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23-Hydroxyphysalolactone, a New Withanolide with a 23-Hydroxyl Group from *Physalis peruviana* (Solanaceae)

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A new withanolide having a hydroxyl group at the C-23 position has been isolated from the leaves of *Physalis peruviana*. The structure of this withanolide has been established to be (20*S*,22*R*,23*R*)-6α-chloro-4β,5β,14α,17β,20,23-hexahydroxy-1-oxowitha-2,24-dienolide (**1**) by spectroscopic evidence.

Keywords——23-hydroxyphysalolactone; withanolide; *Physalis peruviana*; steroidal lactone; oxygenated steroid

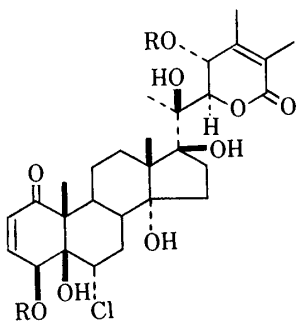
Introduction

Physalis peruviana (Solanaceae) is one of the few sources of highly oxygenated ergostane-type steroids (withanolides and related compounds) possessing an α-oriented side chain. Our interest in these compounds is due not only to their unique structure, but also to the significant biological activities, *viz.*, antineoplastic,[2] insect antifeedant,[3] and antiinflammatory,[4] of some of them. Previous investigations of *P. peruviana* resulted in the isolation and characterization of a large number of withanolides with an α-oriented side chain.[5-12] The present communication describes the isolation of one minor withanolide from the leaves of *P. peruviana* and the determination of its structure as (20*S*,22*R*,23*R*)-6α-chloro-4β,5β,14α,17β,20,23-hexahydroxy-1-oxowitha-2,24-dienolide [(23*R*)-23-hydroxyphysalolactone][13] (**1**).

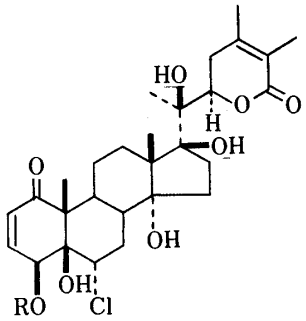
Results and Discussion

Compound **1**, mp 202—204 °C, $[\alpha]_D^{25}$ +60°, was found to possess the molecular formula $C_{28}H_{39}O_9Cl$ on the basis of fast atom bombardment-mass spectrum (FAB-MS) peaks together with the results of elemental analysis, and from its carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum which showed signals for 28 carbons (Table I). A comparison of the molecular formula of compound **1** with that of physalolactone (**3**), $C_{28}H_{39}O_8Cl$, the major withanolide of *P. peruviana* leaves,[8] indicated that **1** may possess a structural resemblance to **3**. This was found to be correct on the basis of the evidence presented below.

The chemical shifts and multiplicity of the carbon signals that originated from the carbocyclic moiety of **1** were found to be in good agreement with those of **3** except for an upfield shift of C-12 (Table I). The similarity of the carbocyclic moiety of both compounds is also evident from a comparison of proton signals due to the C-2, C-3, C-4, C-6 and C-19 hydrogens (Table II). The significant differences in the ^{1}H- and ^{13}C-NMR spectra are: 1) the



1: R=H
2: R=Ac



3: R=H
4: R=Ac

TABLE I. ^{13}C-NMR Data for **1** and Physalolactone (**3**) (in CD_3OD)

Assignment	Compound **1**[a)]	Physalolactone (**3**)	Assignment	Compound **1**[a)]	Physalolactone (**3**)
C-1	203.6 (s)	203.8	C-15	32.8 (t)	31.2
C-2	126.8 (d)	126.9	C-16	37.1 (t)	37.3
C-3	147.5 (d)	147.6	C-17	88.2 (s)	88.3
C-4	65.8 (d)	66.0	C-18	20.9 (q)	21.1
C-5	80.0 (s)	80.0	C-19	9.8 (q)	9.7
C-6	66.0 (d)	66.0	C-20	80.4 (s)	79.7
C-7	35.8 (t)	35.7	C-21	19.3 (q)	19.3
C-8	40.0 (d)	40.1	C-22	85.7 (d)	82.5
C-9	39.9 (d)	39.9	C-23	67.5 (d)	34.0
C-10	58.3 (s)	58.3	C-24	153.8 (s)	153.3
C-11	23.7 (t)	23.8	C-25	122.5 (s)	121.8
C-12	31.0 (t)	35.5	C-26	166.9 (s)	168.9
C-13	55.9 (s)	55.8	C-27	12.7 (q)	12.2
C-14	83.7 (s)	83.5	C-28	16.2 (q)	20.5

a) C-7, C-11, C-15 and C-16 were assigned according to the previous report (ref. 9).

multiplicity of 22-H, a doublet at 4.67 ppm (J=8.5 Hz) for **1** *vs.* a double doublet at 4.76 ppm (J=12.6 and 4.0 Hz) for **3**, 2) the presence of a new oxymethine proton signal at 4.34 ppm (d, J=8.5 Hz) which is coupled to 22-H, and 3) the appearance of an oxymethine carbon signal at 67.5 ppm in **1** with concomitant loss of the C-23 methylene carbon signal of **3**. Thus, it is clear that compound **1** is 23-hydroxyphysalolactone. The ^{1}H-NMR spectrum of the acetate derivative **2** of **1** obtained under standard conditions is in full agreement with the proposed structure (Table III). For comparison, the ^{1}H-NMR data of physalolactone acetate (**4**) are also listed in Table III. The 23-H signal was shifted downfield to 5.81 ppm (6.13 ppm in CD_3OD) upon acetylation.

The use of two-dimensional proton–carbon chemical-shift correlation spectroscopy (COSY) and long-range correlation spectroscopy allowed us to confirm the structure mentioned above, as well as affording a basis for the assignment of carbon signals. The connectivity obtained from the C–H long-range COSY spectrum is shown in Fig. 1.

The stereochemistry of the asymmetric centers in the A—D rings including 17α-side chain and 20*R* configuration could be identical to that of physalolactone as judged from the similarity of ^{1}H- and ^{13}C-NMR spectral data. The configuration at the C-22 position was defined as *R* on the basis of the circular dichroism (CD) spectrum, in which a strong positive Cotton effect was observed at 235 nm ($\Delta\varepsilon$+18.6) [for example, physalolactone was reported to exhibit a positive Cotton effect at 239 nm ($\Delta\varepsilon$+12.35)].[8)] Consideration of the vicinal proton

TABLE II. ^{1}H-NMR Data for **1** and Physalolactone (**3**)[a] (in CD_3OD)

	Compound **1**	Physalolactone (**3**)
2-H	5.90 (dd, 10.2, 2.0) [6.26 (dd, 10.3, 2.0)]	5.90 (dd, 10.2, 2.1)
3-H	6.55 (dd, 10.2, 2.5) [6.82 (dd, 10.3, 2.5)]	6.55 (dd, 10.2, 2.5)
4-H	4.99 (t, 2.3) [5.51 (t, 2.3)]	5.00 (t, 2.4)
6-H	4.38 (m) [4.78 (dd, 13.0, 4.7)]	4.39 (m)
18-H_3	1.04 (s) [1.32 (s)]	1.06 (s)
19-H_3	1.22 (s) [1.64 (s)]	1.22 (s)
21-H_3	1.35 (s) [1.75 (s)]	1.38 (s)
22-H	4.67 (d, 8.5) [5.18 (d, 9.0)]	4.76 (dd, 12.6, 4.0)
23-H	4.34 (d, 8.5) [4.60 (d, 9.0)]	—
27-H_3	1.85 (s) [1.98 (s)]	1.82 (s)
28-H_3	1.97 (s) [2.07 (s)]	1.94 (s)

a) Chemical shifts are in δ units. Coupling constants (in Hz) are given in parentheses. Data recorded in pyridine-d_5 are in brackets.

TABLE III. ^{1}H-NMR Data for the Acetate (**2**) and Physalolactone Acetate (**4**) (in $CDCl_3$)[a]

	Acetate (**2**)	Physalolactone acetate (**4**)
2-H	6.06 (dd, 9.8, 2.1) [6.01 (dd, 10.2, 2.2)]	6.04 (dd, 11.3, 2.5)
3-H	6.34 (dd, 9.8, 2.0) [6.42 (dd, 10.2, 2.2)]	6.34 (dd, 11.3, 3.0)
4-H	6.32 (br s) [6.35 (t, 2.1)]	6.33 (t, 2.7)
6-H	4.34 (dd, 12.5, 5.1) [4.38 (m)]	4.34 (dd, 12.7, 4.5)
18-H_3	1.13 (s) [1.11 (s)]	1.05 (s)
19-H_3	1.20 (s) [1.11 (s)]	1.28 (s)
21-H_3	1.28 (s) [1.25 (s)]	1.41 (s)
22-H	4.93 (s) [4.90 (s)]	4.79 (dd, 10.0, 6.5)
23-H	5.81 (s) [6.13 (s)]	—
27,28-H_3	1.92 (s), 1.95 (s) [1.87 (s), 1.93 (s)]	1.86 (s), 1.92 (s)
Acetate	2.11 (s), 2.13 (s) [2.03 (s), 2.11 (s)]	2.13 (s)

a) Chemical shifts are in δ units. Coupling constants (in Hz) are given in parentheses. Data recorded in CD_3OD are in brackets. Data for **4** are from ref. 9.

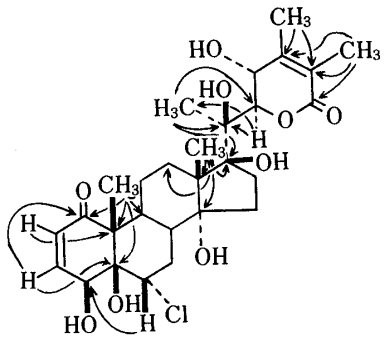


Fig. 1. C–H Connectivities as Revealed by C–H Long-Range COSY Spectrum

coupling constant, $J_{22-23}=8.5$—9.0 Hz, indicated that both the C-22 and C-23 hydrogens are quasiaxial (the dihedral angle being *ca.* 155° according to the Karplus correlation). Thus, the configuration of the C-23 position was settled as *R*. Interestingly, J_{22-23} in the acetate derivative (**2**) is nearly zero, indicating that the dihedral angle is close to 90°. This is probably because the sterically more demanding acetate group could not adopt the quasiequatorial position due to steric interaction. The aforementioned upfield shift of the C-12 carbon signal can be well explained by assuming that the conformation of the side chain of **1** is similar to that reported for withanolide E.[14] In that conformation, the 23-hydroxy group will be sterically close to C-12, which rationalizes the upfield shift of C-12.

On the basis of the data presented above, the structure of compound **1** has been established to be (20*S*,22*R*,23*R*)-6α-chloro-4β,5β,14α,17β,20,23-hexahydroxy-1-oxowitha-2,24-dienolide. To our knowledge, compound **1** is the first reported example of a 23-hydroxylated withanolide from the genus *Physalis*. It is the third example from the family Solanaceae. The first two, withanolides Q and R, were isolated from *Withania simnifera*,[15] and were reported to have 23*S* configuration in contrast to our compound **1**. 23-Hydroxyphysalolactone **1** exhibited cytotoxic activity (IC_{50} value 40 μg/ml) against L-5178Y cells *in vitro*.[16]

Experimental

The melting point was determined on a Yazawa hot stage microscope and is uncorrected. The ultraviolet (UV) spectrum was recorded on a Shimadzu UV 200 spectrometer in methanol solution. The infrared (IR) spectrum was recorded on a Hitachi 260-10 spectrometer. Optical rotation was recorded on a Yanaco OR-50 automatic polarimeter (cell length 1 cm) in methanol solution. The CD spectrum was recorded on a JASCO J-500C spectrometer in methanol solution. FAB-MS was obtained with a JMS-DX 300 spectrometer. ^{1}H-NMR (270 and 400 MHz) spectra were taken with JEOL GX-270 and GX-400 spectrometers, respectively, in the indicated solvent with tetramethylsilane as an internal reference. ^{13}C-NMR spectra were recorded similarly on the same spectrometers. ^{1}H- and ^{13}C-NMR (in CD_3OD) spectra of **3** were recorded in the present study.

Isolation of 1——The work-up of the plant material has been described before.[9] Chromatography (silica gel; elution with benzene–ethyl acetate, 3:7) of the ether extract obtained from air-dried leaves of *P. peruviana* afforded several fractions. The fractions eluted before those containing 4β-hydroxywithanolide E[6] were separated by preparative thin layer chromatography (TLC) to give pure **1** which was crystallized from methanol as fine needles, mp 202—204 °C, $[\alpha]_D^{25}$ +60° ($c=1.4$). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1710, 1670 (CO). UV λ_{max} nm (ε): 216 (16000). CD $\Delta\varepsilon$ (nm): +22 (350), −0.28 (310), +18.6 (235). FAB-MS *m/z*: glycerin matrix, 649, 647 (M+1+glycerin), 631, 629 (M+1−H_2O+glycerin), 539, 537 (M+1−H_2O); NaCl+glycerin matrix, 671, 669 (M+Na+glycerin), 649, 647 (M+1+glycerin), 631, 629 (M+1−H_2O+glycerin), 579, 577 (M+Na), 539, 537 (M+1−H_2O). *Anal.* Calcd for $C_{28}H_{39}O_9Cl\cdot H_2O$: C, 58.68; H, 7.21. Found: C, 58.43; H, 7.11.

Acetylation of 1 to the Acetate (2)——Compound **1** was (3 mg) treated with pyridine (0.2 ml) and acetic anhydride (0.1 ml) at room temperature overnight. Addition of methanol, evaporation of volatile substances by flushing with nitrogen, and separation by preparative TLC afforded the acetate (**2**) (*ca.* 3 mg) as an amorphous solid. This sample was analyzed by ^{1}H-NMR without further purification.

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