Pubescenol, a New Withanolide From *Physalis pubescence*†

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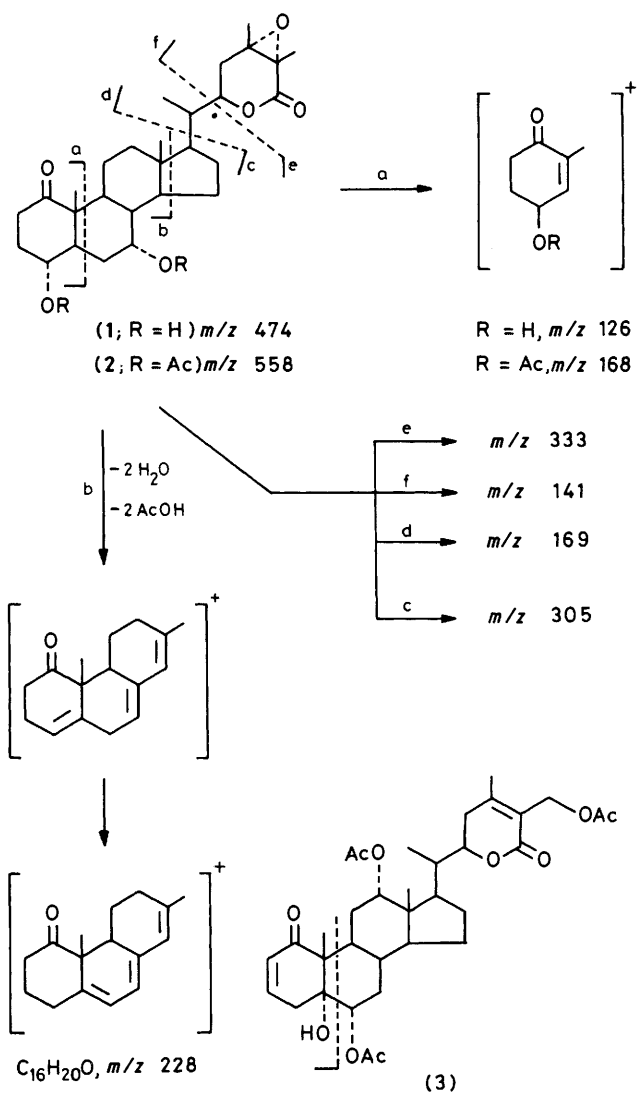
The isolation from *Physalis pubescence* of a new withanolide (now named pubescenol) and physalin E and its acetate is reported. The structure of pubescenol was shown to be 2,3-dihydro-4α,7α-dihydroxy-1-oxo-24α,25α-epoxywithanolide on the basis of its ^{1}H n.m.r., high resolution mass, c.d., and o.r.d. spectra.

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

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, set in benzene and eluted with benzene–ethyl acetate mixtures. Thus, the chloroform extract yielded a new steroid, besides physalin E and its acetate identified by their n.m.r. and mass spectra and by direct comparison with authentic specimens.[2,3]

Since the new steroid did not correspond with any known physalins nor withanolides, it is given a new name pubescenol indicating its origin from *Physalis pubescence*. Pubescenol (**1**) crystallized from acetone as colourless needles, m.p. 180—182 °C; $C_{28}H_{42}O_6$; $\lambda_{max.}$(KBr) 3 410 (OH), 1 728 (δ-lactone), 1 710 (six-membered ring ketone), 1 050, and 1 030 cm^{-1}. It contains two secondary hydroxy groups (δ 3.64 br s and 5.33 d, *J* 4 Hz) and yielded a diacetate (**2**) with pyridine–acetic anhydride at 100 °C, m.p. 201—202 °C; $C_{32}H_{46}O_8$; δ 1.98 s, 2.03 s (two acetyls). Pubescenol is characterized as a steroid resembling a withanolide, the side-chain carrying a δ-lactone system. This is supported by its n.m.r. spectrum in which 18 and 19 methyls appeared as two singlets at δ 0.66 and 0.91 and the 21 methyl as a doublet at 0.87. There is a singlet at δ 1.45 (6 H) accounting for two methyls and leading to the inevitable conclusion that the δ-lactone ring in the side-chain might carry an epoxide ring and the two methyls might be located on the oxirane ring systems[4–6] between C(24) and C(25).

An unequivocal support for the oxirane ring in pubescenol was received from its high-resolution mass spectrum in which there is a prominent peak at *m/z* 458.2992 (rel. int. 25%), 16 atomic mass units less than $M^{+\cdot}$ ion (*m/z* 474) apparently due to the loss of one oxygen atom. Such a loss of elemental oxygen has been noticed among compounds with a oxirane system.[7–9] The mass spectrum contains two more prominent peaks at *m/z* 400.2959 (75%) and 422.2767 (25%) indicating further successive loss of one molecule and two molecules of water. Similarly from the pubescenol diacetate ($M^{+\cdot}$ 588, 5%) the loss of one oxygen atom and two molecules of acetic acid furnished the last mentioned ion *m/z* 422 (100%) as base peak. Furthermore, the ions designated d and f at *m/z* 169 and 141 represent the side-chain and the δ-lactone system carrying an oxirane ring, while *m/z* 153 and 125 denote the same fragments with the loss of one oxygen atom. The tetracyclic fragment designated c is also observed at *m/z* 305. The mass fragmentation of pubescenol and its acetate is represented in the Scheme in which the ions designated *a* at *m/z* 168 (49%) and 126 (27%) are taken to represent ring A carrying one hydroxy or acetoxy group besides the ketonic function.



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

† Taken from the Ph.D. Thesis of Dr. K. Sambi Reddy, Andhra University, Waltair.

The ketone function of the six-membered ring is placed at C-1 as in physalins[10] and withanolides[11] from a consideration of its biogenetic origin. The spectral data of pubescenol (**1**) and its acetate (**2**) do not indicate any α,β-unsaturated ketonic system in pubescenol but a reduced ring A ketone as in dihydrowithanolides.[6,11] One of the two hydroxy groups is now placed at C-4, based upon the natural occurrence of 4-hydroxywithanolides.[11] The dd at δ 5.49 of (**2**) with $J = 2$ and 6 Hz indicates a 4β-equatorial proton and hence the 4α-hydroxy-group in ring A. The second hydroxy group can be placed at C-7 from similar biogentic consideration.[2,11,12] The possibility of a hydroxy group in ring C or D is eliminated from the n.m.r. resonances of the 19 and 18 methyl groups which are at the same positions as in unsubstituted steroids.[12] Pubescenol resembles[13,14] jabarosa lactone F (**3**) and daturalactone[15] in its cleavage pattern in the mass spectrum. For example, the mass ion designated b at m/z 228 (49%) represents A/B/C rings, a single unit from which two molecules of water [two molecules of acetic acid from the acetate (**2**)] are lost. The comparative absence of the effect of acetoxy group on the 10-methyl resonance in the n.m.r. spectrum eliminates the 6β-position for the hydroxy group. From the multiplet at δ 4.98 for the geminal proton on the 7-acetoxy group, the 7-hydroxy group is regarded to be α-axial oriented. The α-configuration of the epoxide ring was finally confirmed by the o.r.d. and c.d. spectra of pubescenol. It showed a strong negative Cotton effect of the saturated δ-lactone n→π* band near 235 nm in accordance with those of 1-oxo-24α,25α-epoxy-2,3-dihydrowithanolides.[6,16] Other Cotton effects and negative c.d. bands were observed at shorter wavelengths, possibly attributable to the cyclohexanone n→π* bands.

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

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 evaporated 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 benzene. The column was eluted with C_6H_6–EtOAc mixtures. β-Sistosterol crystallized from MeOH as colourless prisms, m.p. 135 °C, undepressed by an authentic sample. Physalin E crystallized from MeOH as white shining plates, m.p. 305—307 °C, undepressed with an authentic sample; R_F 0.47 (C_6H_6–EtOAc, 3:7); M^+ 544. Physalin E acetate crystallized from MeOH as colourless shining needles, m.p. 278—279 °C, undepressed with an authentic sample.

Pubescenol (**1**).—This crystallized from acetone as colourless shining needles, m.p. 180—182 °C, R_F 0.57 (C_6H_6–EtOAc, 3:7). $\lambda_{max.}$(EtOH) 212 nm (ε 5 800), $\nu_{max.}$(KBr) 3 400 (OH), 1 728 (δ-lactone), and 1 710 (six-membered ring ketone); δ [$(CD_3)_2SO$, $SiMe_4$ as internal standard] 0.68 (s, 18-CH_3), 0.87 (d, J 7 Hz, 21-CH_3), 0.91 (s, 19-CH_3), 1.45 (6 H, s, 27- and 28-Me), 3.64 (m, 7-H), 4.28 (d, J 4 Hz, 4-H), 4.31 (m, 7-OH), 4.4 (m, 22-H), and 5.33 (d, J 4 Hz, 4-OH) (Found: C, 70.1; H, 8.9. $C_{28}H_{42}O_6$ requires C, 70.22; H, 8.86%); m/z 458.2992 (obs.), 458.3032 (calc., $M^{+\bullet}$ − O), 440.2959 (obs.), 440.2927 (calc., $M^{+\bullet}$ − O − H_2O), 422.2756 (obs.), 422.2821 (calc., $M^{+\bullet}$ − O − 2 H_2O) 348 (rel. int. 4%, $M^{+\bullet}$ − ring A), 330 (9, $M^{+\bullet}$ − ring A − H_2O), 329 (53), 333 (4, $M^{+\bullet}$ − δ-lactone − O), 305 (3, $M^{+\bullet}$ − side chain), 297 (22), 264 (5, $M^{+\bullet}$ − rings A/B/C), 228 (49%, $M^{+\bullet}$ − A/B/C − 2 H_2O), 169 (45, side chain), 153 (25, side chain − O), 141 (45, δ-lactone), 127 (34, ring A + H), 125 (27), 125 (35, δ-lactone − O), 109 (100%, ring A − H_2O + H), and 108 (12); c.d.: $\lambda_{max.}$(dioxane) 295 (Δε + 0.5), 265 (+0.493), 240 (−1.493), 238 (−1.454), 235 (−1 428), and 233 nm (−1.272). Slightly negative at shorter wavelengths; o.r.d. (dioxane): negative Cotton effect $[M]_{288}$ 0° (max.), $[M]_{285}$ 0° (min.), $[M]_{252}$ −1 700 (max.), $[M]_{243}$ −3 300° (min.), $[M]_{244}$ −3 500° (max.), $[M]_{230}$ −4 200° (min.), and $[M]_{255}$ +1 200° nm (min.).

The Acetate (**2**).—This was prepared from (**1**) in pyridine–acetic anhydride by heating on a steam-bath for 1 h; it crystallized from methanol as colourless shining needles, m.p. 201—202 °C, $\nu_{max.}$(KBr) 1 728 (δ-lactone), 1 740 (acetate), 1 710 (six-membered ring ketone), 1 080, and 1 060 cm^{-1}; δ 0.68 s (18-Me), 0.85 (d, J 7 Hz, 21-Me), 1.05 (s, 19-Me), 1.45 (6 H, s, 27- and 28-Me), 1.98 (s, 7-OAc_3), 2.00 (s, 4-OAc_3), 4.40 (m, 22-H), 4.98 (m, 7-H), and 5.49 (dd, J 6, 2 Hz, 4-H); m/z 558 (rel. int. 5%, $M^{+\bullet}$), 438 (3, $M^{+\bullet}$ − 2 AcOH), 422 (100, $M^{+\bullet}$ − 0 − 2 AcOH), 417 (3, $M^{+\bullet}$ − δ-lactone), 390 (4), 389 (9), 348 (5), 330 (10), 297 (9), 269 (20), 288 (17), 229 (50), 210 (15), 169 (100, side chain), 168 (49, ring A), 153 (30, side chain − 0), 141 (100, δ-lactone + 0), 125 (25, δ-lactone − 0); and 108 (100, ring A − AcOH) (Found: C, 68.05; H, 8.15. $C_{32}H_{46}O_8$ requires C, 68.82; H, 8.24%).

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