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Withanolides from Aeroponically Grown *Physalis peruviana* and Their Selective Cytotoxicity to Prostate Cancer and Renal Carcinoma Cells

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Abstract

Investigation of aeroponically grown *Physalis peruviana* resulted in the isolation of 11 new withanolides, including perulactones I–L (**1**–**4**), 17-deoxy-23β-hydroxywithanolide E (**5**), 23β-hydroxywithanolide E (**6**), 4-deoxyphyperunolide A (**7**), 7β-hydroxywithanolide F (**8**), 7β-hydroxy-17-*epi*-withanolide K (**9**), 24,25-dihydro-23β,28-dihydroxywithanolide G (**10**), and 24,25-dihydrowithanolide E (**11**), together with 14 known withanolides (**12**–**25**). The structures of **1**–**11** were elucidated by the analysis of their spectroscopic data, and **12**–**25** were identified by comparison of their spectroscopic data with those reported. All withanolides were evaluated for their cytotoxic activity against a panel of tumor cell lines including LNCaP (androgen-sensitive human prostate adenocarcinoma), 22Rv1 (androgen-resistant human prostate adenocarcinoma), ACHN (human renal adenocarcinoma), M14 (human melanoma), SK-MEL-28 (human melanoma), and normal human foreskin fibroblast cells. Of these, the 17β-hydroxywithanolides (17-BHWs) **6**, **8**, **9**, **11**–**13**, **15**, and **19**–**22** showed selective cytotoxic activity against the two prostate cancer cell lines LNCaP and 22Rv1, whereas **13** and **20** exhibited selective toxicity for the ACHN renal carcinoma cell line. These cytotoxicity data provide additional structure–activity relationship information for the 17-BHWs.

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnat-prod.6b01129. ^{1}H, ^{13}C, and 2D NMR spectra of withanolides **1**–**11**, key HMBC and COSY correlations for **1**, **3**, **5**, and **7**–**11**, key NOESY correlations for **1**, **3**–**5**, and **7**–**11**, and ECD spectra for **1**–**11** (PDF)

The authors declare no competing financial interest.

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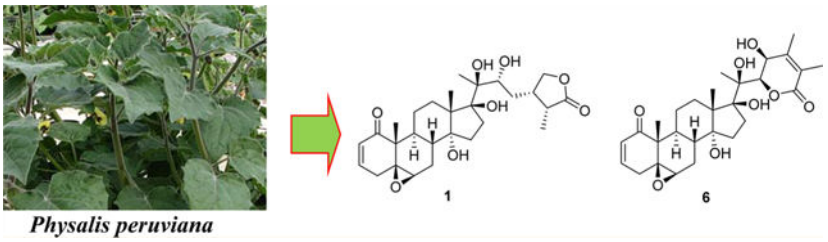
Graphical Abstract



Prostate cancer (PC), a hormone-sensitive cancer that is influenced by androgens such as testosterone,[1] is one of the most frequent solid tumors in older men and the second leading cancer killer of all men in North America.[2] Androgen deprivation is still the standard therapy used to treat the metastatic disease,[3] but patients invariably relapse with more aggressive androgen-insensitive PC. Despite the success of recently approved PC therapies targeting androgen receptor (AR) signaling (e.g., abiraterone)[4] and second-generation antiandrogens (e.g., enzulatamide),[5] durable responses appear to be limited, presumably due to acquired resistance. Moreover, androgen-insensitive PC resumes expression of multiple AR-regulated genes, such as prostate-specific antigen (PSA), suggesting that AR transcriptional activity becomes reactivated at this stage of the disease.[6] Therefore, targeting both androgen-sensitive and androgen-insensitive cancer cells is important in PC drug discovery efforts. Renal carcinoma (RC) is among the 10 most common cancers in the U.S.[7] Current therapies for RC include antiangiogenic and immunotherapies, which have shown only modest success for patients with advanced RC.[8] Thus, the search for effective drugs to treat advanced PC and RC is considered a priority in anticancer drug discovery.

Physalis peruviana L. (family: Solanaceae) is a member of a genus with about 120 species,[9] of which some are employed in traditional medicine in Asia and South America.[10] Over 160 steroidal lactones belonging to the withanolide group have been reported from plants of this genus, and the ethnopharmacological activities of many *Physalis* species used in traditional medicines have been attributed to the presence of these withanolides.[9,10] Previous phytochemical investigations of *P. peruviana* have resulted in the isolation of over 40 withanolides.[11] Of these, 4β-hydroxywithanolide E (**12**), withanolide E (**13**), phyperunolide A (**14**), and withanolide C (**27**) have been reported to be cytotoxic to cell lines derived from lung cancer (A549),[11f] liver carcinomas (Hep G2 and Hep 3B),[11f] and breast adenocarcinomas (MCF-7 and MDA-MB-231).[11f] In addition, **12** was found to inhibit proliferation of MCF-7,[11j] MDA-MB-231,[11j] H1299 (lung cancer),[11h] Ca9–22 (oral cancer),[11k] and HT-29 (colorectal cancer)[11n] cells. We have recently reported that withanolide E (**13**), isolated from wild-crafted *P. peruviana*, was able to eliminate long-term survival of RC cells (ACHN, Caki-1, and SN-12-C) in the presence of TRAIL (tumor necrosis factor-related apoptosis-inducing ligand).[12] In our continuing search for natural-product-based anticancer agents,[13] we have investigated aeroponically grown *P. peruviana*, and herein we report the isolation of 25 withanolides, of which some exhibited selective cytotoxicity for the androgen-sensitive PC (LNCaP), androgen-insensitive PC (22Rv1), and RC (ACHN) cell lines. Eleven of the withanolides encountered are new, and these were identified as perulactones I–L (**1**–**4**), 17-deoxy-23β-hydroxywithanolide E (**5**), 23β-hydroxywithanolide

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E (**6**), 4-deoxyphyperunolide A (**7**), 7β-hydroxywithanolide F (**8**), 7β-hydroxy-17-*epi*-withanolide K (**9**), 24,25-dihydro-23β,28-dihydroxywithanolide G (**10**), and 24,25-dihydrowithanolide E (**11**). Comparison of spectroscopic data with those reported led to the identification of previously known withanolides as 4β-hydroxywithanolide E (**12**),[14] withanolide E (**13**),[14] phyperunolide A (**14**),[11f] withangulatin E (**15**),[15] withanolide S 5-methyl ether (**16**),[16] physalolactone (**17**),[17] withanolide S (**18**),[18] withaperuvin L (**19**),[11i] physapruin A (**20**),[19] withaperuvin J (**21**),[11i] withanolide F (**22**),[20] 4-deoxyphysalolactone (**23**),[17] perulactone B (**24**),[14] and perulactone H (**25**).[14]

RESULTS AND DISCUSSION

Withanolides **1**–**4** were obtained as colorless, amorphous solids. Their ^{1}H NMR and ^{13}C NMR data (Tables 1 and 3) indicated the presence of a γ-lactone side chain similar to those of perulactone and perulactone B (**24**),[11i,14] and therefore **1**–**4** were designated as perulactones I–L. The molecular formula of perulactone I (**1**) was determined to be $C_{28}H_{40}O_8$ based on its HRESIMS and NMR data, suggesting nine degrees of unsaturation. Comparison of its molecular formula and NMR data with those of perulactone B (**24**) ($C_{28}H_{40}O_7$) also encountered in this study suggested that the ring B 5,6-ene moiety in **24** was probably replaced by a 5β,6β-epoxide moiety [δ_H 3.19 (1H, d, J = 2.0 Hz, H-6); δ_C 62.1 (C-5) and 64.0 (C-6)] in **1**. In addition, its HMBC data (Figures S4 and S59, Supporting Information) showed the presence of long-range correlations of H-19 (δ_H 1.26)/C-1 (δ_C 203.8), H-3 (δ_H 6.85)/C-1, H-19/C-5 (δ_C 62.1), H-4β (δ_H 2.96)/C-6 (δ_C 64.0), H-18 (δ_H 1.08)/C-14 (δ_C 83.2), H-18/C-17 (δ_C 88.3), H-21 (δ_H 1.22)/C-17, H-21/C-22 (δ_C 72.3), H-27 (δ_H 1.16)/C-26 (δ_C 180.8), and H-28 (δ_H 4.39 and 4.06)/C-25 (δ_C 37.7), further supporting the presence of a 5,6-epoxide moiety and a perulactone-type side chain in **1**. The β-orientation of the 5,6-epoxide was confirmed by a NOESY correlation between H-6 (δ_H 3.19) and H-4α (δ_H 1.92) (Figures S5 and S60, Supporting Information). The *cis*-linkage of A and B rings in **1** was established by the strong positive Cotton effect at 340 nm in its electronic circular dichrosim (ECD) spectrum[21] (Figure S61, Supporting Information). Epoxidation of perulactone B (**24**) with *m*-CPBA[22] afforded a major product that was identical with **1**. Perulactone I was thus identified as (24*S*,25*R*)-5β,6β-epoxy-14α,17β,20β,22α,28-pentahydroxy-1-oxo-ergosta-2-en-26-oic acid γ- lactone (**1**).

Perulactone J (2) was determined to have the molecular formula $C_{28}H_{40}O_6$, corresponding to nine degrees of unsaturation. The ^{1}H NMR spectrum of **2** contained three olefinic protons [δ_H 6.77 (1H, ddd, J = 10.0, 4.8, 2.4 Hz, H-3), 5.87 (1H, dd, J = 10.0, 2.0 Hz, H-2), 5.59 (1H, brd, J = 5.6 Hz, H-6)], indicating the presence of ring A 2,3-en-1-one and ring B 5,6-ene moieties as in perulactone B (**24**).[14] Comparison of the 13C NMR data of **2** and **24** revealed that the typical tetrasubstituted oxygenated carbon (C-17) signal at δ_C 87.0 in **24**[14] was replaced by a methine signal at δ_C 49.4 in **2**. The long-range correlations observed for H_3-18 (δ_H 1.03)/C-17 (δ_C 49.7) and H_3-21 (δ_H 1.20)/C-17 in the HMBC spectrum of **2** (Figure 2) confirmed the presence of the CH-17 moiety. The NOE correlation between CH_3-18 and CH_3-21 in the 1D NOESY spectrum (Figure 2) suggested that the orientation of the side chain is β. Thus, the orientation of H-17 should be α. The ECD spectrum of **2** showed a negative Cotton effect at 339 nm, which is the opposite of that observed for **1**. This

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difference may be due to the conformational changes of rings A and B of these two compounds caused by the replacement of the 5β,6β-epoxide with a 5,6-ene moiety. The structure of perulactone J was thus elucidated as (17S,24S,25R)-14α,20β,22α,28-tetrahydroxy-1-oxo-ergosta-2,5-dien-26-oic acid γ-lactone (**2**).

The molecular formula of perulactone K (**3**) ($C_{28}H_{38}O_6$) and its ^{1}H and ^{13}C NMR data (Tables 1 and 3) suggested that it is a dehydrated analogue of perulactone I (**1**) ($C_{28}H_{40}O_8$) lacking an oxygen atom. Comparison of its ^{1}H NMR data with those of **1** suggested that **3** contains a signal due to an olefinic proton [δ_H 5.71 (1H, dd, J = 2.4, 1.2 Hz, H-16)] besides signals due to four methyl groups, 2,3-en-1-one, 5β,6β-epoxide, and the side chain γ-lactone moieties. The ^{13}C NMR data (Table 3) of **3** also contained signals typical of other perulactones, including 2,3-en-1-one [δ_C 203.3 (C-1), 144.3 (C-3), 129.3 (C-2)], 5β,6β-epoxide [δ_C 63.2 (C-6), 62.1 (C-5)], and the side chain γ- lactone [δ_C 180.3 (C-26), 72.4 (C-28)]. The 13C NMR spectrum **3** indicated the presence only two oxygenated carbons [δ_C 76.7 (C-20), 74.6 (C-22)]. The HMBC correlations observed for H_3-18 (δ_H 0.94)/C-14 (δ_C 58.1), H_3-21 (δ_H 1.27)/C-17 (δ_C 158.9), and H-16 (δ_H 5.71)/C-13 (δ_C 46.9), while confirming the absence of a hydroxy group at C-14, suggested the presence of a 16,17-ene moiety in **3** (Figures S15 and S59, Supporting Information). The ECD spectrum of **3** showed a positive Cotton effect at 342 nm, which confirmed the stereochemistry of the ring A/B linkage as being the same as that in **1**.[21] On the basis of the foregoing evidence, the structure of perulactone K was determined as (24S,25R)-5β,6β-epoxy-20β,22α,28-trihydroxy-1-oxo-ergosta-2,16-dien-26-oic acid γ-lactone (**3**).

The HREIMS and ^{1}H and ^{13}C NMR data of perulactone L (**4**) were consistent with the molecular formula $C_{28}H_{40}O_7$ and indicated that it is an isomer of perulactone B (**24**). The ^{1}H and ^{13}C NMR spectra of **4** (Tables 1 and 3), assigned with the help of HSQC data (Supporting Information, Figures S19), were almost identical with those of perulactone B (**24**) except for the absence of signals due to the 2(3)-en-1-one moiety in ring A of **24**. Instead, the ^{1}H NMR spectrum of **4** exhibited signals due to a tetrasubstituted 3(4),5(6)-conjugated diene moiety [δ 6.02 (1H, dd, J = 9.6 and 2.8 Hz), 5.61 (1H, ddd, J = 9.6, 4.4, and 2.8 Hz), and 5.66 (1H, t, J = 4.0 Hz)]. These data suggested that **4** is isomeric with **24** and that they differ from each other by virtue of the position of the olefinic double bond in ring A. Finally, treatment of **24** with NaCN–MeOH resulted in the isomerization of its 1-oxo-2(3),5(6)-diene moiety to a 1-oxo-3(4),5(6)-diene unit, affording **4**. Thus, the structure of perulactone L was determined as (24S,25R)-14α,17β,20β,22α,28-pentahydroxy-1-oxo-ergosta-3,5-dien-26-oic acid γ-lactone (**4**).

Withanolide **5** was determined to have the molecular formula $C_{28}H_{38}O_7$, based on its HRESIMS and NMR data. The ^{1}H NMR spectrum of **5** (Table 2) showed signals due to H-3 [δ_H 6.83 (1H, ddd, J = 10.0, 6.4, 2.4 Hz)], H-2 [δ_H 6.00 (1H, dd, J = 10.0, 2.0 Hz)], H-6 [δ_H 3.18 (1H, d, J = 2.0 Hz)], H-22 [δ_H 4.04 (1H, d, J = 8.8 Hz)], five methyl groups, and an oxygenated methine group [δ_H 4.38 (1H, brd, J = 8.8 Hz)]. Comparison of the ^{13}C NMR data of **5** (Table 3) with those of withanolide E (**13**)[14] revealed that the CH_2-23 group in **13** is replaced by an oxygenated methine [δ_C 67.1], and the presence of a methine signal at δ_C 49.0 suggested that C-17 of **5** is not oxygenated. A long-range correlation observed for

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H_3-28 and C-23 in its HMBC spectrum (Figure S25, Supporting Information) further supported the presence of OH-23 in **5**, whereas long-range correlations of H_3-18 (δ_H 1.01)/C-17 and H_3-21 (δ_H 1.33)/C-17 suggested the presence of a methine proton at C-17, confirming that **5** is a 17-deoxy-23-hydroxy analogue of withanolide E (**13**). The large coupling constant (J = 8.8 Hz) observed for H-22/H-23 of **5** was consistent with the relevant dihedral angle of 45° for the favorable conformation adopted by ring E with a β-OH at C-23 as in 23β-hydroxyphysacoztolide.[13] The β-orientation of its side chain at C-17 was deduced from the NOE correlation observed for H_3-18/H_3-21 (Figure S26, Supporting Information). The ECD spectrum of **5** exhibited positive Cotton effects at 265 and 339 nm, suggesting the 22R configuration[21] and the *cis*-linkage of rings A/B.[21] Thus, the structure of **5** was established as 17-deoxy-23β-hydroxywithanolide E [(17S,20S,22R)-5β,6β-epoxy-14α,20,23β-trihydroxy-1-oxowitha-2,24-dienolide].

The HREIMS and NMR data of withanolide **6** were consistent with the molecular formula $C_{28}H_{38}O_8$. Its ^{1}H NMR spectrum (Table 2) exhibited signals due to H-3 [δ_H 6.79 (1H, ddd, J = 10.0, 6.4, 2.4 Hz)], H-2 [δ_H 6.00 (1H, dd, J = 10.0, 2.4 Hz)], H-6 [δ_H 3.17(1H, brs)], H-22 [δ_H 4.76 (1H, d, J = 10.8 Hz)], five methyl groups, and an oxygenated methine group [δ_H 4.27 (1H, brd, J = 10.8 Hz)]. Comparison of the ^{13}C NMR data of **6** (Table 3) with those of withanolide E (**13**)[14] revealed that the CH_2-23 in **13** is replaced by an oxygenated methine (δ_C 66.4) in **6**. Therefore, **6** was suspected to be a 23-hydroxy analogue of withanolide E. In its HMBC spectrum, the presence of a correlation between H_3-28 and C-23 supported the presence of OH-23 in **6** (Figure S31, Supporting Information and Figure 2). The large coupling constant observed for H-22/H-23 (J = 10.8 Hz) of **6** similar to those of **5** and 23β-hydroxyphysacoztolide[13] suggested that the orientation of OH-23 is β. The ECD spectrum of **6** showed positive Cotton effects at 265 and 339 nm, suggesting a 22R configuration[21] and *cis*-linkage of rings A/B.[21] On the basis of the foregoing evidence, the structure of withanolide **6** was elucidated as 23β-hydroxywithanolide E [(20S,22R)-5β,6β-epoxy-14α,17β,20,23β-tetrahydroxy-1-oxowitha-2,24-dienolide].

The molecular formula of withanolide **7** was determined to be $C_{28}H_{36}O_6$ using a combination of HRESIMS and ^{13}C NMR data and accounted for 11 degrees of unsaturation. Comparison of its ^{1}H and ^{13}C NMR data (Tables 2 and 3) with those of withanolide E (**13**) and phyperunolide A (**14**) indicated that **7** contains 2,3-en-1-one, 5β,6β-epoxide, and side chain δ-lactone moieties similar to those in **13** and a 16,17-ene moiety as in **14**. The ^{1}H and ^{13}C NMR data [δ_H 5.77 (1H, dd, J = 3.6, 1.2 Hz); δ_C 124.3 (CH) and 156.4 (C)] further suggested the presence of a 16,17-double bond in **7**, which was confirmed by the correlations of H_3-18 [δ_H 1.12 (3H, s)]/C-17 [δ_C 156.4 (C)] and H_3-21 [δ_H 1.27 (3H, s)]/C-17 observed in its HMBC spectrum (Figure S36, Supporting Information). The absolute configuration of C-22 was determined as R from the positive Cotton effect at 257 nm in its ECD spectrum.[21] Thus, withanolide **7** was identified as 4-deoxyphyperunolide A [(20S,22R)-5β,6β-epoxy-14α,20-dihydroxy-1-oxowitha-2,16,24-trienolide].

Withanolides **8** and **9** were determined to have the same molecular formula ($C_{28}H_{38}O_7$) by the analysis of their HREIMS and ^{13}C NMR data, indicating that they are isomeric with each other and both are mono-oxygenated analogues of withanolide F (**22**),[20] a compound also encountered in the same plant extract. Comparison of the ^{1}H NMR and ^{13}C NMR data

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of **8** (Tables 2 and 3) with those of **22** suggested them to be very similar except that one of the carbocyclic ring CH_2 groups of **22** underwent hydroxylation, leading to a CH-OH [δ_H 4.28 (1H, d, J = 8.8 Hz); δ_C 65.9 (CH)] moiety present in **8**. The HMBC correlations of H-7 (δ_H 4.28)/C-5 (δ_C 136.3) and H_3-19 (δ_H 1.20)/C-5 observed for **8** (Figure S41, Supporting Information) suggested that this OH is attached to C-7, and the NOE correlation of H-7/H-9α (δ_H 2.33) (Figure S42, Supporting Information) helped to determine the orientation of OH-7 as β. The strong positive Cotton effect at 252 nm in the ECD spectrum suggested a 22*R* configuration.[21] The structure of withanolide **8** was thus established as 7β-hydroxywithanolide F [(20S,22R)-7β,14α,17β,20-tetrahydroxy-1-oxowitha-2,5,24-trienolide (**8**)]. The ^{1}H NMR spectrum of withanolide **9** lacked resonances due to the ring A 2,3-en-1-one moiety, but exhibited signals due to a tetrasubstituted 3(4),5(6)-conjugated diene moiety [δ 6.01 (1H, dd, J = 10.0 and 2.4 Hz), 5.67 (1H, ddd, J = 10.0, 4.4, and 2.8 Hz), and 5.50 (1H, d, J = 2.8 Hz)], similar to that of perulactone L (**4**). In its ^{1}H NMR spectrum, **9** showed the presence of a CHOH group [δ_H 4.38 (1H, brd, J = 6.4)], as in **8**. The HMBC correlations of H-7 (δ_H 4.38)/C-5 (δ_C 141.6) and H_3-19 (δ_H 1.36)/C-5 (δ_C 141.6) for **9** (Figure S46, Supporting Information) suggested that this CHOH group is located at C-7, and the NOE correlation of H-7/H-9α (δ_H 2.54) (Figure S47, Supporting Information) confirmed its orientation as β. The absolute configuration of C-22 was determined as *R* from the strong positive Cotton effect at 254 nm in its ECD spectrum[21] (Figure S61, Supporting Information). Thus, the structure of withanolide **9** was elucidated as 7β-hydroxy-17-*epi*-withanolide K [(20*S*,22*R*)-7β,14α,17β,20-tetrahydroxy-1-oxowitha-3,5,24-trienolide].

The HRESIMS and ^{13}C NMR data of withanolide **10** were consistent with the molecular formula $C_{28}H_{40}O_7$. The^1 H and ^{13}C NMR spectra of **10** (Tables 2 and 3), assigned with the help of the HSQC and HMBC data (Figures S51 and S52, Supporting Information), suggested that **10** contains ring A 2,3-en-1-one, ring B 5,6-ene, 14α-OH, and 20-OH moieties similar to those of withanolide G,[20] but the structure of the side chain δ-lactone moiety differed from any of the known withanolides. The presence of only four methyl signals including one doublet and three singlets in its ^{1}H NMR spectrum suggested that ring E of **10** constituted a saturated δ-lactone and that one of the side chain methyl groups (CH_3-27 or CH_3-28) has undergone oxidation to a CH_2OH [δ_H 3.86 (1H, brd, J = 11.0 Hz), 3.65 (1H, dd, J = 11.0, 7.8 Hz)]. The ^{13}C NMR spectrum of **10** (Table 3), assigned with the help of HSQC data, while confirming the presence of ring A 2,3-en-1-one [δ_C 204.2 (C-1), 145.3 (CH-3), 128.0 (CH-2)], ring B 5,6-ene [δ_C 135.1 (C-5), 125.0 (CH-6)], and four methyls [δ_C 20.8 (CH_3-21), 18.9 (CH_3-19), 17.7 (CH_3-18), 14.3 (CH_3-27)], suggested that it contains a saturated δ-lactone [δ_C 177.8 (C-26)], five oxygenated carbons [δ_C 84.4 (C-14), 81.4 (CH-23), 77.2 (C-20), 76.7 (CH-22), 63.2 (CH_2-28)], and a methine carbon (C-17) at 49.8 ppm. These assignments were supported by the long-range correlations of H_3-19/C-1, H_3-19/C-6, H-3/C-1, and H-4/C-6 for rings A/B; H_3-18/C-14, H_3-18/C-17, and H-17/C-21 for ring D; and H_3-27/C-26, H_3-27/C-24, H-23/C-25, H-23/C-28, and H-22/C-21 for ring E in the HMBC spectrum of **10** (Figure S52, Supporting Information). The orientation of the side chain at C-17 was determined to be β by the NOESY correlation between H_3-18 and H_3-21. Additional NOESY correlations observed for the ring E protons, H-23α with H-22α, H_2-28α, and H-25α of **10** (Figure S53, Supporting Information), suggested that OH-23, H-24, and CH_3-25 are β-oriented. The positive Cotton effect at 278 nm in the ECD spectrum

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of **10** (Figure S61, Supporting Information) suggested a 22*R* configuration.[21] Thus, withanolide **10** was identified as 24,25-dihydro-23*β*,28-dihydroxywithanolide G [(20*S*, 22*R*)-14*α*,20,23*β*,28-tetrahydroxy-1-oxowitha-2,5-dienolide].

The molecular formula of withanolide **11** ($C_{28}H_{40}O_7$) and its ^{1}H and ^{13}C NMR data (Tables 2 and 3) indicated that it is an analogue of withanolide E (**13**) in which the double bond of ring E has undergone reduction. The ^{13}C NMR data for **11** suggested the presence of a carbonyl of a saturated *δ*-lactone ring (δ_C 175.4), and its ^{1}H NMR spectrum showed doublets for CH_3-27 [δ_H 1.21 (3H, d, J = 6.8 Hz)] and CH_3-28 [δ_H 1.12 (3H, d, J = 6.8 Hz)]. These assignments for CH_3-27 and CH_3-28 were confirmed by the long-range correlations of H_3-27/C-26, H_2-23/C-25, and H_2-23/C-28 observed in its HMBC spectrum (Figure S57, Supporting Information). The relative configuration of these methyl groups was established by NOESY correlations of H_3-28/H-25 [δ_H 2.16 (1H, m)], H_3-28/H-22, and H-22/H-25 (Figure S58, Supporting Information), suggesting that the saturated *δ*-lactone of ring E of **11** possesses the same twist boat conformation, with equatorial orientations of CH_3-27 and CH_3-28, as in philadelphicalactone A.[23] The absolute configuration of C-22 was determined as *R* from the positive Cotton effect at 278 nm in its ECD spectrum (Figure S61, Supporting Information).[21] On the basis of the foregoing evidence, the structure of withanolide **11** was established as 24, 25 - dihydrowithanolide E [(20*S*,22*R*,24*S*,25*R*)-5*β*,6*β*-epoxy-14*α*,17*β*,20-trihydroxy-1-oxowitha-2-enolide].

Withanolides **1**–**25** were evaluated for their cytotoxic activity in a panel of selected tumor cell lines consisting of LNCaP (androgen-sensitive human prostate adenocarcinoma), 22Rv1 (androgen-resistant human prostate adenocarcinoma), ACHN (human renal adenocarcinoma), M14 (human melanoma), SK-MEL-28 (human melanoma), and normal HFF (human foreskin fibroblast) cells. Physachenolide C (**26**), which we have previously shown to exhibit selective and potent activity for LNCaP cells,[15,22] was used for comparison purposes. Of the withanolides tested, only 17*β*-hydroxywithanolides (17-BHWs), **6**, **8**, **9**, **11**–**13**, **15**, **19**–**22**, and **26**, exhibited cytotoxicity to LNCaP cells at a concentration below 2.0 *μ*M. The IC_{50} data for the active withanolides are presented in Table 4. Interestingly, the order of activity of these 17-BHWs (**13** > **20** > **22** > **12** > **21** > **11** > **8** > **15** > **19** > **9**) was found to be almost the same for both PC cell lines, LNCaP and 22Rv1, except for **6**, with a *β*-OH group at C-23, which was active against 22Rv1 cells with an IC_{50} of 0.89 ± 0.1 *μ*M, but had no activity for LNCaP cells at 2.0 *μ*M. The cytotoxicity data for LNCaP cells for these 17-BHWs suggested that a ring A 2,3-en-1-one and *α*-oriented unsaturated *δ*-lactone side chain and/or a *β*-OH at C-17 are essential for their activity and acetoxylation at C-18 leads to 3-fold enhancement of activity (**26** vs **13**). These data also provided additional structure–activity relationship (SAR) information for 17-BHWs, including decreases in activity due to (i) isomerization of the 2,3-ene to 3,4-ene (**8** vs **9**); (ii) *β*-hydroxylation at C-4 (**12** vs **13**); (iii) *β*-methoxylation at C-4 (**15** vs **13**); (iv) replacement of the 5*β*,6*β*-epoxide with 5,6-ene (**22** vs **13**); (v) *β*-hydroxylation at C-7 (**19** vs **13** and 8 vs 22); (vi) reduction of the 24,25-double bond of the side chain (**11** vs **13**); and (vii) hydroxylation at C-28 (**21** vs **22**). Intriguingly, of the 26 withanolides tested, only three (**13**, **20**, and **26**) showed selectivity for renal carcinoma (ACHN) cells (Table 4). Although there appears to be no direct correlation between cytotoxic activities for ACHN and LNCaP cells, it is noteworthy

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that withanolides **13**, **20**, and **26**, found to be active against ACHN cells, are those with the highest potency for LNCaP cells. Nonetheless, the prostate cancer cell lines were still much more sensitive to the effects of active 17-BHWs. In addition to supporting our previous findings that α-orientation of the side chain and/or the presence of a β-OH at C-17 of withanolides may have a significant influence on their selectivity to certain cancer cell lines, these SAR data for 17-BHWs suggest the importance of the structure of the side chain for their activity and potency.

EXPERIMENTAL SECTION

General Experimental Procedures.

Optical rotations were measured with a JASCO Dip-370 polarimeter using MeOH as the solvent. UV spectra were recorded with a Shimadzu UV 2601 spectrophotometer. ECD spectra were measured with a JASCO J-810 spectropolarimeter. 1D and 2D NMR spectra were recorded in $CDCl_3$ using residual solvent as the internal standard on a Bruker Avance III 400 spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, respectively. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants (J values) are in Hz. Low-resolution MS were recorded on a Shimadzu LCMS-QP8000α and high-resolution MS on a Agilent G6224A TOF mass spectrometer. Analytical thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} aluminum-backed TLC plates (Merck), and preparative TLC was performed on Analtech silica gel 500 μm glass plates. Compounds were visualized with short-wave UV and by spraying with anisaldehyde-sulfuric acid reagent and heating until the spots appeared. Silica gel flash chromatography was accomplished using 230–400 mesh silica gel. Sephadex LH-20 for gel-permeation chromatography was obtained from Amersham Biosciences. HPLC purifications were carried out using a 10 × 250 mm Phenomenex Luna 5 μm C_{18} (2) column for reversed-phase (RP) chromatography and a 10 × 250 mm Econosil Si (10 μ) column for normal-phase (NP) chromatography, with a Waters Delta Prep system consisting of a PDA 996 detector. When required, MM2 energy minimizations of all possible conformers were carried out using Chem3D 15.0 from PerkinElmer, Inc.

Aeroponic Cultivation and Harvesting of *P. peruviana*.

The seeds of *P. peruviana* obtained from Trade Wind Fruit (P.O. Box 1102, Windsor, CA, USA) were germinated in 1.0 in. Grodan rock-wool cubes in a Barnstead Lab-line growth chamber kept at 28 °C with 16 h of fluorescent lighting and maintaining 25–50% humidity. After ca. 4 weeks in the growth chamber, seedlings with an aerial length of ca. 5.0 cm were transplanted to aeroponic culture boxes for further growth, as described previously for *Withania somnifera* and *Physalis crssifolia*.[13,24] A herbarium sample was deposited at the University of Arizona Natural Products Center (accession number NPC-DB-11/5/10). Aerial parts of areoponically grown plants were harvested when fruits were almost mature (ca. 2 months under aeroponic growth conditions). Harvested plant materials were dried in the shade, powdered, and stored at 5 °C prior to extraction.

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Extraction and Isolation of Withanolides.

Dried and powdered aerial parts of aeroponically grown *P. peruviana* (1.2 kg) were divided into four portions, and each portion was extracted with 60% aqueous MeOH (3.0 L) in an ultrasonic bath at 25 °C for 2 h and then allowed to stand for 8 h. After filtration, the resulting filtrates were combined and passed through a column packed with HP-20SS resin (2.0 kg). The column was first eluted with 60% MeOH(aq) (4.0 L) followed by MeOH (5.0 L). The crude withanolide extract (24.5 g) obtained by concentrating the MeOH eluates was subjected to column chromatography on RP C_{18} (400.0 g) and eluted with 2.0 L each of 50%, 60%, and 70% MeOH(aq) and MeOH to afford five fractions, A–E: A (3.5 g) eluted with 50% MeOH(aq); B (4.2 g) with 60% MeOH(aq); C (3.1 g) with 60–70% MeOH(aq); D (4.3 g) with 70% MeOH(aq); and E (8.2 g) with MeOH. Of these, fractions B–D, which contained withanolides, were further purified as described below. Fraction B (4.2 g) on purification by column chromatography over silica gel (200 g) and elution with EtOAc gave 4β-hydroxywithanolide E (**12**) (1.6 g) and an impure fraction (242 mg). This impure fraction was purified by RP HPLC [60% MeOH(aq)] followed by silica gel preparative TLC [$CHCl_3$–MeOH (95:5)] to afford **1** (6.2 mg, R_f 0.4) and additional 4β-hydroxywithanolide E (**12**) (181 mg). A portion of fraction C (2.0 g) was further fractionated by silica gel (250 g) column chromatography and elution with EtOAc (1.0 L), EtOAc–MeOH (98:2) (1.0 L), and MeOH (500 mL), affording seven subfractions, C_1–C_7: C_1 (125.0 mg) eluted with EtOAc; C_2 (610.0 mg) with EtOAc; C_3 (95.0 mg) with EtOAc; C_4 (285.0 mg) with EtOAc; C_5 (195.0 mg) with EtOAc–MeOH (98:2); C_6 (67 mg) with EtOAc–MeOH (98:2); and C_7 (550.0 mg) with MeOH. A portion of subfraction C_2 (400.0 mg) was subjected to further fractionation by silica gel (20 g) column chromatography and elution with $CHCl_3$–MeOH (99:1) (200 mL), $CHCl_3$–MeOH (98:2) (200 mL), and $CHCl_3$–MeOH (95:5) (200 mL). The resulting fractions were combined based on their TLC profiles to afford nine subfractions (C_{2-1}–C_{2-9}). On further purification by RP HPLC, subfraction C_{2-2} (8.5 mg) afforded **14** [4.0 mg, t_R 25.0 min, 55% MeOH(aq)]; subfraction C_{2-4} (37.0 mg) gave **22** [1.4 mg, t_R 33.0 min, 65% MeOH(aq)], **25** [2.0 mg, t_R 22.0 min, 65% MeOH(aq)], and **13** [16.0 mg, t_R 17.0 min, 65% MeOH(aq)]; subfraction C_{2-5} (26.0 mg) afforded **3** [8.8 mg, t_R 24.0 min, 60% MeOH(aq)] and **23** [2.1 mg, t_R 14.0 min, 60% MeOH(aq)]. Subfraction C_{2-6} (95.0 mg) was fractionated by column chromatography on silica gel (20.0 g), and elution with EtOAc provided three subfractions, C_{2-6-1} (40.0 mg), C_{2-6-2} (17.0 mg), and C_{2-6-3} (17.0 mg). Further purification of subfraction C_{2-6-1} (40 mg) by RP HPLC afforded **17** [3.3 mg, t_R 11.0 min, 65% MeOH(aq)] and **24** [20.0 mg, t_R 18.0 min, 65% MeOH (aq)]. When purified by RP HPLC, subfraction C_{2-6-1} gave **5** [1.2 mg, t_R 24.0 min, 55% MeOH(aq)], **6** [3.3 mg, t_R 32.0 min, 55% MeOH(aq)], and **3** [1.5 mg, t_R 36.0 min, 55% MeOH(aq)]. Fraction C_{2-7} (170.0 mg) was subjected to column chromatography on silica gel (20.0 g) and eluted with $CHCl_3$–MeOH (95:5) to afford a withanolide-containing fraction (152.0 mg). Further purification of this fraction (20.0 mg) by silica gel preparative TLC (EtOAc) gave **20** (11.6 mg, R_f 0.4) and **21** (1.8 mg, R_f 0.5). Subfraction C_{2-8} (24.0 mg) was subjected to column chromatography on silica gel (8 g), and elution with EtOAc afforded 8 (15.0 mg) and a fraction (5.2 mg) that was further purified by RP HPLC [60% MeOH(aq)] to give **9** (2.7 mg, t_R 29.0 min). A portion of subfraction C_4 (150.0 mg) was purified by column chromatography over silica gel (8 g) and elution with CH_2Cl_2–MeOH (95:5) to obtain a

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fraction (52.6 mg), which, on further purification by RP HPLC using 60% MeOH(aq), afforded **19** (27.0 mg, t_R 20.0 min) and **8** (11.0 mg, t_R 25.0 min). Purification of subfraction C_5 by column chromatography over silica gel (8 g) and elution with CH_2Cl_2–MeOH (9:1) afforded **18** (35.0 mg). Fraction D (4.0 g) was separated by column chromatography over silica gel (250 g), and elution with EtOAc afforded withanolide E (**13**, subfraction D_2, 2.2 g) and two subfractions, D_1 (245.0 mg) and D_3 (108.0 mg). A portion (100.0 mg) of subfraction D_1 was fractionated by RP HPLC using 65% MeOH(aq) to yield three subfractions, D_{1-1} (35.8 mg, t_R 18.0 min), D_{1-2} (6.2 mg, t_R 22.0 min), and D_{1-3} (4.7 mg, t_R 25.0 min). Subfraction D_{1-1} was further purified by column chromatography on silica gel (8 g), and elution with $CHCl_3$–MeOH (95:5) afforded **3** (1.5 mg), 7 (3.3 mg), and **24** (20 mg). Purification of subfractions D_{1-2} by preparative TLC [silica gel; $CHCl_3$–MeOH (95:5)] gave **2** (4.8 mg), and purification of D_{1-3} by the same procedure afforded **4** (2.1 mg) and **25** (2.0 mg). Further purification of subfraction D_3 by RP HPLC using 65% MeOH(aq) yielded three subfractions: D_{3-1} (50.1 mg), D_{3-2} (25.2 mg), and D_{3-3} (24.4 mg), and subsequent purification of these subfractions by column chromatography over silica gel with $CHCl_3$–MeOH (99:1 and 98:2) gave **16** (10.5 mg) and **18** (6.1 mg) from subfraction D_{3-1}, **10** (3.5 mg) and **11** (1.5 mg) from subfraction D_{3-2}, and **15** (1.4 mg) and **25** (10.2 mg) from subfraction D_{3-3}.

Perulactone I (1): amorphous, colorless powder; $[\alpha]_D^{25}$ + 85 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.81) nm; ECD (MeOH) [θ] −4345 (242 nm), +3766 (340 nm); ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; positive HRESIMS *m*/*z* 527.2620 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_8Na$, 527.2621).

Perulactone J (2): amorphous, colorless powder; $[\alpha]_D^{25}$ + 9 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.76) nm; ECD (MeOH) [θ] +7461 (254 nm), −7491 (339 nm); ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; positive HRESIMS *m*/*z* 495.2720 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_6Na$, 495.2723).

Perulactone K (3): amorphous, colorless powder; $[\alpha]_D^{25}$ + 49 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 222 (3.89) nm; ECD (MeOH) [θ] −2170 (242 nm), +1331 (264 nm), +2031 (342 nm); ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; positive HRESIMS *m*/*z* 493.2554 [M + Na]$^+$ (calcd for $C_{28}H_{38}O_6Na$, 493.2566).

Perulactone L (4): amorphous, colorless powder; $[\alpha]_D^{25}$ + 52 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.84) nm; ECD (MeOH) [θ] +10 095 (245 nm), −1400 (310 nm), −710 (342 nm, sh.); ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; positive HRESIMS *m*/*z* 511.2670 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_7Na$, 511.2672).

17-Deoxy-23β-hydroxywithanolide E (5): amorphous, colorless powder; $[\alpha]_D^{25}$ − 3.0 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 223 (4.02) nm; ECD (MeOH) [θ] −13 854 (231 nm), +440 (266 nm), +1858 (339 nm); ^{1}H and ^{13}C NMR data, see Tables 1 and 3,

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respectively; positive HRESIMS *m*/*z* 509.2502 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_7Na$, 509.2515).

23β-Hydroxywithanolide E (6): amorphous, colorless powder; $[\alpha]_D^{25}$ + 37 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 221 (3.97) nm; ECD (MeOH) [θ] −8240 (239 nm), +187 (264 nm), +2728 (339 nm); ^1H and ^{13}C NMR data, see Tables 2 and 3, respectively; positive HRESIMS *m*/*z* 525.2449 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_8Na$, 525.2464).

4-Deoxyphyperunolide A (7): amorphous, colorless powder; $[\alpha]_D^{25}$ + 65 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.06) nm; ECD (MeOH) [θ] +11 244 (257 nm), +6149 (341 nm); ^1H and ^{13}C NMR data, see Tables 2 and 3, respectively; positive HRESIMS *m*/*z* 491.2408 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_6Na$, 491.2410).

7β-Hydroxywithanolide F (8): amorphous, colorless powder; $[\alpha]_D^{25}$ + 124 (*c* 0.49, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.12) nm; ECD (MeOH) [θ] +27 006 (252 nm), −7184 (338 nm); ^1H and ^{13}C NMR data, see Tables 2 and 3, respectively; positive HRESIMS *m*/*z* 509.2496 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_7Na$, 509.2515).

7β-Hydroxy-17-epi-withanolide K (9): amorphous, colorless powder; $[\alpha]_D^{25}$ + 131 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.15) nm; ECD (MeOH) [θ] +22 189 (254 nm), −2517 (307 nm); ^1H and ^{13}C NMR data, see Tables 2 and 3, respectively; positive HRESIMS *m*/*z* 509.2509 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_7Na$, 509.2515).

24,25-Dihydro-23β,28-dihydroxywithanolide G (10): amorphous, colorless powder; $[\alpha]_D^{25}$ + 4 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.65) nm; ECD (MeOH) [θ] +5674 (254 nm), −8022 (307 nm); ^1H and ^{13}C NMR data, see Tables 2 and 3, respectively; positive HRESIMS *m*/*z* 511.2667 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_7Na$, 511.2672).

24,25-Dihydrowithanolide E (11): amorphous, colorless powder; $[\alpha]_D^{25}$ + 48 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.76) nm; ECD (MeOH) [θ] −2660 (242 nm), +1541 (278 nm), +3916 (339 nm); ^1H and ^{13}C NMR data, see Tables 2 and 3, respectively; positive HRESIMS *m*/*z* 511.2660 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_7Na$, 511.2672).

Epoxidation of Perulactone B (24).—To a stirred solution of **24** (2.0 mg) in CH_2Cl_2 (1.0 mL) was added *m*-chloroperbenzoic acid (2.0 mg), and the mixture was stirred at 25 °C. After 3 h (TLC control), the reaction mixture was partitioned between $CHCl_3$ (5.0 mL) and water (10.0 mL). The $CHCl_3$ layer was washed with water (10.0 mL), dried (Na_2SO_4), and subjected to purification by silica gel preparative TLC [$CHCl_3$−MeOH (95:5)] to afford the major β-epoxidation product as an off-white, amorphous powder (1.0 mg). The ^1H and ^{13}C NMR and APCIMS data of this product were identical with those of **1** obtained above.

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Conversion of Perulactone B (24) to Perulactone L (4).—To a stirred solution of **24** (2.0 mg) in MeOH (1.0 mL) was added NaCN (1.0 mg), and the mixture was stirred at 25 °C. After 2 h (TLC control), the reaction mixture was concentrated using a flow of N_2, and the residue thus obtained was dissolved in $CHCl_3$ (2.0 mL). The $CHCl_3$ solution was concentrated and was separated by silica gel preparative TLC [$CHCl_3$–MeOH (95:5)] to afford the product as an off-white, amorphous powder (1.9 mg). The APCIMS and ^1H and ^{13}C NMR data of this product were identical with those of **4** obtained above.

Cytotoxicity Assay.

The ACHN, M14, and SK-MEL-28 cells were all obtained from the Developmental Therapeutics Program (NCI, Frederick, MD, USA). LNCaP and HFF cells were purchased from ATCC (Manassas, VA, USA), and the 22Rv1 cells were kindly provided by Dr. Len Neckers (NCI, Bethesda, MD, USA). Cell lines were maintained as recommended by the source institution. Cell numbers were estimated using the MTS dye assay (Promega, Madison, WI, USA).[25] The MTS dye assay was used for evaluating cytotoxicity of withanolides **1**–**26** against androgen-sensitive human prostate adenocarcinoma (LNCaP), androgen-resistant human prostate adenocarcinoma (22Rv1), human renal adenocarcinoma (ACHN), human melanoma (M14 and SK-MEL-28) cell lines, and human foreskin fibroblast cells. Doxorubicin and DMSO were used as positive and negative controls, respectively. For LNCaP, ACHN, 22Rv1, and HFF cells, the assay was performed in RPMI with 5% fetal calf serum, 2.0 mM L-glutamine, 1× nonessential amino acids, 1.0 mM sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin, 10 mM HEPES, and 5×10^{-5} M 2-mercaptoethanol. For M14 and SK-MEL-28 cells, DMEM replaced RPMI. Briefly, 10 000 cells (LNCaP) or 5000 cells (22Rv1, ACHN, M14, SK-MEL-28, HFF) were incubated overnight at 37 °C in 96-well microtiter plates. Serial dilutions of compounds in DMSO or vehicle control (DMSO) were added to triplicate wells, and the microtiter plates were incubated for a further 72 h. Viable cell number was determined by the addition of CellTiter 96 AQueous One Solution cell proliferation assay solution (MTS), plates were incubated for 2 h, and then absorbance (A) at 490 nM was measured. Cytotoxicity was calculated as in the following formula: % cytotoxicity = $[(A_{\text{Medium}} - A_{\text{Treatment}})/A_{\text{Medium}})] \times 100$. The IC_{50} values and standard deviations (±) were determined using Microsoft Excel software from dose–response curves obtained from at least three independent experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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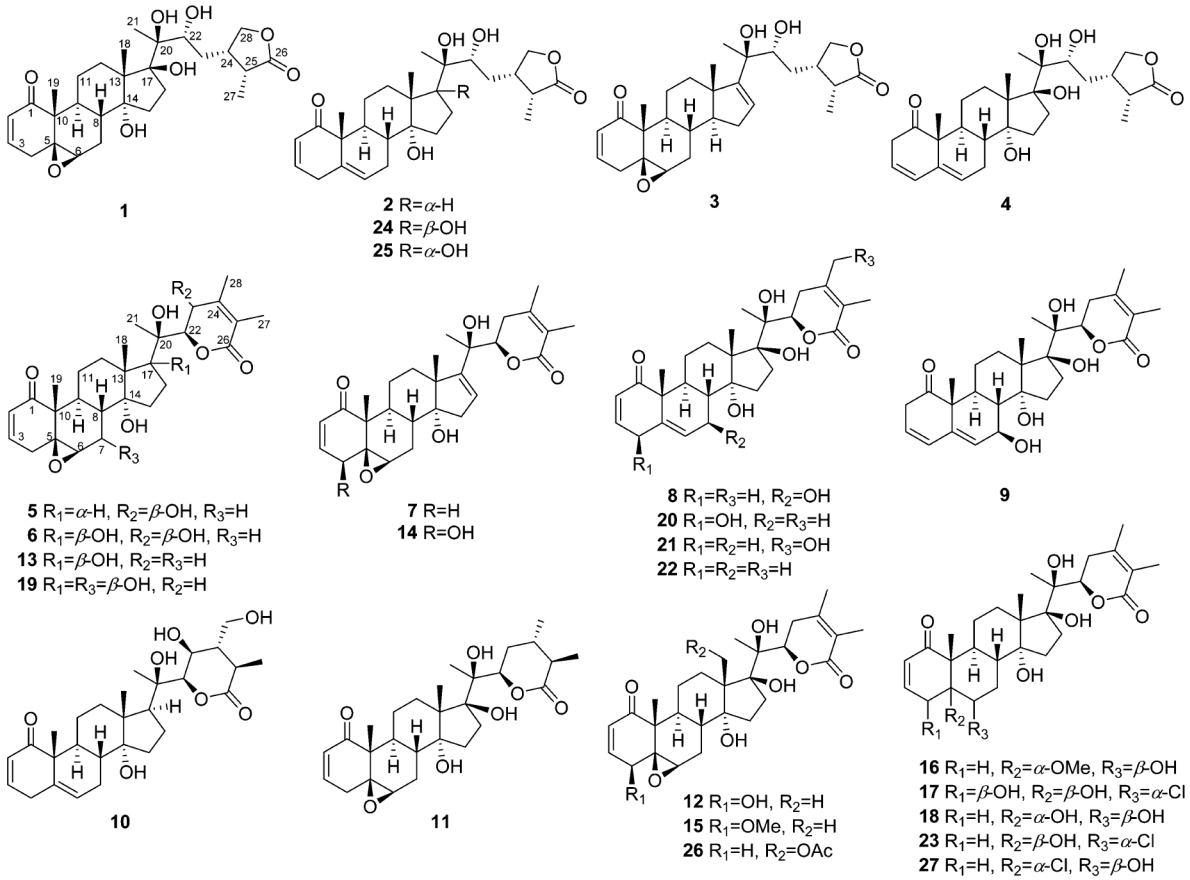

Figure 1.

Structures of withanolides **1**–**25** from aeroponically grown *P. peruviana*, physachenolide C (**26**), and withanolide C (**27**).

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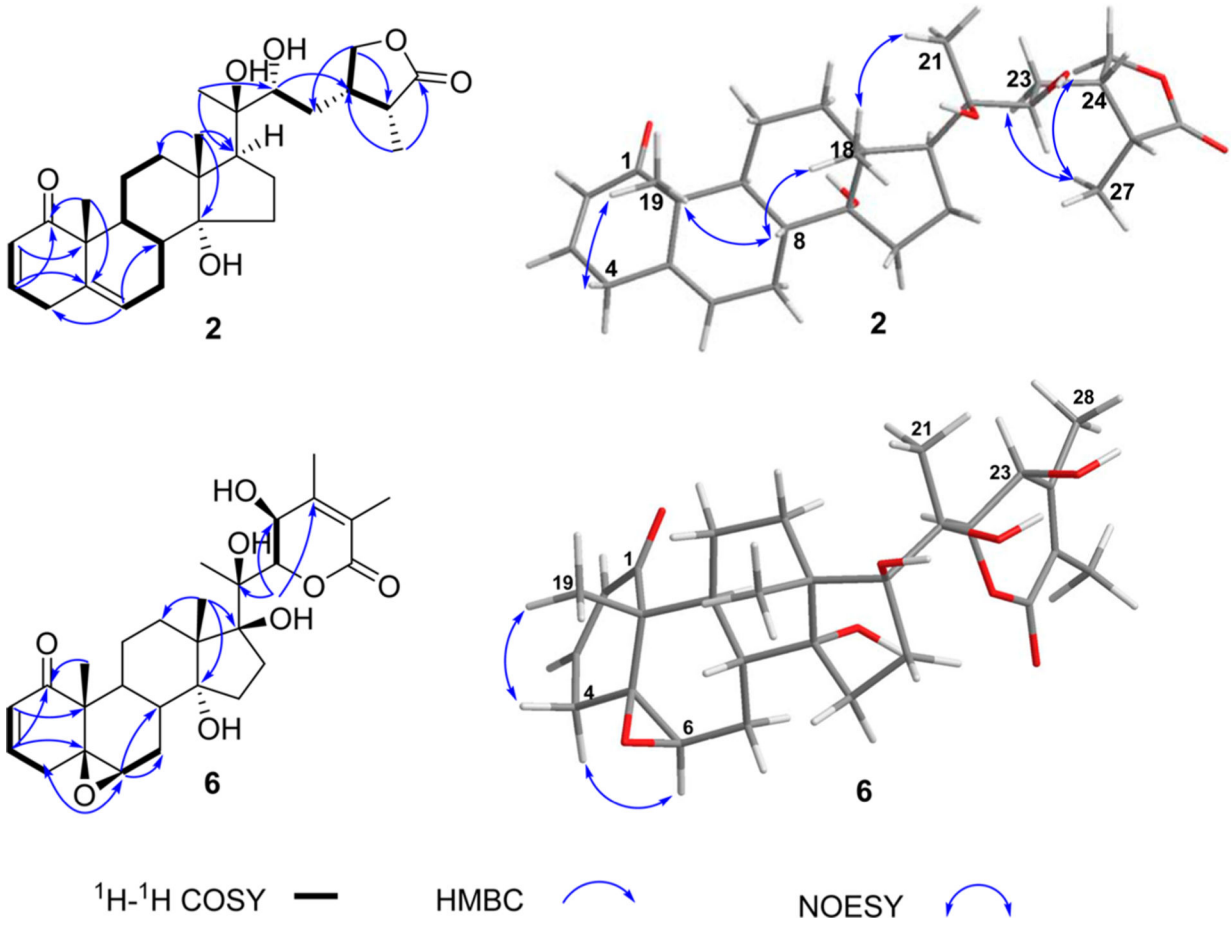

Figure 2.
Key HMBC, ^{1}H–^{1}H COSY, and NOE correlations of withanolides **2** and **6**.

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Table 1.

^{1}H NMR Data (400 MHz, δ, Hz) for Perulactones I–L (1–4) in $CDCl_3$[a]

position	1	2	3	4
2	5.99 dd (10.0, 2.8)	5.87 dd (10.0, 2.0)	6.01 dd (10.0, 2.4)	2.72 dd (20.0, 4.4)
				3.29 brd (20.0)
3	6.85, ddd (10.0, 6.4, 2.0)	6.77 ddd (10.0, 4.8, 2.4)	6.84 ddd (10.0, 6.4, 2.4)	5.61 ddd (9.6, 4.4, 2.8)
4	1.92 m	2.83 dd (21.2, 4.8)	1.90 m	6.02 dd (9.6, 2.8)
	2.96 brd (19.2)	3.27 brd (21.2)	2.98 dt (18.8, 2.8)	
6	3.19 d (2.0)	5.59 brd (5.6)	3.14 d (2.8)	5.66 t (4.0)
7	1.97 m	2.03 m	2.08 m	2.09 m
	1.80 m	1.82 m	1.34 m	2.03 m
8	1.86 m	1.84 m	1.78 m	1.99 m
9	2.33 m	2.00 m	1.19 m	2.36 m
11	2.08 m	2.20 m	2.13 m	1.93 m
	1.62 m	1.54 m	1.54 m	1.48 m
12	2.20 m	1.80 m	1.97 m	1.73 m
	1.47 m	1.55 m	1.49 m	1.56 m
14			1.32 m	
15	1.67 m	1.79 m	2.10 m	2.08 m
	1.52 m	1.52 m	1.89 m	1.27 m
16	2.62 m	1.66 m	5.71 dd (2.4, 1.2)	2.67 m
	1.43 m	1.85 m		1.44 m
17		2.25 m		
18	1.08 s	1.03 s	0.94 s	1.12 s
19	1.26 s	1.23 s	1.25 s	1.38 s
21	1.22 s	1.20 s	1.27 s	1.23 s
22	4.01 brd (10.4)	3.46 dd (10.8, 1.6)	3.65 dt (10.4, 2.0)	4.11 brd (10.4)
23	2.12 m	1.63 m	1.70 m	2.38 m
	1.33 m	1.24 m	1.47 m	1.67 m
24	2.71 q (7.2)	2.70 m	2.60–2.75 m	2.53 m
25	2.65 m	2.65 m	2.60–2.75 m	2.52 m

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position	1	2	3	4
27	1.16 d (7.2)	1.16 d (7.6)	1.16 d (7.2)	1.13 d (7.6)
28	4.06 dd (8.8, 8.4)	4.10 dd (9.2, 8.4)	4.14 dd (9.2, 7.6)	4.03 dd (8.8, 7.6)
	4.39 dd (8.8, 7.2)	4.43 dd (9.2, 7.2)	4.43 dd (9.2, 6.8)	4.31 dd (8.8, 6.8)

[a]Assignments based on DEPT, HSQC, and HMBC data.

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Table 2.

^{1}H NMR Data (400 MHz, δ, Hz) for Withanolides 5–11 in $CDCl_3$[a]

position	5	6	7	8	9	10	11
2	6.00 dd (10.0, 2.0)	6.00 dd (10.0, 2.4)	6.01 dd (10.0, 2.8)	5.81,dd (10.0, 2.0)	2.70 dd (20.2, 4.8)	5.86 ddd (10.0, 3.0, 1.0)	6.01 dd (10.0, 3.0)
					3.24 dt (20.0, 2.4)		
3	6.83 ddd (10.0, 6.4, 2.4)	6.79 ddd (10.0, 6.4, 2.4)	6.80 ddd (10.0, 6.4, 2.0)	6.73 ddd (10, 4.8, 2.4)	5.67 ddd (10.0, 4.4, 2.8)	6.76 ddd (10.0, 5.0, 2.5)	6.80 ddd (10.0, 6.5, 2.2)
4	1.90 m	1.84 m	1.83 dd (18.4, 6.4)	2.81 dd (21.2, 4.8)	6.01 dd (10.0, 2.4)	2.82 dd (21.2, 5.0)	1.84 dd (18.4, 6.5)
	2.96 dt (18.8, 2.8)	2.92 dt (18.4, 2.8)	2.92 ddd (18.4, 2.8, 2.0)	3.24 dt (21.2, 2.4)		3.27 brd (21.2)	2.93 dt (18.4, 2.6)
6	3.18 d (2.0)	3.17 brs	3.17 brs	5.43 brs	5.50 d (2.8)	5.59 brd (5.5)	3.17 brs
7	1.92 m	1.95 m	2.08 m	4.28 d (8.8)	4.38 brd (6.4)	2.08 m	1.94 m
	1.82 m		1.93 m			1.81 m	
8	1.95 m	1.91 m	1.98 m	1.67 dd (12.0, 8.8)	1.79 m	1.82 m	1.89 m
9	1.61 m	1.88 m	1.88 m	2.33 m	2.54 m	2.09 m	1.88 m
11	1.98 m	2.03 m	2.02 m	2.28 m	1.91 m	2.18 m	2.06 m
	1.52 m	1.57 m	1.56 m	1.56 m	1.37 m	1.56 m	1.63 m
12	1.67–1.75 m	2.19 dt (4.0, 12.0)	2.12 m	2.38 m	2.41 m	1.97 m	2.33 m
		1.67 m	1.43, m	1.26 m	1.24 m	1.72 m	1.39 m
15	1.57 m	1.67 m	2.19 dd (14.4, 3.6)	1.89 m	1.93 m	1.52 m	1.63 m
	1.48 m	1.60 m	2.31 dd (14.4, 1.2)	1.78 m	1.75 m		1.54 m
16	2.05 m	2.77 ddd (14.8, 11.6, 8.8)	5.77 dd (3.6, 1.2)	2.62 m	2.66 m	1.82 m	2.68 m
	1.83 m	1.53 m		1.41 m	1.43 m		1.40 m
17	2.50 t (9.2)					2.64 t (9.4)	
18	1.01 s	1.07 s	1.12 s	1.05 s	1.07 s	1.01 s	1.09 s
19	1.24 s	1.21 s	1.23 s	1.20 s	1.36 s	1.23 s	1.24 s
21	1.33 s	1.45 s	1.27 s	1.35 s	1.36 s	1.33 s	1.35 s
22	4.04 d (8.8)	4.76 d (10.8)	4.39 dd (12.8, 3.6)	4.88 dd (12.4, 4.4)	4.87 dd (12.0, 4.8)	3.55 d (7.4)	4.89 dd (11.0, 3.4)
23	4.38 brd (8.8)	4.27 brd (10.8)	2.78 brt (15.2)	2.40–2.55 m	2.40–2.55 m	4.18 t (7.4)	1.92 m
			2.14 m				1.82 m
24						2.32 m	1.70 m
25						2.38 m	2.16 m
27	1.88 s	1.89 s	1.85 brs	1.82 s	1.82 s	1.28 d (6.8)	1.21 d (6.8)

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28	1.95 s	1.97 s	1.95 s	1.89 s	1.89 s	3.86 brd (11.0) 3.65 dd (11.0, 7.8)	1.12 d (6.8)

[a] Assignments based on DEPT, HSQC, and HMBC data.

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Table 3.

^{13}C NMR Data (100 MHz) of Withanolides 1–11 in $CDCl_3$[a]

position	1	2	3	4	5	6	7	8	9	10	11
1	203.8 C	204.1 C	203.3 C	211.0 C	203.5 C	202.9 C	202.8 C	203.7 C	209.9 C	204.2 C	203.0 C
2	129.2 CH	127.9 CH	129.3 CH	39.8 CH_2	129.4 CH	129.8 CH	130.0 CH	127.9 CH	39.6 CH_2	128.0 CH	129.9 CH
3	144.7 CH	145.3 CH	144.3 CH	126.8 CH	144.3CH	143.6 CH	143.5 CH	144.9 CH	123.9 CH	145.3 CH	143.7 CH
4	33.0 CH_2	33.4 CH_2	33.0 CH_2	129.2 CH	33.0 CH_2	32.9 CH_2	32.8 CH_2	32.8 CH_2	128.6 CH	33.5 CH_2	32.8 CH_2
5	62.1 C	135.2 C	62.1 C	141.0 C	61.9 C	62.2 C	62.2 C	136.3 C	141.6 C	135.1 C	62.1 C
6	64.0 CH	124.8 CH	63.2 CH	121.9 CH	64.0 CH	64.1 CH	63.8 CH	129.8 CH	130.7 CH	125.0 CH	64.1 CH
7	26.3 CH_2	25.3 CH_2	30.9 CH_2	25.8 CH_2	26.2 CH_2	26.3 CH_2	26.0 CH_2	65.9 CH	66.3 CH	25.3 CH_2	26.2 CH_2
8	34.0 CH	35.1 CH	28.5 CH	35.6 CH	32.3 CH	34.1 CH	31.2 CH	45.1 CH	44.4 CH	35.3 CH	34.1 CH
9	36.9 CH	36.3 CH	44.9 CH	34.1 CH	37.4 CH	36.7 CH	37.0 CH	34.4 CH	33.3 CH	36.2 CH	36.8 CH
10	48.5, C	50.8 C	48.5 C	52.4 C	48.5 C	48.6 C	48.6 C	50.2 C	52.0 C	50.7 C	48.6 C
11	23.2 CH_2	22.1 CH_2	23.4 CH_2	21.7 CH_2	22.4 CH_2	22.7 CH_2	21.8 CH_2	22.8 CH_2	21.6 CH_2	22.2 CH_2	22.9 CH_2
12	30.0 CH_2	32.5 CH_2	36.0 CH_2	32.3 CH_2	32.1 CH_2	30.1 CH_2	28.4 CH_2	30.2 CH_2	30.0 CH_2	32.3 CH_2	30.4 CH_2
13	54.2 C	47.4 C	46.9 C	54.6 C	47.6 C	54.8 C	52.3 C	54.8 C	54.9 C	47.3 C	54.7 C
14	83.2 C	84.6 C	58.1 CH	83.7 C	84.9 C	82.1 C	84.5 C	81.7 C	81.4 C	84.4 C	81.9 C
15	32.5 CH_2	32.0 CH_2	30.9 CH_2	31.6 CH_2	32.5 CH_2	32.3 CH_2	39.9 CH_2	34.9 CH_2	34.6 CH_2	32.2 CH_2	32.4 CH_2
16	37.6 CH_2	20.9 CH_2	126.9 CH	37.7 CH_2	21.3 CH_2	37.8 CH_2	124.3 CH	37.6 CH_2	37.7 CH_2	20.8 CH_2	37.8 CH_2
17	88.3 C	49.4 CH	158.9 C	88.3 C	49.0 CH	88.1 C	156.4 C	86.7 C	86.9 C	49.8 CH	87.7 C
18	20.6 CH_3	17.4 CH_3	17.9 CH_3	20.6 CH_3	17.5 CH_3	20.4 CH_3	20.6 CH_3	20.5 CH_3	20.5 CH_3	17.7 CH_3	20.6 CH_3
19	15.2 CH_3	18.9 CH_3	14.9 CH_3	20.2 CH_3	15.2 CH_3	14.6 CH_3	14.2 CH_3	18.5 CH_3	19.9 CH_3	18.9 C	14.5 CH_3
20	78.5 C	77.5 CH	76.7 C	79.0 C	77.1 C	79.5 C	74.7 C	78.6 C	78.6 C	77.2 C	79.2 C
21	19.3 CH_3	20.2 CH_3	23.1 CH_3	19.0 CH_3	24.1 CH_3	19.3 CH_3	22.41 CH_3	19.1 CH_3	19.2 CH_3	20.8 CH_3	17.2 CH_3
22	72.3 CH	75.2 CH	74.6 CH	72.6 CH	85.6 CH	82.4 CH	79.4 CH	80.7 CH	80.5 CH	76.7 CH	78.9 CH
23	31.9, CH_2	28.8 CH_2	27.3 CH_2	29.9 CH_2	67.1 CH	66.4 CH	29.8 CH_2	34.3 CH_2	34.3 CH_2	81.4 CH	33.2 CH_2
24	38.1 CH	38.1 CH	37.9 CH	38.1 CH	151.7 C	153.4 C	149.3 C	151.3 C	151.2 C	37.2 CH	31.8, CH
25	37.7 CH	37.7 CH	37.9 CH	37.8 CH	121.9 C	121.1 C	127.6 C	121.1 C	121.2 C	49.6 CH	40.7 CH
26	180.8 C	180.4 C	180.3 C	180.8 C	164.1 C	164.2 C	165.2 C	167.2 C	167.0 C	177.8 C	175.4 C
27	10.5 CH_3	10.5 CH_3	10.5 CH_3	10.6 CH_3	12.9 CH_3	12.8 CH_3	12.4 CH_3	12.1 CH_3	12.2 CH_3	14.3 CH_3	14.4 CH_3

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position	1	2	3	4	5	6	7	8	9	10	11
28	72.5 CH_2	72.2 CH_2	72.4 CH_2	73.0 CH_2	15.7 CH_3	15.5 CH_3	22.4 CH_3	20.6 CH_3	20.6 CH_3	63.2 CH_2	21.4 CH_3

[a] Assignments based on DEPT, HSQC, and HMBC data.

[b] Assignments are interchangeable.

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Table 4.

Cytotoxicity Data of Withanolides from Aeroponically Cultivated *Physalis* peruviana against a Panel of Selected Tumor Cell Lines and Normal Cells[a]

	cell line[b]					
compound	**LNCaP**	**22Rv1**	**ACHN**	**M14**	**SK-MEL-28**	**HFF**
6	>2	0.89 ± 0.10	>2	>2	>2	>2
8	0.33 ± 0.04	0.56 ± 0.01	>2	>2	>2	>2
9	0.94 ± 0.10	0.99 ± 0.08	>2	>2	>2	>2
11	0.29 ± 0.05	0.21 ± 0.02	>2	>2	>2	>2
12	0.16 ± 0.04	0.09 ± 0.01	>2	>2	>2	>2
13	0.06 ± 0.01	0.07 ± 0.01	0.46 ± 0.06	>2	>2	>2
15	0.33 ± 0.02	0.24 ± 0.03	>2	>2	>2	>2
19	0.33 ± 0.02	0.26 ± 0.02	>2	>2	>2	>2
20	0.11 ± 0.01	0.07 ± 0.01	1.0 ± 0.08	>2	>2	>2
21	0.24 ± 0.02	0.21 ± 0.02	>2	>2	>2	>2
22	0.13 ± 0.10	0.07 ± 0.02	>2	>2	>2	>2
26	0.02 ± 0.01	0.03 ± 0.01	0.57 ± 0.06	>2	>2	>2
doxorubicin	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.09 ± 0.01	0.25 ± 0.01	0.15 ± 0.03

[a]Results are expressed as IC_{50} values in μM. Doxorubicin and DMSO were used as positive and negative controls. Withanolides **1**–**5**, **7**, **10**, **14**, **16**–**18**, and **23**–**25** had no activity up to 5.0 μM.

[b]Key: LNCaP = androgen-sensitive human prostate adenocarcinoma; 22Rv1 = androgen-resistant human prostate adenocarcinoma; ACHN = human renal adenocarcinoma; M14 = human melanoma; SK-MEL-28= human melanoma; HFF = human foreskin fibroblast.