Physapubenolide and Pubescenin, Two New Ergostane-type Steroids from *Physalis pubescens* L. (Solanaceae)

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Structures of two new ergostane-type steroids which occur in the leaves of a variety of *Physalis pubescens* L. (Solanaceae) growing in India were established by spectral analysis and chemical transformations. Physapubenolide (**2a**) is (20*S*,22*R*)-15α-acetoxy-5β,6β-epoxy-4β,14β-dihydroxy-1-oxowitha-2,24-dienolide. Pubescenin (**6a**) is (20*S*,22*R*)-24,25-epoxy-1α-hydroxy-3β-(*O*-β-D-glucopyranosyl)with-5-enolide.

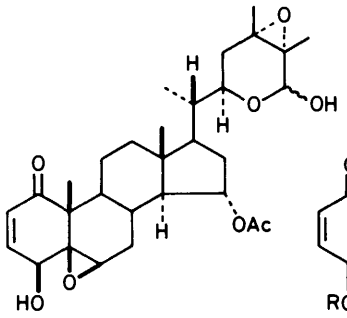
Variously substituted, highly oxidized ergostane-type steroids were isolated from different species of *Withania*, *Acnistus*, *Physalis*, *Nicandra*, and other genera of the Solanaceae family. The first compounds in this series, which possessed a six-membered-ring lactone in the side-chain, were given the generic name withanolides. The chemistry of these and other biogenetically related compounds has been reviewed.[1]

An investigation of the steroidal constituents of *Physalis pubescens* L. conducted a few years ago in our laboratory resulted[2] in the isolation and characterization of physapubescin (**1**). The plants were raised at the experimental farm of the Faculty of Agriculture, Rehovot, Israel, from seeds received from the Botanical Garden of Dijon, France. In connection with an investigation of the insect antifeedant properties of several *Physalis* species,† *P. pubescens* plants were raised again in 1981, this time from seeds collected in the outskirts of Waltair, India. There were small morphological differences between the Dijon and Waltair varieties and, much to our surprise, not even a trace of physapubescin (**1**) could be isolated from the leaves of the latter variety. Instead of compound (**1**), two new ergostane-type steroids, never encountered before, were isolated and characterized: an unsaturated lactone which was given the name physapubenolide (**2a**) and the monoglucoside of an epoxy lactone which was named pubescenin (**6a**). The identity of the plant material was confirmed by Professor S. K. Roy of the Banaras Hindu University, who kindly analysed a herbarium specimen of *P. pubescens* raised from seeds collected in India by Dr. S. S. Nittala.

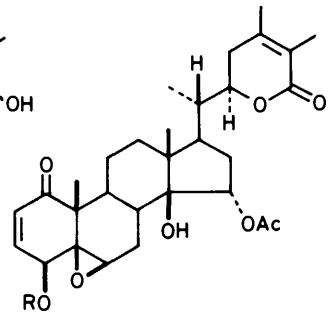
The structural analysis of compounds (**2a**) and (**6a**) is reported in this paper. According to the ^{1}H n.m.r. spectrum (Table 1), rings A and B in physapubenolide (**2a**) have the same substitution pattern (5β,6β-epoxy-4β-hydroxy-2-en-1-one) as in physapubescin (**1**);[2] the nine-carbon-atom side-chain includes an αβ- unsaturated δ-lactone, the same as in withanone[3] and other related compounds.[1] The positive band at 247 nm in the c.d. spectrum of the 15-one derivative (**4**) of physapubenolide is instrumental in assigning the 22*R* (22β$_F$) configuration, the same as in all the withanolides and related compounds studied thus far. Additional n.m.r. signals indicate the presence of a secondary acetoxy group (δ_H 1.99 for CH_3 and δ_H 4.97, doublet, *J* 4.4 Hz, for an α-to-oxygen proton). In view of the molecular formula $C_{30}H_{40}O_8$ of physapubenolide (**2a**), a tertiary hydroxy group should also be present.

Acetylation of the naturally occurring monoacetate (**2a**) afforded the diacetate (**2b**) [downfield shift of the doublet assigned to 4α-H from δ_H 3.79 in (**2a**) to δ_H 4.70 in (**2b**)]. Catalytic hydrogenation of compound (**2a**) afforded the 2,3-

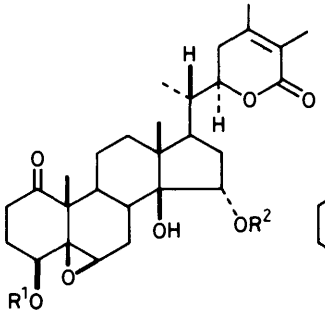
† The results of this investigation will be published elsewhere.



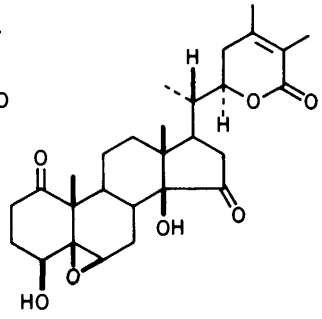
(**1**) Physapubescin



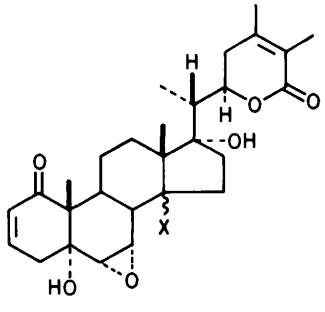
(**2a**) R = H Physapubenolide
(**2b**) R = Ac



(**3a**) R^1 = H, R^2 = Ac
(**3b**) R^1 = R^2 = H
(**3c**) R^1 = R^2 = Ac
(**3d**) R^1 = Ac, R^2 = H



(**4**)



(**5a**) X = α-H
(**5b**) X = β-OH

Table 1. ^{1}H N.m.r. data[a] of physapubenolide (**2a**) and derivatives

Proton	Compound (**2a**)	(**2b**)	(**3a**)	(**3b**)	(**3c**)	(**4**)
2-H	6.18 d (10.0)	6.24 d (9.8)				
3-H	6.95 dd (10.0; 5.5)	7.05 dd (9.8; 6.0)				
4-H	3.79 d (5.5)	4.70 d (6.0)	3.48 t (3.1)	3.45 t (3.1)	4.62 t (3.1)	3.54 t (3.1)
6-H	3.36 m ($w_{\frac{1}{2}}$ 4)	3.34 m ($w_{\frac{1}{2}}$ 4)	3.24 m ($w_{\frac{1}{2}}$ 4)	3.22 m ($w_{\frac{1}{2}}$ 4)	3.20 m ($w_{\frac{1}{2}}$ 4)	3.29 m ($w_{\frac{1}{2}}$ 4)
15-H	4.97 d (4.4)	4.95 d (4.3)	4.98 d (4.4)	3.98 d (4.4)	4.97 d (4.4)	
22-H	4.32 dt	4.32 dt	4.38 dt	4.35 dt	4.37 dt	4.42 dt
18-H_3	1.11 s	1.10 s	1.08 s	1.04 s	1.08 s	1.15 s
19-H_3	1.40 s	1.38 s	1.32 s	1.30 s	1.30 s	1.31 s
21-H_3	1.02 d (6.8)	1.01 d (6.5)	1.00 d (6.9)	1.03 d (6.8)	1.00 d (6.8)	1.03 d (6.5)
27- and 28-H_3	1.87 s 1.94 s	1.86 s 1.94 s	1.86 s 1.93 s	1.86 s 1.93 s	1.86 s 1.93 s	1.87 s 1.94 s
CH_3CO	1.99 s	1.98 s, 2.06 s	2.00 s		2.00 2.03	

[a] Solvent $CDCl_3$; δ values; coupling constants (Hz) in parentheses.

dihydro derivative (**3a**), whose acetate group was hydrolysed under basic conditions to give a compound (**3b**) possessing two secondary hydroxy groups: 4β-OH which was present as such in the dihydro derivative (**3a**) (δ_H 3.45) and a new free hydroxy group identified by the upfield shift of the corresponding C*H*–OR signal from δ_H 4.98 in (**3a**) to δ 3.98 in (**3b**).

Treatment of compound (**3b**) with pyridinium chlorochromate (PCC) in acetone,[4] at room temperature, resulted in the exclusive oxidation of the hydroxy group obtained by hydrolysis of the acetate in compound (**3a**). The obtained ketone (**4**) had the newly formed carbonyl as a cyclopentanone ($\nu_{max.}$ 1 747 cm^{-1}), thus leaving only two possible locations (C-15 or C-16) for the secondary acetate in compound (**3a**).

The rates of acetylation of the two secondary hydroxy groups in compound (**3b**) were significantly different. After 12 h at room temperature in the presence of acetic anhydride and pyridine, acetylation of 4β-OH was complete, whereas that of the ring D secondary hydroxy group proceeded only to an extent of *ca.* 30% to give a mixture of diacetate (**3c**) (minor component) and monoacetate (**3d**) (major component). Acetylation of the secondary hydroxyls was complete only when performed for longer periods of time; as expected, the tertiary alcohol (*vide infra*) was unaffected.

In order to confirm and supplement the ^{1}H n.m.r. information and that obtained by the simple chemical transformations described above, the ^{13}C n.m.r. spectra (Table 2) of compound (**2a**), its dihydro derivative (**3a**), and the hydrolysis product (**3b**) of the latter were taken. For compounds (**2a**) and (**3a**), single-frequency off-resonance decoupled spectra provided information not only on signal multiplicity, but also allowed, *via* residual couplings, a direct link between carbon and proton resonances; this is especially useful in the assignment of the methyl groups. The oxygenation pattern of rings A and B in compound (**2a**) is confirmed by the almost identical carbon shifts of ring A to those found in withanolide D;[5] the signals of the carbons in the side-chain lactone are also easily identified.

The remaining resonances indicate the presence of a tertiary hydroxy group which might be attached at C-14 or C-17, and a secondary acetate which should be at either C-15 or C-16 (see above). The task of assigning the positions of these substituents is made easier by our previous experience with withanolides of a variety of hydroxylation patterns.[5] Of the two possible locations of the tertiary hydroxy group, position 17 (α or β) is excluded because it would have required a higher degree of shielding of C-12 and of deshielding of C-20 than actually found (Table 2). Since a 14α-OH group would also shield C-12 through a γ-effect, we are left only with the 14β-OH possibility. The lack of shielding of C-12 and the deshielding of C-18 in compound (**2a**), as compared with the carbon shifts in 14-unsubstituted steroids, is consistent with the results obtained with 14β-hydroxycardenolides.[6] It is worth mentioning that in contrast to cardenolides and bufadienolides in which the presence of a 14β-OH group is a common feature,[7] there exists,

Table 2. ^{13}C N.m.r. assignments of physapubenolide (**2a**) and derivatives

Carbon	Compound (**2a**)	(**3a**)	(**3b**)[a]	Carbon	Compound (**2a**)	(**3a**)	(**3b**)[a]
1	202.6	211.3	213.2	16	33.8	33.7	36.5
2	131.5	31.7	31.6	17	52.3	52.2	51.9
3	143.5	26.8	26.4	18	15.6	15.7	14.4
4	69.6	72.5	73.1	19	17.2	15.3	15.2
5	63.4	66.0	66.3	20	37.5	37.4	37.8
6	62.7	60.0	59.3	21	17.2	17.2	17.3
7	26.1	26.1	26.2	22	78.5	78.4	78.3
8	40.3	39.0	39.3	23	31.2	31.2	30.6
9	36.1	35.8	37.1	24	149.5	149.3	150.8
10	47.9	50.8	51.1	25	121.9	122.0	121.9
11	21.8	21.1	20.6	26	166.8	166.7	168.1
12	41.4	41.0	41.5	27	12.4	12.4	12.4
13	46.1	46.3	45.7	28	20.5	20.5	20.6
14	84.1	84.2	85.2	$COCH_3$	169.8	169.6	
15	80.8	80.7	79.3	$COCH_3$	21.5	21.6	

[a] A few drops of MeOH were added to improve the solubility.

Table 3. Solvent shifts Δ/p.p.m. [$\delta(CDCl_3) - \delta(C_5D_5N)$] of methyl groups in physapubenolide (**2a**) and related compounds

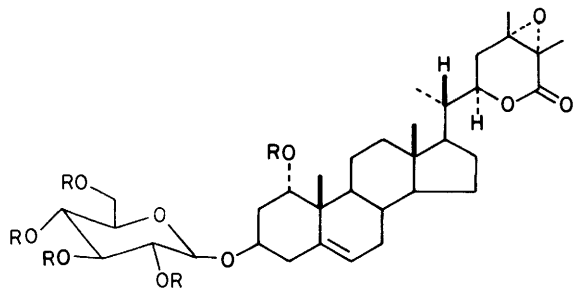
Compound		$\delta(CDCl_3)$	$\delta(C_5D_5N)$	Δ
Withanone (**5a**)[3]	18-H_3	0.85	0.86	−0.01
	21-H_3	1.04	1.20	−0.16
14β-Hydroxywithanone (**5b**)[8]	18-H_3	1.16	1.52	−0.36
	21-H_3	1.06	1.43	−0.37
Physapubenolide (**2a**)	18-H_3	1.11	1.32	−0.21
	19-H_3	1.40	1.82	−0.42
	21-H_3	1.02	1.08	−0.06
(**2b**)	18-H_3	1.10	1.32	−0.22
	19-H_3	1.38	1.66	−0.28
	21-H_3	1.01	1.09	−0.08
(**3a**)	18-H_3	1.08	1.30	−0.22
	19-H_3	1.32	1.68	−0.36
	21-H_3	1.00	1.08	−0.08
(**3b**)	18-H_3	1.04	1.33	−0.29
	19-H_3	1.30	1.72	−0.42
	21-H_3	1.03	1.10	−0.07
(**4**)	18-H_3	1.15	1.28	−0.13
	19-H_3	1.31	1.69	−0.38
	21-H_3	1.03	1.12	−0.09

so far, only one report of such a withanolide [14β-hydroxywithanone (**5b**)[8]]. All other withanolides and related ergostanes are either 14α-H or 14α-OH compounds.[1]

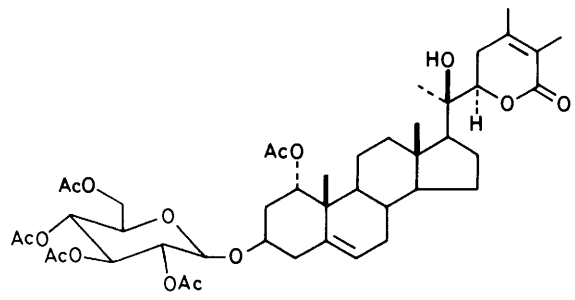
The β-orientation of the 14-OH group is confirmed by pyridine-induced solvent shifts [$\delta(CDCl_3) - \delta(C_5D_5N)$] of the C-18 and C-21 methyl groups in the ^1H spectra of physapubenolide (**2a**) and its derivatives (**2b**), (**3a**), (**3b**), and (**4**) (Table 3). The data were compared with the corresponding shifts in withanone (**5a**)[3] and 14β-hydroxywithanone (**5b**).[8] The pyridine-induced shifts in withanone (14α-H; 17α-OH) indicate a significant deshielding effect of 17α-OH on the signal of 21-H_3, but not on that of 18-H_3. This is in agreement with the relative positon of 18-H_3 with respect to 17α-OH (dihedral angle of *ca.* 150°). In 14β-hydroxywithanone (**5b**) (14β-OH; 17α-OH) the geometry of the molecule is changed by the *cis* junction between rings C and D, and the effect of the two hydroxy groups is clearly indicated by major shifts downfield for both methyl groups (−0.36 p.p.m. for 18-H_3 and −0.37 p.p.m. for 21-H_3).

In physapubenolide (**2a**) (a 17α-H compound) and its derivatives (**2b**) and (**3a**), there is a consistently strong solvent effect on the signal of 18-H_3 (−0.21 to −0.22 p.p.m.) and a weaker influence on the signal of 21-H_3 (−0.06 to −0.08 p.p.m.). Such effects can be interpreted only by assuming a β orientation of the 14-OH group, as indicated by the ^{13}C n.m.r. data.

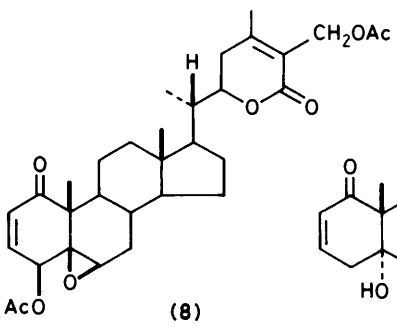
The last problem to solve is the position and orientation of the secondary acetate in ring D. The ^{13}C chemical shift of C-17 in physapubenolide is 52.3 p.p.m., very near to the 51.9 p.p.m. value[5] in withaferin A, a compound in which ring D is unsubstituted. The absence of a β-effect at C-17 implies that the acetate group might be attached at C-15 but not at C-16. Saturation of the 2,3-double bond [(**2**) ⟶ (**3a**)] affects the chemical shifts of ring A carbons and brings about small changes in the resonances of carbons 6, 8, and 11; as expected, the resonances of ring D and side-chain carbons are unaffected (Table 2). Hydrolysis of the acetate group [(**3a**) ⟶ (**3b**)] has, however, more far-reaching effects than usual.[6] Resonances for carbons 6, 9, and 18 change by more than 1 p.p.m. and most other resonances are affected by *ca.* ±0.5 p.p.m. These changes suggest small but significant conformational differences between compounds (**3a**) and (**3b**) which are best explained if the 15-OH group is a α oriented ('*endo*' with respect to the carbocyclic skeleton). Such an orientation is in accord with the



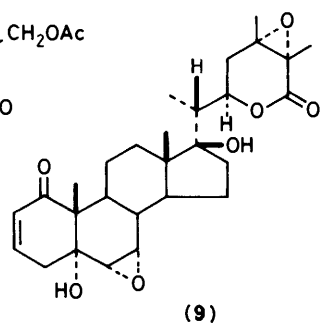
(**6a**) R = H Pubescenin
(**6b**) R = Ac



(**7**)



(**8**)



(**9**)

pattern (doublet) for 15-H in the ^1H n.m.r. spectra of physapubenolide and its derivatives. Such a pattern implies a dihedral angle close to 90° between 15-H and one of the two C-16 protons. Inspection of models indicate that such a situation can exist only when 15-H is β oriented.

The hindrance of a 15α-OH group in a 14β-OH steroid may explain the ease of its oxidation [(**3b**) ⟶ (**4**)] and the difficulty of its acetylation [(**3b**) ⟶ (**3c**) + (**3d**)]. Furthermore, the alternative 15β-OH configuration for compound (**3b**) would have required a significant increase in the pyridine shift of the 21-H_3 group, as compared with that measured in the corresponding 15-acetate (**3a**). In both compounds, the pyridine-induced shifts of 21-H_3 are similar (−0.08 and −0.07 p.p.m., respectively); the difference between the corresponding solvent shifts of 18-H_3 (−0.22 and −0.29 p.p.m., respectively) fits better with a 14β-OH;15α-OH structure than with a 14β-OH;15β-OH structure.

Physapubenolide (**2a**) is therefore (20*S*,22*R*)-15α-acetoxy-5β,6β-epoxy-4β,14β-dihydroxy-1-oxowitha-2,24-dienolide.

In contrast to other steroidal lactones, such as cardenolides and bufadienolides,[7] which exist in Nature mainly as the corresponding 3β-*O*-glycosides, the vast majority of withanolides and biogenetically related compounds are found as free aglycones. A possible explanation is that most compounds of

Table 4. ^{1}H N.m.r. data[a] of pubescenin penta-acetate (**6b**) and physalolactone B glucoside tetra-acetate (**7**)

Proton	Compound (**6b**)	Compound (**7**)	Proton	Compound (**6b**)	Compound (**7**)
1-H	5.05 t (2.5)	5.05 t (2.5)	1′-H	4.58 d (8)	4.58 d (7.9)
3-H	3.82 m	3.82 m	2′-H	4.93 dd (9.3; 8)	4.94 dd (9.4; 7.9)
6-H	5.52 d (5.5)	5.52 d (5.5)	3′-H	5.20 t (9.3)	5.20 t (9.4)
22-H	4.53 dt[b]	4.30 dd (12; 4.5)	4′-H	5.05 dd (9.3; 8.5)	5.05 dd (9.4; 8.6)
18-H_3	0.69 s	0.86 s	5′-H	3.68 ddd (8.5; 4.9; 2.4)	3.67 ddd (8.6; 5.0; 2.5)
19-H_3	1.05 s	1.06 s	6′-H_2	4.09 dd (12.2; 2.4), 4.23 dd (12.2; 4.9)	4.09 dd (12.0; 2.5), 4.23 dd (12.0; 5.0)
21-H_3	0.91 d (6.6)	1.27 s			
27- and 28-H_3	1.56 s, 1.48 s	1.87 s, 1.95 s			

[a] Recorded at 270 MHz on a Bruker WH-270 spectrometer; solvent $CDCl_3$; δ values; coupling constants (Hz) in parentheses. Primed numbers refer to carbohydrate moiety. [b] Partially obscured by the signal of 1′-H.

Table 5. ^{13}C N.m.r. assignments (δ/p.p.m.) of pubescenin penta-acetate (**6b**) and several model compounds

Carbon	Compound (**6b**)	(**7**)[b]	(**8**)[c]	(**9**)[d]
C-1	74.9	74.9	201.1	202.1
C-2	33.7	33.7	133.9	129.0
C-3	74.9	74.9	139.8	139.5
C-4	38.1	38.1	72.2	36.8
C-5	136.7	136.7	61.0	73.2
C-6	124.9	124.7	60.2	56.3
C-7	28.8	31.6	31.1	56.8
C-8	31.7	31.2	29.6	36.7
C-9	42.3	42.1	44.1	35.1
C-10	40.5	40.6	48.1	50.9
C-11	20.7	20.6	21.3	21.7
C-12	39.6	39.9	39.2	32.1
C-13	42.8	43.0	42.6	48.5
C-14	56.5	56.8	56.1	46.1
C-15	24.3	23.9	24.2	22.7
C-16	27.3	22.1	27.3	35.1
C-17	52.1	54.6	51.9	85.1
C-18	11.7	13.6	11.5	14.8
C-19	19.4	19.4	15.7	14.8
C-20	38.6	75.1	38.8	40.5
C-21	13.7	21.1	13.3	13.6
C-22	76.3	81.0	78.2	76.8
C-23	31.7	31.6	30.1	32.7
C-24	59.3	148.9	157.1	59.1
C-25	62.6	122.0	121.9	63.1
C-26	170.6	166.1	165.3	170.5
C-27	13.0	12.5	58.0	12.7
C-28	18.0	20.6	20.6	17.9
C-1′	99.6	99.6		
C-2′	71.4	71.4		
C-3′	72.9	72.9		
C-4′	68.5	68.6		
C-5′	71.8	71.8		
C-6′	62.1	62.1		
5 × OAc	*e*			

[a] Primed numbers refer to atoms in carbohydrate moiety. [b] Physalolactone B 3-*O*-β-glucopyranoside.[10] [c] Withaferin A diacetate.[5] [d] Nic-2 lactone.[5] [e] 170.3; 170.0; 169.4 (×2); 169.2 for C=O. 20.7 (×4); 21.1 for CH_3.

this group are 2-en-1-ones which are formed by stepwise oxidation of a simple 5-en-3β-ol precursor.[1a]

The first withanolide glycosides, dunawithanine A and B, were isolated by Adam and co-workers[9] from *Acnistus australis*. They were followed by physalolactone B glucopyranoside,[10]* isolated from *Physalis peruviana*. The three compounds have the same aglycone, namely 1α-acetoxy-3β,20-dihydroxywitha-5,24-dienolide. An additional glycoside, pubescenin, which was isolated from *Physalis pubescens*, Waltair variety, was identified as compound (**6a**) by ^{1}H and ^{13}C n.m.r. analysis of the corresponding penta-acetate (**6b**). According to this analysis pubescenin is (20*S*,22*R*)-1α-hydroxy-3β-*O*-(β-D-glucopyranosyl)-24,25-epoxywith-5-enolide. The β-glycoside-type linkage is evident from the ^{1}H n.m.r. signal of 1′-H (δ_H 4.58, doublet, *J* 8 Hz) and that of the anomeric carbon in the ^{13}C n.m.r. spectrum (99.6 p.p.m.) in the corresponding penta-acetate (**6b**).

The ^{1}H assignments are made by comparison with those of physalolactone B 3-*O*-β-D-glucopyranoside tetra-acetate (**7**) (Table 4). The only differences are in the signals of 22-H and of three methyl groups, (C-21, -27, and -28). The ^{13}C assignments (Table 5) are based on comparison with the spectra of three known compounds: (a) physalolactone B glucoside (**7**) for the resonances of the sugar moiety carbons and for those of rings A and B; (b) withaferin A diacetate (**8**)[5] for the resonances of rings C and D carbons; and (c) nic-2 lactone (**9**)[5] for the signals of the side-chain carbons.

Experimental

M.p.s were taken with a Fisher–Johns apparatus. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter and refer to solutions in chloroform, unless otherwise stated. C.d. measurements were performed with a Cary 60 instrument for solutions in acetonitrile. I.r. spectra were recorded on a Perkin-Elmer Infracord 137 spectrophotometer and refer to chloroform solutions; u.v. spectra were recorded on a Cary 14 instrument for solutions in ethanol; ^{1}H n.m.r. spectra were recorded at 270 MHz on a Bruker WH instrument, and ^{13}C n.m.r. spectra were recorded at 22.63 MHz on a Bruker WH-90 instrument. Analyses were performed in the Microanalytical Laboratory of the Weizmann Institute under the direction of Mr. R. Heller, and in the Microanalytical Laboratory of the Hebrew University under the direction of Mrs. S. Blum.

Plant Material.—*Physalis pubescens* L. plants were raised in 1981 at the Experimental Farm of the Faculty of Agriculture from seeds collected in India (outskirts of Waltair) by Dr. S. S. Nittala.

Isolation Procedure.—Crushed, dry leaves (1.5 kg) were extracted with methanol (Soxhlet). The extract was concentrated to a volume of 1.5 l, an equal volume of water was slowly added, and the mixture was stirred for several hours. The

* We apologize for not being aware of the work of Professor G. Adam and co-workers (ref. 9*a*) who were the first to isolate and characterize glycosides in this series.

solution obtained after filtration was extracted with light petroleum (60—80 °C) and then with chloroform (3 × 1.5 l). The combined chloroform extracts were washed with water, dried (Na_2SO_4), and the residue obtained after evaporation of the solvent was chromatographed on silica gel 60, 230—400 mesh (Merck).

Elution with light petroleum–ethyl acetate (1:1) afforded *physapubenolide* (**2a**) (1.2 g) which was crystallized from chloroform–benzene as fine needles, m.p. 144—145 °C; $[\alpha]_D$ +76° (*c* 0.16); $\lambda_{max.}$ 223 nm (ε 14 000) (Found: C, 68.1; H, 7.7%; M^+, 528. $C_{30}H_{40}O_8$ requires C, 68.2; H, 7.6%; *M*, 528.6).

Further elution with ethyl acetate–methanol (9.5:0.5) afforded *pubescenin* (**6a**) (0.9 g) as needles from methanol, m.p. 188—189 °C; $[\alpha]_D$ −38.6° (*c* 0.12) (Found: C, 63.8; H, 8.4. $C_{34}H_{52}O_{10}\cdot H_2O$ requires C, 63.9; H, 8.5%).

Acetylation of Physapubenolide (**2a**).—Acetylation was performed with pyridine and acetic anhydride, overnight at room temperature. The product was precipitated with water and collected by filtration. It was purified on a preparative chromatoplate (1 mm thickness) and characterized as compound (**2b**) by its n.m.r. spectrum, $[\alpha]_D$ +84° (*c* 0.2). It could not be induced to crystallize.

Catalytic Hydrogenation of Physapubenolide (**2a**).—Physapubenolide (300 mg) was hydrogenated over 10% Pd–$CaCO_3$ in absolute ethanol (100 ml) at room temperature and atmospheric pressure. The reaction was discontinued after absorption of one mol equiv. of hydrogen, the catalyst was removed, and the product (**3a**) was isolated after evaporation of the solvent and was crystallized from chloroform as *needles*, m.p. 135—136 °C; $[\alpha]_D$ +49.5° (*c* 0.3) (Found: C, 68.0; H, 8.1. $C_{30}H_{42}O_8$ requires C, 67.9; H, 8.0%). Acetylation of compound (**3a**) as described for physapubenolide (**2a**) afforded the acetate (**3c**), $[\alpha]_D$ +54° (*c* 0.1). The product could not be crystallized.

Hydrolysis of Dihydrophysapubenolide (**3a**).—The compound (100 mg) was dissolved in 5% methanolic KOH (20 ml) and the solution was set aside for 12 h. The solution was then brought to pH *ca.* 4 (dil. HCl), water was added, and the product was extracted with chloroform. Work-up gave the *triol* (**3b**), m.p. 282—283 °C (from methanol); $[\alpha]_D$ +23.5° (*c* 0.1); c.d.: $\Delta\varepsilon_{286}$ −4.5; $\Delta\varepsilon_{247}$ +4.7 (Found: C, 68.8; H, 8.4. $C_{28}H_{40}O_7$ requires C, 68.8; H, 8.25%).

Oxidation of the trans-*Glycol* (**3b**) *to the Acyloin* (**4**).—To a stirred solution of compound (**3b**) (60 mg) in acetone (20 ml) was added PCC (2.5 mol equiv.) in small portions. After 2 h at room temperature, the solution was decanted, the residue was washed several times with acetone, and the combined solutions were filtered through a small column of silica gel (10 g). The residue obtained after evaporation of the solvent was crystallized from chloroform, to give the *acyloin* (**4**) m.p. 236—238 °C; $\nu_{max.}$ 1 747 and 1 707 cm^{-1}; c.d.: $\Delta\varepsilon_{280}$ −5.4; $\Delta\varepsilon_{247}$ +4.9 (Found: C, 69.0; H, 7.9. $C_{28}H_{38}O_7$ requires C, 69.1; H, 7.9%).

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