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PUBESCENOL, A WITHANOLIDE FROM *PHYSALIS PUBESCENCE*

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Key Word Index—*Physalis pubescence*; Solanaceae; sitosterol; pubescenol; physalin E; physalin E acetate.

Abstract—A new withanolide, named pubescenol, and also physalin E and physalin E acetate have been isolated from *Physalis pubescence*. The structure of pubescenol is assigned as 4α,7α-dihydroxy-1-oxo-24α,25α-epoxy-2,3-dihydro-withanolide.

INTRODUCTION

Physalis pubescence is a diploid annual of Canadian origin which is cultivated in Puerto Rico as a medicinal herb [1]. It was raised in the Botanic Farm of Andhra University, Waltair, for our chemical examination.

RESULTS AND DISCUSSION

Fresh green plants were collected, shade-dried and extracted with *n*-hexane and chloroform. The dark green *n*-hexane extract contained only plant waxes. The green chloroform extract was separated on a silica gel G column eluted with benzene–ethyl acetate mixtures to yield a new steroid, and also physalin E and its acetate. The latter compounds were identified by their NMR and mass spectra and by direct comparison with authentic samples [2, 3].

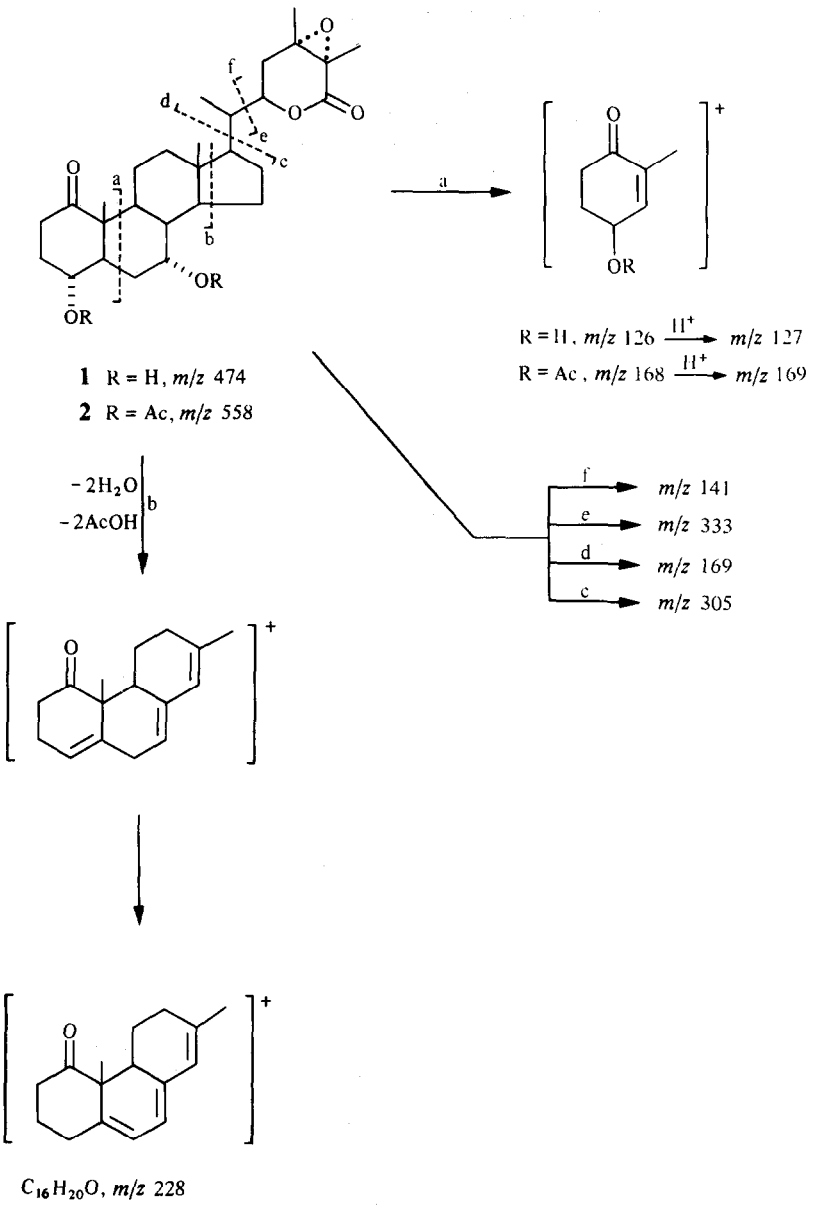
As the new steroid did not correspond with any known physalins or withanolides, it was given the trivial name pubescenol, indicating its origin from *Physalis pubescence*. Pubescenol (**1**) crystallized from acetone as colourless needles; mp 180–182°; $C_{28}H_{42}O_6$; ν_{max}^{KBr} 3410 (OH), 1728 (δ-lactone), 1710 (six-membered ketone) 1050 and 1030 cm^{-1}. It contained two secondary hydroxyls (δ 3.64 *br s* and 5.33 *d*, $J = 4$ Hz) and yielded a diacetate (**2**) with pyridine and acetic anhydride at 100°; mp 201–202°; $C_{32}H_{46}O_8$; δ 1.98 *s*, 2.03 *s* (two acetoxyls). Pubescenol was characterized as a steroid resembling a withanolide, the side-chain carrying a δ-lactone system. This was supported by its ^{1}H NMR spectrum in which the C-18 and C-19 methyls appeared as two singlets at δ 0.66 and 0.91 and the C-21 methyl as a doublet at 0.87. There was a singlet at 1.45 (6H) accounting for two methyls and leading to the inevitable conclusion that the δ-lactone ring system in the side chain might carry an epoxide ring and the two methyls might be located on the oxirane ring system [4, 5] between C-24 and C-25.

Unequivocal support for the oxirane ring in pubescenol was obtained from its mass spectrum in which there was a prominent peak at *m/z* 458.2992 (rel. int. 25%), 16 amu less than the $[M]^+$ ion (474 not observed) apparently due to the loss of one oxygen atom. Such a loss of elemental oxygen is not unusual and has been noticed frequently among compounds with an oxirane system [6]. The mass spectrum contained two more strong peaks at *m/z* 440.2959 (75%) and 422.2756 (25%) indicating further successive losses of 1 mol and 2 mol of water. Similarly from the pubescenol diacetate, $[M]^+$ at *m/z* 558 (5%), the loss of one oxygen atom and 2 mol of acetic acid furnished the last mentioned ion at *m/z* 422 as the base peak. Furthermore, the ion at *m/z* 141 (45%) represented the δ-lactone system carrying an oxirane ring, while *m/z* 305 (4%) denoted the tetracyclic fragment without the side chain. The mass fragmentation of pubescenol and its acetate is represented in Scheme 1 in which the ions at *m/z* 168 (49%) and 125 (27%) are taken to represent ring A carrying one hydroxyl or acetoxyl besides the ketonic function.

The ^{1}H NMR spectral data of pubescenol and its acetate (Table 1) do not indicate any α,β-unsaturated ketonic system but a reduced ring A ketone as in dihydrowithanolides. The ketonic function of a six-membered ring is therefore placed at C-1 as in physalins [7] and withanolides [8]. The signal at δ 5.49 in **2** is a multiplet that can be analysed as a doublet of doublets ($J = 6$, 2 Hz) assignable to the 4-βH and hence the 4α-hydroxyl is indicated to be in ring A. The alternate 3β-position for this hydroxyl is regarded to be improbable because of its stability towards protonic reagents (see below).

The possibility of the second hydroxyl in ring C or D was eliminated by the ^{1}H NMR resonances of the C-19 and C-18 methyls which were noticed at the same positions as in any unsubstituted steroids [9]. Pubescenol resembled Jabarosa lactone F (**3**) [10, 11] in its cleavage pattern in the mass spectrum. For example, the ion at *m/z* 228 (49%) represented the A/B/C rings as a single unit from which 2 mol of water (2 mol of acetic acid from the acetate **2**) were lost. The comparative absence of the effect of the acetoxyl on the C-19 methyl resonance in the ^{1}H NMR spectrum completely eliminated the 6β-position for the second hydroxyl. From the multiplet at δ 4.98 for the geminal proton on the C-7 acetoxyl, the C-7 hydroxyl is regarded to be α-axial oriented.

The structure of pubescenol is thus assigned as 4α,7α-dihydroxy-1-oxo-24α,25α-epoxy-2,3-dihydro-withanolide (**1**), which is different from a new steroid, physapubescin [5], isolated from the same plant.



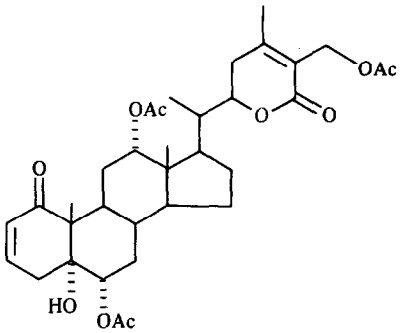
Scheme 1. Mass spectral fragmentation of pubescenol (**1**) and its acetate (**2**).

Further confirmation of the presence of the 4α,7α-hydroxyls in pubescenol (**1**) was secured from a study of its behaviour in the presence of the protonic reagents (a) 5% H_2SO_4 in ethanol and (b) 5% H_2SO_4 in acetic acid. With the former, a trihydroxy derivative, compound **4**, $C_{28}H_{42}O_6$, mp 252°, UV: nil, R_f: 0.38 (benzene–ethyl acetate, 3:7), was produced but with the latter mixture the corresponding triacetate, compound **5**, $C_{34}H_{48}O_9$: mp 136–138°; R_f: 0.29 (benzene–ethyl acetate, 4:1) was formed. Their ^{1}H NMR spectra revealed that one of the two hydroxyls was eliminated with the introduction of a trisubstituted olefinic bond, and also, as in physapubescin [5], the oxirane ring was cleaved to yield the 24β,25α-glycol. The ^{1}H NMR spectra (Table 1) contained no olefinic proton signals characteristic of 2-en-1-one which might be expected if the pubescenol (**1**) contained a 3-hydroxyl group [12]. On the other hand, the spectrum of compound **4** contained a broad triplet at δ 5.60 (compound **5** had a multiplet at δ 5.52) indicating a proton on a trisubstituted olefinic bond in the molecule. Out of the three probable positions, C-4/C-5 or C-5/C-6 can be readily eliminated as these are known to cause a downfield shift of the C-19 methyl signal by *ca* 0.4 ppm [12]. Since it was noticed at δ 1.010 (*s*) in compound **4** and 1.07 (*s*) in compound B without a prominent shift from 0.91 in the parent compound, the double bond was therefore placed at C-7/C-8 and the olefinic proton at C-7. This olefinic bond could have arisen by the proton catalysed *trans*-diaxial elimination of the 7α-hydroxyl in pubescenol (**1**). The 4α-hydroxyl was thus left intact and now the 4β-H appeared at 3.950 (*m*) in compound **4** and at 4.90 (*m*) in its acetate (compound **5**).

Table 1. ^{1}H NMR spectral data for pubescenol (1), its acetate (2) and compounds 4 and 5 produced by dehydration

Assignment	Pubescenol (1)*	Pubescenol acetate (2)*	Compound 4†	Compound 5†
H-18	0.68	0.68 *s*	0.718 *s*	0.704 *s*
H-21	0.84 *d* (J = 6 Hz)	0.86 *d* (J = 6 Hz)	0.997 *d* (J = 6 Hz)	0.987 *d* (J = 6 Hz)
H-19	0.91 *s*	0.91 *s*	1.010 *s*	1.087 *s*
H-27	1.45 *s*	1.45 *s*	1.509 *s*	1.509 *s*
H-28			1.370 *s*	1.388 *s*
H-22	4.38 *m*	4.38 *m*	4.392 *d/t*	4.384 *m*
H-4β	4.28 *m*	5.49 *m*	3.950 *m*	4.90 *m*
H-7β	3.64 *m*	4.98 *m*	—	—
H-7	—	—	5.60 *t*	5.521 *m*
OAc-4α or OH-4α	5.33 *d*	2.00 *s*	—	1.999 *s*
OH-7 or OAc-7	4.31 *m*	1.98 *s*	—	—
OAc-25				2.030 *s*
OAc-24	—	—	—	2.036 *s*

*100 MHz spectrum in DMSO-d_6.
†270 MHz spectrum in $CDCl_3$.



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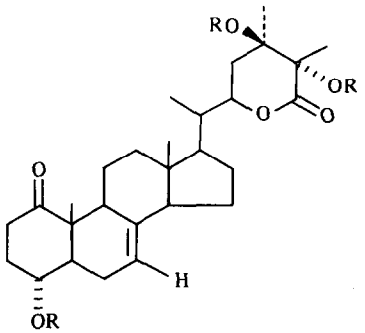

R
4 H
5 Ac

In compound 4, the C-27 and C-28 methyl groups appeared at δ 1.509 and 1.370, respectively (and in 5 at 1.509 and 1.388) suggesting that they each carry a hydroxyl or acetate due to the scission of the oxirane ring in the lactone ring, as observed in physapubescin [5] under similar circumstances. Finally, the C-22 CH proton was noted at δ 4.392 in the spectrum of 4 and at 4.384 in that of 5, values, very close to that observed in the spectrum of pubescenol (4.38) which confirms that the C-23 methylene was unchanged during this reaction.

From the foregoing results, compound 4 is assigned the structure 4α,24β,25α-trihydroxy-1-oxo-2,3-dihydro-7-en-22,26-olide (4) and 5 is its acetate thus providing final support for the structure of pubescenol (1).

EXPERIMENTAL

Isolation. The fresh air-dried plants of *P. pubescence* (500 g) were successively extracted with hexane and $CHCl_3$. The $CHCl_3$ extract was evapd and the residue (10 g) was adsorbed on silica gel (40 g) and after drying placed on the top of a column (60 × 6 cm) of silica gel G in C_6H_6. The column was eluted with C_6H_6–EtOAc mixtures. Sitosterol was crystallized from MeOH as colourless prisms, mp 135°, undepressed by an authentic sample. Physalin E was crystallized from MeOH as white shining plates, mp 305–307°, undepressed with an authentic sample; R_f 0.47 (C_6H_6–EtOAc, 3:7); $[M]^+$ at m/z 544. Physalin E acetate was crystallized from MeOH as colourless shining needles, mp 278–279°, undepressed with an authentic sample.

Pubescenol (1). Crystallized from Me_2CO as colourless shining needles, mp 180–182°, R_f 0.57 (C_6H_6–EtOAc, 3:7). UV λ_{max}^{EtOH} nm: 212; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1728 (δ-lactone), 1710 (six-membered ring ketone). (Found: C, 70.08; H, 8.89. $C_{28}H_{42}O_6$ requires: C, 70.22; H, 8.86%.) MS m/z: 458.2992 (obs.), 458.3032 (calc.) $[M-O]^+$; 440.2959 (obs.), 440.2927 (calc.) $[M-O-H_2O]^+$; 422.2756 (obs.), 422.2821 (calc.) $[M-O-2H_2O]^+$; 348 (rel. int. 4) $[M-$ring A$]^+$; 330 (9) $[M-$ring A $-H_2O]^+$; 329 (53); 333 (4) $[M-\delta$-lactone $-O]^+$; 305 (3) $[M-$side chain$]^+$; 297 (22); 269 (22); 264 (5) $[M-$rings A/B/C$]^+$; 228 (49) $[M-$rings A/B/C $-2H_2O]^+$; 169 (45) [sidechain]$^+$; 141 (45) [δ-lactone $-O]^+$; 127 (34) [rings A + H]$^+$; 126 (27); 109 (100) [ring A $-H_2O+H]^+$; 108 (12). CD (dioxan) λ_{max} nm: 295 ($\Delta\varepsilon$ 0), 265 (+0.493), 240 (−1.493), 238 (−1.454), 236 (−1.428), 233 (−1.272). Slightly negative at shorter wavelengths. ORD (dioxan): Negative Cotton effect. $[M]_{288\,nm}$ 0° (max), $[M]_{285}$ 0° (min), $[M]_{252}$ −1700° (max), $[M]_{248}$ −1700° (max), $[M]_{243}$

$-3300°$ (min), $[M]_{244}$ $-3500°$ (min), $[M]_{242}$ $-3100°$ (max), $[M]_{230}$ $-4200°$ (min), $[M]_{225}$ $+1200°$ (min).

Pubescenol acetate (2). This compound was prepared from 1 in pyridine–Ac_2O by heating on a steam-bath for 1 hr and crystallized from MeOH as colourless shining needles, mp 201–202°. IR ν_{max}^{KBr} cm^{-1}: 1728 (δ-lactone), 1740 (acetate), 1710 (six-membered ring ketone), 1080 and 1060. MS *m/z* (rel. int.): 558 $[M]^+$ (5), 438 $[M-2\ HOAc]^+$ (3), 422 $[M-O-2\ HOAc]^+$ (100), 417 $[M-\delta\text{-lactone}]^+$ (3), 390 (4), 389 (9), 348 (5), 330 (10), 297 (9), 269 (20), 228 (17), 229 (50), 210 (15), 169 [side chain]$^+$ (100), 168 [ring A]$^+$ (49), 141 [δ-lactone + O]$^+$ (100), 108 [ring A $-$ HOAc]$^+$ (100). (Found: C, 68.05; H, 8.15. $C_{32}H_{46}O_8$ requires: C, 68.82; H, 8.24%.]

Reaction of pubescenol with 5% H_2SO_4 *in* EtOH. Pubescenol (40 mg) was treated with 5% H_2SO_4–EtOH at room temp. for 10 min. The reaction mixture was concd *in vacuo* and after dilution extracted with $CHCl_3$. The solvent was removed from the product and the residue crystallized from Me_2CO. Compound 4 (R_f 0.38, EtOAc–C_6H_6, 7:3) separated as colourless cystals, mp 252°. (Found: C, 70.60; H, 9.04. $C_{28}H_{42}O_6$ requires: C, 70.86; H, 8.92%.)

Reaction of pubescenol with 5% H_2SO_4 *in glacial* HOAc. Pubescenol (45 mg) was treated with cold 5% H_2SO_4–HOAc and the mixture was kept at room temp. overnight. The product was extracted with $CHCl_3$ and purified by chromatography over silica gel (finer than 200 mesh). Elution with C_6H_6–EtOAc (4:1) furnished compound 5 (R_f 0.29; C_6H_6–EtOAc, 4:1) as colourless crystals, mp 136–138°. (Found: C, 67.71; H, 8.12. $C_{34}H_{48}O_9$ requires: C, 67.98; H, 8.05%.)

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