17-Epiacnistin-A, a Further Withanolide from the Leaves of *Discopodium penninervium*

Solomon Habtemariam,*,† Brian W. Skelton,‡ Peter G. Waterman,§ and Allan H. White‡

School of Chemical and Life Sciences, University of Greenwich, Woolwich, Wellington Street, London SE18 6PF, U.K., Department of Chemistry, University of Western Australia, Nedlands, WA 6009, Australia, and Centre for Phytochemistry, Southern Cross University, PO Box 157, Lismore, NSW 2480, Australia

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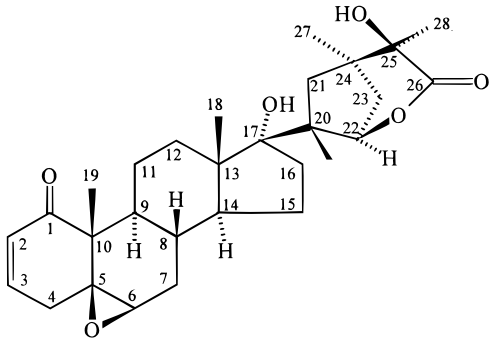
A novel withanolide (**1**) has been isolated from the leaves of *Discopodium penninervium* and identified as 17-epiacnistin-A on the basis of spectroscopic and X-ray diffraction data.

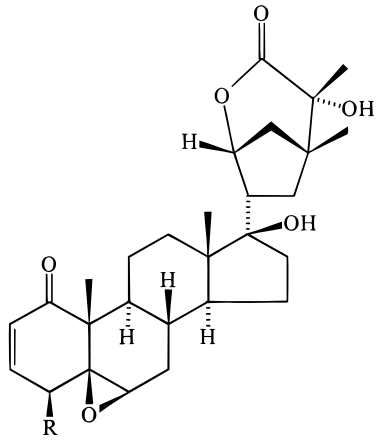
Withanolides are ergostane-based steroidal lactones that have been isolated from a number of species of Solanaceae.[1] Recent phytochemical studies on the leaves[2] and the roots[3] of *Discopodium penninervium* Hochst have resulted in the isolation of typical withanolides with a range of oxidation patterns in the steroidal skeleton. The cytotoxicity and immunosuppresive activity of the *Discopodium* withanolides together with their structure−activity relationships have recently been established.[4] We now wish to report on the structure of a further metabolite (**1**), the structure of which has been resolved by spectroscopic methods and confirmed by an X-ray diffraction study.

The HREI-mass spectrum of **1** indicated a molecular ion at *m*/*z* 470, solving for $C_{28}H_{38}O_6$. The IR spectrum showed a broad band between 3500 and 3000 cm^{-1} (OH) and bands in the carbonyl region at 1726 and 1663 cm^{-1} for lactone and α,*β*-unsaturated carbonyls, respectively. The ^1H and ^{13}C NMR spectra were correlated with each other by means of HMBC ($^2J/^3J$) experiments (Table 1). The observed NMR resonances were in close agreement with those reported for the tetracyclic steroid portion of the 5*β*,6*β*-17α-hydroxy-1-oxowitha-2,24-dienolide jaborosalactone-L, which we had isolated previously from this species.[2] The remainder of the ^1H NMR spectrum of **1** closely resembled that reported for the bicyclic C-17 substituent found in the acnistin series of withanolides, such as acnistin-E (**3**).[5−7] Indeed chemical shift data for **1** was comparable to that obtained for acnistin-A (**2**)[6] except for the 10 ppm shielding observed for C-8 in the ^{13}C NMR spectrum (40.7 ppm to 30.3 ppm) and of 0.15 ppm in Me-18 in the ^1H NMR spectrum (*δ* 0.92 to *δ* 0.77). These (especially the change in ^1H NMR chemical shift data) can be explained by a change in orientation of the C-17 substituents[2] in **1** in comparison to **2**, so that the hydroxyl is α and the side chain *β*.

The relative conformation of **1** was established by an X-ray diffraction study and the results (Figure 1) were consistent with the steriochemistry as shown. Geometrical parameters, although not overtly precise, are in accord with established norms, in view of the small specimen and limited data. Hydrogen bonding involving the hydroxylic species is evident: O(3)(H(17))···O(1) (*x*, *y* $-1/2$, 1 − *z*) 2.909(8) (2.14(7)); O(6)(H(25))···O(2) (1 − *x*, $1/2$ + *y*, 1 − *z*) 2.791(7) (2.06(7)); O(01)(H(01a))···O(5) 2.96(1) (1.8); O(01)-(H01b)···O(6) (*x*, *y* −1, *z*) 2.96(1) (2.3(1)) Å.



(1)



(2) R = H

(3) R = OH

Thus, **1** is a new acnistin type withanolide which differs from the previously isolated acnistins in stereochemistry at C-17 and as a consequence in the orientation of the side chain. We have elected to give this compound the trivial name, 17-epiacnistin-A.

Experimental Section

General Experimental Procedures. Silica gel (Merck 7749) was used for vacuum liquid chromatography (VLC) and silica gel 60 PF_{254} for preparative TLC. Spots and bands were detected by spraying with 5% vanillin in H_2SO_4 and heating to 100 °C. ^1H, ^{13}C, $^1H-^1H$ COSY, $^1H-^1H$ NOESY (mixing time = 0.8 s), HC-COBI,[8] and HMBC (optimized for 7 Hz, long-

* To whom correspondence should be addressed. Phone: +44-0208-331-8302. Fax: +44-0208-331-8305. E-mail: s.habtemariam@greenwich.ac.uk.
† University of Greenwich.
‡ University of Western Australia.
§ Southern Cross University.

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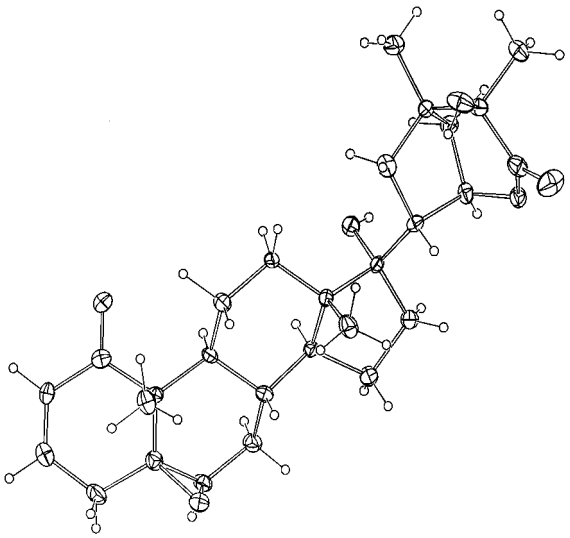

Figure 1. Molecular projection of **1** normal to the fused ring "plane" with 20% thermal ellipsoids for the non-hydrogen atoms, hydrogen atoms having arbitrary radii of 0.1 Å.

Table 1. ^{1}H and ^{13}C NMR Data for **1**

position	^{1}H	^{13}C	$^2J/^3J$ HMBC
1		203.8	
2	6.01 dd (10.1, 2.6)	129.4	C-4
3	6.85 ddd (10.1, 6.2, 2.4)	144.7	C-1, C-5
4	1.90 m	32.2	C-2, C-3, C-5, C-6, C-10
	3.00 dt (19.2, 2.5)		
5		62.2	
6	3.13 d (2.5)	63.5	H-7, C-8
7	1.32 td (9.6, 2.7)	31.4	C-6
	2.09 m		
8	1.59 m	30.3	
9	1.19 m	44.6	
10		48.6	
11	2.16 m	23.9	
12	1.72 m	32.3	C-10, C-11, C-17
	1.48 m		
13		47.4	
14	1.50−2.00	50.5	
15	1.50−2.00	23.5	
16	1.45 m	37.9	C-14, C-17
	2.00 m		
17		86.1	
18	0.77 s	16.0	C-13, C-14, C-17
19	1.21 s	15.4	C-1, C-5, C-9, C-10
20	2.50 m	53.2	C-22
21	2.50 m	34.6	C-17, C-22, C-23, C-25
	1.40 m		
22	4.68 d (2.3)	84.6	C-21, C-23, C-26
23	2.03 d (13.4)	41.7	C-24, C-25
	1.81 dd (13.4, 3.2)		
24		47.0	
25		76.9	
26		179.0	
27	1.46 s	25.7	C-24, C-25, C-26
28	1.26 s	20.1	C-21, C-23, C-24

range coupling) NMR spectra were recorded using a Bruker AMX-400 instrument. EIMS were obtained by direct probe insertion at 70 eV.

Plant Material. *D. penninervium* was collected from an area along the Wolgemo River, ca. 2.5 km north of Kotebe (alt. ca. 2500 m) in March 1992. A voucher specimen (SHM-22) has been deposited at the National Herbarium of Ethiopia, University of Addis Ababa.

Extraction and Isolation. Ground leaves of *D. penninervium* (800 g) were extracted by maceration (cold EtOH, 2 weeks). Removal of the solvent yielded a residue (20 g), which was suspended in water and subjected to VLC over silica gel and eluted with hexane, followed by hexane−EtOAc mixtures of increasing polarity, then EtOAc, and finally Me_2CO. After removal of chlorophylls by gel filtration through Sephadex LH-20 (solvent $CHCl_3$−MeOH, 1:1), the EtOAc fraction was subjected to further column chromatography over silica gel eluting with $CHCl_3$−MeOH mixtures of increasing polarity. The fraction eluted with 5% MeOH, after preparative TLC (solvent $CHCl_3$−MeOH, 1:1), gave **1** (6 mg).

17-Epiacnistin-A (1): needles from MeOH, mp 240−250 °C; IR ν_{max} 3450 br, 2967, 1726, 1663, 1458, 1380, 1254, 1125, 1070, 1025, 954, 753, 665 cm^{-1}; UV λ_{max} 220 (log ϵ 2.9) nm; ^{1}H NMR and ^{13}C NMR (see Table 1); HREIMS *m/z* $[M]^+$ found 470.2669 (calcd for $C_{28}H_{38}O_6$ 470.2668); EIMS *m/z* (rel int) 470 $[M]^+$ (1.5), 283 (35.9), 267 (19.0), 265 (41.4), 240 (67.0), 238 (55.1), 229 (32.4), 227 (32.4), 225 (60.6), 223 (26.1), 213 (24.8), 211 (27.7), 173 (29.6), 171 (36.4), 159 (26.1), 157 (27.4), 151 (100), 138 (81.7).

Single-Crystal X-ray Structure Determination. When originally examined (in 1993), the crystals available (very fine whiskers) were intractable to currently available four-circle/single-counter diffractometer technology. With the advent of a Bruker AXS CCD area detector instrument, a further attempt was successful. A full sphere of data was measured to $2\theta_{max} = 58°$, yielding 11522 total reflections, these merging (including "Friedel pairs", there being no substantial anomalous scatterer) to 3359 unque ($R_{int} = 0.036$), 1361 of these with $F > 4\sigma(F)$ being considered "observed" and used in the full-matrix least-squares refinement without absorption correction. Anisotropic thermal parameters were refind for the non-hydrogen atoms. A difference map residue was modeled as a water molecule oxygen, site occupancy set at unity after trial refinement. (x, y, z, U_{iso}) were refined meaningful for all hydrogen atoms (except those associated with the water molecule, where "thermal motion" was higher) despite the rather limited data. The absolute configuration was assumed from the chemistry. Conventional residuals R, R_w (statistical weights derivative of $\sigma^2(I) = \sigma^2(I_{diff}) + 0.0004\sigma^4(I_{diff})$) at convergence were 0.048, 0.034. Neutral atom scattering factors were employed, computation using the Xtal 3.4 program system.[9] Pertinent results are given in the figures and below; full details are deposited in the Cambridge Crystallographic Data Centre.[10]

Crystal data: $C_{28}H_{38}O_6 \cdot H_2O$, M = 488.6; monoclinic, space group $P2_1$ (C_2^2, No. 4), a = 10.343(7) Å, b = 7.559(5) Å, c = 16.32(1) Å, β = 103.54(1)°, V = 1240 Å^3; D_c (Z = 2) = 1.30$_8$ g cm^{-3}; $F(000)$ = 528; μ_{Mo} = 0.9 cm^{-1}; specimen 0.30 × 0.05 × 0.05 mm; monochromatic Mo Kα radiation, λ = 0.7107$_3$ Å, T ca. 300 K.

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