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2,3-DIHYDROJABOROSALACTONE A, A WITHANOLIDE FROM *ACNISTUS BREVIFLORUS*

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Key Word Index—*Acnistus breviflorus*; Solanaceae; withanolide; 2,3-dihydrojaborosalactone A; 5α-methoxy-4,5-dihydrojaborosalactone B; 5α-ethoxy-4,5-dihydrojaborosalactone B.

Abstract—From *Acnistus breviflorus* the new 27-hydroxy-5β,6β-epoxy-1-oxo-(22*R*)-witha-24-enolide (2,3-dihydrojaborosalactone A) as well as seven known withanolides, withaferin A, 2,3-dihydrowithaferin A, 6α-chloro-5β-hydroxywithaferin A, 5,6-deoxywithaferin A, jaborosalactone A, jaborosalactone D and jaborosalactone E were isolated and characterized by means of spectroscopic (^{1}H NMR, ^{13}C NMR and mass spectral) methods. Depending on the extraction solvent (methanol or ethanol), a known artifact (3β-methoxy-2,3-dihydrowithaferin A) and the new 5α-methoxy-4,5-dihydrojaborosalactone B and 5α-ethoxy-4,5-dihydrojaborosalactone B were also isolated and characterized.

INTRODUCTION

Previous studies carried out on *Acnistus breviflorus* (Griseb.) have shown that this plant contains different withanolides depending on its origin. Thus, Nittala and Lavie [1] have found only withanolides containing an OH-4 group in plants growing in Israel while we have found, in addition, several jaborosalactones in plants growing in Argentina [2, 3].

We wish to report that from extracts of *A. breviflorus* collected in Tucumán (Argentina), we have isolated a new withanolide identified as 27-hydroxy-5β,6β-epoxy-1-oxo-(22*R*)-witha-24-enolide (2,3-dihydrojaborosalactone A, **1a**) besides seven known withanolides that were identified as withaferin A (**3b**), 2,3-dihydrowithaferin A (**1b**), 6α-chloro-5β-hydroxywithaferin A (**6**), 5,6-deoxywithaferin A (**4**) [1], jaborosalactone A (**3a**) [4, 5], jaborosalactone D (**2b**) [6] and jaborosalactone E (**2a**) [6]. Moreover, three artifacts were also obtained depending on whether the initial extraction was performed with methanol or ethanol; the former solvent gave the known 3β-methoxy-2,3-dihydrowithaferin A (**5**) [7, 8] and a new compound, identified as 5α-methoxy-4,5-dihydrojaborosalactone B (**2c**), while the latter solvent afforded a hitherto unknown product that was characterized as 5α-ethoxy-4,5-dihydrojaborosalactone B (**2d**).

RESULTS AND DISCUSSION

The concentrated methanolic extract from *A. breviflorus* was diluted with water and extracted with petrol and with ethyl ether. The latter extract was chromatographed on silica gel affording crude fractions of the main withanolides, i.e. withaferin A and jaborosalactones A, D and E as previously described [3]. The intermediate chromatographic fractions contained mixtures that were further separated by prep. RP-HPLC, as described elsewhere [9]. Compound **1a** had a slightly smaller R_f than jaborosalactone A. The structure of **1a** was established as 2,3-dihydrojaborosalactone A based on the following spectroscopic evidence. The ^{13}C NMR spectrum (Table 1) showed two carbonyl carbons plus two additional sp^2 carbons. These two carbons and one of the carbonyl carbons corresponded to the unsaturated δ-lactone ring. The second carbonyl was assigned to the keto group at C-1 which, in the absence of the 2,3-double bond, resonates *ca* δ9 to lower field than the C-1 of jaborosalactone A. The rest of the spectrum presented only small differences with that of jaborosalactone A (Table 1). The ^{1}H NMR spectrum of **1a** (Table 2) did not present olefinic protons but, otherwise, it was very similar to that of jaborosalactone A [5]. The mass spectrum of **1a** showed the molecular ion at *m/z* 456 and fragments at *m/z* 153 and 140 which were attributed to ring A plus C-6, C-7 and C-19, and C-6 and C-19, respectively [5]. The proposed structure was confirmed by selective hydrogenation of the 2,3-double bond of jaborosalactone A which afforded a product identical to natural **1a**.

Compound **2c** presented in the mass spectrum a molecular ion at *m/z* 486 and fragments at *m/z* 468 $[M-18]^+$, 454 $[M-32]^+$ and 436 $[M-18-32]^+$ indicating a methoxylated withanolide derivative. The ^{13}C NMR spectrum was very similar to that of jaborosalactone D (Table 1) except for the resonances of C-4–C-6 and the presence of an extra signal at δ49.7 which was assigned to the methoxyl carbon at C-5. This carbon showed the typical deshielding (*ca* δ4) due to methylation of the OH-5 group. The low chemical shift value observed for this methyl group may be explained by a spatial influence by ring A. This finding is of particular interest since magnetic anisotropy of vicinal groups rarely affects ^{13}C NMR chemical shifts. The ^{1}H NMR spectrum was also very similar to that of jaborosalactone D [6] except for the extra resonance at δ3.02 of the shielded methoxyl protons. Hence, compound **2c** was identified as 5α-methoxy-4,5-dihydrojaborosalactone B. Treatment of jaborosalactone A (**3a**) with methanol–sulphuric acid afforded a product

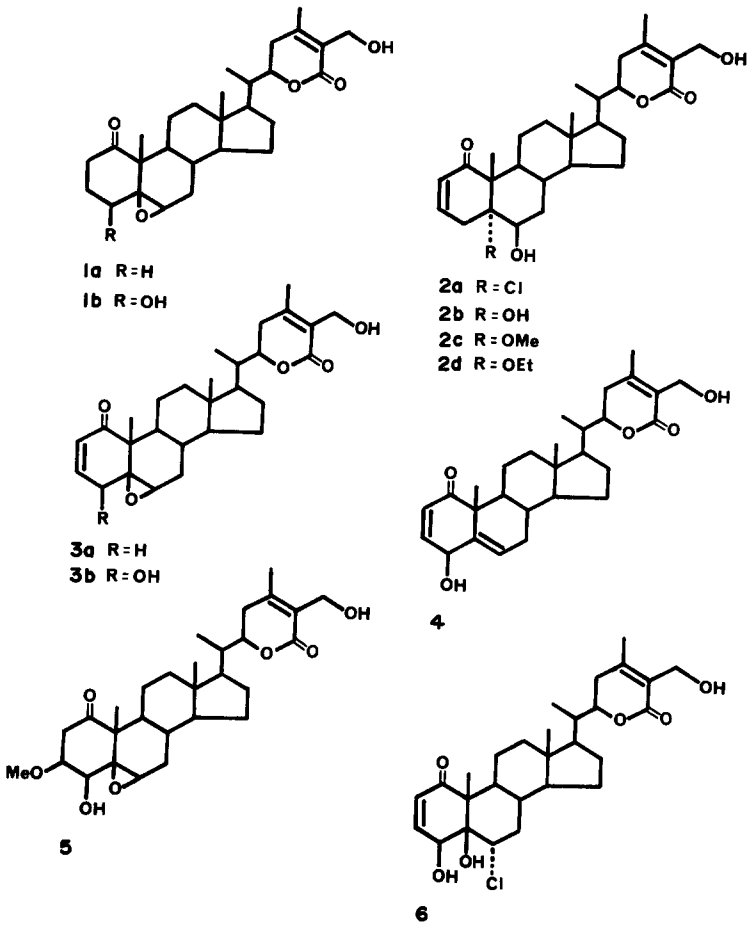

Table 2. ^{1}H NMR spectral data of compounds **1a**, **2c** and **2d** (100 MHz, $CDCl_3$–TMS, δ-values)

Proton No.	1a	2c	2d
Me-18	0.67 (*s*)	0.76 (*s*)	0.77 (*s*)
Me-21	0.97 (*d*, J = 6 Hz)	1.01 (*d*, J = 6 Hz)	1.03 (*d*, J = 7 Hz)
Me-19	1.16 (*s*)	1.31 (*s*)	1.29 (*s*)
Me-28	2.03 (*s*)	2.06 (*s*)	2.05 (*s*)
H-27	4.37 (*s*)	4.38 (*s*)	4.39 (*s*)
H-22	4.42 (*dt*, $J_{22,23}$ = 3 Hz, $J_{22,20}$ = 12 Hz)	4.46 (*dt*, $J_{22,23}$ = 3 Hz, $J_{22,20}$ = 12 Hz)	4.46 (*dt*, $J_{22,23}$ = 3 Hz, $J_{22,20}$ = 12 Hz)
H-2	—	5.82 (*dd*, $J_{2,3}$ = 10 Hz, $J_{2,4\beta}$ = 2 Hz)	5.84 (*dd*, $J_{2,3}$ = 10 Hz, $J_{2,4\beta}$ = 2 Hz)
H-3	—	6.56 (*ddd*, $J_{3,2}$ = 10 Hz, $J_{3,4\alpha}$ = 5 Hz, $J_{3,4\beta}$ = 2 Hz)	6.60 (*ddd*, $J_{3,2}$ = 10 Hz, $J_{3,4\alpha}$ = 5 Hz, $J_{3,4\beta}$ = 2 Hz)
H-4	—	2.40 (*m*)	2.60 (*m*)
H-6	—	3.92 (*br s*)	3.92 (*br s*)
OMe	—	3.02 (*s*)	—
$OCH_2\underline{Me}$	—	—	1.02 (*t*, J = 7 Hz)
$OC\underline{H}_2Me$	—	—	3.14 (*q*, J = 7 Hz)

identical to **2c** confirming the assignment of the structure proposed for this compound.

Compound **2d**, $C_{30}H_{44}O_6$ ($[M]^+$ at m/z 500) presented in its mass spectrum fragments at m/z 482 $[M-18]^+$, 454 $[M-46]^+$ and 436 $[M-18-46]^+$ indicating the presence of an ethoxyl group in a withanolide structure. The ^{13}C NMR and ^{1}H NMR spectra of compound **2d** were almost identical to those of compound **2c** except that the

Table 1. ^{13}C NMR spectral data of compounds **1a**, **2a–2d**, **3a** and **6** (25.2 MHz, $CDCl_3$–TMS, δ-values)

Carbon No.	1a	2a	2b	2c	2d	3a	6 (SFORD)
1	212.87	203.04	204.96	203.79	203.80	203.32	200.39 (*s*)
2	30.34	128.33	128.32	129.28	129.18	129.12	127.28 (*d*)
3	29.16	142.59	141.47	138.70	138.76	144.22	143.37 (*d*)
4	35.22	37.57	35.55	27.60	28.22	32.96	65.75 (*dd*)
5	64.25	81.34	77.23	81.51	81.23	63.23	78.23 (*s*)
6	60.45	74.09	73.99	68.71	69.26	61.97	66.09 (*d*)
7	29.83	30.51	29.98	30.01	29.88	29.88	39.02 (*t*)
8	31.87	33.33	33.30	33.92	34.18	31.14	35.11 (*d*)
9	42.97	43.33	43.03	43.21	43.09	44.66	45.99 (*d*)
10	51.94	53.11	51.95	52.50	52.44	48.39	57.29 (*s*)
11	21.96	23.49	23.17	23.41	23.38	23.59	22.72 (*t*)
12	39.22	40.22	39.97	40.18	40.04	39.61	39.33 (*t*)
13	42.69	42.47	41.31	41.20	41.08	42.59	43.15 (*s*)
14	55.95	56.28	55.51	55.63	55.61	55.85	55.28 (*d*)
15	24.26	24.38	24.21	24.33	24.32	24.21	23.99 (*t*)
16	27.27	27.45	27.19	27 34	27.28	27.23	27.34 (*t*)
17	52.27	55.77	52.06	52.29	52.22	52.00	51.77 (*d*)
18	11.62	12.39	12.15	12.25	12.24	11.75	11.85 (*q*)
19	18.36	16.53	15.64	15.76	15.75	15.00	9.91 (*q*)
20	38.74	39.25	38.86	39.02	38.91	38.76	38.75 (*d*)
21	13.34	13.40	13.23	13.32	13.31	13.29	13.31 (*q*)
22	78.76	79.17	78.84	78.90	78.83	78.67	78.62 (*d*)
23	29.83	30.16	29.84	29.90	28.88	29.88	29.91 (*t*)
24	152.62	155.43	153.92	152.72	152.68	152.99	153.34 (*s*)
25	125.58	125.53	125.33	125.72	125.57	125.50	125 56 (*s*)
26	166.75	167.49	167.10	166.82	166.86	166.76	166.87 (*s*)
27	57.34	56.29	56.73	57.45	57.44	57.18	56.90 (*t*)
28	19.98	20.21	20.00	19.93	19.97	19.99	20.04 (*q*)
OMe	—	—	—	49.71	—	—	—
OCH_2Me	—	—	—	—	57.30	—	—
OCH_2Me	—	—	—	—	15.61	—	—

methoxyl signal observed for **2c** was replaced by signals from the ethoxyl group in **2d** (see Tables 1 and 2). Again the shielding effect of ring A enone on the methylene group was evident in both spectra. Therefore, the 5α-ethoxy-4,5-dihydrojaborosalactone B identity was assigned to compound **2d**. Another 5α-ethoxywithanolide derivative, namely 5α-ethoxy-1-oxo-6β,14α,17β,20α_F-tetrahydroxy-(20*S*,22*R*)-witha-2,24-dienolide, has been described in the lit. [10].

Table 1 also shows the ^{13}C NMR spectral data of the known jaborosalactones A, D and E which have not been previously reported. It also presents the ^{13}C NMR data for 6α-chloro-5β-hydroxywithaferin A, previously described in the lit. [11], where we have reassigned the signals for C-10, C-14, C-17 and C-27 based on SFORD experiments.

EXPERIMENTAL

Plant material and isolation procedure. Similar to those previously described [2, 3, 9].

2,3-Dihydrojaborosalactone A (**1a**). White crystals from EtOAc, mp 180–182°. MS *m/z* (rel. int.): 456 $[M]^+$ (5), 438 $[M-18]^+$ (3), 420 $[M-36]^+$ (5), 315 $[M-141]^+$ (3), 153 $[C_9H_{13}O_2]^+$ (44), 141 $[C_7H_9O_3]^+$ (61), 140 $[C_8H_{12}O_2]^+$ (17), 123 $[141-18]^+$ (35), 95 $[123-28]^+$ (23).

5α-Methoxy-4,5-dihydrojaborosalactone B (**2c**). Crystals from EtOAc, mp 200–201°. MS *m/z* (rel. int.): 486 $[M]^+$ (4), 468 $[M-18]^+$ (6), 454 $[M-32]^+$ (10), 436 $[M-32-18]^+$ (5), 345 $[M-141]^+$ (2), 139 $[C_8H_{11}O_2]^+$ (10).

5α-Ethoxy-4,5-dihydrojaborosalactone B (**2d**). Crystals from EtOAc, mp 203–204°. MS *m/z* (rel. int.): 500 $[M]^+$ (40), 482 $[M-18]^+$ (63), 454 $[M-46]^+$ (19), 436 $[M-46-18]^+$ (38), 418 $[M-46-36]^+$ (34), 303 $[M-197]^+$ (11), 197 $[C_{11}H_{17}O_3]^+$ (53), 141 $[C_7H_9O_3]^+$ (100).

Preparation of **1a** *from jaborosalactone A* (**3a**). Compound **3a** (10 mg) in dioxane (2 ml) was hydrogenated over PtO_2 (6 mg) at room temp. and atm. pres for 1 hr. The catalyst was filtered off and the residue, obtained by evaporation of the solvent, was purified by RP-HPLC affording a product identical (^1H NMR) to natural 2,3-dihydrojaborosalactone A (**1a**).

Preparation of **2c** *from jaborosalactone A* (**3a**). Compound **3a** (6 mg) in MeOH (4 ml) was treated with 4 M H_2SO_4 (0.03 ml) at room temp., with occasional shaking, for 30 min. It was poured into aq. $NaHCO_3$ soln and extracted with CH_2Cl_2. The product, obtained by evaporation of the solvent, was identical (^1H and ^{13}C NMR) to compound **2c**.

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