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ACNISTOFERIN, A NEW WITHANOLIDE FROM *ACNISTUS BREVIFLORUS*

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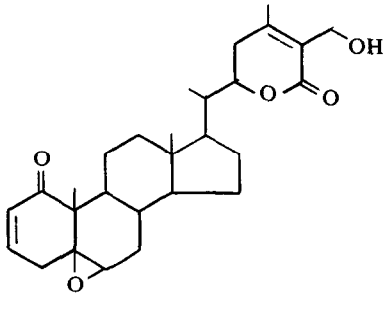
Key Word Index—*Acnistus breviflorus*; Solanaceae; withanolide; withaferin A; 3-methoxy-2,3-dihydro-withaferin A; jaborosalactone A; acnistoferin.

INTRODUCTION

Previous investigation on the plant *Acnistus breviflorus* (Griseb.) has resulted in the isolation and identification of withaferin A [1]. From the same source we have now isolated, besides withaferin A, three other withanolides. Two of them were identified as jaborosalactone A (**1**) [2, 3] and 3-methoxy-2,3-dihydro-withaferin A [4]; the third withanolide was characterized as the 1-oxo-5α,6β,27-trihydroxy-witha-2,24-dienolide (**2**) and named acnistoferin.

RESULTS AND DISCUSSION

The methanolic extract of dry leaves of the plant was diluted with water and extracted with petrol; it was then extracted with ether and the residue obtained by evaporation of the ether was chromatographed on Si gel. The fractions eluted with CH_2Cl_2–MeOH (96:4) gave a product to which the 1-oxo-5α,6β,27-trihydroxy-witha-2,24-dienolide structure was assigned from the following evidence. Compound **2**, $C_{28}H_{40}O_6$, mp 285–288°, presented in its IR spectrum bands at 3300, 1700, 1050, 960



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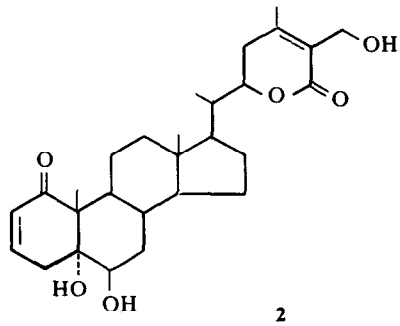

2

and 870 cm^{-1}. The ^1H NMR (DMSO-d_6) showed signals at δ 0.71 (3H, *s*, C-18), 0.95 (3H, *d*, J = 6 Hz, C-21), 1.18 (3H, *s*, C-19), 2.03 (3H, *s*, C-28), 4.12 (1H, *brs*, C-6), 4.17 (2H, *s*, C-27), 4.62 (1H, *m*, C-22), 5.65 (1H, *dd*, J = 10 and 2 Hz, C-2), 6.58 (1H, *m*, C-3). Comparing this spectrum with that of withaferin A [4, 5], it was evident that both have an identical lactonic structure in the side chain but differed in the substituents at rings A and B. There were also differences with the ^1H NMR spectrum of jaborosalactone A [2, 3] indicating that the new withanolide did not possess an epoxy function because it lacked the typical signal from the C-6 proton of the 5β,6β-epoxy ring which normally appears at δ 3.2. The MS (70 eV) presented the parent ion at *m/e* 472 with four successive losses of water (454, 436, 418, 400); the important ions at 331 and 301 could be assigned to M − lactone ring and M − (lactone ring + C-20 and C-21), respectively; the fragment *m/e* 331 loses two moles of water successively giving ions at *m/e* 313 and 295. The ions at *m/e* 197 (lactone ring + C-16, C-17, C-20, C-21), 171 (lactone ring + C-20 and C-21) and 141 (lactone ring) indicated the assignment of the lactonic structure to the side chain. The ion at *m/e* 125 could be formed from ring A by a McLafferty rearrangement similar to the one postulated for withaferin A derivatives [4]. Acetylation of acnistoferin produced a diacetate whose IR spectrum showed the presence of a free hydroxyl group which was tertiary. The ^1H NMR spectrum ($CDCl_3$) presented signals at δ 0.76 (3H, *s*, C-18), 1.01 (3H, *d*, J = 6 Hz, C-21), 1.30 (3H, *s*, C-19), 2.05 (3H, *s*, C-28), 2.08 (3H, *s*, —CO—C$\underline{H}_3$), 2.12 (3H, *s*, —CO—C$\underline{H}_3$), 4.43 (1H, *m*, C-22), 4.82 (1H, *brs*, C-6), 4.87 (2H, *s*, C-27), 5.88 (1H, *dd*, J = 10 and 2 Hz, C-2), 6.57 (1H, *ddd*, J = 10, 4 and 2 Hz, C-3). The shift to lower field of the signals from the C-6 and C-27 protons indicated the sites of acetylation; moreover, the signals from the olefinic protons had a pattern similar to the one observed for jaborosalactone A [2, 3] indicating that there was no hydroxyl group at C-4. In addition, the signals from the C-2, C-3, C-6 and C-19 protons resembled those in the corresponding spectra from withanolides with a 5α,6β-dihydroxy arrangement [6], giving a clue to the configuration of both hydroxyl groups. These results and the failure of acnistoferin to form an isopropylidene derivative upon treatment with acetone in several conditions led to the conclusion that it had hydroxyl groups at C-5 and C-6 in a *trans*-diaxial configuration. Conclusive proof for the proposed structure came from the opening of the epoxy ring of jaborosalactone A. Thus, compound **1** on reaction with diluted sulphuric acid in acetone [6] led nearly quantitatively to a product identical to natural acnistoferin. Hence, the proposed structure for acnistoferin (**2**) as the 5α,6β-dihydroxy analogue of jaborosalactone A is firmly established.

Althoubh 3-methoxy-2,3-dihydro-withaferin A is an artifact produced as a consequence of extraction with methanol [4, 5], acnistoferin seems to be a truly natural compound since its yield does not change using either methanol or ethanol as the extraction solvent.

EXPERIMENTAL

Mps were determined on a heating block and are uncorr. TLC was performed on Si gel G. IR were recorded in Nujol, ^1H NMR at 60 MHz, and MS at 70 eV with a direct insertion probe. Microanalyses were performed by Dr. B. B. de Deferrari.

Plant material. A. breviflorus was collected in the Tucumán province in August and December. Voucher specimens have been deposited in the Instituto Miguel Lillo (Tucumán, Argentina) under No. 3245.

Extraction of plant and isolation procedure. Dry leaves of *A. breviflorus* (3 kg) were continuously extracted with MeOH (12 l.) for 5 days. The extract was concd to 1.5 l., H_2O (1.5 l.) was added and the mixture was extracted with petrol. The methanolic aq. soln was then extracted with Et_2O. Evapn of the dried extract produced 80 g of a dark-green residue. A portion of the residue (20 g) was chromatographed on a Si gel column; elution with CH_2Cl_2–MeOH (99:1) gave crude jaborosalactone A (3.4 g) which after rechromatography and recrystallization from EtOH yielded pure compound, mp 222–223°; IR ν_{max}^{Nujol} cm^{-1}: 3500, 1695, 1670, 1135, 1000, 970, 850, 800; ^1H NMR ($CDCl_3$): δ 0.72 (3H, *s*, C-18), 1.01 (3H, *d*, J = 6 Hz, C-21), 1.27 (3H, *s*, C-19), 2.07 (3H, *s*, C-28), 3.17 (1H, *brs*, C-6), 4.40 (3H, *m*, C-22 and C-27), 6.08 (1H, *dd*, J = 10 and 2.5 Hz, C-2), 6.92 (1H, *ddd*, J = 10, 6 and 2.5 Hz, C-3); MS *m/e* (rel. int.): 454 (10.1), 436 (10.5), 418 (7.4), 400 (5.3), 313 (15.1), 305 (16.1), 304 (17.2), 295 (8.4), 225 (45.1), 197 (33.3), 151 (60.2), 141 (100), 138 (33.4), 123 (58.1), 119 (33.7), 107 (35.8), 105 (36.8), 95 (50.5), 93 (35.8), 91 (36.8). Elution with CH_2Cl_2–MeOH (97.5:2.5) afforded crude fractions of a mixture of withaferin A and 3-methoxy-2,3-dihydro-withaferin A (9.17 g) which after rechromatography and recrystallizations gave pure samples of both compounds; withaferin A, mp 248–250°; IR ν_{max}^{Nujol} cm^{-1}: 3400, 1680, 1670, 1220, 1020, 920, 800; ^1H NMR ($CDCl_3$): δ 0.70 (3H, *s*, C-18), 1.00 (3H, *d*, J = 6 Hz, C-21), 1.42 (3H, *s*, C-19), 2.06 (3H, *s*, C-28), 3.25 (1H, *brs*, C-6), 3.80 (1H, *d*, J = 6 Hz, C-4), 4.40 (3H, *m*, C-22 and C-27), 6.25 (1H, *d*, J = 10 Hz, C-2), 7.00 (1H, *dd*, J = 10 and 6 Hz, C-3); MS *m/e* (rel. int.): 470 (3.4), 452 (5.7), 434 (4.6), 416 (2.3), 347 (13.8), 197 (23.1), 141 (67.8), 131 (73.6), 124 (100), 123 (64.4), 95 (86.2). 3-Methoxy-2,3-dihydro-withaferin A, mp 241–243°; IR ν_{max}^{Nujol} cm^{-1}: 3400, 1680, 1670, 1090, 1010, 780; ^1H NMR ($CDCl_3$): δ 0.68 (3H, *s*, C-18), 1.00 (3H, *d*, J = 6 Hz, C-21), 1.29 (3H, *s*, C-19), 2.05 (3H, *s*, C-28), 2.40–3.00 (2H, *m*, C-2), 3.23 (1H, *brs*, C-6), 3.37 (3H, *s*, —OMe), 3.50 (1H, *brs*, C-4), 3.70 (1H, *m*, C-3), 4.40 (3H, *m*, C-22 and C-27); MS *m/e* (rel. int.): 502 (3.1), 484 (5.7), 470 (4.5), 347 (16.3), 199 (31.0), 197 (42.8), 141 (100), 133 (38.0), 131 (59.5), 125 (69.0), 123 (71.4), 95 (99.1). Fractions eluted with CH_2Cl_2–MeOH (96.5:3.5 and 96:4) gave crude compound **2** (5.4 g) which was recrystallized from EtOH, mp 285–288°; $[\alpha]_D$ +99.6° (*c* 2.7, $CHCl_3$–MeOH 6:4); UV λ_{max}^{EtOH} 221 nm (log ε 4.20); IR ν_{max}^{Nujol} cm^{-1}: 3300, 1700, 1050, 960, 870; ^1H NMR (DMSO-d_6): δ 0.71 (3H, *s*), 0.95 (3H, *d*, J = 6 Hz), 1.18 (3H, *s*), 2.03 (3H, *s*), 4.12 (1H, *brs*), 4.17 (2H, *s*), 4.62 (1H, *m*), 5.65 (1H, *dd*, J = 10 and 2 Hz), 6.58 (1H, *m*); MS *m/e* (rel. int.): 472 (1.6), 454 (3.2), 436 (7.1), 418 (6.8), 400 (2.6), 331 (27.2), 313 (18.2), 301 (31.8), 295 (22.7), 269 (31.8), 225 (31.8), 197 (45.4), 171 (31.8), 141 (45.4), 125 (56.8), 123 (45.4), 121 (40.9), 119 (36.4), 109 (40.9), 107 (59.1), 105 (50.0), 95 (72.7), 93 (63.6), 91 (59.1), 81 (68.2), 79 (59.1), 67 (81.8), 55 (100). (Found: C, 71.29; H, 8.49. $C_{28}H_{40}O_6$ requires: C, 71.16; H, 8.53%).

Diacetylacnistoferin. Compound **2** (100 mg) was treated with Ac_2O (2 ml) in Py (2 ml) and the soln left overnight. After the usual work-up, the acetyl derivative was crystallized from EtOH yielding 105 mg of diacetylacnistoferin, mp 255–258°; $[\alpha]_D$ +52.2° (*c* 2.9, $CHCl_3$); UV λ_{max}^{EtOH} 220 nm (log ε 4.29); IR ν_{max}^{Nujol} cm^{-1}: 3400, 1720, 1680, 1240, 1040; ^1H NMR ($CDCl_3$): δ 0.76 (3H, *s*), 1.01 (3H, *d*, J = 6 Hz), 1.30 (3H, *s*), 2.05 (3H, *s*), 2.08 (3H, *s*), 2.12 (3H, *s*), 4.43 (1H, *m*), 4.82 (1H, *brs*), 4.87 (2H, *s*), 5.88 (1H, *dd*, J = 10 and 2 Hz), 6.57 (1H, *ddd*, J = 10, 4 and 2 Hz); MS *m/e* (rel. int.): 556 (1.2) (M^+), 496 (7.7) (M − 60), 478 (19.2) (M − 18 − 60), 436 (11.6) (M − 2 × 60), 418 (55.4) (M − 18 − 2 × 60), 373 (40.3) (M − lactone ring), 313 (15.4) (M −

lactone ring − 60), 295 (43.4) (M − lactone ring − 60 − 18), 239 (94.8) (lactone ring + C_{16} − C_{17} − C_{20} − C_{21}), 225 (100) (lactone ring + C_{17} − C_{20} − C_{21}), 183 (49.6) (lactone ring), 125 (51.2) ($C_7H_9O_2$, McLafferty on ring A). (Found: C, 69.13; H, 8.14. $C_{32}H_{44}O_8$ requires: C, 69.04; H, 7.97%).

Preparation of acnistoferin from jaborosalactone A. Compound **1** (60 mg) was dissolved in Me_2CO (43 ml), treated with 8 N H_2SO_4 (0.33 ml) and the soln was stirred for 4 hr at room temp. It was then poured into dil $NaCO_3H$ soln and extracted with $CHCl_3$. Evapn of the solvent gave a residue that was crystallized from EtOH. The product (45 mg) was identical (mp, IR, ^{1}H NMR) to natural acnistoferin.

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29-HYDROXYLUPEOL FROM *GYMNOSPORIA WALLICHIANA**

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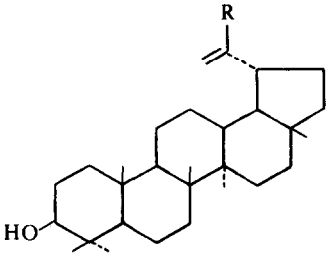
Key Word Index—*Gymnosporia wallichiana*; Celastraceae; wallichianol; wallichenol; 29-hydroxylupeol; triterpenoids.

A chemical investigation of *Gymnosporia wallichiana* reported [1] that the mixture of compound F_1 (wallichianol) and compound F_2 (hereafter referred to as wallichenol), could not be resolved by any chromatographic means. However, it was observed that MS of the mixture of wallichianol and wallichenol contained M^+, M − 15 and M − 18 peaks of wallichianol as well as the corresponding peaks due to wallichenol, two mass units lower than those of wallichianol and it was anticipated that wallichenol had a double bond in the molecule. Wallichianol was therefore isolated by Br_2 oxidation of wallichenol to a mixture of products of higher R_f value followed by chromatography. The structure of wallichianol was elucidated as (20*S*)-lupan-3β,29-diol.

The present paper deals with the structure elucidation of wallichenol. The mixture of wallichianol and wallichenol was silylated and subjected to GC–MS, which furnished separate MS for silylated wallichianol and wallichenol. Both the MS showed common fragment ions involving rings A and B at *m/e* 279, 202, and 189 whereas M^+, M − Me, M − $(Me)_3SiOH$ and M − $(Me)_3$-SiOH − Me peaks of silylated wallichenol were two mass units lower than the corresponding peaks of wallichianol. This indicated a skeletal similarity between the two compounds.



1 R = CHO
2 R = CH_2OH

The ^{1}H NMR spectrum of the wallichianol and wallichenol mixture showed a pair of broad singlets at δ 4.13 and 4.93 ppm, whereas these signals were absent in the ^{1}H NMR spectrum of wallichianol. These signals were therefore assigned to the olefinic protons of wallichenol. On addition of trichloroacetyl isocyanate (TAI) [2], the olefinic signals suffered a downfield shift to δ 4.76 and 5.05 ppm, indicating the presence of an allylic OH group in wallichenol. This inference was confirmed by MnO_2 oxidation of the mixture of wallichianol and wallichenol which resulted in the formation of a product of higher R_f value (TLC) leaving wallichianol intact. The new product was separated from wallichianol by chromatography over Si gel. This product (1), $C_{30}H_{48}O_2$ (M^+ 440), mp

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