Physapruins A and B, Two New Withanolides from *Physalis pruinosa* BAILEY[1)]

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Two new withanolides, physapruins A and B, were isolated from the methanolic extract of the fresh aerial parts of *Physalis pruinosa* (Solanaceae), and their structures have been determined to be 4β,14α,17β,20β-tetrahydroxy-1-oxo-20*S*,22*R*-witha-2,5,24-trienolide (1) and 6β-acetoxy-5α-butoxy-14α-17β-20β-trihydroxy-1-oxo-20*S*,22*R*-witha-2,24-dienolide (2) on the basis of spectral evidence.

Keywords *Physalis pruinosa*; Solanaceae; withanolide; physapruin A; physapruin B

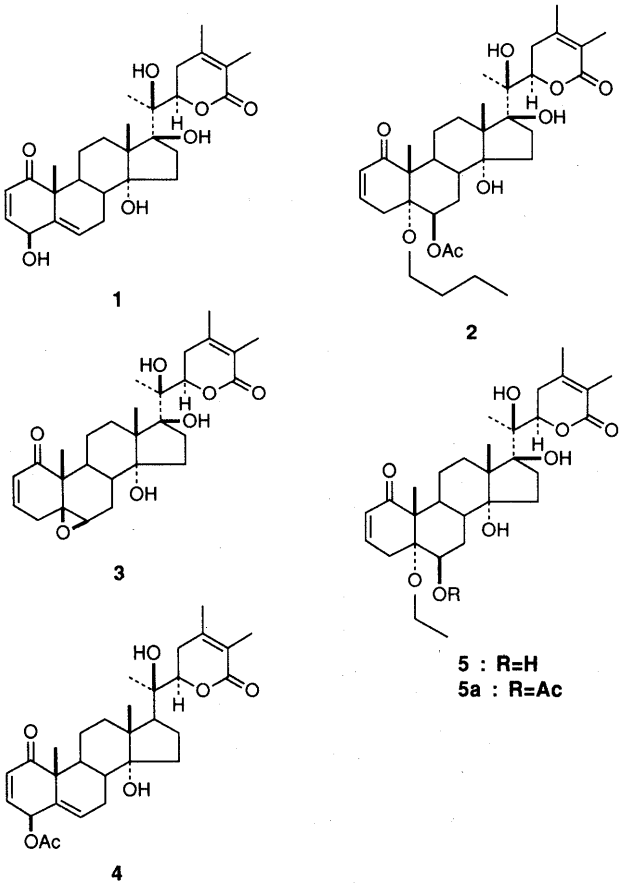
In our previous papers,[2)] we reported the structure elucidation of seven withanolides, physagulins A, B, C, D, E, F and G, isolated from *physalis angulata* L. As an extended search for withanolides from the same genus, we have now isolated two new withanolides from the fresh aerial parts of *Physalis pruinosa* and determined their structures.

Physapruin A (**1**), obtained as colorless needles, with a mp of 207—209 °C and $[\alpha]_D$ +159.1° (MeOH), exhibited the absorption bands at 3408 cm^{-1} (hydroxyl), 1696 cm^{-1} (α,β-unsaturated δ-lactone) and 1664 cm^{-1} (α,β-unsaturated ketone) in the IR spectrum (KBr). The positive fast atom bombardment mass spectrum (FAB-MS) displayed peaks due to $[M+Na]^+$ at *m/z* 509 and the stepwise elimination of four hydroxy groups at *m/z* 469 $[M-H_2O+H]^+$, 451 $[M-2H_2O+H]^+$, 433 $[M-3H_2O+H]^+$ and 415 $[M-4H_2O+H]^+$. The mass fragments at *m/z* 125 and 165 were characteristic of side chain cleavage between C-20 and C-22 and between C-17 and C-20.[3)] These peaks suggested that **1** was a 17,20-dihydroxywithanolide.

The ^{1}H-NMR spectrum, as listed in Table I, showed features typical of a withanolide. Five singlet signals at δ 1.13, 1.42, 1.45, 1.88 and 1.94 could be assigned to five methyl groups, and the signals of two olefinic methyl groups at δ 1.88 and 1.94, as well as the characteristic H-22 signal (dd, *J*=10.8, 5.7 Hz) at δ 4.92 suggested the presence of a typical α,β-unsaturated δ-lactone side chain. The coupling pattern and constants of the H-22 signal and the chemical shifts of two singlet methyl signals, H_3-21 and H_3-18 at δ 1.45 and 1.13, were indicative of a 14α,17β,20β-trihydroxyl substitution by comparison with the analogous substituted withanolides, withanolide E (**3**) and related compounds.[3,4)] The presence of these hydroxy groups at C-17 and 20 was supported by cleavage between C-17 and C-20, and between C-20 and C-22 in the FAB-MS.

On the other hand, in the low field, *cis* olefinic proton signals at δ 6.78 (1H, dd, *J*=10.2, 4.5 Hz) and 5.93 (1H, d, *J*=10.2 Hz), an olefinic proton signal at δ 5.92 (1H, d, *J*=5.9 Hz) and a hydroxymethine proton signal at δ 4.62 (d, *J*=4.5 Hz) could be assigned to H-3, H-2, H-6 and H_α-4, respectively. These signals suggested a 2,5-diene-1-one-4β-hydroxyl system for rings A and B.

In the ^{13}C-NMR spectrum of **1**, as listed in Table II, signals for rings C and D and the side chain moiety were similar to those of **3**. Moreover, the remaining signals due to rings A and B could be reasonably assigned, taking into consideration the effect of an acetyl group, by comparison with those of withanolide U monoacetate (**4**).[4,5)] Based on these spectral data and the positive Cotton effect curve at 256 nm[6)] in the CD spectrum, the structure of **1** having a 22*R*-configuration could be expressed as shown in the formula.



Physapruin B (**2**), was isolated as an acetyl derivative, $C_{34}H_{50}O_9$, an amorphous powder, $[\alpha]_D$ +47.0° (MeOH), in order to achieve easy separation. In the IR spectrum of **2**, absorption bands appeared at 3452 cm^{-1} (hydroxy), 1740 cm^{-1} (acetyl carbonyl), 1714 cm^{-1} (α,β-unsaturated δ-lactone) and 1690 cm^{-1} (α,β-unsaturated ketone). The positive FAB-MS of **2** gave $[M+Na]^+$, $[M+H]^+$ and $[M-H_2O+H]^+$ ion peaks at *m/z* 625, 603 and 585, respectively, and peaks at *m/z* 165 and 125 indicated the

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TABLE I. ^{1}H-NMR Data (δ/ppm, in $CDCl_3$) for Physapruin A (**1**), B (**2**), Withanolide E (**3**), and 5α-Ethoxy-6β,14α,17β,20-tetrahydroxy-1-oxo-20*S*,22*R*-witha-2,24-dienolide Monoacetate (**5a**)

H	1	3	2	5a
2	5.93 d (10.2)	6.03 dq (10.0, 2.5, 1.0)	5.82 dd (10.0, 2.7)	5.82 dd (10, 3)
3	6.78 dd (10.2, 4.5)	6.87 dq (10.0, 5.5, 2.5)	6.43 ddd (10.0, 5.3, 2.0)	6.44 dq (10, 3)
4	4.62 d (4.5)		2.33 dd (19.7, 5.7)	2.34 dd (22, 6)
			2.55 d (19.7)	2.55 br d (22, 4)
6	5.92 d (5.9)	3.20 m	5.15 d (2.7)	5.15 br s ($W_{1/2}$ 8.2)
18	1.13 s	1.10 s	1.14 s	1.15 s
19	1.42 s	1.25 s	1.26 s	1.27 s
21	1.45 s	1.42 s	1.41 s	1.41 s
22	4.92 dd (10.8, 5.7)	4.88 m	4.93 m	4.94 br t ($W_{1/2}$ 20)
27	1.88 s	1.88 s	1.88 s	1.88 s
28	1.94 s	1.93 s	1.94 s	1.94 s
–OAc			2.11 s	2.11 s
5α-Butoxyl, ethoxyl			3.05 dd (14.2, 6.6)	3.14 dq (8, 7)
			3.28 dd (14.2, 6.1)	3.34 dq (8, 7)
			0.81 t (7.3)	1.05 t (7)

Chemical shifts are in δ/ppm; coupling constants in Hz are in parentheses; abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad.

TABLE II. ^{13}C-NMR Data (δ/ppm, in $CDCl_3$) for Physapruin A (**1**), B (**2**), Withanolide E (**3**), Withanolide U Monoacetate (**4**) and 5α-Ethoxy-6β,14α,17β,20-tetrahydroxy-1-oxo-20*S*,22*R*-witha-2,24-dienolide (**5**)

Carbon	1	3	4	2	5
C- 1	203.4	203.2	202.9	203.1	204.9
C- 2	129.2	129.8	134.2	129.6	129.4
C- 3	143.2	143.9	140.0	138.0	139.6
C- 4	69.3	32.9	70.0	32.1	31.0
C- 5	138.2	62.2	133.9	77.0	81.5
C- 6	131.5	64.2	130.1	71.6	69.9
C- 7	25.9	26.2	25.6	26.5	28.5
C- 8	35.8	34.1	34.4	33.4	33.9
C- 9	36.9	36.9	36.4	34.8	34.1
C-10	49.5	48.9	49.4	52.8	53.2
C-11	22.5	22.9	21.8	22.6	22.9
C-12	34.2	34.3	32.4	37.7	37.7
C-13	54.5	54.5	47.5	54.9	55.1
C-14	81.9	82.3	84.9	82.3	83.0
C-15	30.2	30.1	32.0	30.8	30.1
C-16	37.7	37.7	20.6	34.2	34.5
C-17	88.0	87.8	49.4	87.9	88.2
C-18	20.5	20.6	17.4	21.0	21.2
C-19	22.6	14.6	21.8	13.8	15.6
C-20	78.9	80.0	75.3	79.0	79.2
C-21	19.7	19.5	21.2	20.1	20.1
C-22	80.1	80.3	81.3	79.9	80.5
C-23	32.5	32.5	31.8	32.7	32.8
C-24	150.7	151.1	149.1	150.5	151.3
C-25	121.4	121.4	122.0	121.5	121.5
C-26	166.2	166.6	166.2	165.8	166.6
C-27	12.3	12.3	12.5	12.4	12.5
C-28	20.6	20.6	20.6	20.6	20.9
5α-Butoxy, ethoxy				61.7	57.5
				28.0	16.0
				19.3	
				15.0	
–OAc			170.3	170.2	
			21.4	21.4	

presence of the hydroxy groups at C-17 and 20.

A comparative study of the ^{1}H-NMR spectrum of **2** (Table I) with that of **1** suggested that **2** has the same C and D rings and side chain moiety as **1**. Signals at δ 2.33 (1H, dd, J=19.7, 5.7 Hz), δ 2.55 (1H, d, J=19.7 Hz) and δ 5.15 (1H, d, J=2.7 Hz) were similar to those of the acetate (**5a**) of an analog withanolide, 5α-ethoxy-6β,14α,17β,20-tetrahydroxy-1-oxo-20*S*,22*R*-witha-2,24-dienolide (**5**)[7] obtained from *Withania somnifera*, and were attributable to methylene protons at C-4 and an acetoxymethine proton at C-6. Moreover, a deshield signal due to H_3-19 at δ 1.26 suggested the presence of a hydroxy group at C-5. Furthermore, the existence of a 1-butoxy group was suggested by the ^{1}H–^{1}H correlated spectroscopy (COSY) of **2**. It was determined that the 1-butoxy group was connected to C-5 by comparing the ^{13}C-NMR spectrum with that of **5**.

In comparing the ^{13}C-NMR spectrum of **2** with that of **5**, the chemical shifts, except for those at C-3—7, 9, 10 and 19 on the A, B rings and the 1-butoxy group were in good correlation. These chemical shifts of C-3—7, 9, 10 and 19 were presumed to occur because of the introduction of an acetoxy group at C-6. The positive Cotton effect at 255 nm in the CD spectrum of **2** indicated a 22*R* configuration, and the negative Cotton effect at 334 nm showed a *trans* fusion of rings A and B.[6] Thus, the structure of **2** was concluded to be as shown in the formula. The 1-butoxy group at C-5 was suspected to be artificially introduced into the 5β,6β-epoxy ring during separation.

Experimental

The melting point was measured using a Yanagimoto micromelting point apparatus and was uncorrected. Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter and CD spectrum on a JASCO J-50A spectropolarimeter. The IR spectra were recorded with a Hitachi IR spectrometer, model 270-30. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM-GX 400 NMR spectrometer and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The FAB-MS were measured with a JEOL DX-303 HF spectrometer and taken in a glycerol matrix containing NaI. Thin layer chromatography was performed on pre-coated Kieselgel 60 F_{254} (Merck) and detection was achieved by spraying it with 10% H_2SO_4, followed by heating. Column chromatography was carried out on Kieselgel (270—300 mesh and 230—400 mesh, Merck) and Sephadex LH-20 (Pharmacia Fine Chemicals).

Extraction and Separation The fresh aerial parts (2.2 kg) of *Physalis pruinosa* BAILEY (Solanaceae), harvested at the botanical garden of Kyushu University in December 1988, were extracted with MeOH, and the extract was partitioned between 1-BuOH and H_2O, then the 1-BuOH layer was partitioned between 1-BuOH–EtOAc and H_2O. The organic layer (30 g) was subjected repeatedly to column chromatography over silica gel using $CHCl_3$–MeOH–H_2O = 1 : 0 : 0 → 8 : 3 : 0.5 and Sephadex LH-20 using MeOH to give physapruins A (**1**, 34.6 mg), B (**2**, 13.7 mg), withanolide E (**3**, 113.0 mg), 4β-hydroxywithanolide E[8] (76.9 mg) and 2,3-dihydro-3β-methoxy-4β-hydroxywithanolide E monoacetate[9] (21.3 mg). During this separation procedure, the free compound of **2** and 2,3-dihydro-3β-methoxy-4β-hydroxywithanolide E monoacetate were difficult to separate and were converted to acetyl derivatives to facilitate separation.

Physapruin A (1) Colorless needles from $CHCl_3$–MeOH, mp 207—209 °C, $[\alpha]_D$ +159.1° (c=0.46, MeOH). Positive FAB-MS (m/z): 509 $[M+Na]^+$, 469 $[M-H_2O+H]^+$, 451 $[M-2H_2O+H]^+$, 433 $[M-3H_2O+H]^+$, 415 $[M-4H_2O+H]^+$, 283, 265, 169, 125. IR (KBr): 3408, 1696, 1664 cm^{-1}. CD (c=0.60, MeOH) [θ] (nm): −28000 (340) (negative max.), +83000 (256) (positive max.).

Physapruin B (2) Amorphous powder, $[\alpha]_D$ +47.0° (c=0.47, MeOH). Positive FAB-MS (m/z): 625 $[M+Na]^+$, 603 $[M+H]^+$, 585 $[M-H_2O+H]^+$, 549 $[M-3H_2O+H]^+$, 417, 357, 283, 265, 169, 125. IR (KBr): 3452, 1740, 1714, 1690 cm^{-1}. CD (c=0.047, MeOH) [θ] (nm): −15000 (334)

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(negative max.), +51000 (255) (positive max.).

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References and Notes

1) This work is Part XXVII in the series of studies on the constituents of solanaceous plants.
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