Journal of Natural Products
Vol. 48, No. 3, pp. 474-476, May-Jun 1985

PUBESENOLIDE, A NEW WITHANOLIDE FROM *PHYSALIS PUBESCENS*

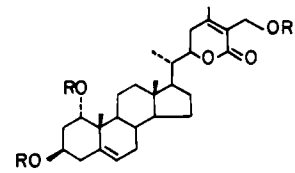
MAHENDRA SAHAI[1]

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel

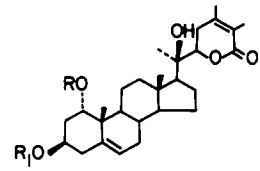
One of the important genera of the family Solanaceae is *Physalis*, which has earned the reputation of elaborating different varieties of C_{28}-steroidal lactones built on an ergostane framework, viz., physalins (1), withanolides (2), withaphysalins (3), ixocarpalactones (4,5), and the recently isolated perulactones (6,7). The multiplicity and diversity of structural types of C_{28}-steroids witnessed in this genus and the reported biological activity (8,9) for this class of compounds prompted the extension of our studies to a variety of *Physalis pubescens* L., an annual herb which, so far, has not been investigated.

Extraction of the whole plant and chromatographic resolution of the $CHCl_3$-soluble fraction afforded a new steroidal lactone, the structure of which was determined mainly on the basis of spectroscopic analysis. In contrast to typical withanolides, which bear a Δ^2-1-one system in ring A of their molecules, pubesenolide (**1a**) bears a 1α,3β-dihydroxy system which may be regarded as the immediate precursor for the Δ^2-1-one system. In this respect, pubesenolide is a close relative of physalolactone B (**2a**) which bears the traits of its genesis from 24-methylenecholesterol, the known precursor (10) for this class of compounds.

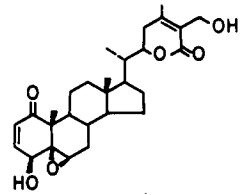
Pubesenolide (**1a**), $C_{28}H_{42}O_5$ (M^+ 458), mp 145-146°, showed ir absorption bands attributable to hydroxyl group (3590) and an α,β-unsaturated-δ-lactone (1685 cm^{-1}). The uv spectrum (λ max 228 nm; ε, 7,400) of pubesenolide is also suggestive of an α,β-unsaturated-δ-lactone chromophore.



1a R=H (Pubesenolide)
1b R=Ac (Pubesenolide triacetate)



2a R=Ac, R_1=H (Physalolactone B)
2b R=R_1=H (Deacetylphysalolactone B)
2c R=R_1=Ac (Physalolactone B monoacetate)



3 (Withaferin A)

The substitution patterns of rings A and B were deduced from ^{1}H-nmr spectral comparison of (**1a**) with that of deacetylphysalolactone B (**2b**) (11). The chemical shifts and splitting patterns of the signals originating from the carbinyl hydrogens at C-1 (δ 3.85, 1 H, narrow triplet) and C-3 (δ 3.98, 1 H, seven lines) and the lone olefinic proton at C-6 (δ 5.59, 1 H, broad doublet, J=5.4 Hz) are in perfect agreement with those of deacetylphysalolactone B. Likewise, the chemical shifts of these hydrogens in pubesenolide triacetate (**1b**) are comparable with the corresponding signals of physalolactone B monoacetate (**2c**). However, unlike deacetylphysalolactone

[1]Present address: Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India.

B (**2b**), the ^{1}H-nmr spectrum of pubesenolide exhibited only one vinyl methyl singlet at δ 2.03 for the C-28 protons and a hydroxymethyl group as a quartet centered at δ 4.36 (J=14 and 2 Hz, C-27-H_2) which moved downfield to δ 4.88 in its triacetate (**1b**). The characteristic signals for the C-22-H of withanolides (δ 4.45, 1 H, dt, J=14.3 and 3.4 Hz) and a secondary methyl group at C-21 were also discernible. These observations suggest that the side chain of pubesenolide (**1a**) is similar to that of withaferin A (**3**) (12,13), and the structure of pubesenolide may be formulated as 1α,3β,27-trihydroxyergosta-5,24-dien-26,22-olide (**1a**).

The structure (**1a**) is consistent with ^{13}C-nmr data (see Table 1). The signals, originating from the carbons of rings A and B, are comparable with those of physalolactone B (14), and those of rings C, D and the side chain are in perfect agreement with the corresponding signals of withaferin A (**3**) (15).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter. Ir spectra were recorded on a Perkin-Elmer infracord 137 spectrophotometer and refer to KBr pellets; uv spectra were recorded on a Cary 14 instrument for solutions in EtOH: ^{1}H-nmr spectra were recorded at 270 MHz on a Bruker WH instrument, and ^{13}C-nmr spectra were recorded at 22.63 MHz on a Bruker WH-90 instrument. The multiplicities of the signals were determined by a single-frequency off-resonance decoupled (SFORD) spectrum and were confirmed by partially-relaxed Fourier-Transform experiment. Mass spectra were determined with a Varian MAT 731 high resolution mass spectrometer.

TABLE 1. ^{13}C-nmr Data

Atom	Compound		
	Pubesenolide (**1a**)	Withaferin A diacetate	Physalolactone B (**2a**)
C-1	72.8 d	201.1	75.2
C-2	38.3 t	133.9	35.5
C-3	66.1 d	139.8	66.4
C-4	41.4 t	72.2	41.4
C-5	137.8 s	61.6	137.5
C-6	125.6 d	60.2	124.7
C-7	31.9 t	31.1	31.7
C-8	31.4 t	29.6	31.2
C-9	41.5 d	44.1	41.8
C-10	41.5 s	48.1	40.9
C-11	22.4 t	21.3	20.2
C-12	39.6 t	39.2	39.8
C-13	42.8 s	42.6	43.0
C-14	56.3 d	56.1	56.8
C-15	24.5 t	24.2	23.9
C-16	27.4 t	27.3	22.0
C-17	52.0 d	51.9	54.7
C-18	11.8 q	11.5	13.6
C-19	15.5 q	15.7	19.4
C-20	38.8 d	38.8	75.2
C-21	13.5 q	13.3	20.8
C-22	78.7 d	78.2	81.0
C-23	29.8 t	30.1	31.5
C-24	154.0 s	157.1	149.1
C-25	124.9 s	121.9	122.0
C-26	167.2 s	165.3	166.2
C-27	56.9 t	58.6	12.5
C-28	20.2 q	20.6	20.5

PLANT MATERIAL.—The plant material used for this study was raised, from the seeds of a variety of *P. pubescens* in the experimental farm of the Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel. The seeds were provided by Dr. S.S. Nittala, Lecturer, Department of Chemistry, Andhra University, Waltair, Andhra Pradesh, India. A herbarium specimen of the plant material is being preserved in the laboratory.

EXTRACTION AND CHROMATOGRAPHY.—Air-dried and powdered whole plant of *P. pubescens* (1.5 kg) was extracted with MeOH in a soxhlet extractor for 48 h. The alcoholic extract was concentrated to a small volume (1.5 liter) and diluted with an equal volume of H_2O. The mixture was stirred, then filtered, The filtrate was extracted successively with *n*-hexane (3×1 liter) and $CHCl_3$ (3×1 liter). The combined $CHCl_3$ extract was washed, dried (anhydrous Na_2SO_4), and evaporated to give a solid residue (10.0 g).

The residue was chromatographed over 0.5 kg of silica gel S and eluted with hexane-EtOAc mixtures of increasing polarity. Eluates from hexane-EtOAc (7:3) were mixed and evaporated to give a solid residue (0.2 g), which was a mixture of two components. This fraction was rechromatographed over silica gel (mesh 230-400) (50 g) and eluted with hexane-EtOAc (3:2). Fractions of 25 ml each were collected.

PUBESENOLIDE (**1a**).—Fractions 19-31 of the above column gave a solid residue, 0.15 g (0.01%), which crystallized from MeOH as colorless micro needles, mp 145-146°; $[\alpha]_D$ +28.5° (c 1.8 $CHCl_3$); ir ν max 3590, 1685 cm^{-1}; uv λ max (EtOH) 228 nm (ϵ, 7,400); ^1H nmr δ 5.59 (1H, br d, J=5.4 Hz, H-6), 4.45 (1H, dt, J=14.3 and 3.4 Hz, H-22), 4.36 (2H, q, J=14 and 2 Hz, H-27), 3.98 (1H, seven lines, H-3), 3.85 (1H, t, H-1), 2.03 (3H, s, H-28), 1.04 (3H, s, H-19), 1.03 (3H, d, J=6.5 Hz, H-21), and 0.73 (3H, s, H-18); ms *m/z* (rel. int.) 458 (M^+, 0.86%) 440 (13%), 211 (29%), 209 (14%), 185 (14%), 173 (22%), 159 (38), 145 (29%), 131 (38%), 125 (20%), 121 (31%), 109 (28%), 105 (57%), 95 (60%), and 67 (100%). *Anal.* calcd for C, 73.28% and H, 9.29%; Found: $C_{28}H_{42}O_5$ requires C, 73.33% and H, 9.23%.

ACETYLATION OF **1a**.—A solution of **1a** (30 mg) in pyridine (0.2 ml) was treated with Ac_2O (1 ml) and left overnight at 0°. The reagents were removed in vacuo and the residue was crystallized from Me_2CO-hexane as fine needles, mp 96-97°; ir ν max 1725, 1240, 1230, and 1210 cm^{-1}; ^1H nmr δ 5.58 (1H, br d, J=5.4 Hz, H-6), 5.06 (1H, t, 1-H), 4.93 (1H, *seven lines*, H-3), 4.88 (2H, d, J=2.1 Hz, H-27), 4.43 (1H, dt, J=13.4 and 3.4 Hz, H-22), 2.03 (3H, s, H-28), 1.09 (3H, s, H-19), 1.01 (3H, d, J=6.5 Hz, H-21), 0.71 (3H, s, H-18), 2.07, 2.06, and 2.05 (3H, s, 3×-OCOCH_3); ms, *m/z* (rel. int.) 584 (M^+, 0.5%), 464 (7%), 422 (6%), 280 (14%), 279 (30%), 189 (21%), 175 (30%), 167 (34%), 150 (35%), 149 (15%), 85 (60%), 83 (60%), 79 (68%), and 57 (100%). *Anal.* calcd for C, 69.78% and H, 8.32; Found: $C_{34}H_{46}O_8$ requires C, 69.84% and H, 8.27%.

ACKNOWLEDGMENTS

Sincere thanks are due to Dr. Ehud Keinan, Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel, for nmr spectral data, and to Professor Anil B. Ray and Dr. I. Kirson for discussions. The author is grateful to Professor S.K. Roy, Department of Botany, Banaras Hindu University, Varanasi-221005, India, for identification of plant material.

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Received 18 September 1984