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PHYSALOLACTONE C, A NEW WITHANOLIDE FROM *PHYSALIS PERUVIANA*

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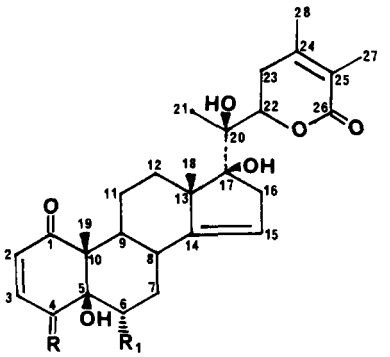
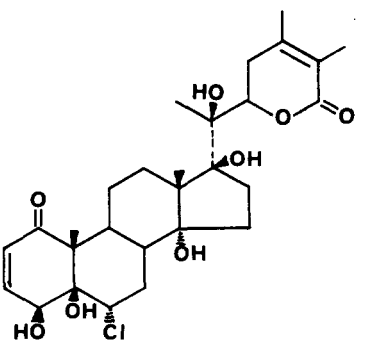
ABSTRACT.—A new withanolide, physalolactone C (17*S*, 20*S*, 22*R*-6α-chloro-4β, 5β, 17β, $20\alpha_F$-tetrahydroxy-1-oxo-ergosta-2,14,24-trienolide) (**1**), has been isolated and characterized by a study of its physicochemical data and its semisynthesis from physalolactone (**2**).

In continuation of our work on the steroidal lactones (1-8) of *Physalis peruviana* L. (Solanaceae), we report herein the structure elucidation of physalolactone C, a minor steroidal constituent isolated from the leaves of this plant. Physalolactone C (**1**), $C_{28}H_{37}O_7Cl$, mp 271°, showed a positive Beilstein test and exhibited molecular ion peaks at *m/z* 520 and 522, with an abundance ratio of 3:1 in its mass spectrum. Its close relationship with its companion physalolactone (**2**) (1) was revealed from its spectral data.

The ir spectrum of physalolactone C (**1**) displayed a band at 1680 cm^{-1} due to the presence of an α,β-unsaturated δ-lactone. Its pmr spectrum showed signals for two vinylic methyl groups at δ1.76 and 1.89 in addition to three tertiary methyl groups at δ 1.25 (6H) and 1.12 (3H) and the characteristic withanolide 22-H at δ 4.83. Physalolactone C (**1**) showed in its mass spectrum diagnostic ion peaks at *m/z* 169 (**3**), 152 (**4**), and 125 (**5**) for 20-hydroxywithanolides and yielded, on chromic acid oxidation, the δ-lactone methyl ketone (**6**) identical to that obtained by similar oxidation of physalolactone (**2**) (1). The presence of an enone chromophore indicated from its uv spectrum [λ max 238 nm (log ε 3.81)] became further evident in its pmr spectrum, which showed signals for two olefinic hydrogens at δ 5.83 and 6.50 as an AB part of an ABX system; the hydrogen for the X part appearing at δ 4.86 in physalolactone C (**1**) and at δ 6.27 in its monoacetate (**1a**). This observation indicated the presence of a γ-hydroxy-





1 R=◄OH,▪▪▪H; R_1=Cl

1a R=◄OAc,▪▪▪H; R_1,=Cl

1b R=O; R_1=Cl

1c R=◄OH,▪▪▪H; R_1=OH

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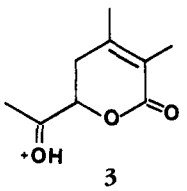
α,β-unsaturated ketone system in physalolactone C (**1**) same as that in physalolactone (**2**). In conformity with this observation, physalolactone C (**1**) gave an enedione (**1b**) on MnO_2 oxidation. The identity of the substitution pattern of the A and B rings in physalolactone C and physalolactone (**2**) was obvious from the observation that the cmr resonance signals of the carbon atoms associated with this part of the molecules were virtually identical in physalolactone (**2**) (3) and physalolactone C monoacetate (**1a**), taking into consideration the expected changes effected by the acetoxy function at C-4 in **1a** instead of a hydroxy function in **1**.

The cmr spectrum of physalolactone C monoacetate (**1a**) indicated the presence of a trisubstituted double bond [δ_C 150.2 (s) & 115.3 (d)] in addition to the double bonds due to the enone and α,β-unsaturated-δ lactone systems. An isolated olefinic hydrogen signal at δ 5.22 (broad singlet) in the pmr spectrum not only substantiated the presence of a trisubstituted double bond but suggested its location between C-14 and C-15 as in withanolide L (9) and withaperuvin B (5). Further, a comparison of cmr data (Table 1) of physalolactone C acetate (**1a**) with withaperuvin B (**1c**) revealed the perfect identity of rings C and D and side chains of the two molecules. Physalolactone C (**1**) was therefore formulated as 17*S*, 20*S*, 22*R*-6α-chloro-4β, 5β, 17β, $20\alpha_F$-tetrahydroxy-1-oxoergosta-2,14,24-trienolide (**1**), chemical evidence for which was provided by selective dehydration (10) of physalolactone (**2**) to physalolactone C (**1**).

TABLE 1. ^{13}C-Shieldings in Physalolactone C Acetate, Physalolactone, and Withaperuvin B

Carbon number	Physalolactone C acetate (**1a**)[a]	Physalolactone (3)	Withaperuvin B (5)
1	198.6	[b]	201.1
2	128.2	126.8	127.2
3	140.9	145.5	146.9
4	66.9	66.0	67.8
5	78.6	78.8	79.7
6	63.8	65.5	74.4
7	35.3	34.5	37.0
8	33.1	39.3	33.6
9	48.2	38.4	48.3
10	57.8	57.0	56.9
11	23.4	22.8	24.1
12	42.1	34.5	42.9
13	55.1	54.9	55.5
14	150.2	82.3	150.7
15	115.3	30.9	114.8
16	35.9	37.2	35.7
17	86.7	87.4	86.9
18	17.4	20.7	17.6
19	9.3	9.5	10.5
20	77.5	78.8	77.9
21	20.3	19.0	20.2
22	79.9	80.9	80.8
23	34.6	32.2	34.5
24	150.3	152.0	151.9
25	121.6	121.3	121.6
26	165.7	[b]	166.5
27	12.4	12.2	12.6
28	20.6	20.7	20.6

[a]δ_C ($MeCO_2$) 21.5 and 169.8.
[b]Not observed due to low signal-to-noise ratio.



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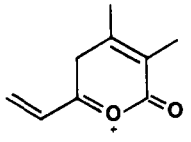

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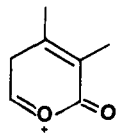

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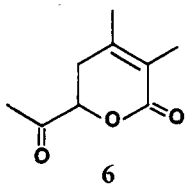

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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were recorded on a Toshniwal melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer model 241 automatic polarimeter. Uv spectra were taken on a Perkin-Elmer model 202 spectrophotometer. Ir spectra were obtained on a Perkin-Elmer model 257 spectrophotometer. The 270 MHz pmr spectra were recorded on a Bruker WH spectrometer and 60 MHz spectra on a Perkin-Elmer R24 spectrometer. Cmr spectra were obtained at 22.63 MHz on a Bruker WH-90 spectrometer. Chemical shifts are relative to TMS and reported in δ (ppm) units.

PLANT MATERIAL.—The leaves of *P. peruviana* used in this study were collected from fields in the suburbs of Varanasi, India, during May 1979, and authenticated by Dr. S.K. Roy, Department of Botany, Banaras Hindu University, Varanasi, India. A specimen sample is retained in the Department of Medicinal Chemistry, Banaras Hindu University.

EXTRACTION AND CHROMATOGRAPHY.—The air-dried leaves (5 kg) were ground and extracted with 95% EtOH by cold percolation. The extract was concentrated under reduced pressure to a small volume (3 liters) and then diluted with an equal volume of H_2O with stirring. The solution was extracted successively with petroleum ether (bp 60-80°) and Et_2O. The total Et_2O extract was washed and dried (anhydrous Na_2SO_4) to give, on removal of solvent, a greenish, gummy mass (52 g). This was chromatographed over silica gel and eluted first with C_6H_6 and then with increasing amounts of EtOAc in C_6H_6.

PHYSALOLACTONE C (**1**).—The later fractions of C_6H_6-EtOAc (7:3) yielded, on removal of solvent, a homogeneous solid (0.4 g) that crystallized from MeOH as colorless shining needles; $C_{28}H_{37}O_7Cl$, mp 271°, $[\alpha]^{25}D$ +174° (c, 0.2, DMSO), uv λ max (MeOH) 238 nm (log ε 3.81); ir ν max (KBr) 3578, 3540, 3350, 1680 (br) cm^{-1}; 60 MHz pmr (DMSO-d_6) 1.02 (3H, s, 18-Me), 1.25 (6H, s, 19 and 21-Me), 1.76 and 1.89 (3H, each s, 27 and 28-Me), 5.83 (1H, d, J=10 Hz, 2-H), 6.50 (1H, dd, J=10, 2.5 Hz, 3-H), 4.83 (1H, br, 22-H); ms (ei, 70ev) *m/z* 522, 520, 486, 484, 464, 448, 286, 284, 170, 169, 152 (base peak), 125, 109.

Anal. calcd. for $C_{28}H_{37}O_7Cl$: C, 64.55; H, 7.10%. Found: C, 64.28; H, 7.06%.

PHYSALOLACTONE C MONOACETATE (**1a**).—To a solution of physalolactone C (**1**) (35 mg) in pyridine was added Ac_2O (1 ml), and the mixture was left under anhydrous conditions for 12 h. The residue, on removal of reagents *in vacuo*, crystallized from $CHCl_3$-hexane as colorless needles, mp 259-261°, ir ν max KBr 3575, 3525, 2940, 1748, 1700, 1670 cm^{-1}; 270 MHz pmr ($CDCl_3$) 1.14, 1.30, 1.32, 1.88, 1.95 (3H, each s), 2.16 (3H, s, OAc), 4.40 (1H, dd, J=10.5, 6 Hz, 6-H), 4.61 (1H, t, 8.2 Hz, 22-H), 5.33 (1H, br s, 15-H) 6.05 (1H, dd, J=8.5, 3.2 Hz, 3-H), 6.27 (1H, br, 4-H), 6.29 (1H, dd, J=8.5, 2.4 Hz, 2-H); ms (ei, 70 ev) *m/z* 564, 563, 562, 547, 544, 522, 520, 508, 484, 466, 448, 421, 419, 394, 170, 169, 152 (base), 125, 110, 109, 43, 41.

MANGANESE DIOXIDE OXIDATION OF PHYSALOLACTONE C TO **1b**.—A solution of physalolactone C (**1**) (50 mg) in dry Me_2CO was stirred with active MnO_2 (0.2 g) for 48 h and the reaction mixture was filtered. The filtrate, on removal of solvent, afforded a residue which was separated by preparative tlc to furnish a light yellow crystalline solid (19 mg); mp 230-232°; uv λ max (MeOH) 231 nm (log ε 4.06); ms (ei, 70 ev) *m/z* 520, 518, 482, 466, 464, 446, 377, 375, 339, 170, 169, 152 (base), 125, 109.

JONES' OXIDATION OF PHYSALOLACTONE C (**1**) TO **6**.—A solution of physalolactone C (**1**) (100 mg) in Me_2CO (25 ml) was titrated with Jones' reagent (0.25 ml) with stirring at room temperature. The reaction mixture was left for 30 min when the green chromium salt settled. The supernatant liquid was decanted off, mixed with Me_2CO washings, and evaporated to dryness at room temperature. The residue was chromatographed over silica gel (5 g) and eluted first with C_6H_6 and then with C_6H_6-EtOAc mixtures. C_6H_6-EtOAc (19:1) eluates furnished a homogeneous oil that gave a crystalline DNP derivative as silky needles; mp 198-200° (dec.), identical with the DNP derivative of the δ-lactone methyl ketone (**6**), obtained by Jones' oxidation of physalolactone (**2**) (mmp and pmr).

CONVERSION OF PHYSALOLACTONE (**2**) TO PHYSALOLACTONE C (**1**).—To an ice-cold solution of physalolactone (**2**) (0.1 g) in Me_2CO (10 ml) was added 8 N H_2SO_4 (2 ml) and left at room temperature for 48 h. The reaction mixture was diluted with H_2O (100 ml) and extracted with EtOAc. The extract was washed with H_2O, dried (anhydrous Na_2SO_4), evaporated to dryness, and twice crystallized from MeOH to yield crystals; mp 271° (67 mg), identical in all respects to physalolactone C (mmp and ir).

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