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28-HYDROXYWITHANOLIDE E FROM *PHYSALIS PERUVIANA*

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Key Word Index—*Physalis peruviana*; Solanaceae; Cape gooseberry; calyx; 28-hydroxywithanolide E; 4β-hydroxywithanolide E; withanolides; ecdysteroid antagonist; ^{13}C PENDANT.

Abstract—Two withanolides, 28-hydroxywithanolide E and 4β-hydroxywithanolide E, the former being new, have been isolated from the calyces of *Physalis peruviana*. The structure of the new compound was determined primarily on the basis of extensive 1D and 2D NMR spectral analysis, notably ^{13}C PENDANT, COSY 45, HMBC and HMQC. Copyright © 1997 Elsevier Science Ltd

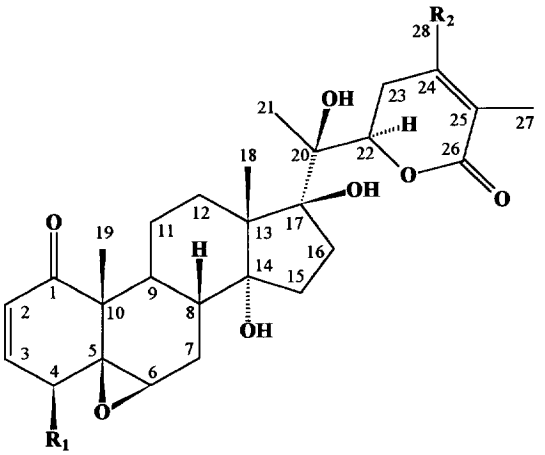
INTRODUCTION

The withanolides are a group of steroidal lactones which have been isolated from the genera *Acnistus*, *Datura*, *Jaborosa*, *Lycium*, *Physalis* and *Withania* of the family Solanaceae [1]. They have also been found in a soft coral [2]. *Physalis peruviana* L. (Solanaceae), commonly known as 'Cape gooseberry' is a tropical hairy plant with fuzzy, slender-pointed, heart-shaped leaves, bearing yellowish flowers followed by orange, edible fruits [3]. The presence of a number of withanolides in different parts of this plant has been reported earlier [1, 4–13]. We now wish to record the isolation and characterization of a new withanolide from calyces of this plant.

RESULTS AND DISCUSSION

The RP-HPLC analysis of the methylene chloride phase after partitioning of an aqueous extract of calyces of *P. peruviana* L. yielded two withanolides, one of which was readily identified as 4β-hydroxywithanolide E (**1**) [14] by direct comparison of its physical and spectroscopic data with those published in the literature. The other one, on the basis of extensive spectroscopic analysis, was identified as 28-hydroxywithanolide E (**2**), which is new.

Compound **2**, $C_{28}H_{38}O_8$, gave a UV absorption peak at 222 nm attributable to the α,β-unsaturated carbonyl chromophores. The ^{1}H and ^{13}C NMR spectra (Table 1) of **2** were very similar to those of 5β,6β-epoxy-2,24-diene-1-one withanolides [14–17]. The chemical shifts and multiplicities of two mutually coupled olefinic protons at δ 6.02 and 6.82 (Table 1) and their further coupling with methylene protons at δ 1.92 and 2.95 are typical for the H-2, H-3 and H_2-4 spin system of a steroidal Δ^2-1-one in which C-5 is quaternary [18]. A ^{1}H signal at δ 3.19 together with ^{13}C signals for quaternary (δ 61.8) and oxymethine (δ 64.1) suggested a 5β,6β-epoxy group in **2**. A COSY-45 spectrum revealed all possible ^{1}H–^{1}H correlations and led us to compare the ^{1}H NMR data for **2** with that for withanolide E (**3**) [14] where all the signals were found to be almost identical, with only the exception that in **2**, one (δ 1.89) instead of two (as in **3**) 3H singlets (owing to the methyls on the α,β-unsaturated δ-lactone ring) was present and the other one was replaced by a



	R_1	R_2
1	-OH	Me
2	-H	$-CH_2OH$
3	-H	Me

Table 1. ^{1}H NMR (400 MHz) data for compounds **2** and **3** (coupling constant J = Hz in parentheses) and ^{13}C PENDANT NMR (100 MHz) data for **2**

	δ^1H		δ^{13}C
C	**2**	**3**†	(**2**)
1	—	—	203.0
2	6.02 *dd* (2.8, 10.0)	6.03 *dq* (10.0)	129.8
3	6.82 *ddd* (2.3, 6.4, 10.0)	6.87 *dq*	143.7
4_{ax}	1.92*	‡	32.9
4_{eq}	2.95 *dt* (2.6, 19)	‡	
5	—	—	61.8
6α	3.19 *d* (2.1)	3.20	64.1
7a	1.99*	‡	26.2
7b	1.96*	‡	
8β	1.96 *m**	‡	34.2
9	1.85 *m**	‡	36.9
10	—	—	48.6
11a	2.10 *m**	‡	22.9
11b	1.60 *m**	‡	
12a	2.30 *m**	‡	30.0
12b	1.35 *m**	‡	
13	—	—	54.6
14	—	—	82.2
15a	1.70 *m**	‡	32.5
15b	1.60 *m**	‡	
16a	2.72 *m**	‡	37.7
16b	1.48 *m**	‡	
17	—	—	87.8
Me-18	1.11 *s*	1.10 *s*	20.5
Me-19	1.24 *s*	1.25 *s*	14.6
20	—	—	79.2
Me-21	1.43 *s*	1.42 *s*	20.0
22	4.90 *dd* (3.0, 13.4)	4.88 *m*	80.7
23a	2.91 *m*	‡	28.7
23b	2.45 *br t*	‡	
24	—	—	151.7
25	—	—	121.8
26	—	—	165.8
Me-27	1.89 *s*	1.93 *s*	11.9
Me-28	—	1.88 *s*	—
28	4.43 *d* (14.1)	—	62.1
	4.31 *d* (14.3)	—	

Spectra obtained in $CDCl_3$ referenced to $CHCl_3$ at δ 7.27 (^{1}H) and 77.23 (^{13}C).

*Assignments obtained from COSY-45.

†Data (60 MHz) obtained from Kirson *et al.* [14].

‡Data not available.

-CH_2OH group (δ_H 4.43, 4.31, J = 14 Hz; δ_C 62.1). This was also evident from the EI mass spectrum fragment ion m/z 141 (35%) $[C_7H_9O_3]^+$ indicative of such a side-chain where cleavage occurred between C-20 and C-22. The HMBC spectrum (Table 2) revealed a ^2J correlation from the oxymethylene group (δ 4.43, 4.31) to C-24 (δ 151.7) and ^3J to C-25 (δ 121.8) and C-23 (δ 28.7). There was a ^2J correlation from Me-27 (δ 1.89) to C-25 (δ 121.8) and ^3J to the carbonyl carbon (C-26, δ 165.8) and C-24 (δ 151.7). Thus, the placement of the oxymethylene group as C-28 was confirmed. The ^{13}C PENDANT [19] (Table 1) showed all 28 carbons, HMQC displayed all ^{1}H–^{13}C direct ^1J couplings, and HMBC revealed all major long-range ^{1}H–^{13}C correlations, leading to the unambiguous assignment of all protons and carbons. The relative configuration was determined by direct comparison of the NMR data for **2** with that for **3** [14] and other related withanolides [4–18, 20]. Thus, the structure of this new withanolide was confirmed as **2**. It is worth mentioning that the presence of a C-28 hydroxyl in withanolides is rare in comparison with the occurrence of a C-27 hydroxyl group [1]. However, two compounds (perulalactone and perulalactone B) derived from precursors with C-28 hydroxyls, which possess five-membered 26,28-γ-lactone rings, rather than six-membered 26,22-δ-lactone rings as in **2**, have been isolated from *P. peruviana* [21, 22].

Certain withanolides have recently been shown to antagonize the action of 20-hydroxyecdysone on an ecdysteroid-responsive insect cell line [23]. Consequently, it was of interest to determine if the two withanolides isolated from *P. peruviana* calyces also possessed activity. Although both compounds were clearly cytotoxic at concentrations $>10^{-5}$ M, neither compound showed any specific agonistic or antagonistic activities, in accord with the hypothesis that a C-3 hydroxyl or methoxyl group is required for the antagonistic activity of withanolides towards ecdysteroids [23].

EXPERIMENTAL

UV spectra: in MeOH. NMR spectra: Bruker AVANCE DRX400 instrument using standard Bruker microprograms. Chemical shifts: in ppm. EIMS: Kratos Profile HV3 dual focusing magnet sector mass spectrometer. HPLC: Gilson model 802C HPLC coupled with Gilson UV–VIS detector. RP: reversed-phase Spherisorb 5 ODS-2 semiprep. C_{18} column; RP sepns monitored at 220 nm.

Bioassay. Ecdysteroid agonist and antagonist activities of the compounds were assessed with a microplate-based bioassay using the *Drosophila melanogaster* B_{II} cell line [24]. The withanolides were tested at concns from 10^{-8} to 10^{-4} M. For the antagonist assay, a concn of 20-hydroxyecdysone of 5×10^{-8} M was used.

Plant material. Fruits (and associated calyces) of *Physalis peruviana* L. were purchased from J. Sainsbury p.l.c., Epsom, U.K.

Extraction. Finely cut calyces (5.4 g) were extracted ×3 with 200 ml H_2O by heating from ambient temp. to 90°. Filtered extracts were extracted with CH_2Cl_2 (2 × 200 ml). The CH_2Cl_2 extract was concd using a rotary evaporator at a max. temp. of 45°.

Isolation of compounds. Withanolides were purified from the CH_2Cl_2 extract by RP-HPLC with MeOH–H_2O (13:7) at 2 ml min^{-1}. Two major UV-absorbing peaks were present at 10.6 (**2**) and 12.9 min (**1**). Purity of the withanolide samples was verified by normal-phase HPLC [APEX II DIOL analyt. column, eluted with CH_2Cl_2–MeOH (24:1), monitored at 235 nm].

28-Hydroxywithanolide E (**2**) (1.6 *mg*). Amorphous. UV λ_{max} nm (log ε): 222 (4.20). ^{1}H and ^{13}C NMR:

Table 2. $^{1}H-^{13}C$ HMQC direct correlation (^{1}J) and $^{1}H-^{13}C$ HMBC long-range correlations (^{2}J and ^{3}J) in **2**

	δ ^{13}C		
H	^{1}J	^{2}J	^{3}J
H-2	129.8 (C-2)		32.9 (C-4)
H-3	143.7 (C-3)		203.0 (C-1), 61.8 (C-5)
H_2-4	32.9 (C-4)	143.7 (C-3)	64.1 (C-6)
H-6	64.1 (C-6)	26.2 (C-7)	34.2 (C-8)
H_2-7	26.2 (C-7)		
H-8	34.2 (C-8)		
H-9	36.9 (C-9)	48.6 (C-10)	61.8 (C-5)
H_2-11	22.9 (C-11)		
H_2-12	30.0 (C-12)	22.9 (C-11)	
H_2-15	32.5 (C-15)		
H_2-16	37.7 (C-16)		
H-22	80.6 (C-22)		
H_2-23	28.7 (C-23)		121.8 (C-25), 62.1 (C-28)
Me-18	20.5 (C-18)	54.6 (C-13)	82.2 (C-14), 87.8 (C-17), 30.0 (C-12)
Me-19	14.6 (C-19)	48.6 (C-10)	61.8 (C-5), 203.0 (C-1)
Me-21	20.0 (C-21)	79.2 (C-20)	87.8 (C-17), 80.7 (C-22)
Me-27	11.9 (C-27)	121.8 (C-25)	151.7 (C-24), 165.8 (C-26)
H_2-28	62.1 (C-28)	151.7 (C-24)	28.7 (C-23), 121.8 (C-25)

Spectra obtained in $CDCl_3$.

Table 1. Found: $[M]^+$ 502.2567; $C_{28}H_{38}O_8$ requires 502.58; EIMS *m/z* (rel. int.): 502 $[M]^+$ (0.3), 484 (2), 466 (5), 448 (2), 361 (1), 343 (6), 325 (4), 299 (6), 282 (3), 281 (5), 265 (3), 255 (4), 238 (6), 225 (6), 223 (5), 213 (6), 211 (5), 197 (6), 189 (6), 185 (12), 171 (12), 169 (14), 168 (34), 167 (10), 165 (8), 163 (8), 161 (10), 159 (13), 157 (12), 151 (16), 150 (10), 147 (20), 145 (18), 143 (14), 142 (13), 141 $[C_7H_9O_3]^+$ (35), 139 (20), 138 (16), 135 (26), 134 (14), 125 (45), 124 (31), 123 (47), 122 (22), 119 (31), 109 (52), 108 (30), 105 (34), 97 (38), 95 (60), 91 (42), 85 (75), 83 (100), 82 (20), 81 (35), 71 (32), 69 (38), 55 (48).

4β-Hydroxywithanolide E (**1**) (2.0 *mg*). Amorphous. UV, ^{1}H NMR and EIMS data as reported in ref. [14].

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