18,20-Hemiacetal-type and Other Withanolides from *Dunalia brachyacantha*

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Two *Dunalia brachyacantha* specimens collected at different locations in Argentina were investigated separately. The 18,20-hemiacetal-type withanolides (17*S*,20*R*,22*R*,24*S*,25*R*)-4β-acetyloxy-5β,6β:18,20-diepoxy-18-hydroxy-1-oxowitha-2-enolide (**1**) and (17*S*,20*R*,22*R*)-4β-acetyloxy-5β,6β:18,20-diepoxy-18-hydroxy-1-oxowitha-2,24-dienolide (**2**) were isolated from the first collection, both as mixtures epimeric at C-18. The second collection contained instead (17*R*,20*S*,22*R*)-5β,6β-epoxy-4β,16α-dihydroxy-1-oxowitha-2,24-dienolide (**3**); (17*R*,20*S*,22*R*)-5β,6β-epoxy-4β,18-dihydroxy-1-oxowitha-2,24-dienolide (**4**), and (17*R*,-20*S*,22*R*)-5β,6β-epoxy-4β,16α-dihydroxy-1,18-dioxowitha-2,24-dienolide (**5**). All five new withanolides (**1**−**5**) bear an A and B ring substitution pattern similar to that of withaferin A, and their structural elucidation was achieved by spectroscopic techniques.

The withanolides are a group of steroidal lactones restricted primarily to the family Solanaceae,[1] although a few related compounds have been found in a *Cassia* species.[2] To date, these compounds have been isolated from members of the subfamily Solanoideae but not from the subfamily Cestroideae. The presence of withanolides thus appears to be a chemotaxonomic distinction between these subfamilies. From the 25 species originally described under *Dunalia* Kunth, only seven have been recognized as belonging to this genus *sensu stricto*.[3] The remaining 18 species were placed in three other genera: *Acnistus*,[4] *Vassobia*,[5] and *Iochroma*.[4] Among the seven authentic *Dunalia* species, *D. brachyacantha* Miers, the only one native to Argentina, has been studied chemically, and it afforded withaferin A.[6] On the other hand, *D. solanaceae* Kunth, a species distributed from Colombia to Ecuador, has been shown to contain acnistins A−H.[7−9] It should be noted that the correct name for *D. australis* (Griseb.) Sleumer, also reported in the chemical literature,[10,11] is *Iochroma australe* Griseb.[4] As part of our ongoing project focused on the chemotaxonomic study of species in the Solanaceae, we have investigated two collections of *D. brachyacantha* from different locations, from which five new withanolides (**1**−**5**) were isolated; their structures were elucidated by spectroscopic data interpretation and by chemical transformations.

Results and Discussion

A collection of *D. brachyacantha* leaves and flowers, gathered in Catamarca Province, Argentina, was extracted with EtOH at room temperature. The crude extract, after chromatographic separation on Si gel 60, afforded an amorphous white solid that appeared as one spot by TLC and by FABMS gave a quasimolecular ion at *m*/*z* 567 [M + K]$^+$ (100%). The general profile for the A- and B-ring resonances in the ^{1}H and ^{13}C NMR spectra, resembled that for withaferin A;[12,13] it also showed duplicate resonances for several protons and carbons, indicating that it was a nearly 1.5:1 binary mixture of structurally related compounds of **1** (Tables 1 and 2). The individual components could not be separated. Resonances for H-2, H-3, H-4, and H-6 for both isomers had chemical shifts identical to those described for the diacetate of withaferin A;[12] the singlet at δ 2.05 and the absence of an allylic coupling between H-4 and H-2 (1H, d, J = 9.8 Hz) gave conclusive evidence that the acetyl group was attached to C-4 in a β orientation.[12] This was confirmed by the NOE cross-peak observed for the pair H-4/H-6α in the NOESY spectrum. The absence of a signal for CH_3-18 and the presence of two proton signals at δ 5.12 (1H, br d, J = 5.7 Hz) (63%) and 5.25 (1H, br d, J = 5.7 Hz) (37%) (Table 1) were indicative of a lactol function at C-18[14] and were assigned to the 18*R* and 18*S* epimers, respectively. In accordance with this, the ^{13}C NMR spectrum showed methine signals at 101.0 and 103.4 ppm (Table 2) and only five methyl groups (C-19, C-21, C-27, C-28, and AcO-4β). To avoid the inconvenience of working with an unresolved epimeric mixture, **1** was oxidized with Jones's reagent to give dilactone **6** as a single product in 77% yield. The ^{1}H NMR spectrum of **6** showed the disappearance of the signals for the epimeric H-18 protons (Table 1), while the ^{13}C NMR spectrum exhibited a new carbonyl signal at 177.0 ppm that was assigned to C-18 (Table 2). Furthermore, the resonances of C-13 and C-17, comprising a five-membered lactone ring, were shifted at 55.1 ppm ($\Delta\delta$ = −2.7) and 52.6 ppm ($\Delta\delta$ = −4.6), respectively, when compared with the spectrum of the major compound of mixture **1** (Table 2). Two proton doublets at δ 1.16 (H_3-28) and 1.26 (H_3-27), together with a carbon signal at 174.5 ppm (C-26), indicated that the side chain δ-lactone was saturated. The stereochemistry for the methyl groups attached to C-24 and C-25 was assigned by comparison with the spectra of withaphysacarpin[15] and confirmed by a NOESY experiment in which the methine signal at δ 4.48 (H-22) showed correlation peaks with the proton signals at δ 1.16 (H_3-28), 1.46 (H_3-21), 1.53 (H-23eq), and 2.18 (H-25 and/or H-17). The chemical shift of H-25 was assigned by a COSY experiment and was coincident with that found in the literature.[15] Assuming the *R* configuration at C-22 as in all known natural withanolides, the NOE cross-peak observed for H-22/H-28 is possible only in the 24*S* stereoisomer. Furthermore, the NOE cross-peak observed for H-22/H-25 is possible only in the 25*R* stereoisomer, if the six-membered lactone ring adopts a half-boat or twist-boat conformation. According to X-ray and confor-

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Table 1. ^{1}H NMR Spectral Data of Compounds **1** to **7** ($CDCl_3$, 200.13 MHz)[a]

proton(s)	**1**[b]	**2**[b]	**3**	**4**	**5**	**6**	**7**
2	6.24 d [6.25 d] (9.8)	6.23 d [6.24 d] (10.0) [(9.8)]	6.21 d (9.8)	6.21 d (10.0)	6.18 d (10.0)	6.24 d (9.8)	6.25 d (10.0)
3	7.02 dd [7.05 dd] (9.8; 6.0)	7.01 dd [7.04 dd] (10.0; 6.0) [(9.8; 6.0)]	6.94 dd (9.9; 5.8)	6.93 dd (10.0; 5.8)	6.94 dd (10.0; 5.8)	7.04 dd (9.8; 6.1)	7.04 dd (10.0; 6.0)
4	4.69 d [4.66 d] (6.0)	4.69 d [4.66 d] (6.0)	3.77 dd (5.8; 2.6)	3.76 d (5.8)	3.76 d (5.8)	4.68 d (6.1)	4.68 d (6.0)
6	3.23 br d (5.6)	3.22 br d (5.7)	3.25 br s	3.24 br s	3.24 br s	3.23 br s	3.23 br s
7α	1.37 m	1.38 m	1.32 m	obsc	1.30 m	1.30 m	1.30 dd (13.7; 11)
		2.27 m	2.11 m		2.15 m	2.28 m	2.27 dd (13.7; 5.5)
7*β*	2.26 m						
8*β*	1.90 m	1.56 m [1.90 m]	1.51 m	obsc	1.48 m	2.45 m	obsc
9α	0.94 td (10.3; 3.2)	0.96 td [0.95 m] (10.0; 3.0)	1.04 m	obsc	obsc	0.92 td (11.2; 5.0)	0.92 td (10.0; 4.0)
11a	1.93 m	1.91 m [1.50 m]	1.78 m	obsc	2.06 m	1.87 m	1.87 m
11b	1.78 m	1.78 m			1.40 m		
12a	2.55 m	2.55 m [2.00 m]	1.94 m		2.56 m	2.20 m	obsc
12b	1.38 m	1.36 m	1.14 m		1.33 m	obsc	obsc
14	1.18 m	1.28 m [1.17 m]	1.13 m		1.70 m	1.19 m	obsc
15a		1.95 m	1.85 m		2.20 m	obsc	
15b		1.73 m	1.67 m		1.67 m	obsc	
16α		1.60 m [1.61 m]				1.09 m	
16*β*		1.16 m	4.13 br t (6.2)		4.38 br t (6.6)	1.73 m	
17α		2.04 m	1.35 m		1.56 m	2.20 m	
18	5.12 d [5.25 d] (5.7)	5.14 s [5.24 s]	0.73 s	3.72 d (10.6) 3.57 d (10.6)	9.80 s		
19	1.36 s [1.38 s]	1.36 s [1.38 s]	1.41 s	1.42 s	1.33 s	1.47 s	1.46 s
20			2.10 m		2.05 m		
21	1.44 s [1.26 s]	1.46 s [1.27 s]	1.01 d (6.6)	1.12 d (6.6)	0.95 d (6.6)	1.46 s	1.49 s
22	4.38 dd [4.51 dd] (11.0; 3.3) [(10.9; 3.2)]	4.42 dd [4.48 dd] (13.0; 3.0)	4.63 dt (11.3; 3.3)	4.40 dt (13.1; 4.0)	4.52 dt (10.6; 5.5)	4.48 dd (10.2; 3.8)	4.51 dd (12.0; 4.0)
23α	1.40 m [1.52 m]	2.03 m [2.15 m]	2.32 m		2.31 m	1.53 m	2.16 m
23*β*	1.79 m [1.85 m]	2.40 m [2.49 m]	2.32 m		2.31 m	1.88 m	2.48 m
24*β*	1.76 m					1.82 m	
25α	2.18 m					2.18 m	
27	1.22 d (6.7)	1.86 s	1.88 br s	1.88 br s	1.85 br s	1.26 d (6.6)	1.88 s
28	1.15 d [1.14 d] (6.5)	1.92 s	1.92 br s	1.92 br s	1.92 br s	1.16 d (6.3)	1.95 s
OAc	2.05 s	2.04 s				2.06 s	2.05 s

[a] Chemical shifts are in ppm downfield from TMS; *J* couplings (in parentheses) are in Hz. [b] Chemical shift data corresponds to the major epimer (18*R*). Distinct resonances observed in the spectrum for the 18*S* epimer are shown in square braquets.

mational studies on closely related withanolides, these forms have been found in the crystalline state and in solution.[16] Molecular modeling of the six-membered lactone ring of compound **6** (using the AM1 semiempirical method) showed that several conformers with similar energies may coexist, including boat and twist-boat forms in which the H-22/H-25 distance is ca. 2.5 Å and H-22/H_3-28 is ca. 2.8 Å. The H-17 (δ 2.20) signal, which partially overlaps the H-25 signal and is fairly close in space to H-22 (ca. 3.1 Å) in one of the rotamers around the C-20/C-22 bond, may contribute to the observed NOE cross-peak. Therefore, the relative configuration for the side chain lactone ring was established as H-22*R*, H-24*S*, and H-25*R*.[13,15,16] The CH_3-19 and CH_3-21 ^{1}H NMR signals were assigned unambiguously by a HETCOR experiment. On the basis of these results, the structure of **1** was established as a mixture of the 18*R* and 18*S* isomers of (17*S*,20*R*,22*R*,24*S*,25*R*)-4*β*-acetyloxy-5*β*,6*β*:18,20-diepoxy-18-hydroxy-1-oxowitha-2-enolide, with the former being the major component. The latter fact was revealed by the downfield shift observed for H-21 of the major epimer to δ 1.44, $\Delta\delta = +0.18$ (Table 1), due to the deshielding effect of the OH group with α orientation at the chiral center C-18. The cross-correlation peak observed between H-18 (δ 5.25) and CH_3-21 (δ 1.26) in the NOESY experiment for the 18*S*-epimer (**1**, minor component), supported these assignments. The ^{13}C NMR spectrum signals of **1** were assigned unambiguously to each of the isomers by their relative heights and by HETCOR and DEPT experiments (Table 2). Acetylation of mixture **1** with $(Ac)_2O$ in pyridine afforded a mixture (5:1) of diacetyl derivatives (**8**). The ^{1}H NMR spectrum showed that the H-18 singlets were shifted downfield to δ 6.03 and 6.23, supporting the presence of a lactol function at C-18; also four acetyl proton singlets appeared at δ 2.03 and 2.05 for (18*S*)-**8**, and δ 2.01 and 2.06 for (18*R*)-**8**.

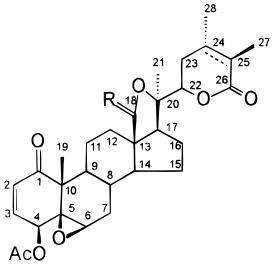
Compound **2** was obtained as a white amorphous powder. The FABMS gave a quasimolecular peak at *m*/*z* 565 (100%) corresponding to the $[M + K]^+$ ion. Similar to **1**, compound **2** was demonstrated to be an epimeric mixture from its ^{1}H NMR spectrum, which showed the characteristic H-18 epimeric protons resonating at δ 5.14 (s) and 5.24 (s). The ^{1}H and ^{13}C NMR signals were mostly superimposable on those of the components of mixture **1** (Tables 1 and 2), whereas clear differences were found for the resonances of the δ-lactone side chain. Treatment of mixture **2** with $CrO_3-H_2SO_4$ afforded dilactone **7** (yield 76%). The NMR spectral data of **6** and **7** were almost identical, except for those signals belonging to the side chain which, in the

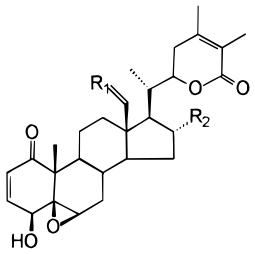
Table 2. ^{13}C NMR Spectral Data of Compounds **1** to **8** ($CDCl_3$, 50.32 MHz)

	1[a]		**2**[a]							**8**	
carbon	(18*R*)	(18*S*)	(18*R*)	(18*S*)	**3**	**4**	**5**	**6**	**7**	(18*S*)	(18*R*)
1	201.2	201.3	201.2	201.4	202.1	202.2	202.4	201.5	201.5	201.0	201.1
2	133.8	133.8	133.8	133.8	132.3	132.2	131.8	133.8	133.7	133.5	133.5
3	139.7	139.9	139.7	139.9	143.0	141.9	142.4	140.0	140.0	139.9	139.7
4	72.0	72.1	72.0	72.2	69.9	69.8	69.6	72.0	72.0	71.7	71.6
5	61.0	60.8	60.0	60.5	63.9	63.9	63.6	60.8	60.8	60.5	60.7
6	60.4	60.3	60.3	60.4	62.4	62.6	62.5	60.1	60.1	59.8	60.1
7	31.3	31.3	31.3	31.3	31.1	31.4	31.1	31.5	31.5	31.3	31.3
8	30.6	30.1	30.7	30.0	29.2	29.9	31.1	27.9	27.8	30.9[b]	30.7[b]
9	43.9	44.4	44.0	44.5	44.0	44.4	43.7	43.5	43.5	43.2	44.0
10	48.2	48.2	48.2	48.2	47.7	47.7	47.5	48.2	48.2	47.8	47.8
11	23.6	23.6	23.6	23.7	21.8	22.5	23.8	21.3	21.3	22.8	23.4
12	34.5	36.8	34.5	36.9	39.4	34.3	34.1	35.1	35.0	34.3	36.6
13	57.8	58.5	58.8	58.8	43.9	46.8	60.1	55.1	55.1	57.2	58.2
14	56.4	54.6	56.4	54.9	61.2	55.5	53.2	55.9	55.9	56.2	54.0
15	25.3[d]	25.2[d]	25.4	25.5	37.6	24.3	37.1	25.7	26.0	25.1[c]	24.7
16	26.1[e]	27.3[e]	26.2	27.5	76.4	27.2	76.1	27.3	27.3	26.1[c]	26.9
17	57.2	56.8	57.0	57.0	53.4	52.7	60.8	52.6	52.3	56.2	56.5
18	101.0	103.4	100.9	103.5	13.0	60.1	206.2	177.0	177.2	98.8	101.0
19	15.9	15.8	16.0	15.8	17.3	17.5	17.6	15.8	15.9	15.3	15.7
20	85.2	85.1	85.2	84.8	37.8	39.1	38.1	84.1	83.5	86.6	86.8
21	21.3	23.5	21.8	24.7	13.7	14.1	14.0	22.5	23.6	20.5	23.8
22	79.4	80.0	80.5	80.4	78.8	78.4	78.6	77.8	76.9	78.6	79.0
23	31.4[f]	32.1[f]	31.3	32.1	29.9	29.6	30.8	31.3	31.2	31.0	31.0
24	31.2[g]	31.3[g]	147.6	148.5	150.1	148.8	149.8	30.9	147.3	30.3[h]	30.2[k]
25	40.6	40.6	122.4	122.0	121.5	122.2	121.6	40.8	122.6	40.3	40.8
26	175.2	175.5	165.0	165.0	167.4	166.9	166.9	174.5	165.0	175.2	175.0
27	14.2	14.2	12.4	12.5	12.8	12.5	12.4	14.2	12.5	14.0	14.1
28	21.2	21.2	20.3	20.4	20.5	20.5	20.5	21.2	20.4	21.1	20.9
OAc	20.8,170.1	20.8,170.1	20.7,170.1	20.7,170.1				20.8,170.2	20.8,170.1	20.6,169.6[i]	20.9,169.7[i]

[a] Chemical shifts determined in mixture; isomer 18*R* being the major component in both mixtures. [b–i] Assignments may be interchanged.

latter, had a conjugated double bond that was revealed by the proton singlets at δ 1.88 (H_3-27) and 1.95 (H_3-28), together with the olefinic carbons at 147.3 ppm (C-24) and 122.6 ppm (C-25) (Tables 1 and 2). Proton and carbon resonances were assigned as for compounds **1** and **6**. Based on this evidence, the structure of **2** was determined to be (17*S*,20*R*,22*R*)-4β-acetyloxy-5β,6β:18,20-diepoxy-18-hydroxy-1-oxowitha-2,24-dienolide, isolated as an epimeric mixture at C-18, with the 18*R* isomer being the major component (ca. 1.5:1).





1 R= H, OH; 18(*R*/*S*)

2 R= H, OH; 18(*R*/*S*), 24,25-dehydro

6 R= =O

7 R= =O; 24,25-dehydro

8 R= H, OAc, 18(*R*/*S*)

3 R_1= H; R_2= OH

4 R_1= H, OH; R_2= H

5 R_1= =O; R_2= OH

The second *D. brachyacantha* sample, leaves and flowers, was collected in Córdoba Province; it was extracted in the same manner as the initial sample, and the withanolides were purified by column chromatography on Si gel. Compound **3** precipitated as colorless needles. Its HREIMS was consistent with a molecular formula of $C_{28}H_{38}O_6$; the EIMS showed a base peak at *m*/*z* 125 ($C_7H_9O_2$), indicating the presence of an α,β-unsaturated δ-lactone ring that was further confirmed by ^1H and ^{13}C NMR (Tables 1 and 2). The pattern of substitution of the A and B rings was the same as those of compounds **1** and **2**, except that C-4 (69.9 ppm) bore a free OH group.[12] The proton signal of the latter (δ 2.30) as well as that for a second hydroxyl group (δ 3.49) disappeared after addition of D_2O, and the corresponding methine protons were simplified to a doublet (δ 3.77, H-4) and to a broad triplet (δ 4.13, H-16), respectively (Table 1). The stereochemistry of the OH-16 group was determined to be α by a NOESY experiment, which showed cross-correlation peaks of H-16 with H-15β, CH_3-18, and H-22 and by the distances measured in the geometry calculated by AM1 of both epimers at C-16; H-16 also correlated with C-16 at 76.4 ppm in the HETCOR spectrum. The ^{13}C NMR spectrum signals were in agreement with those of its 16-*O*-acetyl derivative iochromolide isolated from *Iochroma coccineum*.[13] Based on these results, the structure of compound **3** was assigned as (17*R*,20*S*,22*R*)-5β,6β-epoxy-4β,16α-dihydroxy-1-oxowitha-2,24-dienolide or deacetyliochromolide.

Compound **4** failed to give a molecular ion by EIMS, but the base peak at *m*/*z* 125 revealed the presence of an α,β-unsaturated δ-lactone group; the ^1H and ^{13}C NMR spectra were similar to those of **3** (Tables 1 and 2); however, the absence of the CH_3-18 signal and an AB system appearing at δ 3.57 (1H, d, J = 10.6 Hz) and 3.72 (1H, d, J = 10.6 Hz) indicated the presence of a hydroxyl group at C-18. The NMR spectral data of **4** were similar to those of its 18-*O*-acetyl analogue withacnistine, isolated from *I. coccineum*;[13] the H-18 resonances appearing at higher field supported the presence of a free OH group at C-18. Compound **4** was therefore structurally determined as (17*R*,20*S*,22*R*)-5β,6β-epoxy-4β,18-dihydroxy-1-oxowitha-2,24-dienolide or deacetylwithacnistine.

The ^{1}H NMR spectrum of compound **5** resembled that of **3**, but a singlet at δ 9.80 and the lack of a signal for the CH_3-18 protons indicated that C-18 was oxidized to an aldehyde function. A carbinol signal at δ 4.38 (br t) was assigned to H-16, indicating the presence an α hydroxyl group attached to C-16. A COSY experiment showed cross-correlation peaks of H-16 with signals at δ 2.20, 1.67, and 1.56 assigned to H-15α, H-15β, and H-17α, respectively. The chemical shift of H-17 was further confirmed by the cross-correlation peak at δ 2.05 (H-20) which, at the same time, correlated with a signal at δ 0.95 (H-21). The stereochemistry of the hydroxyl group at C-16 was established as α due to the NOE effect observed in a NOESY experiment among H-15β, H-16, H-18, and H-22; also, the coupling constant calculated from the AM1 optimized geometries using the Altona equation[18] were close to those of the ^{1}H NMR spectrum, hence reinforcing this conclusion. On this basis, compound **5** was determined as (17*R*,20*S*,-22*R*)-5β,6β-epoxy-4β,16α-dihydroxy-1,18-dioxowitha-2,24-dienolide.

The five new compounds **1**−**5** isolated from the two *D. brachyacantha* collections share the pattern of substitution of the A and B rings as found in withaferin A, previously isolated from this same species;[6−9] however, the rare 18,20-lactols **1** and **2** were present only in the Catamarca collection (northwestern Argentina). In contrast to previous findings,[7−9] acnistins were not isolated from our collections. To our knowledge, only one 18,20-hemiacetal-type withanolide derivative has been isolated, also as a mixture, from *Physalis minima*[14] and a related family of compounds has been isolated from *Vassobia lorentzii* (unpublished data). The more austral *D. brachyacantha* collection (central Argentina) was shown to contain as major components withanolides **3**−**5**, with **3** and **4** being the deacetyl derivatives of iochromolide and withacnistin, respectively, previously isolated from *Iochroma coccineum*.[13] The chemical results obtained in this work are in agreement with the close taxonomic affinity exhibited by the genera *Dunalia*, *Iochroma*, and *Vassobia*.[5]

Experimental Section

General Experimental Procedures. UV and IR spectra were recorded on a Shimadzu UV 260 and a Nicolet 5-SXC−FTIR spectrometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer at 200.13 and 50.32 MHz, respectively, using $CDCl_3$ as solvent and TMS as an internal standard. Chemical shifts are given in parts per million downfield from TMS, and coupling constants are measured in Hertz. DEPT, HETCOR, COSY, and NOESY experiments were obtained using standard Bruker software. EIMS were collected on a Finnigan 3300 F-100 instrument at 70 eV by direct inlet; FABMS and HREIMS (70 eV) were measured on a VG ZAB-BEqQ mass spectrometer. Melting points were obtained in sealed capillaries and are uncorrected. Chromatographic separations were performed by vacuum liquid chromatography, column chromatography on Si gel 60 (40−63 μm), filtration over Sephadex LH-20, and preparative TLC on Si gel 60 G F_{254} plates (0.2 mm thick).

Plant Material. First collection: leaves and flowers of *D. brachyacantha* were collected near Río Potrero bridge, Andalgalá, Catamarca Province, Argentina, in December 1995. Second collection: leaves and flowers were collected in Pampa de Achala, near Paradero de la Posta, Córdoba Province, Argentina, in December 1996. Voucher specimens are deposited at the Museo Botánico, Universidad Nacional de Córdoba, under nos. A. T. Hunziker 25527 and Barboza 124, respectively.

Extraction and Isolation. Catamarca collection: the dried and powdered leaves and flowers (51.7 g) were extracted with EtOH (3 × 500 mL) at room temperature and concentrated at reduced pressure (40 Torr). The residue was taken with MeOH−H_2O 20% and defatted with hexane. The aqueous layer was extracted with EtOAc and $CHCl_3$, consecutively. The $CHCl_3$ extract was dried with $CaCl_2$, filtered, and concentrated in vacuo, affording 3.03 g of extract. The latter was chromatographed on Si gel 60 (120 g) using hexane, hexane−$CHCl_3$ (7:3, 1:1), $CHCl_3$, $CHCl_3$−MeOH (99:1, 49:1, 19:1, 9:1), and MeOH as eluents. The fractions were pooled according to their TLC profiles. Fraction 11 (81.8 mg) was processed by column chromatography and Sephadex LH-20 to give 17.7 mg of a binary mixture (ca. 1.5:1) of closely related compounds (**1**); further efforts to separate these were unsuccessful. Fraction 13 (565.5 mg) was chromatographed on Si gel eluting with hexane, hexane−EtOAc (9:1, 4:1, 7:3, 3:2), EtOAc, and EtOAc−MeOH (99:1, 95:1). Subfraction 11 afforded 178 mg of the mixture of lactols **1**. Subfraction 51 (80 mg) was purified on Si gel eluting with hexane−EtOAc (3:2, 1:1, 2:3, 3:7), EtOAc, and MeOH, to give 20 mg of the mixture of epimers **2**. The remaining fractions were analyzed by TLC and ^{1}H NMR, which indicated the presence of mainly lactols **1** and **2**.

Córdoba collection: dried and powdered leaves and flowers (260 g) were extracted with EtOH (4 × 2.5 L) at room temperature, giving 44 g of a residue. The latter was partitioned between hexane (700 mL) and MeOH−H_2O 10% (700 mL), and, after the usual work up, 18 g of $CHCl_3$ extract were obtained. This extract was fractionated by vacuum liquid chromatography eluting with hexane, $CHCl_3$−EtOAc (1:1), EtOAc, EtOAc−MeOH (9:1, 4:1, 7:3), and MeOH. A total of 11 fractions was collected. Fraction 4 (1.5 g) was purified through column chromatography with $CHCl_3$−MeOH (99.9:0.1 to 65:1) and $CHCl_3$−MeOH (49:1, 19:1); fractions showing similar TLC behavior were pooled. Subfraction 3 (290 mg) gave colorless needles, and the compound was structurally determined as 16-deacetyliochromolide (**3**, 80 mg). Subfraction 4 (123.6 mg) was processed in the same way as fraction 4, affording 6.7 mg of a mixture that was purified by preparative TLC (hexane−Me_2CO 2:3) to give 18-deacetylwithacnistin (**4**, 3 mg) and **5** (1.5 mg). Fraction 5 (2.58 g) was fractionated by vacuum liquid chromatography, successive column chromatographic steps, and preparative TLC to give **3** (87.3 mg) and **5** (11 mg).

(17*S*,20*R*,22*R*,24*S*,25*R*)-4β-Acetyloxy-5β,6β:18,20-diepoxy-18-hydroxy-1-oxowitha-2-enolide (1, 18*R* and 18*S*): white amorphous powder; UV (MeOH) λ_{max} (log ϵ) 212 (3.5) nm; IR film (AgCl) ν_{max} 3472 (hemiacetal OH), 2952, 1745 (C=O δ-lactone and acetyl groups), 1683 (α,β-unsaturated ketone), 1457, 1372, 1230, 1097, 1008, 755 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; FABMS (*m*-nitrobenzyl alcohol−K_2CO_3) *m*/*z* 567 (100), $[M + K]^+$.

Acetylation of a mixture of **1** (178 mg) with $(Ac)_2O$ (3 mL) and pyridine (3 mL) for 15 h at room temperature, afforded 154 mg (87%) (ca. 5:1, by ^{1}H NMR) of a mixture of epimers (**8**, 18*S*/18*R*). The individual compounds could not be purified, and they were analyzed as a mixture. White powder: UV λ_{max} (log ϵ) 205 (3.0), 212 (3.0) nm; IR film (AgCl) ν_{max} 2961, 1739, 1683, 1457, 1374, 1233, 1096, 1050, 999, 756 cm^{-1}; ^{1}H NMR for compound **8** (18*S*, major component) δ 7.03 (1H, dd, J = 9.8, 6.1 Hz, H-3), 6.22 (1H, d, J = 9.8, H-2), 6.03 (1H, s, H-18*R*), 4.64 (1H, d, J = 6.1, H-4α), 4.42 (1H, br d, J = 2.3, H-22α), 3.21 (1H, br s, H-6α), 2.05 (3H, s, OAc), 2.03 (3H, s, OAc), 1.54 (1H, m, H-24), 1.37 (3H, s, H-21), 1.24 (3H, s, H-19), 1.19 (3H, d, J = 6.6, H-27), 1.13 (3H, d, J = 6.2, H-28), and 0.91 (1H, m, H-9α); ^{13}C NMR, see Table 2; ^{1}H NMR for compound **8** (18*R*, minor component) δ 6.99 (1H, dd, J = 9.8, 6.3 Hz, H-3), 6.23 (1H, s, H-18*S*), 6.22 (1H, d, J = 9.8, H-2), 4.68 (1H, d, J = 6.3, H-4α), 4.37 (1H, br s, H-22α), 3.21 (1H, br s, H-6α), 2.06 (3H, s, OAc), 2.01 (3H, s, OAc), 1.43 (1H, m, H-24), 1.32 (3H, s, H-19), 1.27 (3H, s, H-21), 1.20 (3H, d, J = 6.6, H-27), 1.10 (3H, d, J = 6.2, H-28), and 0.94 (1H, m, H-9α); ^{13}C NMR, see Table 2; EIMS *m*/*z* 511 (6) $[M - C_2H_3O_2]^+$, 468 (1), 444 (41), 443 (47), 384 (4), 341 (4), 313 (10), 171 (8), 128 (6), 127 (9), 124 (16), 44 (100).

Oxidation of Mixture 1. Mixture **1** (17 mg) was dissolved in degassed Me_2CO (1.5 mL) and treated with 8N CrO_3−

H_2SO_4 (0.2 mL) at 0 °C for 4 min while bubbling He into the reaction mixture. 2-Propanol (excess) and H_2O (1 mL) were added and the mixture extracted with $CHCl_3$. Evaporation of the solvent afforded dilactone **6** (13 mg, 76.5%): white crystals, mp 260−261 °C; UV λ_{max} (log ϵ) 205 (3.0), 213 sh (2.9) nm; IR film (AgCl) ν_{max} 3063, 2955, 2871, 1761, 1687, 1460, 1381, 1228, 1092, 956 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* $[M]^+$ absent, 467 (14) $[M - C_2H_3O_2]^+$, 466 (15) $[-C_2H_4O_2]$, 400 (3.6) $[-C_7H_{11}O_2]$, 399 (3.5), 362 (17), 293 (3), 207 (6), 128 (5), 127 (10), 124 (51), 56 (37), 44 (100); HREIMS *m*/*z* $[M - C_2H_4O_2]^+$ 466.2358 (calcd for $C_{28}H_{34}O_4$ 466.2355).

(17*S*,20*R*,22*R*)-4*β*-Acetyloxy-5*β*,6*β*:18,20-diepoxy-18-hydroxy-1-oxowitha-2,24-dienolide (2, 18*R* and 18*S*): white amorphous powder, UV λ_{max} (log ϵ) 207 (3.5), 215 (3.5) nm; IR film (AgCl) ν_{max} 3472 (hemiacetal OH), 2944, 1741 (C═O acetyl group), 1701 (C═O α,*β*-unsaturated *δ*-lactone), 1690 (α,*β*-unsaturated ketone), 1461, 1381, 1323, 1230, 1133, 1013, 760 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; FABMS *m*/*z* 565 (100), $[M + K]^+$.

Oxidation of Mixture 2. The mixture of lactols **2** (13 mg) was treated as above with Jones's reagent to give dilactone **7** (10 mg, 76%): white powder, mp 146−147 °C; UV λ_{max} (log ϵ) 210 (4.6) nm; IR film (AgCl) ν_{max} 3089, 2929, 2863, 1763 (C═O acetyl group), 1726 (C═O α,*β*-unsaturated *δ* lactone), 1690 (α,*β*-unsaturated ketone), 1653, 1464, 1384, 1238, 1092, 1027, 968 cm^{-1};^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 464 (20) $[M - C_2H_4O_2]^+$, 446 (11), 400 (6), 360 (27), 359 (23), 294 (4), 153 (14),125 (47), 124 (48), 97 (28), 44 (100); FABMS (NOBA − K_2CO_3) *m*/*z* 563 (27) $[M + K]^+$, 345 (43), 269 (30), 231 (100); HREIMS *m*/*z* $[M - C_2H_4O_2]^+$ 464.2195 (calcd for $C_{28}H_{36}O_6$ 464.2199).

(17*R*,20*S*,22*R*)-5*β*,6*β*-Epoxy-4*β*,16α-dihydroxy-1-oxowitha-2,24-dienolide (3): colorless needles, mp 250−251 °C; UV λ_{max} (log ϵ) 213 (2.7) nm; IR film (AgCl) ν_{max} 3448, 2957, 1689, 1457, 1402, 1300, 1143, 1041, 1000, 972, 755 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 470 (0.3) $[M]^+$, 425 (3), 330 (0.2), 300 (0.9), 281 (1.6), 150 (42), 125 (100), 97 (21), 43 (24); HREIMS *m*/*z* $[M]^+$ 470.2665 (calcd for $C_{28}H_{38}O_6$ 470.2668).

(17*R*,20*S*,22*R*)-5*β*,6*β*-Epoxy-4*β*,18-dihydroxy-1-oxowitha-2,24-dienolide (4): white crystals, mp 150 °C (dec); UV λ_{max} (log ϵ) 211 (2.7) nm; IR film (AgCl) ν_{max} 3280, 2936, 2875, 1689, 1464, 1402, 1130, 1041, 925, 761 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* $[M]^+$ absent, 440 (1) $[M - CH_2O]^+$, 422 (1), 404 (0.4), 343 (0.5), 267 (2), 249 (2), 125 (79), 109 (15), 97 (19), 91 (18), 69 (21), 67 (30), 55 (59), 43 (99), 41 (100); FABMS (glycerol) *m*/*z* 471 (100), $[M + 1]^+$.

(17*R*,20*S*,22*R*)-5*β*,6*β*-Epoxy-4*β*,16α-dihydroxy-1,18-dioxowitha-2,24-dienolide (5): white crystals, mp 238−240° (dec); UV λ_{max} (log ϵ) 212 (3.7) nm; IR film (AgCl) ν_{max} 3527, 2946, 1715, 1685, 1461, 1388, 1218, 1139, 1042, 921 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 484 (0.2) $[M]^+$, 466 (0.2) $[M - H_2O]^+$, 441 (2), 423 (1), 267 (3), 249 (2), 153 (7), 125 (71), 109 (22), 97 (17), 91 (16), 69 (19), 67 (30), 55 (58), 43 (100); FABMS (glycerol) *m*/*z* 485 (6) $[M + 1]$; 467 (100) $[M + 1 - H_2O]$.

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