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WITHANOLIDES OF *ACNISTUS BREVIFLORUS*

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Key Word Index—*Acnistus breviflorus*; Solanaceae; withanolides; steroidal lactones.

Abstract—The chemical examination of *Acnistus breviflorus* afforded nine withanolides, four of which are new and were established as 2,3,24,25-tetrahydro-27-desoxywithaferin A (4β-hydroxy-5β,6β-epoxy-1-oxo-22*R*-withanolide), 2,3-dihydro-27-desoxywithaferin A (4β-hydroxy-5β,6β-epoxy-22*R*-witha-24-enolide), 5,6-desoxywithaferin A (4β,27-dihydroxy-1-oxo-22*R*-witha-2,5,24-trienolide) and 2,3-dihydro-5,6-desoxywithaferin A (4β,27-dihydroxy-1-oxo-22*R*-witha-5,24-dienolide). The five known compounds were: withaferin A; 2,3-dihydrowithaferin A; 24,25-dihydro-27-desoxywithaferin A and withaferin A-6α,5β-chlorohydrin.

INTRODUCTION

As part of our continued interest in the withanolides of the plants belonging to the Solanaceae [1], we undertook a systematic chemical examination of *Acnistus breviflorus* with the objective of isolating their components and laying the groundwork for understanding their biosynthesis. Nine withanolides were obtained during liquid chromatography of the chloroform extract, four of which are new compounds. The compounds occurring in the largest amounts were withaferin A (**1a**) [2] and 2,3-dihydrowithaferin A (**2a**) [2, 3]. Other known compounds present in minor quantities were 24,25-dihydro-27-desoxywithaferin A (**3a**) [4], 27-desoxywithaferin A (**4**) [4] and 6α-chloro-5β-hydroxywithaferin A (**5**) [5, 6]. The four new compounds were established by spectral analysis as: 2,3,24,25-tetrahydro-27-desoxywithaferin A (**6a**); 2,3-dihydro-27-desoxywithaferin A (**7a**); 5,6-desoxywithaferin A (**8a**) and 2,3-dihydro-5,6-desoxywithaferin A (**9a**).

RESULTS

Among the members of the Solanaceae family, *Withania sommifera* is so far outstanding in the elaboration of a variety of withanolides [1]. This species occurs as different chemotypes and was found to be a good model for the study of chemogenetics [7]. Along these lines, we undertook the chemical analysis of other members of this family. The genus *Dunalia* is sometimes synonymous with *Acnistus* [8] and it is possible that a member of this genus could produce such a model. *Acnistus australis* from Argentina [9], *A. arborescens* from Brazil [10] and, more recently, *A. ramiflorum* [11] have already been investigated. Hence, we chose *A. breviflorus*, originating from Argentina and grown in our experimental plots, for our study. The structure elucidation of the new compounds is presented in the sequel.

Compound **6a** ($C_{28}H_{42}O_5$) was established as 2,3,24,25-tetrahydro-27-desoxywithaferin A. In the UV, this compound did not show the usual absorptions for the unsaturated ketone of ring A and δ-lactone of the side-chain; however, the IR showed their carbonyl groups, the frequencies suggesting them to be saturated. The ^{13}C NMR spectrum showed no sp^2 carbons in addition to these two carbonyls, thus double bonds are absent. The protons of the two secondary methyl groups of the reduced lactone (Table 1) are in accord with similar ones in the known compound **3a** [4, 12] and, by correlation, have also similar conformations, 27-Me is β-eq and 28-Me is α-eq, as drawn. The 4β-hydroxyl gave an acetate (**6b**), a compound also obtained by catalytic hydrogenation of the known **3b**. The mass spectrum of **6a** showed a fragment at m/z 331 $[M - 127]^+$ which was attributed to the loss of the saturated side-chain by cleavage of the C-17,C-20 bond [4].

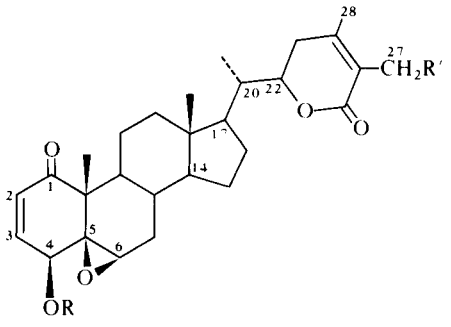
Compound **7a** ($C_{28}H_{40}O_5$) was established as 2,3-dihydro-27-desoxywithaferin A. Four sp^2 carbons were shown by the ^{13}C NMR spectrum, while the ^1H NMR spectrum indicated the absence of the usual enone system of ring A but revealed a close similarity with the structure of **6a**. The acetate **7b** was compared with a synthetic sample obtained from withaferin A diacetate (**1b**) by careful hydrogenation (over 5% Pd–$CaCO_3$), whereby the Δ^2 was saturated and the allylic 27-OAc underwent hydrogenolysis [13]. Compound **7a** was hydrogenated (over 10% Pd–C) and the product (**10**) had the two lactonic secondary methyl groups in a *cis*-orientation ($J_{24,25}$ = 14.4 Hz) in contrast to their *trans* stereochemistry observed ($J_{24,25}$ = 10 Hz, [12]) in the natural compounds. In fact, the various possible conformers of the saturated lactones in withanolides have been discussed [14] and by correlation, in **10**, the 27-Me is β-eq and 28-Me is β-ax.

Compound **8a** ($C_{28}H_{38}O_5$) was established as 5,6-desoxywithaferin A. The ^{13}C NMR spectrum showed eight sp^2 carbons. Thus, a trisubstituted double bond (corresponding to one singlet and one doublet in the SFORD spectra) could be present in addition to the usual unsaturated systems of ring A and the side-chain (UV and IR spectra). This double bond could be placed at C-5,C-6, as deduced from an analysis of the ^1H NMR spectrum (Table 1) which compared well with that of withanolide U(**11**) [15] for protons in the A/B rings system. For confirmation, **11** was reacted with MnO_2. The double allylic 4β-hydroxyl was oxidized to the ketone **12** thus resulting in a diene–dione in rings A/B, λ_{max} 225 and 278 nm (ε 21 000 and 2200). A similar oxidation of **8a**

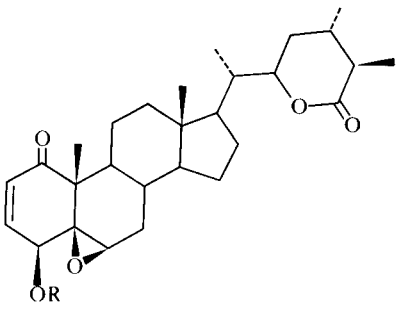
Table 1. ^{1}H NMR signals of relevant protons in withanolides

Compound	2-H	3-H	4-H	6-H	22-H	Methyl groups: 18	19	21	27	28	Other signals
6a	—	—	3.52 (*t*, 3.1)	3.14 (*br s*)	4.32 (*dt*, 11.6, 3.2)	0.67	1.32	0.94 (*d*, 6.7)	1.12 (*d*, 6.7)	1.22 (*d*, 6.7)	
6b	—	—	4.34 (*m*)	3.18 (*br s*)	4.36 (*dt*, 11.6, 3.2)	0.67	1.33	0.95 (*d*, 6.7)	1.14 (*d*, 6.7)	1.23 (*d*, 6.7)	2.06 (4-OAc)
7a	—	—	3.52 (*m*)	3.20 (*br s*)	4.35 (*dt*, 13.2, 3.4)	0.67	1.32	0.99 (*d*, 6.7)	1.88	1.93	
7b	—	—	4.57 (*t*, 3.2)	3.15 (*br s*)	4.35 (*dt*, 13.2, 3.4)	0.67	1.28	0.98 (*d*, 6.7)	1.88	1.93	2.06 (4-OAc)
10	—	—	3.51 (*m*)	3.14 (*br s*)	4.33 (*m*)	0.66	1.32	0.95 (*d*, 7)	1.14 (*d*, 7)	0.93 (*d*, 7)	
8a	5.96 (*d*, 9.8)	6.77 (*dd*, 10.1, 4.6)	4.63 (*br d* 4.2)	5.93 (*br d* 3.7)	4.45 (*dt*, 13.1, 3.5)	0.76	1.57	1.04 (*d*, 6.8)	—	2.05	4.38 (*m*, 27-C$\underline{H}_2$OH)
8b	6.02 (*d*, 10)	6.71 (*dd*, 10, 4.6)	5.88 (*d*, 4.6)	6.11 (*br d* 4.5)	4.43 (*dt*, 13.1, 3.5)	0.75	1.39	1.03 (*d*, 6.4)	—	2.06	2.09 (27-OAc and 4-OAc) 4.90 (*d*, 2.1, 27-C$\underline{H}_2$OAc)
9a	—	—	3.64 (*m*)	5.75 (*dd*, 4.4, 0.6)	4.43 (*dt*, 13.2, 2.4)	0.73	1.52	1.02 (*d*, 6.8)	—	2.05	4.36 (*d*, 4.4, 27-C$\underline{H}_2$OH)
9b	—	—	5.43 (*m*)	5.93 (*br d*, 4.5)	4.43 (*dt*, 13.2, 2.4)	0.72	1.42	1.02 (*d*, 6.6)	—	2.06	2.09 (27-OAc), 2.08 (4-OAc) 4.90 (*d*, 2.1, 27-C$\underline{H}_2$OAc)
11	6.91 (*dd*, 5.2, 2.4)		—	6.71 (*dd*, 10.4, 4.3)	4.22 (*dd*, 13.1, 3.7)	1.08	1.41	1.30	1.89	1.96	
13	6.86 (*d*, 0.6)		—	3.42 (*br d* 2.1)	4.35 (*dt*, 13.2, 3.5)	0.72	1.01 (*d*, 6.4)	1.38	1.88	1.94	
14	6.70 (*dd*, 20.3, 6.2)		—	6.84 (*dd*, 5.8, 2.4)	4.35 (*dt*, 13.2, 3.4)	0.76	1.39	1.04 (*d*, 6.4)	—	2.05	4.38 (*d*, 4.3, 27-C$\underline{H}_2$OH)
15	6.70 (*dd*, 20.3, 6.2)		—	6.83 (*dd*, 5.6, 2.6)	4.35 (*dt*, 13.2, 3.4)	0.78	1.39	1.33 (*d*, 7)	—	2.55	10.26 (*s*, 27-C$\underline{H}$O)
16	—	—	4.34 (*m*)	—	4.36 (*dt*, 13.1, 3.3)	0.67	1.14	0.96 (*d*, 6.3)	1.88	1.94	2.08 (4-OAc)

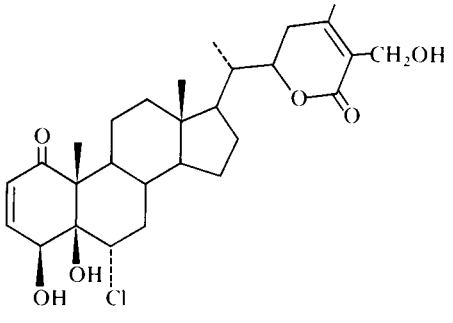
Chemical shifts are in δ units; coupling constants (in Hz) are in parentheses.



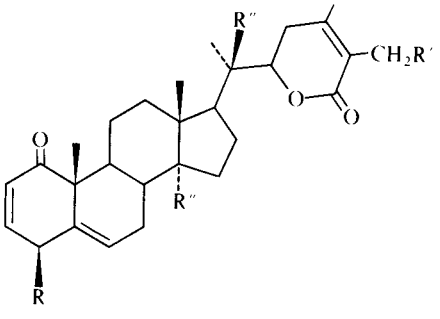
1a R = H, R′ = OH
1b R = Ac, R′ = OAc
2a 2,3-dihydro, R = H, R′ = OH
2b 2,3-dihydro, R = Ac, R′ = OAc
4 R = R′ = H
7a 2,3-dihydro, R = R′ = H
7b 2,3-dihydro, R = Ac, R′ = H



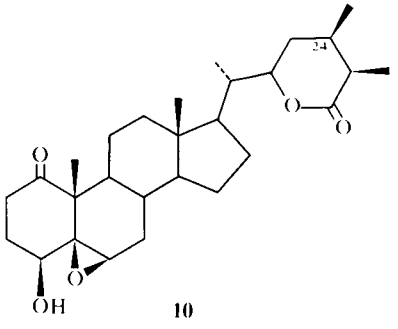
3a R = H
3b R = Ac
6a 2,3-dihydro, R = H
6b 2,3-dihydro, R = Ac



5



8a R = R′ = OH, R″ = H
8b R = R′ = OAc, R″ = H
9a 2,3-dihydro, R = R′ = OH, R″ = H
9b 2,3-dihydro, R = R′ = OAc, R″ = H
11 R = R″ = OH, R′ = H



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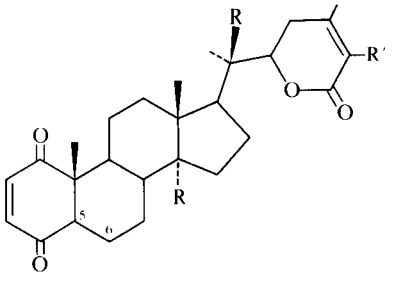
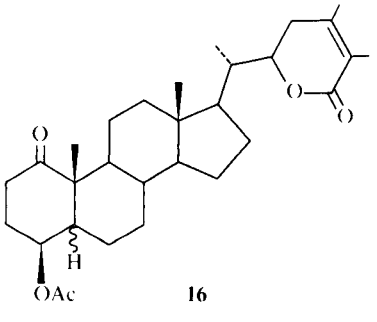


12 Δ^5, R = OH, R′ = Me
13 5,6β-epoxy, R = H, R′ = Me
14 Δ^5, R = H, R′ = CH_2OH
15 Δ^5, R = H, R′ = CHO



16

produced compounds **14** and **15**, in which an identical system is present. However, the latter also contained an aldehyde, resulting from the oxidation of the allylic 27-CH_2OH (ν_{max} 2878 and 1690 cm^{-1} for the –CHO group). This aldehyde also induces a deshielding of the 28-Me by 0.6 ppm.

When the diacetate **8b** was hydrogenated over Pd, 3 mol of H_2 were absorbed resulting in the product **16** with saturation of the Δ^2 bond, hydrogenolysis of 27-CH_2OAc and Δ^5 reduction in the given sequence.

Compound **9a** ($C_{28}H_{40}O_5$) was established as 2,3-dihydro-6,7-desoxywithaferin A. This compound was lacking the usual enone system of ring A, as shown by the ^1H NMR spectrum. The ^{13}C NMR spectrum indicated six sp^2 carbons assigned to the two carbonyl and two double bonds, one being at Δ^5. The 4β-OH is allylic to the latter, which is exo-trisubstituted and as such could not be oxidized with MnO_2, but was easily acetylated. The diacetate **9b** absorbed 2 mol of H_2, producing a product (**16**) found identical in all respects with that obtained from **8b**.

DISCUSSION

It was found that all the compounds isolated from *Acnistus breviflorus* (**1**–**9**) have a hydroxyl group at C-4 but none at C-20, C-17 and C-14, and that the substitution pattern in rings A/B, unlike the situation in *Withania somnifera* [1], lacks variety. A relatively new feature seems to be dominant in this plant, namely the reduction of the double bonds. For example, the yield of 2,3-dihydrowithaferin A (**2a**) (50 g), compared to that of withaferin A (**1a**) (90 g), from 12 kg of the plant material indicates the predominance of this reaction in this plant. The occurrence of a saturated lactone (in compounds **3a** and **6a**) is a characteristic which is not widespread among the withanolides and has so far been encountered only in a South African type of *W. somnifera* [4]. Interestingly, during a hybridization of this plant with chemotype II (Israel), this reduction became a dominant character [12, 16]. These results indicate that an appropriate genetic combination may lead to such a dominant character as encountered in *Acnistus breviflorus*. The occurrence of a reduced lactone, together with a non-reduced ring A, indicates a biosynthetic process which cannot be duplicated in the laboratory, the sequence of reduction being inverse.

The isolation of 5,6-desoxywithaferin A (**8a**) which seems to be a direct precursor of the naturally abundant withaferin A (**1a**) in this plant, lends support to the biogenetic scheme presented earlier [1].

EXPERIMENTAL

Mps were measured on a Fischer–Johns apparatus and are uncorr. Optical rotations were determined in $CHCl_3$. IR spectra were recorded in KBr pellets; UV spectra were recorded for EtOH solns; ^1H NMR spectra were determined on a Bruker WH270 instrument and ^{13}C NMR spectra on a Brucker WH90, operating at 22.63 MHz in $CDCl_3$ with TMS as internal standard. For LC, a 500-g Si gel G 60 (E. Merck) column was eluted with hexane–EtOAc mixtures and 1 l. fractions were collected. Mass spectra were determined under the direction of Dr. Z. Zaretskii, and microanalyses carried out by Mr. R. Heller of our Institute.

Isolation of the withanolides from Acnistus breviflorus *Griseb.* The extraction procedure, carried out on 12 kg leaves, was described earlier [6]. The withanolides were isolated from the various fractions by CC of the $CHCl_3$ extract as presented below. The fractions were worked up separately to yield the indicated compounds:

Fractions	Eluant (hexane–EtOAc)	Compound	Total yield (g)
17–25	1:1	**5**	0.02
29–35	1:1	Mixture A (**4** + **6a**)	0.50
33–48	1:1	**7a**	0.75
45–60	2:3	Mixture B (**8a** + **9a**)	0.42
53–69	2:3	**5**	0.45
67–95	2:3	**1**	90.0
88–120	2:3	**2a**	50.0

4β-Hydroxy-5β,6β-epoxy-1-oxo-22R-withanolide (**6a**). Mixture A contained, in equal proportions, **4** and **6a**, which had identical R_f 0.65 value on TLC plates (EtOAc). A small quantity of pure **4** (100 mg) was obtained after several rapid crystallizations from hot MeOH. The mother liquors were evapd to dryness (400 mg), dissolved in EtOAc–$CHCl_3$ (2:1, 200 ml) and shaken for 6 hr. with freshly prepared MnO_2 (300 mg). The residue from the filtrate, upon separation by prep. TLC gave two products: unreacted compound **6a** (210 mg) and compound **13** (85 mg), the oxidation product of **4**. Compound **6a** mp 209–210° from EtOAc; $[\alpha]_D$ +17.4° (*c* 0.2); IR ν_{max}^{KBr} cm^{-1}: 3420, 1730, 1705, 1440, 1370, 1190, 1020, and 920; ^{13}C NMR: δ 211.6 (C-1), 72.9 (C-4), 66.7 (C-5), 58.8 (C-6), 11.6 (C-18), 15.4 (C-19), 14.5 (C-21), 78.3 (C-22), 176.6 (C-26), 12.8 (C-27) and 21.5 (C-28); MS *m/z* (rel. int.): 458 $[M]^+$ (5.7), 440 $[M - H_2O]^+$ (7.5), 442 $[M - 2H_2O]^+$ (3.9), 331 $[M - 127]^+$ (5.1), 313 $[M - 127 - H_2O]^+$ (4.2) and 127 (63.2). (Found: C, 73.3; H, 9.35; $C_{28}H_{42}O_5$ requires C, 73.42 and H, 9.24%.) The acetate **6b**, obtained by pyridine–Ac_2O; crystals, mp 215–217° from EtOAc; $[\alpha]_D$ +25.2° (*c* 0.1). (Found: C, 71.5; H, 8.98; $C_{30}H_{44}O_6$ requires C, 72.06 and H, 8.87%.) Compound **3b** on hydrogenation (5% Pd–$CaCO_3$) in EtOH also gave **6b**, compared by IR and NMR spectra.

5β,6β-Epoxy-1,4-dioxo-22R-witha-2,24-dienolide (**13**). Mp 252–253° from EtOAc; UV λ_{max}^{EtOH} nm: 287.5 and 223 (ε 1900 and 20100); IR ν_{max}^{KBr} cm^{-1}: 1698, 1685, 1670, 1440, 1260, 1110 and 900; MS *m/z* (rel. int.): 452 $[M]^+$ (0.9), 284 (22), 270 (5.5), 256 (16), 185 (7.5) and 125 (13.5). (Found: C, 74.19; H, 8.42; $C_{28}H_{36}O_5$ requires C, 74.4 and H, 8.03%.)

4β-Hydroxy-5β,6β-epoxy-1-oxo-22R-witha-24-enolide (**7a**). Mp 236–237° from EtOAc; $[\alpha]_D$ –3.0° (*c* 0.12); UV λ_{max}^{EtOH} nm: 224 (ε 7800); IR ν_{max}^{KBr} cm^{-1}: 3400, 1715, 1697, 1380, 1185, 942, and 915; ^{13}C NMR: δ 66.7 (C-5), 58.5 (C-6), 11.5 (C-18), 15.2 (C-19), 13.4 (C-21), 78.3 (C-22), 149.2 (C-24), 121.9 (C-25), 167.1 (C-26), 12.5 (C-27) and 20.5 (C-28); MS *m/z* (rel. int.): 456 $[M]^+$ (12), 438 $[M - H_2O]^+$ (18), 420 $[M - 2H_2O]^+$ (8.5), 331 $[M - 125]^+$ (12), 313 $[M - 125 - H_2O]^+$ (6), 301 (8.5), 288 (51.5), 225 (12.5), 181 (88) and 125 (100). (Found: C, 73.42; H, 8.98; $C_{28}H_{40}O_5$ requires C, 73.75 and H, 8.35%.) The acetate **7b**, obtained by pyridine–Ac_2O treatment; crystals, mp 246–247° from EtOAc; $[\alpha]_D$ –3.8° (*c* 0.1); UV λ_{max}^{EtOH} nm: 224.5 (ε 7200); IR ν_{max}^{KBr} cm^{-1}: 1735, 1720, 1706, 1235, 1120, 1020 and 950. (Found: C, 72.12; H, 8.72; $C_{30}H_{42}O_6$ requires C, 72.35 and H, 8.5%.)

4β-Hydroxy-5β,6β-epoxy-1-oxo-22R-24-isowithanolide (**10**). Compound **7a** (50 mg) in EtOH (25 ml) was hydrogenated over 10% Pd–C (50 mg) for 8 hr and the product (45 mg), mp 227–228° crystallized from EtOAc. (Found: C, 73.27; H, 9.45; $C_{28}H_{42}O_5$ requires C, 73.42 and H, 9.24%.)

4β,27-*Dihydroxy*-1-*oxo*-22R-*witha*-2,5,24-*trienolide* (**8a**). Mixture B contained, in equal proportions, **8a** and **9a** with identical R_f 0.53 value on TLC (EtOAc) which co-crystallized from various solvents. Compound **8a** was obtained pure after several rapid crystallizations from hot MeOH (50 mg); mp 218–220°; $[\alpha]_D$ +42.5° (*c* 0.17); UV λ_{max}^{EtOH} nm 215: (ε 17 000); IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1665, 1393, 1265, 1130, 1010 and 963; ^{13}C NMR: δ 202.6 (C-1), 131.0 (C-2), 142.9 (C-3), 69.2 (C-4), 129.1 (C-5), 126.1 (C-6), 11.8 (C-18), 22.8 (C-19), 13.3 (C-21), 78.8 (C-22), 153.0 (C-24), 125.7 (C-25), 167.1 (C-26), 57.3 (C-27) and 20.0 (C-28); *m/z* (rel. int.): 454 [M] (25.4%), 436 $[M - H_2O]^+$ (26.2), 283 $[M - 151 - H_2O]^+$ (16), 265 $[M - 151 - 2H_2O]^+$ (13), 239 (13.3), 225 (31.1), 169 (13.2) and 157 (31.7). (Found: C, 74.01; H, 8.48; $C_{28}H_{38}O_5$ requires C, 74.07 and H, 8.44%.) The diacetate **8b**, obtained by pyridine–Ac_2O treatment, mp 252–253° from EtOAc; $[\alpha]_D$ +48.2° (*c* 0.11); UV λ_{max}^{EtOH} nm: 214.5 (ε 18 000). (Found: C, 71.26; H, 8.09; $C_{32}H_{42}O_7$ requires C, 71.43 and H, 7.85%.)

4β,27-*Dihydroxy*-1-*oxo*-22R-*witha*-5,24-*dienolide* (**9a**). The mother liquors from mixture B were evapd and the residue oxidized with MnO_2 as before. The product separated into three components by prep. TLC with EtOAc as eluant: unreacted compound **9a** (190 mg) and the two oxidation products **14** (45 mg) and **15** (15 mg). Compound **9a**, mp 212–213° from EtOAc; $[\alpha]_D$ +27.4° (*c* 0.2); UV λ_{max}^{EtOH} nm: 225 (ε 8800); IR ν_{max}^{KBr} cm^{-1}: 3400, 1718, 1680, 1410, 1200, 1025 and 976; ^{13}C NMR: δ 214.8 (C-1), 73.5 (C-4), 143.7 (C-5), 128.0 (C-6), 12.0 (C-18), 21.4 (C-19), 13.5 (C-21), 79.1 (C-22), 154.1 (C-24), 125.8 (C-25), 167.5 (C-26), 57.2 (C-27) and 12.0 (C-28); MS *m/z* (rel. int.): 456 $[M]^+$ (20), 438 $[M - H_2O]^+$ (15.5), 285 (16), 267 (18), 227 (16.6), 199 (20.7), 185 (17) and 159 (33.6). (Found: C, 73.42; H, 8.98; $C_{28}H_{34}O_5$ requires C, 73.75 and H, 8.84%.) The diacetate, **9b**, obtained by pyridine–Ac_2O treatment; crystals, mp 228–230° from EtOAc; $[\alpha]_D$ +35.7° (*c* 0.13); UV λ_{max}^{EtOH} nm: 224 (ε 8600). (Found: C, 71.42; H, 8.38; $C_{30}H_{42}O_6$ requires C, 71.17 and H, 8.21%.)

27-*Hydroxy*-1,4-*dioxo*-22R-*witha*-2,5,24-*trienolide* (**14**). Mp 245–247° from EtOAc; UV λ_{max}^{EtOH} nm: 287 and 222 (ε 1800 and 22 000); IR ν_{max}^{KBr} cm^{-1}: 3400, 1698, 1676, 1668, 1625, 1450, 1260 and 1020. (Found: C, 74.12; H, 8.23, $C_{28}H_{36}O_5$ requires C, 74.4 and H, 8.03%.)

1,4,27-*Trioxo*-22R-*witha*-2,5,24-*trienolide* (**15**). Mp 215–216° from EtOAc; UV λ_{max}^{EtOH} nm: 289 and 222 (ε 3200 and 25 400); IR ν_{max}^{KBr} cm^{-1}: 2878, 1700, 1690, 1678, 1668, 1530, 1450 and 1375. (Found: C, 74.33; H, 7.81, $C_{28}H_{34}O_5$ requires C, 74.73 and H, 7.62%.)

14α,20α_F-*Dihydroxy*-1,4-*dioxo*-22R-*witha*-2,5,24-*trienolide* (**12**). Withanolide U (**11**) (100 mg) was oxidized with MnO_2 (300 mg) as before and the product was recrystallized from EtOAc, mp 274–275°. $[\alpha]_D$ +35.1° (*c* 0.11); UV λ_{max}^{EtOH} nm: 225 and 278 (ε 21 000 and 2200); IR ν_{max}^{KBr} cm^{-1}: 3510, 1700, 1685, 1662, 1620, 1600, 1430 and 1120. (Found: C, 71.62; H, 7.82; $C_{28}H_{36}O_6$ requires C, 71.85 and H, 7.75%.) MS *m/z* (rel. int.): 468 $[M]^+$ (21), 450 $[M - H_2O]^+$ (6), 325 $[M - 125 - H_2O]^+$ (99.5), 307 $[M - 125 - 2H_2O]^+$ (16), 187 (44.5), 169 (28), 126 (100), 125 (90).

4-*Acetoxy*-1-*oxo*-22R-*witha*-24-*enolide* (**16**). Compounds **8b** and **9b** (40 mg) in EtOH (30 ml) were hydrogenated in two separate batches over 5% Pd–$CaCO_3$ for 10 hr when **8b** absorbed 3 mol and **9b**, 2 mol of H_2. From both, the same product **16**, was obtained (35 mg), mp 229–230° from EtOAc; MS *m/z* (rel. int.) 486 $[M]^+$ (0.1), 444 $[M - COCH_2]^+$, (0.5), 426 $[M - AcOH]^+$ (52), 408 $[M - AcOH - H_2O]^+$ (16.6), 305 $[M - 125 - AcOH]^+$ (11), 287 $[M - 125 - AcOH - H_2O]^+$ (17), 125 (100). (Found: C, 74.22; H, 9.25. $C_{30}H_{44}O_5$ requires C, 74.44; H, 9.16%.)

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NOTE ADDED IN PROOF

Recently it has been reported that jaborosalactone A and acnistoferin have been isolated from a specimen of *A. breviflorus* collected in Tucumán, Argentina, which is probably a different chemotype. These withanolides have no hydroxyl group at C-4 [17].