Withaperuvin and 4-Deoxyphysalolactone, Two New Ergostane-type Steroids from *Physalis peruviana* (Solanaceae)

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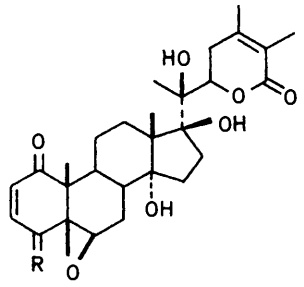
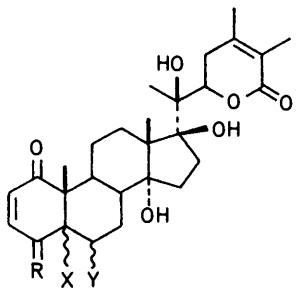
Withaperuvin (17*S*,20*S*,22*R*-4β,5β,6α,14α,17β,20α_F-hexahydroxy-1-oxoergosta-2,24-dien-22,26-olide) was isolated from the roots of *Physalis peruviana* (Varanasi variety). The structure of the carbocyclic moiety was solved by *X*-ray analysis of the androstane derivative obtained by CrO_3 oxidation of withaperuvin-4,6-diacetate. The structure of the side-chain was inferred by spectral analysis. 4-Deoxyphysalolactone (17*S*,20*S*,22*R*-6α-chloro-5β,14α,17β,20α_F-tetrahydroxy-1-oxoergosta-2,24-dien-22,26-olide) is a minor steroidal constituent of the leaves of the same plant variety. Its structure was deduced by spectral analysis.

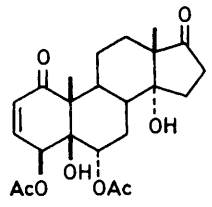
THE main steroidal constituents of *Physalis peruviana* (Solanaceae) growing in Southern India (the Ooty Hills, near Pondicherry) are withanolide E (1),[1a] withanolide S (2),[1a,b] and 4β-hydroxywithanolide E (3).[1c] These compounds are practically absent in the variety growing near Varanasi (Uttar Pradesh). Previous investigations on this population of *P. peruviana* resulted in the isolation of perulactone,[2] which represents an early stage in the biogenetic oxidative process leading to the withanolides, and of physalolactone (4a),[3] a *trans*-diequatorial chlorohydrin related to (3).



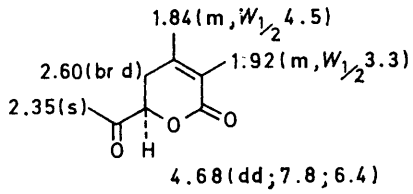
(1) R = H_2
(3) R = β-OH, α-H



(2) R = H_2 ; X = α-OH ; Y = β-OH
(4a) R = β-OH, α-H ; X = β-OH ; Y = α-Cl
(4b) R = β-OAc, α-H ; X = β-OH ; Y = α-Cl
(5a) R = β-OH, α-H ; X = β-OH ; Y = α-OH
(5b) R = β-OAc, α-H ; X = β-OH ; Y = α-OAc
(8) R = H_2 ; X = β-OH ; Y = α-Cl



(6)



(7)

We present now the structures of two new ergostane-type steroids which were isolated from the Varanasi variety of *P. peruviana*: withaperuvin (5a) (17*S*,20*S*,22*R*-4β,5β,6α,14α,17β,20α_F-hexahydroxy-1-oxoergosta-2,24-dien-22,26-olide) was found in the roots, and 4-deoxyphysalolactone (8) (17*S*,20*S*,22*R*-6α-chloro-5β,14α,17β,20α_F-tetrahydroxy-1-oxoergosta-2,24-dien-22,26-olide) is a minor companion of perulactone and of physalolactone in the leaves of the plant. The structure of (5a) was solved by crystallographic analysis of the androstane derivative (6) obtained by CrO_3 oxidation of the diacetate (5b), whereas that of (8) was deduced by analysis of its spectral data.

RESULTS AND DISCUSSION

Withaperuvin (5a) $C_{28}H_{40}O_9 \cdot H_2O$, m.p. 225—227 °C, has ^1H and ^{13}C n.m.r. spectra which indicate clearly the

TABLE 1

^{1}H N.m.r. data *

Compound	2-H	3-H	4-H	6-H	22-H	18-Me	19-Me	21-Me	27- and 28-Me	MeCO
(3b)	6.30d (10.0)	7.08dd (10.0; 6.5)	4.66d (6.5)	3.31br,s ($W_{\frac{1}{2}}$ 4)	4.78dd (10.0; 6.5) [5.15]	1.08s [1.32]	1.41s [1.72]	1.41s [1.73]	1.95s; 1.89s [1.91; 1.82]	2.03s
(4b)	6.04dd (11.3; 2.5)	6.34dd (11.3; 3.0)	6.33t (2.7)	4.34dd (12.7; 4.5)	4.79dd (10.0; 6.5)	1.05s	1.28s	1.41s	1.92s; 1.86s	2.13s
(5a)	[6.28dd]	[6.89dd]	[5.77t]	[4.53dd]	[5.16dd]	[1.31s]	[1.60s]	[1.78s]	[1.98s; 1.82s]	
(5b)	6.04dd (10.4; 2.1) [6.29]	6.36dd (10.4; 2.3) [6.65]	6.44t (2.2) [6.92]	5.21dd (11.9; 4.9) [5.56]	4.80dd (10.0; 6.5) [5.18]	1.06s [1.29]	1.30s [1.57]	1.41s [1.75]	1.93s; 1.86s	2.17s; 2.03s
(6)	6.10dd (10.4; 2.1)	6.40dd (10.4; 2.3)	6.42t (2.2)	5.14dd (11.9; 4.9)		0.99s	1.32s			2.20s; 2.08s
(8)	6.01dd (10.0; 2.5)	6.75ddd (10.0; 5.5; 2.5)	H_{ax} 3.00dt (20.0; 2.5) H_{eq} 2.55dd (20.0; 5.5)	4.34dd (11.5; 7.0)	4.81dd (10.0; 6.5)	1.07s	1.23s	1.42s	1.93s; 1.86s	

* Recorded at 270 MHz on a Bruker WH-270 spectrometer; solvent $CDCl_3$; δ values; data for solutions in C_5D_5N in square brackets; coupling constants (Hz) in parentheses; estimated error ±0.1 Hz.

presence of the same 17α-oriented side-chain as in compounds (1)—(4) (Tables 1 and 2). In addition, the ^{1}H n.m.r. spectrum of (5a) shows an ABX spectrum for three protons, at δ 6.28 (2-H), 6.89 (3-H), and 5.77 (4-H), as well as a signal at δ 4.53 (6-H). In the diacetate (5b) the signal of 6-H was shifted to δ 5.56, and the already low-field signal of 4-H was shifted to δ 6.92. In both compounds, 4-H resonates at positions which are unusually low for C*H*OH and C*H*OAc protons; on initial inspection, this may have suggested a hemiacetal type structure for this proton. Such an assignment was not confirmed, however, by the ^{13}C n.m.r. data of (5a); in addition to four oxycarbons (14, 17, 20, and 22) which were readily assigned, the spectrum pointed to the presence of three other oxycarbons (δ 67.0, 74.5, and 79.4), neither one being of the hemiacetal type.

TABLE 2

^{13}C N.m.r. data [a]

Carbon	(5a)	(5b) [b]	(4a)	(8)	(1)
1	202.4	201.1	*c*	*c*	204.2
2	127.2	128.2	126.8	127.2	129.7
3	145.9	142.3	145.5	144.4	142.3
4	67.0	67.8	66.0 [d]	34.6 [d]	33.1
5	79.4	78.8	78.8	77.8	62.6
6	74.5	76.6	65.5 [d]	66.9	64.5
7	32.0	28.6	34.5	31.9	26.4
8	37.4	38.0	39.3	39.3	34.2
9	37.8	38.0	38.4	37.8	37.0
10	55.6	57.0	57.0	55.8	48.9
11	22.7	22.9	22.8	23.0	23.2
12	34.5	34.5	34.5	34.5 [d]	34.7
13	54.0	54.8	54.9	55.0	54.7
14	82.8	82.1	82.3	82.2	82.7
15	30.1	30.2	30.1	30.2	30.5
16	37.4	37.3	37.2	37.8	37.2
17	87.6	87.6	87.4	87.7	87.7
18	20.7	20.7	20.7	20.9	20.7
19	9.7	9.6	9.5	8.7	14.7
20	78.8	78.8	78.8	78.8	78.9
21	19.1	19.1	19.0	19.2	19.0
22	80.9	81.1	80.9	81.0	81.5
23	32.2	32.2	32.2	32.5	32.6
24	151.9	152.0	152.0	152.0	152.3
25	121.2	121.2	121.3	121.4	121.3
26	167.7	167.8	*c*	167.7	168.1
27	12.2	12.3	12.2	12.4	12.2
28	20.7	20.7	20.7	20.9	20.7

[a] In p.p.m. from internal $SiMe_4$ for $CDCl_3$–MeOH solutions. [b] δ($MeCO_2$) = 21.2, 21.3, 169.5, and 171.2. [c] Signal not observed due to low signal-to-noise ratio. [d] Signals within a vertical column may be interchanged.

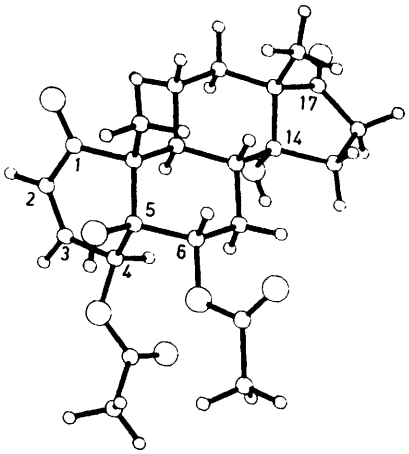
This apparent discrepancy between the ^{1}H and ^{13}C n.m.r. data was solved by *X*-ray analysis of compound (6), which disclosed its structure to be 4β,6α-diacetoxy-5β,14α-dihydroxyandrost-2-ene-1,17-dione. The lattice parameters and the relevant data are as follows: space group $P2_1$; $a = 6.176(2)$, $b = 11.190(3)$, $c = 15.033(4)$ Å; $\beta = 95.14°$; $U = 1\,034.7$ Å^3; $D_m = 1.30$ g cm^{-3}; $Z = 2$; $D_c = 1.30$ g cm^{-3}. Intensity data (2 373 reflections) were collected at 83 K with Mo-K_α radiation on a CAD-4 automatic diffractometer. The structure was solved by the MULTAN computer program, and refined anisotropically by full-matrix least squares to $R = 0.052$, $R' = 0.061$. Full crystallographic details will be published elsewhere.

According to this analysis ring A is in a twisted boat conformation with C-1, C-2, and C-3 nearly in the same plane; C-4 is slightly below the plane and is held in this position by hydrogen bonding between 5β-OH and the ether oxygen of 4β-OAc. Rings B and C are in the chair conformation. The dihedral angles between the best planes of the four rings are: A/B 67°; B/C 3°; C/D 9° (see Figure).

The *cis* relationship between rings A and B in withaperuvin introduces a *gauche* interaction between C-19 and the 5β-OH group, which results in a considerable shielding of the C-19 resonance [δ 9.6 in (5b) as compared to 15.2 in (3)]. Furthermore, C-4 is axial with respect to ring B and its signal appears at *ca.* 5 p.p.m. to higher field relative to other 4β-acetoxylated withanolides [*cf.* δ 72.5 in the 4-acetate of (3)]; this is due mainly to the close proximity between 4α-H and 7α-H. The resulting steric compression is expressed also in the unusual low-field resonance of 4α-H in (5b). The preferred conformation of (5b) in solution should be that in which 4α-H enters into the deshielding region of the carbonyl group of the 6α-acetate [in the crystal (compound 6), the carbonyl oxygen is directed toward C-7]. This geometry seems to be favoured by the distortion introduced in

ring A by the hydrogen bond between 5β-OH and 4β-OAc.

The Cotton effect at *ca.* 340 nm is usually considered as a reliable diagnostic tool for the recognition of the junction between rings A and B in 2-en-1-one steroids: this effect is negative in the 5α-isomers, and positive in the 5β-counterparts.[4] The conformational differences between rings AB in withaperuvin (5a) and other 5β-steroids are dramatically expressed in their chiroptical properties. The curves are positive and of the same magnitude in withanolide E (1) and 4β-hydroxywithanolide E (3) ($\Delta\varepsilon_{340}$ +1.69,[1a] and $\Delta\varepsilon_{340}$ +1.42,[1c] respectively). The effect is still positive, but of half the intensity, in 4-deoxyphysalolactone (8) (5β-hydroxy-6α-chloro-substituents; $\Delta\varepsilon_{342}$ +0.89; $\Delta\varepsilon_{334}$ +0.85). The conformational change begins to be evident in physalolactone (4a) (4β,5β-dihydroxy-6α-chloro-substituents) in which the Cotton effect remains positive, but it is of very low intensity ($\Delta\varepsilon_{365}$ +0.20; $\Delta\varepsilon_{345}$ +0.28; $\Delta\varepsilon_{336}$ +0.25).



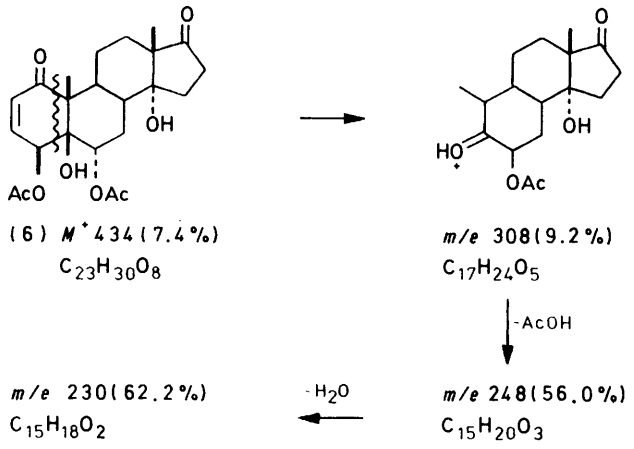
X-Ray structure of compound (6)

The tendency toward negative values is fully developed in withaperuvin (4β,5β,6α-trihydroxy-substituents) which has a negative curve ($\Delta\varepsilon_{370}$ −0.25; $\Delta\varepsilon_{354}$ −0.53; $\Delta\varepsilon_{339}$ −0.67; $\Delta\varepsilon_{322}$ −0.60) comparable to that present in withanolide S (5α,6β-dihydroxy-substituents; $\Delta\varepsilon_{336}$ −1.32).[1a] The fine structure in the Cotton effect of (5a) confirms the strong compression due to the interaction between the substituents of carbons 4, 5, and 6.

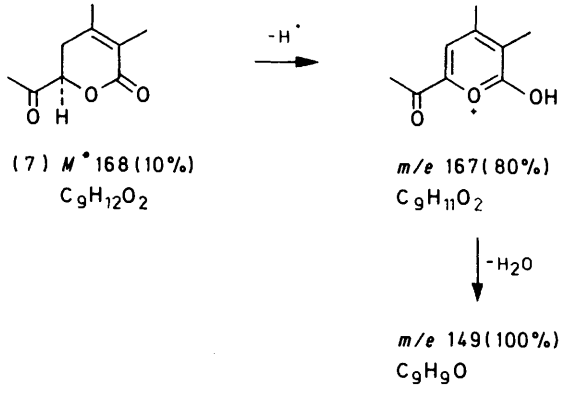
In addition to the androstane derivative (6), the oxidation of (5b) resulted in the δ-lactone methyl ketone (7) which was previously obtained by a similar degradation of 4β-hydroxywithanolide E (3),[5] and of physalolactone (4a).[2]

The fragmentation under electron impact of compound (6), M^+ 434, is dominated by cleavage of the 1–10 and 4–5 bonds, leading to the fragment $C_{17}H_{24}O_5$, *m/e* 308 (9.2%), which subsequently loses AcOH and H_2O to give the most important fragments $C_{15}H_{20}O_3$, *m/e* 248 (56%) and $C_{15}H_{18}O_2$, *m/e* 230 (62.2%).

Most interesting is the fragmentation of the δ-lactone methyl ketone (7). The molecular ion of this compound is of low relative intensity, M^+ 168 (10%), because it easily eliminates a hydrogen to give a fragment of *m/e* 167 (80%); the latter subsequently loses water to give the base peak, *m/e* 149 (100%).



The structure of 4-deoxyphysalolactone (8), m.p. 207—208 °C, was deduced by comparing its ^1H and ^{13}C n.m.r. data with those of withanolide E (1) and physalolactone (4a). The molecular ion could not be obtained by mass spectrometry; under electron impact, the compound easily loses HCl to give the highest peak of significant intensity at *m/e* 486. The only signal ($M^+ - H_2O$ at *m/e* 504) which could have eventually confirmed the presence of chlorine was of such low intensity that it was impossible to detect the isotopic peak at *m/e* 506. Thus the evidence for chlorine was obtained by classical means (Beilstein's test and microanalysis). The chemical shift of C-6 (δ 66.9) was in good agreement with a C–Cl structure (δ 65.5 in physalolactone), whereas the pattern and the position of 6-H [δ 4.34 (dd, *J* 11.5, 7.0 Hz) was similar to that found in physalolactone acetate (4b), and confirmed the equatorial orientation of the adjacent chlorine. Furthermore, the chemical shift of C-19 (δ 8.7), which was similar to that found in (5a), confirmed the strong interaction of this carbon with 5β-OH.



EXPERIMENTAL

M.p.s were taken with a Fisher-Johns apparatus. Optical rotations were recorded with an automatic Perkin-Elmer polarimeter and refer to solutions in acetonitrile. C.d.

measurements were performed in the same solvent with a Cary 60 instrument. I.r. spectra were recorded on a Perkin-Elmer Infrared 137 spectrophotometer and refer to KBr pellets; 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; ^{13}C n.m.r. spectra were recorded at 22.63 MHz on a Bruker WH-90 instrument operating in the Fourier-transform mode. Mass spectra were determined with a Varian MAT 731 high-resolution mass spectrometer, under the supervision of Dr. Z. V. Zaretskii. Analyses were performed in the Microanalytical Laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

Plant Material.—Leaves and roots of *Physalis peruviana* were collected in April 1979, in the suburbs of Varanasi City where it is cultivated for its edible berries.

Isolation of Withaperuvin (5a).—Air-dried roots (2 kg) were crushed and extracted with 95% ethanol by cold percolation. The extract was concentrated under reduced pressure to a small volume (1 l) and then diluted with an equal volume of water. The solution was extracted successively with light petroleum (60—80 °C) and ether. The residue from the ether extract was chromatographed over silica gel (B.D.H.). Elution with benzene–ethyl acetate (1 : 4) furnished a near-homogeneous fraction which was purified by passing through a short bed of silica gel G (Centron) and eluting with ethyl acetate. The residue from this eluate crystallised from methanol (0.05% from the dry roots), needles, m.p. 225—227 °C, $[\alpha]_D$ +156.8° (*c* 0.15); $\nu_{max.}$ 1 710 and 1 675 cm^{-1}; $\lambda_{max.}$ 214 nm (ε 18 000) (Found: C, 62.0; H, 7.5. $C_{28}H_{40}O_9 \cdot H_2O$ requires C, 62.43; H, 7.86%).

Acetylation of withaperuvin was performed with acetic anhydride and triethylamine, overnight at room temperature. The diacetate (5b) had m.p. 219—221 °C (from chloroform); $[\alpha]_D$ +146.3° (*c* 0.20); $\nu_{max.}$ 1 720, 1 670, 1 250, and 1 210 cm^{-1}; $\lambda_{max.}$ 218 nm (ε 17 000) (Found: M^+ — AcOH, 544; C, 61.2; H, 7.15. $C_{32}H_{44}O_{11} \cdot H_2O$ requires C, 61.72; H, 7.45%).

CrO_3 *Oxidation of Withaperuvin Diacetate.*—A solution of withaperuvin diacetate (150 mg) in acetone (40 ml) was treated with Jones reagent (0.3 ml) at room temperature. The reaction mixture was left for 30 min when the green chromium salts settled. The supernatant was decanted off, mixed with the acetone washings of the residue, and evaporated to dryness at room temperature. Chromatography over silica gel [benzene–ethyl acetate (9 : 1)] gave an oil (7), identical with that obtained by similar oxidation of physalolactone.[3] Further elution with a more polar mixture [benzene–ethyl acetate (7 : 3)] afforded a solid (6) which crystallised from benzene as stout needles, m.p. 279—280 °C, $[\alpha]_D$ +105.3° (*c* 0.2); $\nu_{max.}$ 3 530, 1 745, 1 720, and 1 668 cm^{-1}, $\lambda_{max.}$ 216 nm (ε 8 850) (Found: M^+, 434.192 3. Calc. for $C_{23}H_{30}O_8$: M^+, 434.193 2).

4-*Deoxyphysalolactone* (8).—Extraction of the leaves of the same variety of *P. peruviana* and subsequent column chromatography,[2] afforded, *inter alia*, perulactone (20*R*,-22*R*,24*S*,25*R*-1α-acetoxy-3β,20,22-trihydroxyergost-5-en-26,28-olide). Re-chromatography of the crude product (100 mg) on 1-mm preparative layer chromatoplates of silica gel PF_{254} (Merck 20 × 40 cm) gave (8); (5 mg), m.p. 207—208 °C (from ethyl acetate), $[\alpha]_D$ +103° (c 0.1); $\nu_{max.}$ 1 710 and 1 760 cm^{-1}; $\lambda_{max.}$ 220 nm (ε 17 900) (Found: Cl, 6.65. $C_{28}H_{39}ClO_7$ requires Cl, 6.78%).

We thank Dr. A. Gedanken (Bar-Ilan University) for c.d. measurements.

[0/998 *Received*, 27*th June*, 1980]

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