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Three New Withanolides, Physagulins A, B and D from *Physalis angulata* L.[1)]

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Three new withanolides, physagulins A (1), B (2) and D (3), were obtained from the methanolic extract of the fresh leaves and stems of *Physalis angulata* L. (Solanaceae). Their structures were established as (20*S*,22*R*)-15α-acetoxy-5β,6β-epoxy-14α-hydroxy-1-oxowitha-2,16,24-trienolide (1), (20*S*,22*R*)-15α-acetoxy-5α-chloro-6β,14α-dihydroxy-1-oxowitha-2,16,24-trienolide (2) and (20*S*,22*R*)-1α,3β,27-trihydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranoside (3) by spectroscopic means.

Keywords *Physalis angulata*; Solanaceae; withanolide; physagulin

Several physalin derivatives were previously isolated from *Physalis angulata* L.[2)] Withagulatin A (4),[3)] also reported by Chen's group, was an ergostane-type steroidal lactone possessing a characteristic partial structure, 14α-hydroxy-15α-acetoxy-16-ene system on ring D. In addition, withaminimin (5)[4)] from *Physalis minima* was reported by Ray's group.

During a search for constituents possessing antitumor

TABLE I. ^{1}H-NMR Spectra of Physagulins A (**1**), B (**2**), D Hexaacetate (**3a**), Withagulatin A (**4**), Withaminimin (**5**), Withanolide E (**7**), Daturataturin B Heptaacetate (**8a**) and Pubescenin Pentaacetate (**9**)

H (Solvent)	**1** ($CDCl_3$)	**1** (Pyridine-d_5)	**4** ($CDCl_3$)	**7** ($CDCl_3$)	**2** ($CDCl_3$)	**5** ($CDCl_3$)	**3a** ($CDCl_3$)	**8a** ($CDCl_3$)	**9** ($CDCl_3$)
1							5.05 m	5.07 br s	5.05 t (2.5)
2	6.02 dd (9.9, 2.7)	6.18 dd (10.0, 2.4)	6.14 d (10)	6.03 dq (10, 2.5, 1)	5.90 dd (10.3, 2.6)	5.87 dd (10.4, 2.5)			
3	6.88 ddd (9.9, 6.2, 2.2)	6.84 ddd (10.0, 5.5, 1.8)	6.97 dd (10, 5.8)	6.58 ddd (10, 5, 2)	6.62 ddd (10.3, 5.1, 3.0)	6.58 ddd (10, 5, 2)	3.82 m	3.88 m	3.82 m
4	2.99 dt (19.1, 2.6)	3.01 br d (19.1)	3.76 dd (5.8, 2.0)		3.33 d (19.8)	3.19 dt (20, 2.5)			
6	3.25 d (2.6)	3.25 br s	3.34 br s	3.20 br s	3.96 br s	3.60 br t (2)	5.53 m	5.77 d (4.0)	5.52 d (5.5)
15	5.23 d (2.8)	5.81 d (2.5)	5.19 d (2.7)	5.33 d (2.5)	5.29 d (2.6)	5.33 d (2.5)			
16	5.67 d (2.8)	6.07 d (2.5)	5.63 d (2.7)	5.60 br d (2.5)	5.65 d (2.6)	5.60 br d (2.5)			
18	1.09 s	1.32 s	1.06 s	1.16 s	1.15 s	1.16 s	0.71 s	0.70 s	0.69 s
19	1.27 s	1.39 s	1.40 s	1.42 s	1.27 s	1.21 s	1.06 s	1.05 s	1.05 s
21	1.13 d (7.0)	1.22 d (7.0)	1.07 d (7.0)	1.12 d (7)	1.12 d (6.6)	1.12 d (7)	1.00 d (6.6)	1.03 d (6.6)	0.91 d (6.6)
22	4.26 ddd (12.8, 6.2, 3.7)	4.37 dt (12.8, 3.7)	4.21 ddd (12.0, 7.0, 3.0)	4.34 ddd (12, 7, 4)	4.33 m	4.34 ddd (12, 7, 4)	4.42 br d (9.5)	4.41 br d (9.9)	4.53 dt
27	1.86 s	1.83 s	1.81 s	1.87 s	1.85 s	1.87 s	4.90 d (11.7) 4.86 d (11.7)	4.91 d (11.5) 4.86 d (11.5)	1.56 s
28	1.93 s	1.51 s	1.90 s	1.98 s	2.00 s	1.98 s	2.05 s	2.05 s	1.48 s
15-*O*-Ac	1.96 s	2.00 s	1.91 s		2.05 s				
Glc-1							4.58 d (8.0)	4.58 d (8.0)	4.58 d (8)
Glc-2							4.94 dd (9.5, 8.0)	4.94 dd (9.6, 8.0)	4.93 dd (9.3, 8)
Glc-3							5.20 t (9.5)	5.18 t (9.6)	5.20 t (9.3)
Glc-4							5.05 dd (9.9, 9.5)	5.05 t (9.6)	5.05 dd (9.3, 8.5)
Glc-5							3.68 ddd (9.9, 4.9, 2.5)	3.68 ddd (9.6, 5.0, 2.5)	3.68 ddd (8.5, 4.9, 2.4)
Glc-6							4.09 dd (12.1, 2.5) 4.24 dd (12.1, 4.9)	4.10 dd (12.3, 2.5) 4.23 dd (12.3, 5.0)	4.09 dd (12.2, 2.4) 4.23 dd (12.2, 4.9)
Other-OAc							2.00, 2.02, 2.05 2.06, 2.07, 2.08	2.00, 2.02, 2.04 2.05, 2.08	

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.

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activities in solanaceous plants, we found a new withanolide, physagulin C (**6**),[5] from the fresh leaves and stems of *Physalis angulata* L. This compound was a new type of withanolide having a ring D substituted by many oxygen atoms. In a continuing study on the constituents of this plant, we have now isolated three new withanolides, physagulins A (**1**), B (**2**) and D (**3**) along with two already known withagulatin A (**4**) and withaminimin (**5**) from the fresh leaves and stems of the title plant. We here report the isolation and structure elucidation of the new withanolides.

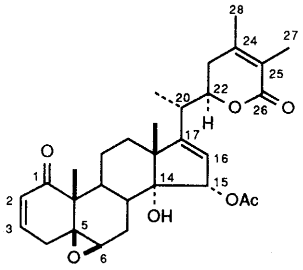
Physagulin A (**1**), a white powder, exhibited absorption bands at 1738 cm^{-1} (acetyl group), 1712 cm^{-1} (α,β-unsaturated δ-lactone) and 1676 cm^{-1} (α,β-unsaturated carbonyl) in the infrared (IR) spectrum. The positive fast atom bombardment mass spectrum (FAB-MS) displayed peaks at *m/z* 533 $[M+Na]^+$, 511 $[M+H]^+$, 493 $[M-H_2O+H]^+$, 451 $[M-AcOH+H]^+$, 433 $[M-H_2O-AcOH+H]^+$ and 415 $[M-2H_2O-AcOH+H]^+$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (in $CDCl_3$, Table I) of **1** was compared with that of withangulatin A (**4**), and the respective signals could be assigned as follows: six methyl protons [δ 1.09 (s, H_3-18), δ 1.13 (d, $J=7.0$ Hz, H_3-21), δ 1.27 (s, H_3-19), δ 1.86 (s, H_3-27), δ 1.93 (s, H_3-28) and δ 1.96 (s, 15-*O*-acetylmethyl)], three oxygen methine protons [δ 3.25 (d, $J=2.6$ Hz, H-6), δ 4.26 (ddd, $J=12.8$, 6.2, 3.7 Hz, H-22) and δ 5.23 (d, $J=2.8$ Hz, H-15)], three olefinic protons [δ 5.67 (d, $J=2.8$ Hz, H-16), δ 6.02 (dd, $J=9.9$, 2.7 Hz, H-2) and δ 6.88 (ddd, $J=9.9$, 6.2, 2.2 Hz, H-3)]. However, the H-4 proton signal observed at δ 3.76 (dd, $J=5.8$, 2.0 Hz) in **4** disappeared and a new signal occurred at δ 2.99 (dt, $J=19.1$, 2.6 Hz) in **1**. Moreover, since the H_3-19 proton signal shifted from δ 1.40 in **4** into δ 1.27 in **1**, **1** was assumed to be lacking in the hydroxy group at C-4. In comparing the carbon-13 nuclear magnetic resonance (^{13}C-NMR) data (Table II) for **1** with those of **4** and withanolide E (**7**),[6] signal data for **1** were coincident with those of rings A and B in **7**, and with those of rings C and D and side chain moiety in **4**. All assignments of ^{1}H-NMR and ^{13}C-NMR spectra could be supported by proton–proton correlation spectroscopy (^{1}H–^{1}H COSY) and carbon-13–proton correlated spectroscopy (^{13}C–^{1}H COSY) of **1**. The stereochemistry of many asymmetric centers has been established by nuclear Overhauser effect (NOE) difference measurements (in pyridine-d_5). Irradiation at the signal due to H-15 (δ 5.81) enhanced the signal intensities of H_3-18 and H-16, and irradiation at the signal of H-18 (δ 1.32) resulted in higher signal intensities of H-15 and H-8. These NOE experiments led to establishment of the relative configurations at C-14 and C-15. Moreover, the circular dichroism (CD) spectrum of **1** showed a positive Cotton effect at 253 nm to confirm a 22*R* configuration, and a negative Cotton effect at 350 nm indicated *cis*-fusion of rings A and B.[7] Based on these spectral data, the structure of physagulin A (**1**) was determined as shown in the formula.

Table II. ^{13}C-NMR Data (δ/ppm) for Physagulins A (**1**), B (**2**), D (**3**), Withagulatin A (**4**), Withaminimin (**5**), Withanolide E (**7**) and Daturataturin B (**8**)

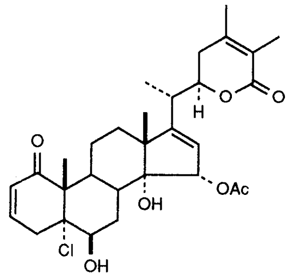
Carbon	**1** ($CDCl_3$)	**4** ($CDCl_3$)	**7** ($CDCl_3$)	**2** ($CDCl_3$)	**5** ($CDCl_3$)	**3** (C_5D_5N)	**8** (C_5D_5N)
C-1	203.7	202.4	203.2	201.0	204.1	72.3	72.3
C-2	128.8	131.4	129.8	128.4	128.7	37.8	37.6
C-3	144.9	142.8	143.9	141.7	141.3	73.9	73.8
C-4	32.9	69.6	32.9	37.0	36.0	39.1	38.9
C-5	61.5	63.2	62.2	81.1	77.2	139.2	142.7
C-6	63.8	63.2	64.2	74.0	74.3	124.0	127.8
C-7	24.9	24.7	26.2	26.6	26.5	32.2	64.7
C-8	34.9	34.7	34.1	35.2	35.4	32.1	34.0
C-9	39.3	39.3	36.9	36.9	35.4	41.5	39.2
C-10	48.3	47.6	48.9	52.8	52.2	42.1	42.9
C-11	23.4	21.9	22.9	22.8	23.2	20.5	20.4
C-12	37.8	37.5	34.3	38.5	38.8	39.6	39.4
C-13	52.1	52.0	54.5	52.1	52.2	42.8	42.7
C-14	81.3	81.5	82.3	82.1	82.3	56.4	52.2
C-15	83.6	83.6	30.1	83.4	83.4	24.6	24.6
C-16	121.1	120.8	37.7	120.6	120.4	27.3	27.4
C-17	161.9	162.4	87.8	160.8	161.3	52.1	49.7
C-18	16.0	17.7	20.6	16.7	16.8	11.7	11.7
C-19	15.1	15.9	14.6	15.6	15.1	19.6	18.3
C-20	35.3	35.4	80.0	35.4	36.1	39.1	38.2
C-21	17.5	17.3	19.5	17.0	17.2	13.5	13.5
C-22	77.9	79.4	80.3	78.3	78.5	78.1	78.2
C-23	32.7	33.1	32.5	32.1	32.4	29.8	29.8
C-24	148.7	148.4	151.1	150.6	150.3	153.9	153.9
C-25	122.1	122.2	121.4	121.3	121.4	127.3	122.9
C-26	166.3	166.4	166.6	167.6	167.5	166.3	166.4
C-27	12.4	12.4	12.3	12.3	12.4	56.1	56.2
C-28	20.4	20.3	20.6	20.7	20.6	20.1	20.1
15-OAc	21.3	21.3		21.4	21.4		
	169.8	170.0		170.5	170.6		
glc-1						102.7	102.9
glc-2						75.2	75.2
glc-3						78.4	78.5
glc-4						71.4	71.5
glc-5						78.3	78.4
glc-6						62.5	62.7

Physagulin B (**2**), a white powder, showed peaks due to $[M]^+$ (*m/z* 546), $[M-H_2O+H]^+$ (*m/z* 531, 529), $[M-AcOH+H]^+$ (*m/z* 489, 487), $[M-H_2O-AcOH+H]^+$ (*m/z* 471, 469) and $[M-2H_2O-AcOH+H]^+$ (*m/z* 451) in the positive FAB-MS. The above set of pair peaks suggested the occurrence of chlorine in the molecule. Beilstein Cu reaction was positive. The IR spectrum of **2** indicated the presence of an acetyl group (1734 cm^{-1}), an α,β-unsaturated δ-lactone group (1714 cm^{-1}) and an α,β-unsaturated carbonyl group (1694 cm^{-1}). Comparison of the ^{1}H-NMR and ^{13}C-NMR spectral data (Tables I and II, respectively) of **2** with those of withaminimin (**5**) showed that the two were almost similar except the signals around C-5. Therefore, it was deduced that a chlorine atom was located at C-5. Furthermore, the signals around C-5 could be assigned as listed in Tables I and II by the ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY measurements. The stereochemistries at C-14 and C-15 in **2** were established as *R* and *S* since they showed the same chemical shifts and coupling constants as those of **1**. The positive Cotton effects at 247 and 340 nm in the CD spectrum of **2** indicated the 22*R* configuration and *trans*-fusion in rings A and B. Thus, the structure of physagulin B (**2**) was characterized as shown in the formula.

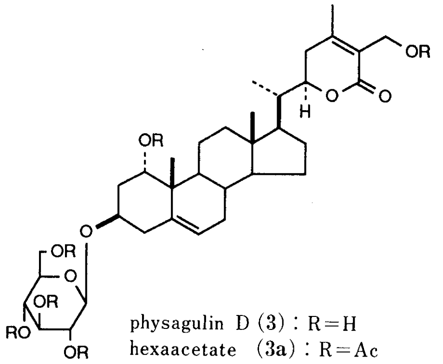
Physagulin D (**3**), a white powder, had a strong absorption band at 3432 cm^{-1} (hydroxyl) and a characteristic band at 1698 cm^{-1} (α,β-unsaturated δ-lactone) in its IR spectrum. The positive FAB-MS showed a $[M+H]^+$ peak at *m/z* 621 and a $[M-\text{glucose}+H]^+$ peak at *m/z* 459. The hexaacetate (**3a**) of **3** showed peaks due to $[M+Na]^+$ (*m/z* 895), $[M+H]^+$ (*m/z* 873), $[M+Na-AcOH+H]^+$ (*m/z* 835) and $[M-AcOH+H]^+$ (*m/z* 813) in the positive FAB-MS. Assignments of the respective ^{1}H-NMR signals in **3a** were made by comparison with those of rings C, D and side chain moiety of daturataturin B heptaacetate (**8a**),[8] and with those of rings A, B and the sugar moiety of pubescenin



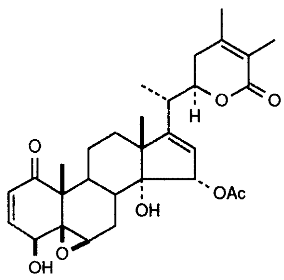
physagulin A (1)



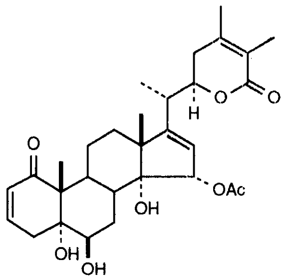
physagulin B (2)



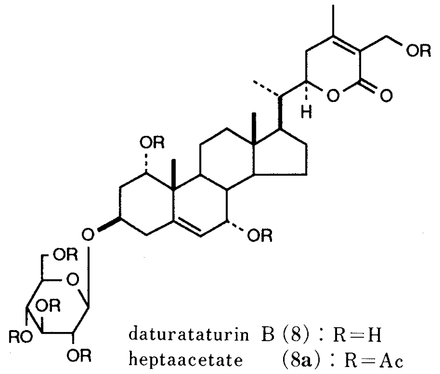
physagulin D (3) : R=H
hexaacetate (3a) : R=Ac



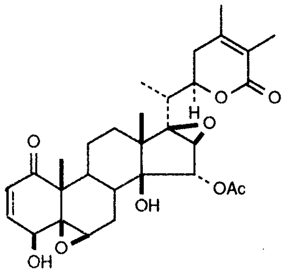
withagulatin A (4)



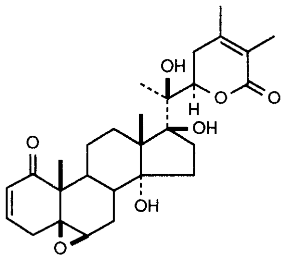
withaminimin (5)



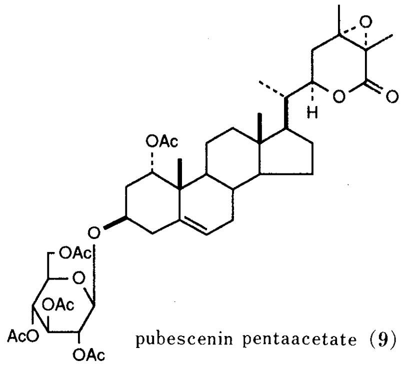
daturataturin B (8) : R=H
heptaacetate (8a) : R=Ac



physagulin C (6)



withanolide E (7)



pubescenin pentaacetate (9)

pentaacetate (9).[9] Namely, the signals in ^{1}H-NMR spectrum for 3a could be assigned as follows: four methyl groups [δ 0.71 (s, H_3-18), 1.00 (d, $J=6.6$ Hz, H_3-21), 1.06 (s, H_3-19) and 2.05 (s, H_3-28)], one hydroxymethyl group [δ 4.86 (d, $J=11.7$ Hz, H-27) and 4.90 (d, $J=11.7$ Hz, H′-27], and two oxygen methine protons [δ 4.42 (br d, $J=9.5$ Hz, H-22), 3.82 (m, H-3)]. These proton signals indicated that the sugar moiety combined with C-3 as well as with daturataturin B (**8**). Furthermore, signals due to an acetoxymethine proton [δ 5.05 (m, H-1)], an olefinic proton [δ 5.53 (m, H-6)] and tetra-*O*-acetyl β-D-glucopyranosyl residue [δ 4.58 (d, $J=8.0$ Hz, glc H-1), 4.94 (dd, $J=9.5$, 8.0 Hz, glc H-2), 5.20 (t, $J=9.5$ Hz, glc H-3), 5.05 (dd, $J=9.9$, 9.5 Hz, glc H-4), 3.68 (ddd, $J=9.9$, 4.9, 2.5 Hz, glc H-5), 4.09 (dd, $J=12.1$, 2.5 Hz, glc H-6) and 4.24 (dd, $J=12.1$, 4.9 Hz, glc H′-6)] were in good agreement with those of pubescenin pentaacetate (**9**). The ^{1}H-NMR spectrum, therefore, suggested that **3a** was a withanolide having an 1α-acetoxy-3β-*O*-tetraacetyl-β-D-glucopyranosyl-5-ene system. The ^{13}C-NMR spectrum (Table II) of **3** was also in good accordance with that of **8**, except for signals around C-7. Based on these spectral data and the observation of a positive Cotton effect at 253 nm suggesting a 22*R* configuration, the structure of physagulin D (**3**) was concluded as shown in the formula.

Regarding the three compounds here obtained, it is summarized that physagulin A (**1**) corresponds to the 4-deoxy derivative of **4**, that physagulin B (**2**) can be derived by fission of the 5β,6β-epoxy compound accompanied by introduction of naturally occurring chlorine atom and that physagulin D (**3**) is an intermediate substance as is **8** of the 1-one-2-ene withanolide.

Experimental

The 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

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a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The FAB-MS were measured with JEOL DX-303 HF spectrometer and taken in a glycerol matrix containing NaI. Thin layer chromatography was performed on precoated Kieselgel 60 F_{254} (Merck) and detection was achieved by spraying 10% H_2SO_4 following by heating. Column chromatography was carried out on Kieselgel (270—30 mesh and 230—400 mesh, Merck) and Sephadex LH-20 (Pharmacia Fine Chemicals).

Extraction and Separation The fresh leaves and stems of *Physalis angulata* L. (Solanaceae) (2.0 kg), harvested at the botanical garden of Fukuoka University in September 1988, were extracted with MeOH and the extract was partitioned between 1-BuOH and H_2O. The 1-BuOH layer (38 g) was subjected repeatedly to column chromatography over silica gel using $CHCl_3 : MeOH : H_2O = 1:0:0 \rightarrow 8:2:0.1 \rightarrow 0:1:0$ and Sephadex LH-20 using MeOH to give physagulins A (**1**, 28.6 mg), B (**2**, 200.0 mg), D (**3**, 150.0 mg), withaminimin (**4**, 70.0 mg) and withagulatin A (**5**, 90 mg).

Physagulin A (1) A white powder, $[\alpha]_D$ +134.9° (c=0.56, MeOH). Positive FAB-MS m/z: 533 $[M+Na]^+$, 511 $[M+H]^+$, 493 $[M-H_2O+H]^+$, 451 $[M-AcOH+H]^+$, 433 $[M-H_2O-AcOH+H]^+$, 415 $[M-2H_2O-AcOH+H]^+$, 391, 355. IR (KBr): 3496, 1738, 1712, 1676 cm^{-1}. CD (c=0.056, MeOH) $[\theta]$ (nm): −6800 (350) (negative max.), +57800 (253) (positive max.).

Physagulin B (2) A white powder, $[\alpha]_D$ +159.5° (c=0.87, $CHCl_3$). Positive FAB-MS m/z: 529 $[M-H_2O+H]^+$, 487 $[M-AcOH+H]^+$, 469 $[M-H_2O-AcOH+H]^+$, 451 $[M-2H_2O-AcOH+H]^+$, 433, 345. IR (KBr): 3464, 1734, 1714, 1694 cm^{-1}. CD (c=0.052, MeOH) $[\theta]$ (nm): +17800 (340) (positive max.), +75400 (247) (positive max.).

Physagulin D (3) A white powder, $[\alpha]_D$ +21.0° (c=0.80, MeOH). Positive FAB-MS (m/z): 621 $[M+H]^+$, 459 $[M-glc+H]^+$, 441 $[M-glc-H_2O+H]^+$, 423 $[M-glc-2H_2O+H]^+$, 405 $[M-glc-3H_2O+H]^+$, 351, 251. IR (KBr): 3432, 2940, 2932, 1698, 1464, 1436, 1400, 1346, 1322, 1298 cm^{-1}. CD (c=0.08, MeOH) $[\theta]$ (nm): +69750 (253) (positive max.). ^{1}H-NMR (pyridine-d_5) δ: 0.62 (3H, s, H_3-18), 0.97 (3H, d, J=6.6 Hz, H_3-21), 1.01 (3H, s, H_3-19), 2.13 (3H, s, H_3-21), 2.64 (1H, t, J=13.1 Hz, H-4), 2.88 (1H, dd, J=13.1, 3.3 Hz, H′-4), 3.83—5.02 (12H, m, methine and methylene protones bearing oxygen atoms), 5.57 (d, J=4.8 Hz, H-6).

Acetylation of 3 A solution of **3** (53 mg) in pyridine–acetic anhydride (2 : 1, 1.5 ml) was left overnight at r.t. and the reaction mixture was chromatographed on silica gel (benzene : acetone = 20 : 1) to afford physagulin D hexaacetate (**3a**, 23.4 mg), a white powder, $[\alpha]_D$ +19.7° (c=0.44, $CHCl_3$). Positive FAB-MS m/z: 895 $[M+Na]^+$, 873 $[M+H]^+$, 835 $[M+Na-AcOH+H]^+$, 813 $[M+H-AcOH]^+$, 525, 465, 405, 331, 211.

References and Notes

1) This work is Part 21 in the series of studies on the constituents of solanaceous plants.

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