.97 (s)	1.09 (s)	0.97 (s)	1.11 (s)
28	0.98 (s)	1.09 (s)	0.99 (s)	1.13 (s)
MeCOO		2.08 (s)		2.05 (s)
4-OH	5.43 (d, 4.4)		5.58 (d, 4.2)	
14-OH	5.34 (d,6.3)	3.16 (s)	5.39 (s)	3.02 (s)
17-OH	6.43 (t, 5.1))	6.18 (s)	6.84 (s)	6.18 (s)
24-OH	4.93 (d, 7.2)	5.21 (s)	4.90 (s)	5.21 (s)

[a] DMSO-d_6. [b] $CDCl_3$. Scalar coupling constants, were determined from HOMO-2D-J and ROESY spectroscopy.

The **DEPT** spectrum of withajardin C (4) (Table I) indicates three carbonyls at 201.10, 178.37 and 170.1 ppm (the latter corresponding to the acetate), five oxygenated carbons, two of which are attributed to an epoxide (60.91 s and 60.73 d), five methyls (one the acetate methyl) and an oxygenated methine among other signals. **HMQC** established that the oxygenated methine corresponds to C-22 which is shown in ^{1}H NMR as a doublet shifted to δ 4.62 (J=1.7Hz) and in ^{13}C NMR as a doublet at 77.76[17,18]. Using a combination of **HMQC** and **HMBC** (Table IV) and starting from the lactone carbonyl at 178.37 three bond correlations could be observed with H-22, H-27 and H-21ax; the latter is also coupled with C-17 and an oxygented quaternary carbon at 70.37 ppm which was assigned to C-24 since this is also connected by two and three bonds to H-23ax and H-28. The latter correlation is very significant as it places a hydroxyl γ to the lactone carbonyl and so the homocyclic system closure in the side chain could be established between C-21 and C-25 and not between C-21 and C-24 as in the acnistins. This fact was confirmed beyond possibility of doubt by the

two and three bond correlations observed for C-20, C-24, C-25 and those for H-27 and H-28. These observations also hold good for the side chain of the withajardins A, B and D (Table IV).

The hydroxylations sites were also easy to deduce by **HMBC**; one was established at C-14 by correlation with Me-18 and another at C-17 by connection with the C-18, C-20 and C-21 protons. The acetate group was positioned at C-4 given the correlations of the acetate methyl with C-4 and those of the H-4 with the carbonyl at C-4. Similarly the epoxide could be sited between C-5, C-6. In the same way connectivities between the other atoms in the molecule could be established (Table IV):

Table IV. Three and Two Bond Correlations (HMBC Experiment) of Withajardins A-D (1-4)[a]

C	Withajardin A(1)[b]	Withajardin D(2)[c]	Withajardin B(3)[b]	WithajardincC(4)[c]
1	19-Me	3, 19-Me	2, 3, 19-Me	19-Me
2				4
3		4	4, 5, 4-OH	4
4		2	2, 3, 4-OH	2, 3
5	19-Me	3, 4, 19-Me	4, 4-OH, 19-Me	3, 4, 7, 19-Me
6		8		
7			6	6
8			6	6
9	19-Me	19-Me	7, 19-Me	19-Me
10	2, 6, 19-Me	2, 4, 6, 19-Me	2, 4, 19-Me	2, 4, 19-Me
12		18-Me	18-Me	18-Me
13	14-OH, 18-Me	14-OH, 18-Me	14-OH, 18-Me	18-Me
14		18-Me	18-Me	18-Me
15		8, 14-OH		
16		20		15-β
17	18-Me	17-OH, 20, 21-ax	17-OH, 21-ax	18-Me, 21-ax
20	17-OH	17-OH, 21ax	17-OH	
21	28-Me	27-Me	27-Me	27-Me
23	28-Me	28-Me	28-Me	20,-24-OH
24	23-ax, 25-OH, 27-Me	21-ax, 23, 24-OH	23-ax, 24-OH. 28-Me	21-eq, 21-ax, 23-ax, 28-Me
25	28-Me	21-ax, 28-Me	21-ax, 23-ax, 24-OH, 27-Me	28-Me
26	27-Me	21-ax,27-Me	21-ax,22	21-ax, 22, 27-Me
28	24-OH	24-OH	24-OH	
Me-COO-		4, Me-COO-		
Me-COO-				4, Me-COO

[a]: Experiment optimized to 10.0 MHz. [b]: DMSO-d_6. [c]: $CDCl_3$

The relative configurations was deduced from the analysis of the **ROESY** spectrum of withajardins A-D in which effects between H-4 and H-6, H-8 and H-19 and H-23ax and H-18 among others could be appreciated (Table V).

The absolute configuration of Withajardin C was established by means of X-ray diffraction; a perspective view of the structure is shown in Figure 3. The central ring system is formed by the fusion of one five-membered and three six membered rings characterized by two *trans*-connected rings B/C and C/D and the quasi *cis* connected A/B rings, four axial substituents, two methyl groups at C-10 and C-13 (β-position)

and two hydroxy substituents at C-14 and C-17 (α-oriented); the protonated carbons C-8 and C-9 are α and β positioned, respectively. Rings A and B adopted a half-chair conformation and the C ring is chair conformed. The five-membered D-ring adopts a conformation between half-chair and envelope. This moity is linked to another ring system with three six-membered rings (C-20, C-21, C-25, C-26, O-5, C-22; C-20, C-21, C-25, C-24, C-23, C-22; C-22, C-23, C-24, C-25, C-26, O-5) all of which adopt a boat conformation; the protonated C-20 is β oriented and the molecule has several asymmetric centres with the following configurations: C-4 S, C-8 R, C-9 S, C-10 R, C-13 R, C-14 R, C-17 S, C-22 R, C-24 R, C-25 R.

Table V. ROESY Correlations for Withajardins

Proton	withaiardin A(1)	withaiardin D(2)	withaiardin B(3)	withaiardin C(4)
H-2	H-3	H-3	H-3	H-3
H-3	H-2. 4-OH	H-2. H-4. 4-OH		
H-4	H-6. 4-OH	H-3. H-6. 4-OH		
H-6	H-4. H-7ax	H-3	H-4	H-3
H-8	19-Me	19-Me	19-Me	19-Me
H-11ax	19-Me	19-Me.H-12eq		
H-16 α	H-21eq. H-23eq	H-23eq	H-21eq	H-23eq
H-20	18-Me. H-21eq	H-23eq	18-Me.H-21eq	H-23eq
H-21ax	H-21eq. H-22	H-21eq. H-22. 24-OH	H-22. 17-OH. 24-OH	H-21eq. H-22
H-21eq	H-16α. 27-Me	H-16α. 27-Me	H-16α. 27-Me	
H-22	H-20. H-23eq. H-23ax	H-20. H-21ax.H-21eq	H-20. H23eq. H-23ax	H-20. H-21ax. H-21eq
H-23ax	H-16α. 24-OH	H-22. H-23eq. 18-Me	H-16α. 24-OH	H-21eq. H-23eq. 18-Me
24-OH	H-21ax. H-23ax. 17-OH. 27-Me	H-21ax. H-23ax. 17-OH	H-21ax. H-23ax	H-21ax. H-23ax
17-OH	14-OH. 24-OH	14-OH. 24-OH		
4-OH	H-3. H-4	H-3. H-4		

The molecules are linked together in the crystal by hydrogen bonds between hydroxy groups and the water molecule. All hydroxy H atoms acts as donors in one intermolecular H-bond and two intramolecular H-bonds. On the other hand, the water molecule acts as both donor and aceptor. The geometry of the H-bonds is described below:

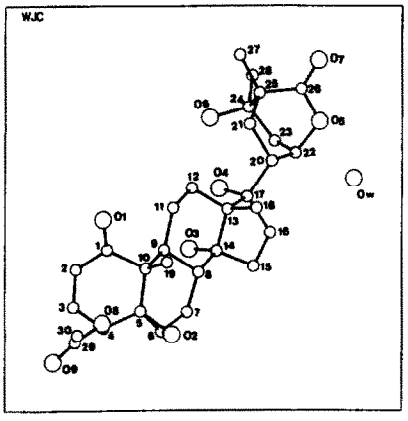


Figure 3. PLUTO drawing of Withajardin C (4)

D--H.....A	D--H	D.....(Å)	H.....A	D--H.....A	.....Symetry
O3-H3....Ow	0.89 (3)	2.736(6)	1.85(3)	171(3)	1-x, 1/2+y, 1.5-z
O4-H4....O3	0.87 (3)	2.560(2)	1.75(3)	154(3)	x, y, z
O6-H6....O4	0.89 (4)	2.744(3)	1.89(4)	159(3)	x, y, z
Ow-H1w.O2	0.98 (4)	2.803(3)	1.83(4)	171(3)	1-x, y-1/2, 1/2-z
Ow-H2z..O7	0.85 (4)	2.768(3)	1.92(4)	178(4)	1-x, 1/2+y,1.5-z

Structure solution

The structure was solved by direct methods SIR88[19] and refined by full-matrix least-squares methods with anisotropic thermal parameters for non-H atoms. All H-atoms were found in a Fourier difference map and were included as isotropics contributors and refined. A weighting scheme was selected to prevent dependence in $< w \Delta^2 F >$ vs $< | Fo | >$ and $< \sin \theta / \lambda >$. After several cycles of weighted mixed refinement, the final R and Rw values were 3.5 and 4.0, respectively. The final difference synthesis showed the residual electron density as no greater than 25 $e\text{Å}^{-3}$; number of variables 525, ratio of freedom 4.98, degree of freedom 2094. The absolute configuration was determined with Bijvoet differences with $\Delta Fc > 0.15$ and with the least experimental error Fo $>10\sigma$ (Fo). 37 more relevant Bijvoet pairs from the raw data (hkl and -h -k -l alternately measured intensities with no extra care on recentering, scan speed, etc) gave the following results: $R1 = \Sigma[\Delta Fo - \Delta Fc] / N = 0.316$ and $R2 = \Sigma[\Delta Io - \Delta Ic] / \Sigma[\Delta Io] = 0.879$ for the right enantiomer model, the R1 and R2 values for the wrong enantiomer were 0.476 and 1.273, respectively, thus the absolute configuration was established for the molecule.

Withajardin B (**3**) $C_{28}H_{38}O_8$, is a very similar compound to withajardin C which can be deduced from the similarity of their ^1H and ^{13}C NMR spectra (Tables I and III), with only a few differences in the chemical shift, chiefly of H-4 and C-2, C-5; moreover the ^1H and ^{13}C NMR spectra of withajardin B do not have the characteristic signals of the acetate group which in withajardin C was placed at C-4 by HMBC; therefore, withajardin B would seem to be 4-Hydroxy-4-deacetoxy-withajardin C. This is supported by the correlation of 4-OH with C-3, C-4 and C-5 (Table IV). To check out this theory, withajardin B was cold-acetylated and formed a monoacetate which proved identical to withajardin C in its IR and NMR spectra.

Withajardins A and D (**1** and **2**) seem also to be a very similar pair of compounds and are distinguished from the other two withajardins only by the presence of a double bond instead of an epoxide[17], the rest of the molecule being the same. The position of the double bond between C-5, C-6 was established by means of HMBC since two and three-bond correlations of C-5 with H-3, H-4, H-8 and H-19 could be discerned (Table IV). The β configuration of the substituent at C-4 in both withanolides is similar to that of withajardins B and C and could be deduced by the chemical shift of Me-19 to a relatively low field[17,18] and by the presence of a ROESY effect between H-4 and H-6. The ROESY effect between Me-27 and Me-28 was no clearly visible due to the fact that both groups have a very similar chemical shift.

The above discussion established that withajardins have a bicyclic lactone side chain with a six-membered homocycle which is different from the bicyclic system of the acnistins which consists of a five-membered homocycle, although the class of carbon atoms on ^{13}C NMR is exactly the same for both types of substances. There are other differences, too, between withajardins and acnistins: the first have an extra hydroxyl at C-14 and the configuration of the side chain is 17 β. The fact that acnistins have 17 α configuration is abnormal in the withanolide field but has been accounted for by a biogenetic mechanism of dehydratation-rehydroxylation[22] similar to that postulated[23] for the formation of a 14 α-OH. The compounds obtained from *Deprea orinocensis* and *Dunalia solanaceae* suggest the existence of very specific enzymatic biogenetic mechanisms since, starting from a common precursor, only one type of substances forms in each species, which has been demostrated in our case by means of intensive phytochemical screening of both species. In no instance have withajardins and acnistins been encountered together. The biogenesis of all of

these compounds may be explained as owing to a mechanism such as that shown in Figure 4, which requires prior oxidation of the Me-21 (very possibly in glycoside form) followed by an ^{-}OH electrophilic attack on C-24 or C-25 depending on the species, and then a shift of the double bond on the oxidized Me-21.

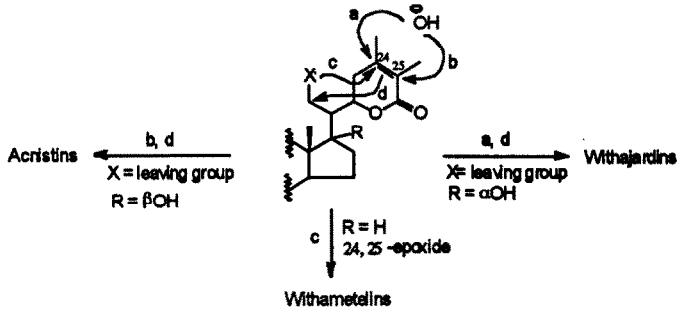


Figure 4. Possible biogenetic routes to differents bicyclic side-chain lactones in withanolides

This mechanism follows a different path from that for the formation of the withametelins[2] in which an electrophilic attack on 24,25-epoxy-withanolide takes place from the 21-CH_2 OH. Paradoxically, and in spite of a withanolide which does have 21-CH_2 OH having been obtained from *Datura metel*[12] (Whence the term withametelins), no other type of substance with a bicyclic system equal to that of the withajardins or acnistins has been reported to date from this species.

Physalin B, a type of substance obtained from other Solanaceae species such as *Physalis* or *Witheringia*[2] has also been found in *D. orinocensis* thus indicating an extra oxidative mechanism in this species at the Me-18 level.

Despite the different positions of the hydroxy groups in relation to the lactone carbonyl group in acnistins and withajardins, the chemical shift in this latter is very similar in ^{13}C NMR in both types of compounds (Table I), although it is different from that of normal withanolides in which it appears at 166 ppm. In the case of acnistins this shift could be accounted for neighbouring β-hydroxylation. However in the withajardins there would seem to be other factors which notably influence the shift of the lactone carbonyl since the hydroxyl at C-24 in theory should exercise a effect on it, shifting it further upfield than normal. Such an effect may be cancelled out by the formation of H-bonding between the hydroxyls of C-24, C-17 and C-14 the presence of which has been firmly established, as seen above, by X-ray diffraction of withajardin C; in the same way, the generation of a bicyclic system with two six-membered rings apparently reduces the tension on the carbonyl which does not occur in the case of the acnistins. The chemical shift of Me-28, further upfield than expected for a hydroxy geminal group can be accounted for by it being situated in the unshielded zone of the lactone carbonyl.

Studies both with acnistins and with withajardins are under way to determine their immunomodulatory activity.

Experimental

^{1}H NMR spectra were recorded on a Bruker AM400 spectrometer at 400 MHz and ^{13}C NMR at 100 MHz; chemical shift are given in parts per million (δ) in relation to the solvent peaks. IR spectra were taken on a Perkin-Elmer Model 1600 spectrometer. Low-resolution mass spectra were run on a Hewlett-Packard 5995 and HRMS on a VG Micromass ZAB-2F, EI, 70 eV for withajardins A, B and D and 15 eV for withajardin C.

Column chromatography was carried out on silica gel (Merck 0.2-0.5 mm) and TLC on silica gel plates, 0.25 mm, with visualization on oleum, heated to 120°C. The NMR data are given in tables I, III, IV and V

Extraction and Isolation Procedures. *Deprea orinocensis* leaves (1.2 Kg) collected in El Jardin, Colombia in July 1991 were extracted by Soxhlet with hexane and then MeOH. The MeOH extract was evaporated almost to dryness, diluted with water and re-extracted with hexane (4 x 1l) and EtOAc (6 l). The EtOAc extracts were chromatographed on silica gel with hexane-EtOAc (4:1-1:8) mixtures and 180 fractions, of 100 ml each were collected. Withajardin D (60 mg) and withajardin C (180 mg) were obtained from the less polar fractions and withajardins A (1.2 g) and B (800 mg) appeared after repeated column chromatography and/or crystallization on MeOH. The withajardins were detected by TLC due to the colour developed (blue or red) when the plates were sprayed and heated.

Withajardin A (1): white powder; HRMS m /z (rel int. %) 448.2238 ($C_{28}H_{32}O_5$) (25), 450.2574 (M^+-$2H_2O$) (25), 432.2396 (35), 417.2125 (15), 280.1439 ($C_{19}H_{20}O_2$) (10), 238.1316 ($C_{17}H_{18}O$) (90), 125.1468 ($C_7H_9O_2$) (60), 55.00993 (C_4H_7) (100).

Withajardin B (3): white powder; LRMS m/z (rel int. %) 487 (M^+- Me) (7), 450 (15), 432 (60), 399 (15), 249 (40), 238 (37), 171 (65), 125 (30), 107 (90), 91 (100), 55 (80).

Withajardin C (4): crystallized from EtOH; HRMS m / z (rel int. %) 545.2747 (M^++ H^+, $C_{30}H_{41}O_9$) (1), 484.2450 ($C_{28}H_{36}O_7$) (1), 254.1263 ($C_{17}H_{18}O_2$) (42), 238.1247 ($C_{13}H_{18}O_4$) (29), 173.0982 ($C_{12}H_{13}O$) (39), 124.0545 ($C_7H_8O_2$) (93), 55.0995 (C_4H_7) (100).

Crystal data. Formula $C_{30}H_{39}O_9.H_2O$, Molecular weight 545.648, calculated density 1.2971 $g.cm^{-1}$, μ =7.428 cm^{-1}, z = 4. The compound crystallizes in the space group P212121. A suitable crystal of 0.35 x 0.30 x 0.20 mm. was used for data collection. Cell dimensions were determined by least-squares from setting 38 reflections with $10^o < \theta < 40^o$: a = 27.934 (3), b = 12.344, c = 8.1032 (2) Å. The data were collected on a Philips PW 1100 diffractometer with graphite monochromated CuKα radiation. A total of 2753 independent reflections were measured and 2619 were considered as observed when $I>2\sigma$ (I), and were used for the structure determination and refinement. Two reference reflections were checked every 90 treflections and they showed no intensity variation. The intensities measurements was performed up $\theta = 65^o$, $\omega/2\theta$ scan technique, scan speed 0.050 $s.g^{-1}$, scan width 1.60. With the same measurement time for both backgrounds as for the peak. The data were corrected for Lorentz and polarization effects, but not for absorption. The atomic scattering factors and the anomalous dispersion corrections were taken from the literature [24].
All calculations performed on a VAX 6410 and the programs from reference[20,21] and several local programs.

Withajardin D (2): white powder; LRMS m /z (rel int. %) 450 (M^+ - HOAc - H_2O) (15), 432 (16), 249 (24), 238 (68), 223 (34), 197 (27), 171 (65), 125 (32), 109 (43), 69 (76), 55 (100).

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