New Withanolide Glycosides from *Physalis peruviana* L.

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Four new withanolide glycosides (1—4) have been isolated from *Physalis peruviana*. Their structures were established as (20*R*,22*R*)-1α-acetoxy-14α,20-dihydroxywitha-5,24-dienolide-3β-(*O*-β-D-glucopyranoside) (1), (20*S*,22*R*)-1α-acetoxy-27-hydroxywitha-5,24-dienolide-3β-(*O*-β-D-glucopyranoside) (2), (20*R*,22*R*)-20,27-dihydroxy-1-oxowitha-5,24-dienolide-3β-(*O*-β-D-glucopyranoside) (3), and (20*R*,22*R*)-14α,20,27-trihydroxy-1-oxowitha-5,24-dienolide-3β-(*O*-β-D-glucopyranoside) (4) on the basis of spectroscopic data including two dimensional NMR techniques and through chemical transformations.

Key words *Physalis peruviana*; Solanaceae; withanolide glycoside

The broad spectrum of biological activities exhibited by withanolides is responsible for the undiminishing interest in them.[1,2] A search of the literature has revealed that withanolide glycosides have only been marginally explored in plants elaborating withanolides and related ergostane-type steroids. The first withanolide glycosides, dunawithanine A and B, were isolated by Adam and co-workers[3] from *Acnistus australis*, and about 24 compounds of this type have appeared in the literature to date. Due to our interest in the biological activities of glycosidic derivatives of withanolides, we carried out phytochemical investigations of the polar fractions of *Physalis peruviana* (Solanaceae).[4] It is a tropical hairy plant with fuzzy, slender-pointed, heart-shaped leaves, bearing yellowish flowers and orange edible fruits.[4,5] The plant has a variety of medicinal uses in the traditional system of medicine.[6—8] The presence of a number of withanolides, many of which are biologically active,[1,2] in different parts of this plant has been reported.[1,9] In the present paper, we wish to describe the isolation and characterisation of four new withanolide glycosides (**1**—**4**) along with the known compounds physalolactone B[10] and its glucoside.[11]

Results and Discussion

Chromatographic resolution of the methanol soluble fraction of *P. peruviana* afforded new withanolide glycosides (**1**—**4**). Compound (**1**), an amorphous solid, exhibited absorption bands at 3464 (hydroxyl), 1736 (acetyl group) and 1710 cm^{-1} (α,β-unsaturated δ-lactone) in the infrared (IR) spectrum. The ultraviolet (UV) absorption at 228 nm further supported the presence of an α,β-unsaturated δ-lactone. The positive fast atom bombardment mass spectrum (FAB-MS) displayed peaks at *m/z* 679 $[M+H]^+$, 661 $[M-H_2O+H]^+$, 643 $[M-2H_2O+H]^+$, 583 $[M-2H_2O-AcOH+H]^+$, 517 [M−162 (hexosyl unit)+H]$^+$, 457 $[M-162-AcOH+H]^+$, 439 $[M-162-AcOH-H_2O+H]^+$. The molecular formula $C_{36}H_{54}O_{12}$, derived from positive HR-FAB-MS, showed ten double bond equivalents. Four of these were accounted for by the tetracyclic steroidal skeleton, and the other six by double bonds, δ-lactone, carbonyl of the acetoxyl group and a hexose moiety. The acetylation of naturally occurring monoacetate **1** afforded a pentaacetate **1a** which still showed hydroxyl absorption at 3431 cm^{-1}, confirming the presence of tertiary hydroxyls in **1**. Acid hydrolysis of **1** yielded an aglycone along with sugar, the latter being identified as glucose by paper chromatography (PC) and also through the retention time of its trimethylsilyl (TMS) ether in gas chromatography (GC).[12] Compounds **2**—**4** also yielded the same sugar moiety on acid hydrolysis. The 20-hydroxywithanolide skeleton of the aglycone of **1** was revealed from mass spectral peaks at *m/z* 125.0603 ($C_7H_9O_2$) and 169.0863 ($C_9H_{13}O_3$) and from its ^{1}H-NMR signals, which included those of five methyl groups including two vinyl methyls and one of H-22 appearing as a dd at δ 4.43.[13] The substitution patterns of rings A, B and the C-17 side chain were deduced from ^{1}H-NMR spectral comparison of the aglycone with that of deacetylphysalolactone B (1α,3β,20-trihydroxy-20*R*,22*R*-witha-5,24-dienolide).[10,13] The chemical shifts and splitting pattern of the signals originating from the carbinyl hydrogens at C-1 (δ 3.84, 1H, t) and C-3 (δ 3.96, 1H, m) and the lone olefinic proton at C-6 (δ 5.61, 1H, br d) were in perfect agreement with those of deacetylphysalolactone B.[10,13] Similarly, signals due to vinyl methyls (δ 1.84, 1.96), Me-21 (δ 1.31) and H-22 (δ 4.43) were also in conformity with those of deacetylphysalolactone B. Comparison of the ^{13}C-NMR data of the aglycone with those of deacetylphysalolactone B[10,13] further supported the above substitution patterns of rings A, B, and the C-17 side chain. However, chemical shifts of the carbons of rings C and D showed significant differences. The most striking difference observed was the downfield shift of the C-14 signal from δ 56.8 (CH) to 84.8 (C) in the aglycone of **1**. Such a substantial downfield shift is caused by the presence of a 14-OH group.[14] This was supported by the molecular formula, $C_{28}H_{42}O_6$, which is 16 units heavier than deacetylphysalolactone B. It has been observed that 14β-OH does not cause the shielding of C-12,[15] while 14α-OH shields C-7, C-9 and C-12 through γ-effects and deshields C-8 through a β-effect.[16] On this basis, α-orientation was assigned to 14-OH in the aglycone of **1**. This assignment was further supported by comparison of ^{13}C-NMR chemical shifts of rings C and D of the aglycone with those of similarly substituted withanolides.[17] The aglycone of **1** was thus characterized as 1α,3β,14α,20-tetrahydroxy-20*R*,22*R*-witha-5,24-dienolide.

The ^{1}H-NMR spectrum of **1** showed a striking resemblance to that of physalolactone B glucopyranoside,[11] indicating the same substitution patterns in rings A, B and the C-17 side chain. The spectrum included signals for three tertiary methyls (δ 1.29, 1.06, 1.02), two vinylic methyls (δ 1.94, 1.85), and one acetyl methyl (δ 2.10) besides signals

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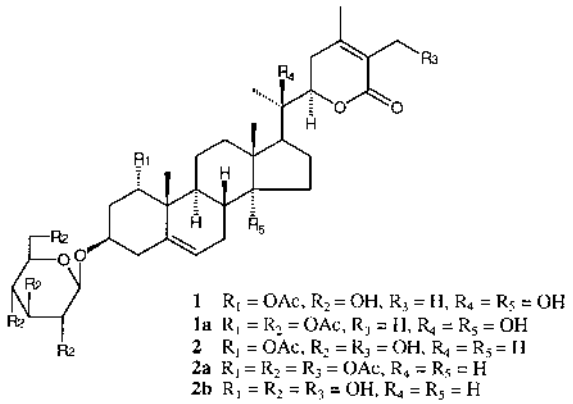

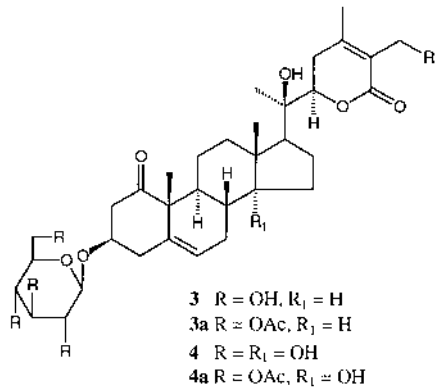

for three oxymethine hydrogens at δ 4.53 (1H, d, J=7.9 Hz), 4.25 (1H, dd, J=12.4, 3.5 Hz) and 3.91 (1H, m) attributable to H-1′, H-22 and H-3, respectively. Furthermore, signals due to an olefinic H-6 and an acetoxymethine H-1 at δ 5.46 (1H, br d, J=5.3 Hz) and 5.07 (1H, t, J=2.6 Hz) were discernible in the spectrum. The latter signal shifted upfield to δ 3.84 in the hydrolyzed product (the aglycone of **1**).

The β-glycosidic-type linkage was inferred from the ^{1}H-NMR signal of H-1′ (δ 4.53, d, J=7.9 Hz) and that of the anomeric carbon in the ^{13}C-NMR spectrum (δ 101.2, CH).[15,18] The attachment of sugar was located through an heteronuclear multiple-bond correlation spectroscopy (HMBC) experiment in which the anomeric proton (δ 4.53) showed long-range correlation ^3J with C-3 (δ 73.8) of the aglycone, while H-3 (δ 3.91) of the aglycone displayed a ^3J interaction with the anomeric carbon (δ 101.2). The same type of β-glycosidic linkage was found in all four of the compounds **1**—**4**. The position of the acetoxyl group was confirmed at C-1 since a ^3J correlation of 1β-H (δ 5.07) to acetoxyl carbonyl at δ 170.3 was observed in the HMBC spectrum. All the assignments in **1**—**4** were confirmed by correlation spectroscopy (COSY)-45, heteronuclear multiple quantum coherence (HMQC) and HMBC experiments.

It has been found that when C-22 has an *S*-configuration, H-22 resonates as a broad singlet with $W_{1/2}\approx$5 Hz, while in the *R*-configuration it appears in the ^{1}H-NMR spectrum as a double doublet with two coupling constants characteristic for axial–axial and axial–equatorial interactions with H_2-23.[19] In the case of compounds **1**, **3** and **4**, H-22 resonated as a double doublet, revealing an *R*-configuration at C-22. The compound **1** was, therefore, assigned the structure (20*R*,22*R*)-1α-acetoxy-14α,20-dihydroxywitha-5,24-dienolide-3β-(*O*-β-D-glucopyranoside).

Compound (**2**), $C_{36}H_{54}O_{11}$, was isolated as an amorphous powder. The IR, ^{1}H- and ^{13}C-NMR data of **2** showed close resemblance to those of **1**. Detailed ^{1}H-NMR study revealed the same substitution in rings A and B, with a major difference in the signals of rings C, D and the C-17 side chain. The oxymethine H-22 appeared as a double triplet at δ 4.46 (1H, J=13.3, 3.4 Hz), showing the absence of hydroxyl group at C-20. This was supported by a doublet at δ 1.02 (3H, J=6.5 Hz) for a secondary methyl at C-20. Four singlets at δ 2.06, 2.01, 1.05 and 0.74 (3H, each) were attributed to acetoxy methyl, vinylic methyl at C-24 and two tertiary methyls at C-10 and C-13, respectively. The absence of another vinylic methyl singlet and the appearance of AB doublets at δ 4.16 and 4.31 (J=12.6 Hz) suggested that C-27 was present as a hydroxylmethyl. Acetylation of naturally occurring monoacetate **2** afforded hexaacetate **2a** in which the aforementioned AB doublets moved to δ 4.76 and 4.84, confirming the presence of a primary hydroxyl at C-27. The mass spectrum of **2** showed a peak at *m/z* 141.0553 ($C_7H_9O_3$) which further supported a hydroxy-substituted α,β-unsaturated δ-lactone moiety at C-20.[17] The spectral data of **2a** coincided with those of physagulin D hexaacetate.[20] Alkaline hydrolysis of **2** with 0.1 N methanolic NaOMe (48 h at 20 °C) furnished the deacetyl derivative **2b** which was identified as physagulin D.[19] Acid hydrolysis (1 N methanolic HCl, 4 h reflux) afforded glucose and an aglycone which was identical to the pubesenolide reported earlier by Sahai.[21] Hence, compound **2** was formulated as (20*S*,22*R*)-1α-acetoxy-27-hydroxywitha-5,24-dienolide-3β-(*O*-β-D-glucopyranoside).

Compound (**3**) $C_{34}H_{50}O_{11}$, an amorphous solid, bears close resemblance to compounds **1** and **2** as revealed by its IR, ^{1}H- and ^{13}C-NMR spectral data. However, the presence of an absorption band at 1702 (cyclohexanone) instead of 1736 cm^{-1} (acetyl) in the IR spectrum and the absence of signals for acetoxymethine and acetoxy methyl protons in its ^{1}H-NMR, together with the existence of a signal at δ 209.9 in its ^{13}C-NMR spectrum, indicated the presence of a carbonyl at C-1 instead of an acetoxyl group. The hydroxyl-substituted α,β-unsaturated δ-lactone moiety was evident from a fragment ion at *m/z* 141.0553 ($C_7H_9O_3$), which originated by the cleavage of a C-20/C-22 bond.[17] The signals at δ 2.03 (3H, s, Me-28), 4.32 (1H, dd, J=13.2, 3.6 Hz, H-22), 4.12, 4.26 (2H, AB d, J=12.0 Hz, H-27) in the ^{1}H-NMR supported this argument. Acetylation of **3** provided pentaacetate **3a** in which the signals of AB doublets moved downfield to δ 4.19 and 4.36, confirming the presence of a primary hydroxyl at C-27. The multiplicity of H-22 (dd) and Me-21 (δ 1.28, 3H, s) showed the presence of a hydroxyl group at C-20. This was confirmed by the fragment ion at *m/z* 185.0816 ($C_9H_{13}O_4$) in the mass spectrum of **3**.[17] Acid hydrolysis of **3** afforded glucose and an aglycone which was shown to be a 20,27-dihydroxywithanolide from mass spectral peaks at *m/z* 141.0553 ($C_7H_9O_3$) and 185.0816 ($C_9H_{13}O_4$) and ^{1}H-NMR signals at δ 4.29 (1H, dd, J=13.0, 4.0 Hz, H-22), 4.27, 4.15 (2H, AB d, J=12.0 Hz, H-27), 2.03 (3H, s, H-28), 1.28 (3H, s, H-21).[17] The substitution patterns of rings A and B were deduced by comparing the oxymethine signal at δ 3.87 (1H, m, H-3) and the olefinic signal at δ 5.56 (1H, br d, J=5.4 Hz, H-6) with those of 3β,14α,20,27-tetrahydroxy-1-oxo-20*R*,22*R*-witha-5,24-dienolide.[17] Comparison of ^{13}C-NMR data of the aglycone with those of the latter compound confirmed the above

substitution patterns of rings A, B and the C-17 side chain. However, the signals due to rings C and D showed significant differences. The most important difference was in the signals of C-13 and C-14 which appeared upfield at δ 43.3 (C) and 56.6 (CH), respectively, revealing the absence of 14-OH. This was in agreement with the molecular formula, $C_{28}H_{40}O_6$, which is 16 units less heavy than 3β,14α,20,27-tetrahydroxy-1-oxo-20*R*,22*R*-witha-5,24-dienolide.[17] This was further confirmed by comparison of the ^{13}C-NMR signals of rings C and D with those of deacetylphysalolactone B[13] and physalolactone B[21] having unsubstituted C and D rings. These evidences led to the structure 3β,20,27-trihydroxy-1-oxo-20*R*,22*R*-witha-5,24-dienolide for the aglycone. Based on these observations, the structure of **3** was deduced to be (20*R*,22*R*)-20,27-dihydroxy-1-oxowitha-5,24-dienolide-3β-(*O*-β-D-glucopyranoside).

Compound (**4**), $C_{34}H_{50}O_{12}$, was isolated as an amorphous powder. The IR, UV, ^{1}H- and ^{13}C-NMR of **4** were similar to **3** with particular reference to the substitution pattern of rings A, B and the C-17 side chain. Acetylation provided pentaacetate **4a**, which still showed hydroxyl absorption at 3451 cm^{-1} in the IR spectrum, confirming the presence of tertiary hydroxyl groups in **4**. Moreover, the chemical shift values of the carbons of rings C and D differed significantly from those of **3**. The ^{13}C-NMR chemical shift values of C-14 (δ 85.4), C-13 (δ 48.1), C-12 (δ 33.2), C-9 (δ 35.2), C-8 (δ 35.7), and C-7 (δ 26.4) were indicative of a 14α-OH group.[14—17] The chemical shifts due to rings C and D were identical to those of **1**. The acid hydrolysis yielded glucose and an aglycone which was found to be identical to 3β,14α,20,27-tetrahydroxy-1-oxo-20*R*,22*R*-witha-5,24-dienolide.[17] The structure (20*R*,22*R*)-14α,20,27-trihydroxy-1-oxowitha-5,24-dienolide-3β-(*O*-β-D-glucopyranoside) was, therefore, confirmed for **4**.

Experimental

Optical rotations were measured on a JASCO DIP-360 polarimeter. IR and UV spectra were recorded on JASCO 302-A and Hitachi U 3200 spectrophotometers, respectively. EI, FAB and HR-FAB-MS were recorded on JMS HX 110 with a data system and on JMS-DA 500 mass spectrometers. The ^{1}H-,^{13}C-NMR, COSY, HMQC and HMBC spectra were recorded on Bruker spectrometers operating at 500 and 400 MHz for ^{1}H-NMR. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz.

Chromatographic Conditions For TLC precoated aluminium sheets, Silica gel 60 F-254 (20×20 cm, 0.2 mm thick) (E. Merck) were used. Visualization of the TLC plates was achieved under UV at 254 and 366 nm and by spraying with saturated chloroform solution of $SbCl_3$ (with heating) or Dragendorff's reagent.

Plant Material *Physalis peruviana*, whole plant, collected from District Chitral, N.W.F.P (Pakistan) in May, 1996 was identified by Mr. Iftikhar Shah, Plant Taxonomist, Department of Pharmacy, Gomal University, D. I. Khan, where a voucher specimen is deposited in the Herbarium.

Extraction and Separation The air-dried ground plant (30 kg) was exhaustively extracted with 90% EtOH at room temperature. The extract was concentrated and the residue (1.5 kg) was dissolved in MeOH and defatted with petrol. The defatted extract was evaporated and divided into $CHCl_3$-soluble and MeOH-soluble fractions. The latter fraction was concentrated and subsequently loaded on a silica gel column. The elution was carried out with *n*-hexane, *n*-hexane–$CHCl_3$, and $CHCl_3$–MeOH mixtures, gradually increasing the polarity. The fractions obtained in $CHCl_3$–MeOH (8.8 : 1.2) were subjected to flash chromatography (fcc) on silica gel using C_6H_6–$CHCl_3$–MeOH (2 : 5 : 3) as a mobile phase. The first ten fractions were finally purified on TLC using C_6H_6–MeOH (8 : 2) as the solvent system to afford physalolactone B, while final purification of the last twenty fractions by TLC in the solvent system C_6H_6–$CHCl_3$–MeOH (1 : 6 : 3) furnished pure compounds **1**—**4** along with physalolactone B glucopyranoside. Physalolactone B (38.7 mg) and its glucoside (46.6 mg) were identified by comparison of their spectral data with those reported in literature.[10,11]

Compound (1) Amorphous powder (67.4 mg); $[\alpha]_D^{25}$ +16.5° (*c*=0.49, MeOH); IR (KBr) cm^{-1}: 3464, 1736, 1710; UV λ_{max} (MeOH) nm (log ε): 228 (3.89). HR-FAB-MS $[M+H]^+$ *m/z*: 679.3691 (Calcd for $C_{36}H_{55}O_{12}$: 679.3693). EI-MS: *m/z* (rel. int. %): 516 (2), 498 (4), 438 (3), 373 (15), 329 (7), 313 (13), 295 (18), 269 (19), 251 (31), 196 (19),169 (100), 126 (59), 125 (48), 118 (16). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.02 (3H, s, H-18), 1.06 (3H, s, H-19), 1.29 (3H, s, H-21), 1.85 (3H, s, H-27), 1.94 (3H, s, H-28), 2.10 (3H, s, OCOC<u>H</u>$_3$), 3.91 (1H, m, H-3), 4.25 (1H, dd, *J*=12.4, 3.5 Hz, H-22), 4.53 (1H, d, *J*=7.9 Hz, H-1′), 5.07 (1H, t, *J*=2.6 Hz, H-1), 5.46 (1H, br d, *J*=5.3 Hz, H-6). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 12.6 (C-27), 17.5 (C-18),19.5 (C-19), 20.6 (C-28), 20.8 (C-16), 21.1 (OCO<u>C</u>H$_3$), 21.3 (C-21), 21.7 (C-11), 27.6 (C-7), 31.8 (C-23), 32.4 (C-12), 32.7 (C-15), 35.4 (C-8), 35.7 (C-2), 38.4 (C-9), 39.3 (C-4), 40.4 (C-10), 47.8 (C-13), 49.5 (C-17), 62.4 (C-6′), 71.6 (C-4′), 73.8 (C-3), 74.6 (C-1), 74.9 (C-2′), 75.4 (C-20), 78.3 (C-3′), 78.7 (C-5′), 81.3 (C-22), 84.7 (C-14), 101.2 (C-1′), 122.0 (C-25), 125.6 (C-6), 137.4 (C-5), 148.9 (C-24), 166.4 (C-26), 170.3 (O<u>C</u>OCH$_3$).

Acid Hydrolysis of (1) Compound **1** (30.4 mg) was refluxed for 4 h with 1 N methanolic HCl (5 ml). The solution was concentrated under reduced pressure and diluted with 5 ml H_2O. It was extracted with EtOAc and the residue from the organic phase was subjected to preparative TLC which afforded an aglycone (12.7 mg), needles, mp 198—199 °C; $[\alpha]_D^{25}$ +29.3° (*c*=0.36, $CHCl_3$); IR (KBr) cm^{-1}: 3451, 1709, 1650; UV λ_{max} (MeOH) nm (log ε): 230 (3.91). HR-FAB-MS $[M+H]^+$ *m/z*: 475.3055 (Calcd for $C_{28}H_{43}O_6$: 475.3059). EI-MS: *m/z* (rel. int. %): 474 (3), 456 (7), 438 (11), 349 (19), 331 (18), 313 (25), 305 (18), 295 (31), 287 (25), 269 (33) 251 (45),169 (48), 125 (100). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.01 (3H, s, H-18), 1.04 (3H, s, H-19), 1.31 (3H, s, H-21), 1.84 (3H, s, H-27), 1.96 (3H, s, H-28), 3.84 (1H, t, *J*=2.8 Hz, H-1), 3.96 (1H, m, H-3), 4.43 (1H, dd, *J*=12.3, 3.4 Hz, H-22), 5.61 (1H, br d, *J*=5.4 Hz, H-6). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 12.5 (C-27), 17.3 (C-18), 19.5 (C-19), 20.6 (C-28), 20.7 (C-16), 21.2 (C-21), 22.2 (C-11), 26.4 (C-7), 31.6 (C-23), 32.3 (C-12), 32.5 (C-15), 36.3 (C-8), 37.2 (C-9), 38.2 (C-2), 40.8 (C-4), 41.4 (C-10), 47.6 (C-13), 49.4 (C-17), 66.4 (C-3), 73.2 (C-1), 75.5 (C-20), 81.2 (C-22), 84.8 (C-14), 122.0 (C-25), 125.3 (C-6), 137.5 (C-5), 149.2 (C-24), 166.3 (C-26).

For detection of the sugar moiety, the aqueous phase was concentrated and glucose was identified by PC using Schliecher & Schull 2043b chromatographic paper and solvent system *n*-BuOH–HOAc–H_2O (4 : 1 : 5); detection was with aniline–phthalic acid. It was further confirmed by comparing the retention time of its TMS ether with the standard sample in GC.[12]

Acetylation of (1) Compound **1** (20.8 mg) was acetylated with Ac_2O (2 ml) in pyridine (2 ml) at room temperature for 24 h. The solvent was removed *in vacuo* and prep. TLC of the residue afforded pentaactate **1a** (14.2 mg); $[\alpha]_D^{25}$ +21.8° (*c*=0.47, MeOH); IR (KBr) cm^{-1}: 3431, 1733, 1710; UV λ_{max} (MeOH) nm (log ε): 229 (3.82). HR-FAB-MS $[M+H]^+$ *m/z*: 847.4112 (Calcd for $C_{44}H_{63}O_{16}$: 847.4115). EI-MS: *m/z* (rel. int. %): 516 (2), 498 (4), 438 (3), 313 (10), 295 (21), 277 (19), 269 (35), 251 (41), 169 (46), 126 (47), 125 (100). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.01 (3H, s, H-18), 1.05 (3H, s, H-19), 1.30 (3H, s, H-21), 1.86 (3H, s, H-27), 1.95 (3H, s, H-28), 2.02, 2.04, 2.07, 2.08, 2.10 (3H, s, each, 5×OCOC<u>H</u>$_3$), 3.89 (1H, m, H-3), 4.42 (1H, dd, *J*=12.5, 3.7 Hz, H-22), 4.58 (1H, d, *J*=7.8 Hz, H-1′), 5.05 (1H, t, *J*=2.5 Hz, H-1), 5.53 (1H, br d, *J*=5.4 Hz, H-6).

Compound (2) Amorphous powder (71.4 mg); $[\alpha]_D^{25}$ +29.3° (*c*=0.89, MeOH); IR (KBr) cm^{-1}: 3461, 1731, 1710; UV λ_{max} (MeOH) nm (log ε): 218 (3.89). HR-FAB-MS $[M+H]^+$ *m/z*: 663.3741 (Calcd for $C_{36}H_{55}O_{11}$: 663.3744). EI-MS: *m/z* (rel. int. %): 500 (3), 482 (2), 422 (3), 313 (3), 281 (6), 142 (34), 141 (100), 124 (31). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.74 (3H, s, H-18), 1.02 (3H, d, *J*=6.5 Hz, H-21), 1.05 (3H, s, H-19), 2.01 (3H, s, H-28), 2.06 (3H, s, OCOC<u>H</u>$_3$), 3.89 (1H, m, H-3), 4.16, 4.31(2H, AB d, *J*=12.6 Hz, H-27), 4.46 (1H, dt, *J*=13.3, 3.4 Hz, H-22) 4.64 (1H, d, *J*=7.8 Hz, H-1′), 5.06 (1H, t, *J*=2.5 Hz, H-1), 5.53 (1H, br d, *J*=5.4 Hz, H-6). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 12.4 (C-18), 13.3 (C-21), 19.3 (C-19), 20.4 (C-28), 21.4 (OCO<u>C</u>H$_3$), 21.7 (C-11), 24.4 (C-15), 27.3 (C-16), 30.1 (C-23), 31.3 (C-8), 31.7 (C-7), 34.8 (C-2), 38.8 (C-4), 38.9 (C-20), 39.3 (C-12), 41.3 (C-10), 42.1 (C-9), 42.8 (C-13), 51.8 (C-17), 56.5 (C-14), 57.6 (C-27), 62.4 (C-6′), 71.5 (C-4′), 73.4 (C-3), 74.9 (C-2′), 75.4 (C-1), 77.2 (C-3′), 77.6 (C-5′), 78.4 (C-22), 101.4 (C-1′), 122.9 (C-25), 124.9 (C-6), 136.8 (C-5), 154.4 (C-24), 166.6 (C-26), 170.7 (O<u>C</u>OCH$_3$).

Acid Hydrolysis of (2) The procedure described earlier for **1** furnished glucose and an aglycone (10.6 mg), mp 146 °C (lit. 145—146 °C), which was identified as pubesenolide.[21]

Acetylation of (2) Compound **2** (15.5 mg) was acetylated with Ac_2O (2

ml) in pyridine (2 ml) at room temperature for 24 h. Analogous work-up yielded a hexaactate **2a**, an amorphous solid (12.7 mg) which was identical to physagulin D hexaacetate.[20)]

Alkaline Hydrolysis of (2) Compound **2** (25.6 mg) in 5 ml 0.1 N absolute methanolic NaOMe was left for 48 h at room temperature. After neutralization with dil. HOAc, the solution was evaporated under reduced pressure and the residue was purified on TLC to afford a deacetyl derivative **2b** (12.7 mg) which showed spectral data identical to those of physagulin D.[20)]

Compound (3) Amorphous powder (56.8 mg); $[\alpha]_D^{25}$ +73.4° (c=0.43, MeOH); IR (KBr) cm^{-1}: 3454, 1712, 1702, 1650 ; UV λ_{max} (MeOH) nm (log ε): 218 (3.91). HR-FAB-MS $[M+H]^+$ *m/z*: 635.3430 (Calcd for $C_{34}H_{51}O_{11}$: 635.3431). EI-MS: *m/z* (rel. int. %): 472 (4), 454 (3), 436 (8), 331 (36), 287 (9), 246 (6), 185 (18), 141 (41), 124 (100), 123 (13), 95 (13). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.84 (3H, s, H-18), 1.24 (3H, s, H-19), 1.28 (3H, s, H-21), 2.03 (3H, s, H-28), 3.86 (1H, m, H-3), 4.12, 4.26 (2H, AB d, J=12.0 Hz, H-27), 4.28 (1H, d, J=7.9 Hz, H-1′), 4.32 (1H, dd, J=13.2, 3.6 Hz, H-22), 5.47 (1H, br d, J=5.5 Hz, H-6). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 13.6 (C-18), 19.3 (C-19), 20.5 (C-11), 20.6 (C-28), 21.1 (C-21), 22.1 (C-16), 23.8 (C-15), 31.2 (C-8), 31.5 (C-23), 31.6 (C-7), 38.1 (C-4), 39.8 (C-12), 42.1 (C-9), 42.9 (C-13), 45.5 (C-2), 48.5 (C-10), 54.6 (C-17), 56.5 (C-14), 57.8 (C-27), 62.2 (C-6′), 71.4 (C-4′), 74.6 (C-2′), 74.8 (C-3), 75.8 (C-20), 77.4 (C-3′), 77.8 (C-5′), 80.8 (C-22), 100.5 (C-1′), 122.1 (C-25), 124.5 (C-6), 136.7 (C-5), 154.5 (C-24), 164.8 (C-26), 209.9 (C-1).

Acid Hydrolysis of (3) The procedure described earlier for **1** afforded glucose and an aglycone (11.8 mg), mp 186—188 °C; $[\alpha]_D^{25}$ +69.4° (c=0.51, $CHCl_3$); IR ($CHCl_3$) cm^{-1}: 3451, 1710, 1703, 1648; UV λ_{max} (MeOH) nm (log ε): 218 (3.96). HR-FAB-MS $[M+H]^+$ *m/z*: 473.2902 (Calcd for $C_{28}H_{41}O_6$: 473.2902). EI-MS: *m/z* (rel. int. %): 472 (3), 454 (5), 436 (7), 331 (7), 313 (15), 295 (31), 287 (13) 269 (41), (14),185 (34), 167 (36), 142 (41), 141 (46), 124 (100). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.86 (3H, s, H-18), 1.14 (3H, s, H-19), 1.28 (3H, s, H-21), 2.03 (3H, s, H-28), 3.87 (1H, m, H-3), 4.15, 4.27 (2H, AB d, J=12.0 Hz, H-27) 4.29 (1H, dd, J=13.0, 4.0 Hz, H-22), 5.56 (1H, br d, J=5.4 Hz, H-6). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 13.6 (C-18), 19.0 (C-19), 20.4 (C-28), 21.1 (C-21), 21.2 (C-11), 22.3 (C-16), 24.2 (C-15), 31.3 (C-8), 31.9 (C-7), 32.1 (C-23), 39.7 (C-12), 41.2 (C-4), 42.0 (C-9), 43.3 (C-13), 48.7 (C-10), 49.8 (C-2), 54.7 (C-17), 56.6 (C-14), 57.2 (C-27), 68.6 (C-3), 75.6 (C-20), 81.8 (C-22), 124.9 (C-25), 126.4 (C-6), 135.3 (C-5), 154.8 (C-24), 166.7 (C-26), 212.0 (C-1).

Acetylation of (3) Compound **3** (16.4 mg) was acetylated through a procedure similar to that described for compound **1** to obtain the pentaacetate **3a** (12.3 mg); $[\alpha]_D^{25}$ +75.2° (c=0.45, MeOH); IR (KBr) cm^{-1}: 3441, 1735, 1710, 1705; UV λ_{max} (MeOH) nm (log ε): 216 (3.97). HR-FAB-MS $[M+H]^+$ *m/z*: 845.3956 (calcd for $C_{44}H_{61}O_{16}$: 845.3959). EI-MS: *m/z* (rel. int. %): 514 (3), 496 (4), 478 (7), 454 (11), 418 (15), 331 (7), 313 (15), 295 (21), 287 (3) 269 (31), 227 (8), 209 (15), 167 (14),184 (64), 183 (25), 124 (100), 123 (16). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.85 (3H, s, H-18), 1.25 (3H, s, H-19), 1.28 (3H, s, H-21), 2.04 (3H, s, H-28), 2.00, 2.02, 2.05, 2.07, 2.08, (3H, s, each, 5×OCOC$\underline{H}_3$), 3.88 (1H, m, H-3), 4.19, 4.36 (2H, AB d, J=11.8 Hz, H-27) 4.28 (1H, dd, J=12.6, 3.8 Hz, H-22), 4.56 (1H, d, J=7.9 Hz, H-1′), 5.46 (1H, br d, J=5.3 Hz, H-6).

Compound (4) Amorphous powder (66.8 mg); $[\alpha]_D^{25}$ +86.3° (c=0.49, MeOH); IR (KBr) cm^{-1}: 3554, 1710, 1703, 1649; UV λ_{max} (MeOH) nm (log ε): 219 (3.96). HR-FAB-MS $[M+H]^+$ *m/z*: 651.3378 (Calcd for $C_{34}H_{51}O_{12}$: 651.3380). EI-MS: *m/z* (rel. int. %): 488 (5), 470 (7), 452 (13), 434 (12), 347 (3), 329 (9), 311 (5), 303 (6), 285 (14), 185 (21), 142 (34), 141 (100), 124 (85). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.01 (3H, s, H-18), 1.27 (3H, s, H-19), 1.29 (3H, s, H-21), 2.02 (3H, s, H-28), 3.86 (1H, m, H-3), 4.14, 4.27 (2H, AB d, J=12.1 Hz, H-27), 4.34 (1H, d, J=7.8 Hz, H-1′), 4.43 (1H, dd, J=12.6, 3.3 Hz, H-22), 5.64 (1H, br s, H-6). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 18.1 (C-18), 19.0 (C-19), 20.5 (C-28), 20.7 (C-11), 20.9 (C-16), 21.0 (C-21), 26.4 (C-7), 32.1 (C-23), 32.8 (C-15), 33.2 (C-12), 35.2 (C-9), 35.7 (C-8), 38.4 (C-4), 46.2 (C-2), 48.1 (C-13), 49.3 (C-17), 52.1 (C-10), 56.7 (C-27), 62.3 (C-6′), 71.5 (C-4′), 74.4 (C-3), 74.8 (C-2′), 76.2 (C-3′), 76.3 (C-20), 77.2 (C-5′), 81.2 (C-22), 85.4 (C-14), 100.3 (C-1′), 124.6 (C-25), 126.4 (C-6), 134.1 (C-5), 154.2 (C-24), 165.6 (C-26), 209.6 (C-1).

Acid Hydrolysis of (4) Compound **4** was hydrolyzed by repeating the procedure given for compound **1** to obtain glucose and an aglycone (13.9 mg), mp 191—192 °C, which was identified as 3β,14α,20,27-tetrahydroxy-1-oxo-20*R*,22*R*-witha-5,24-dienolide by comparison of its spectral data with those of literature.[17)]

Acetylation of (4) It was performed as described for compound **1** to obtain pentaacetate **4a** (13.4 mg); $[\alpha]_D^{25}$ +81.6° (c=0.47, $CHCl_3$); IR (KBr) cm^{-1}: 3451, 1734, 1708, 1704, 1648; UV λ_{max} (MeOH) nm (log ε): 217 (3.98). HR-FAB-MS $[M+H]^+$ *m/z*: 861.3904 (Calcd for $C_{44}H_{61}O_{17}$: 861.3908). EI-MS: *m/z* (rel. int. %): 530 (2), 512 (5), 494 (9), 470 (11), 434 (17), 347 (9), 329 (11), 311 (19), 303 (12), 293 (31), 285 (15), 267 (21), 184 (67),183 (29), 124 (100), 123 (16). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.02 (3H, s, H-18), 1.29 (3H, s, H-19), 1.31 (3H, s, H-21), 2.04 (3H, s, H-28), 2.01, 2.03, 2.07, 2.08, 2.09 (3H, s, each, 5×OCOC$\underline{H}_3$), 3.89 (1H, m, H-3), 4.24, 4.39 (2H, AB d, J=11.9 Hz, H-27), 4.45 (1H, dd, J=13.0, 4.0 Hz, H-22), 4.53 (1H, d, J=7.9 Hz, H-1′), 5.46 (1H, br d, J=5.3 Hz, H-6).

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