Published in final edited form as:
J Nat Prod. 2011 December 27; 74(12): 2532–2544. doi:10.1021/np200635r.

Cytotoxic Withanolide Constituents of *Physalis longifolia*

Huaping Zhang[†], **Abbas K. Samadi**[‡], **Robert J. Gallagher**[†], **Juan J. Araya**[†], **Xiaoqin Tong**[†], **Victor W. Day**[§], **Mark S. Cohen**[‡], **Kelly Kindscher**[⊥], **Rao Gollapudi**[†], and **Barbara N. Timmermann**[*,†]

[†]Department of Medicinal Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045, United States

[‡]Department of Surgery, School of Medicine, University of Kansas, Medical Center, Kansas City, KS 66160, United States

[§]The Small-Molecule X-ray Crystallography Laboratory, University of Kansas, Lawrence, KS 66047, United States

[⊥]Kansas Biological Survey, University of Kansas, Lawrence, Kansas 66047, United States

Abstract

Fourteen new withanolides **1-14**, named withalongolides A-N, respectively, were isolated from the aerial parts of *Physalis longifolia* together with eight known compounds (**15-22**). The structures of compounds **1-14** were elucidated through spectroscopic techniques and chemical methods. In addition, the structures of withanolides **1, 2, 3**, and **6** were confirmed by X-ray crystallographic analysis. Using a MTS viability assays, eight withanolides (**1, 2, 3, 7, 8, 15, 16**, and **19**) and four acetylated derivatives (**1a, 1b, 2a**, and **2b**) showed potent cytotoxicity against human head and neck squamous cell carcinoma (JMAR and MDA-1986), melanoma (B16F10 and SKMEL-28), and normal fetal fibroblast (MRC-5) cells with IC_{50} values in the range between 0.067 and 9.3 μM.

Classically-defined withanolides are a group of C_{28} ergostane-type steroids with a C-22,26 δ-lactone group, first isolated from the genus *Withania*.[1] They are present primarily in the Solanaceae family, which includes the genera *Acnistus*, *Datura*, *Dunalia*, *Jaborosa*, *Nicandra*, *Physalis*, and *Withania*.[2-9] Withanolides have attracted interest in recent years mainly due to their exhibition of significant biological activities, inclusive of antimicrobial, antitumor, anti-inflammatory, immunomodulatory, and insect-antifeedant activities.[2,3, 6] It has been reported that those withanolides displaying the most promising antitumor characteristics contain an α,β-unsaturated ketone in ring A, a $5\beta,6\beta$-epoxy group in ring B, and a nine-carbon side chain with an α,β-unsaturated δ-lactone group.[10] The typical withanolide, withaferin A (**16**) (Figure 1) contains these three moieties and has been shown in vitro and in vivo to suppress the growth of an array of tumor cells, including breast, pancreatic, prostate, lung, leukemia, and head and neck squamous cell carcinoma (HNSCC), by inducing apoptosis,[11] thus possessing potential application as an antiproliferative agent. As part of an ongoing study of withanolides from plant sources,[11,12] a library of 224 native

[*]Corresponding Author To whom corresponding should be addressed. Tel: +01-785-864-4844. Fax: +01-785-864-5326. btimmer@ku.edu.

Supporting Information. ^{1}H and ^{13}C NMR spectra of withanolides **1-21** and the bioassay data of the samples (CH_2Cl_2-MeOH crude extract, hexane soluble fraction, EtOAc soluble fraction, and *n*-BuOH soluble fraction) are available free of charge via the Internet at http://pubs.acs.org. Crystallographic data for the structures of **1, 2, 3**, and **6** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, under reference numbers CCDC 840311, CCDC 840312, CCDC 840313 and CCDC 840314, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or deposit@ccdc.cam.ac.uk).

plant extracts from the U.S. Great Plains was evaluated for cytotoxic activities against HNSCC and melanoma cell lines using the MTS viability assay. One of the most promising leads, *Physalis longifolia* Nutt. (Solanaceae), commonly known as "long leaf groundcherry", was subjected to a phytochemical investigation and the results are presented herein, including the details of the isolation, and structure elucidation of fourteen new withanolides (**1-14**), four acetylated derivatives (**1a, 1b, 2a**, and **2b**), and eight known compounds (**15-22**). Their cytotoxicity was determined against HNSCC (JMAR and MDA-1986), melanoma (B16F10 and SKMEL-28), and normal fetal fibroblast (MRC-5) cells. This constitutes the first report of a phytochemical and bioactivity study of *P. longifolia*.

RESULTS AND DISCUSSION

The CH_2Cl_2–MeOH (1:1) extract of the aerial parts of the title plant, and the EtOAc-soluble and *n*-BuOH soluble fractions, showed cytotoxicity against the above-mentioned cells with IC_{50} values in the range between 0.7 and 9.8 μg/mL using a MTS assay. All compounds (**1-22**) were isolated from the EtOAc-soluble or *n*-BuOH soluble fractions (see Experimental Section).

Compound **1** was isolated as colorless cuboid crystals obtained from a CH_2Cl_2–CH_3CN mixture, a major metabolite in the EtOAc-soluble fraction. Its molecular formula, $C_{28}H_{38}O_7$, was determined by HRESIMS and NMR experiments, equating to ten double-bond equivalents. Its IR absorptions revealed the presence of hydroxy (3431 and 3233 cm^{-1}), keto (1671 cm^{-1}), and ester (1706 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed the presence of three methyl groups at δ 0.60 (3H, s), 0.90 (3H, d, J = 6.6 Hz), 1.97 (3H, s), seven protons attached to oxygenated carbons at δ 3.18 (1H, brs), 3.52 (1H, d, J = 6.1 Hz), 3.65 (1H, d, J = 9.6 Hz), 4.17 (1H, d, J = 9.6 Hz), 4.23 (1H, d, J = 12.5 Hz), 4.28 (1H, d, J = 12.5 Hz), and 4.33 (1H, dt, J = 13.3, 3.4 Hz), and two olefinic methine groups at δ 6.16 (1H, d, J = 10.0 Hz) and 6.95 (1H, dd, J = 10.0, 6.1 Hz). The ^{13}C NMR (APT) and HSQC spectra for **1** (Table 2) displayed 28 carbon signals differentiated as three CH_3, eight CH_2 (including two oxygenated at δ 61.0 and 56.7), ten CH (including two olefins at δ 145.7 and 132.9, three oxygenated at δ 78.8, 68.0 and 61.8), and seven C (including one keto carbonyl at δ 200.7, one ester carbonyl at δ 167.4, two olefins at δ 154.3 and 125.6, and one oxygenated at δ 61.5), corresponding to $C_{28}H_{35}$. The remaining three hydrogen atoms were therefore assigned to three OH groups, indicating that six rings must be present in the structure.

The NMR data of **1** were very close to those obtained for withaferin A **16**,[1,13] a six-ring withanolide isolated as another major compound in this study (Tables 1-3 and Figure 1). Compound **1** was found to contain the following moieties also observed in **16**: an α,β-unsaturated ketone in ring A ([^{13}C: δ 200.7 (C-1), 132.9 (C-2), 145.7 (C-3); ^1H: δ 6.16 (H-2, d, J = 10.0 Hz), 6.95 (H-3, dd, J = 10.0, 6.1 Hz)]; an epoxy group in ring B [^{13}C: δ 61.5 (C-5), 61.8 (C-6); ^1H: δ 3.18 (brs, H-6)]; a nine-carbon side chain with an α,β-unsaturated δ-lactone group [^{13}C: δ 78.8 (C-22), 154.3 (C-24), 125.6 (C-25), 167.4 (C-26); ^1H: δ 4.33 (H-22, dt, J = 13.3, 3.4 Hz)]), as supported by ^1H-^1H COSY and HMBC experiments. The obvious differences between **1** and **16** were the presence of an oxygenated methylene [C-19, ^{13}C: δ 61.0; ^1H: δ 3.65 (1H, d, J = 9.6 Hz), 4.17 (1H, d, J = 9.6 Hz)] in **1** and a methyl carbon [C-19, ^{13}C: δ 17.6; ^1H: 1.38 (3H, s)] in the latter, suggesting that **1** is a 19-hydroxy derivative of **16**. This observation was supported by the high-frequency shift of C-10 (δ 54.3 in **1** and δ 47.9 in **16**), the low-frequency shifts of C-1 (δ 200.7 in **1** and δ 202.5 in **16**), C-5 (δ 61.5 in **1** and δ 64.1 in **16**), and C-9 (δ 43.9 in **1** and δ 44.3 in **16**) in the ^{13}C NMR spectra, and the HMBC correlations between H_2-19 [3.65 (1H, d, J = 9.6 Hz), 4.17 (1H, d, J = 9.6 Hz] and C-1, C-5, C-9, and C-10.

Acetylation of **1** with acetic anhydride in pyridine gave two derivatives: the 4,19,27-triacetate (**1a**) and the 4,27-diacetate (**1b**) (Tables 1 and 2), which proved the presence of hydroxy groups at C-4, C-19, and C-27 by a high frequency shift of H-4 (from δ 3.52 in **1** to δ 4.79 in **1a** and to δ 4.73 in **1b**), of H_2-19 (from δ 3.65, 4.17 in **1** to δ 4.32, 5.07 in **1a**), and of H_2-27 (δ 4.23, 4.28 in **1** to δ 4.84, 4.88 in **1a** and to δ 4.85, 4.88 in **1b**), and by HMBC correlations between H-4 to the ester carbonyl, between H_2-27 and the ester carbonyl, and between H_2-19 and the ester carbonyl in **1a** and **1b**.

Finally, the structure of **1** was confirmed through a single-crystal X-ray diffraction experiment (Figure 2). Thus, **1** (withalongolide A) was established as 19-hydroxywithaferin A. The full assignments of NMR data of **1**, measured in $CDCl_3$ with trace amount of CD_3OD and in C_5D_5N (Tables 1 and 2), were obtained by 2D-NMR methods including ^1H-^1H COSY, HSQC, HMBC, and ROESY spectra.

Compound **2** was isolated as colorless rod crystals from a CH_2Cl_2–$CH_3C_6H_5$ mixture, and was also a major metabolite from the EtOAc-soluble part. Its molecular formula, $C_{28}H_{38}O_6$, was determined by HRESIMS and NMR experiments. The IR and NMR (^1H and ^{13}C) data (Tables 1 and 2) were similar to those of **1**. Analysis of the 1D- and 2D-NMR data for **2** identified resonances consistent with an *α,β*-unsaturated ketone in ring A [^{13}C: δ 200.2 (C-1), 133.1 (C-2), 145.4 (C-3); ^1H: δ 6.23 (H-2, d, J = 10.4 Hz), 7.01 (H-3, dd, J = 10.4, 6.4 Hz)], an epoxy in ring B [^{13}C: δ 61.7 (C-5), 62.1 (C-6); ^1H: δ 3.25 (brs, H-6)], a nine-carbon side chain with a *δ*-lactone group [^{13}C: δ 78.4 (C-22), 149.2 (C-24), 122.2 (C-25), 167.3 (C-26); ^1H: δ 4.31 (H-22, dt, J = 13.3, 3.4 Hz)], and an oxygenated C-19 [^{13}C: δ 62.1; ^1H: δ 4.32, 3.77 (d, J = 9.7 Hz)]. The obvious differences between **2** and **1** were the presence of a 27-methyl carbon [^{13}C: δ 12.7; ^1H: 1.85 (3H, s)] in **2** and an oxygenated methylene [C-27, ^{13}C: δ 56.7; ^1H: δ 4.28 (1H, d, J = 12.5 Hz), 4.23 (1H, d, J = 12.5 Hz)] in **1**, suggesting that **2** is a 27-deoxy derivative of **1**. This observation was supported by the ^{13}C NMR chemical shift values of the *δ*-lactone moiety [δ 149.2 (C-24), 122.2 (C-25), 167.3 (C-26) in **2** and δ 154.3 (C-24), 125.6 (C-25), 167.4 (C-26) in **1**], by the NMR data comparison of the side chain moiety of **2** to those of 27-deoxywithanolides,[14,15] and by HMBC correlations of H_3-27/C-24, C-25, and C-26.

Acetylation of **2** with acetic anhydride in pyridine yielded the 4,19-diacetate (**2a**) and the 4-monoacetate (**2b**) (Tables 1 and 2), which confirmed the presence of hydroxy groups at C-4 and C-19 by a high frequency shift of H-4 (from δ 3.63 in **2** to δ 4.79 in **2a** and to δ 4.74 in **2b**) and H_2-19 (from δ 3.77, 4.32 in **2** to δ 4.32, 5.07 in **2a**).

Finally, the structure of **2** was confirmed through a single-crystal X-ray diffraction experiment (Figure 3). Thus, **2** (withalongolide B) was determined as 27-deoxy-19-hydroxywithaferin A.

Compound **3** was isolated as colorless cube crystals from a CH_2C_{l2}–CH_3CN mixture, a minor component from the EtOAc-soluble fraction. Its molecular formula, $C_{28}H_{38}O_7$, was determined by HRESIMS and NMR experiments. The NMR data of **3** (Tables 1 and 2) were also akin to those of withaferin A **16**.[1,13] Analysis of the 1D- and 2D-NMR data of **3** identified resonances consistent with an *α,β*-unsaturated ketone in ring A [^{13}C: δ 204.0 (C-1), 131.6 (C-2), 143.2 (C-3); ^1H: δ 6.22 (H-2, d, J = 10.0 Hz), 6.96 (H-3, dd, J = 10.0, 6.5 Hz)], an epoxy in ring B [^{13}C: δ 64.5 (C-5), 63.0 (C-6); ^1H: δ 3.23 (brs, H-6)], and a nine-carbon side chain with a *δ*-lactone group [^{13}C: δ 78.9 (C-22), 153.0 (C-24), 125.9 (C-25), 167.2 (C-26); ^1H: δ 4.39 (H-22, dt, J = 13.2, 3.5 Hz)]. The obvious differences between **3** and **16** were the presence of an oxygenated methine [C-11, ^{13}C: δ 69.5; ^1H: 4.15 (1H, brs)] in **3** and a low-frequency methylene [C-11, ^{13}C: δ 22.3; ^1H: δ 1.27 (1H, m), 1.18 (1H, m)] in **16**, implying that **3** is 11-hydroxywithaferin A. This observation was supported by the high-frequency shift of C-9 (*δ* 48.1 in **3** and *δ* 44.3 in **16**) and C-12 (*δ* 47.8 in **3** and *δ* 39.5 in **16**), and the low-frequency shift of C-8 (*δ* 27.0 in **3** and *δ* 29.9 in **16**) in the ^{13}C NMR spectrum; by the presence of a fragment -CH_2–CH(OH)–CH–CH(CH_2)–CH- (starting with C-12 and ending with C-14) deduced from ^{1}H-^{1}H COSY and HSQC experiments; and by the HMBC correlations between H_α-12 (*δ* 2.17, dd, *J* = 13.8, 2.8 Hz) and C-9 (*δ* 48.1), 11 (*δ* 69.5), 13 (*δ* 42.1), 14 (*δ* 58.2). The orientation of the hydroxy group at C-11 was deduced as *β* due to the broad single peak pattern of H-11 (*δ* 4.15, brs), the small coupling constant of 3.3 Hz between H-9 (*δ* 1.25, dd, *J* = 10.8, 3.3 Hz) and H-11, and ROESY correlations of H-11/H-9 and H-11/H_α-12. Finally, the structure of **3** was confirmed through a single-crystal X-ray diffraction experiment (Figure 4). Thus, the new withanolide **3** (withalongolide C) was established as 11*β*-hydroxywithaferin A.

Compounds **4** and **5** were isolated as two presumed artifacts. These two compounds were probably formed from withalongolide A (**1**) and withalongolide C (**3**), respectively, by a Michael-type addition due to the use of CH_3OH during the extraction procedure. It is possible that these compounds are formed in a similar fashion to 2,3-dihydro-3*β*-methoxywithaferin A (**17**)[1] (Tables 2 and 3), which was most likely derived from withaferin A (**16**) during this study. Comparing the NMR data of the methoxy group in **17** [-OCH_3 group at C-3: ^{1}H: *δ* 3.32 (3H, s); ^{13}C: *δ* 57.0; H-3: *δ* 3.68 (1H, ddd, *J* = 6.3, 3.2, 2.2 Hz); C-3: *δ* 77.7] with both **4** and **5** [-OCH_3: ^{1}H: *δ* 3.35 (3H, s); ^{13}C: *δ* 57.6; H-3: *δ* 3.70 (1H, ddd, *J* = 8.3, 3.4, 2.6 Hz); C-3: *δ* 77.7 in **4** and -OCH_3: ^{1}H: *δ* 3.34 (3H, s); ^{13}C: *δ* 57.4; H-3: *δ* 3.75 (1H, ddd, *J* = 6.5, 4.0, 2.2 Hz); C-3: *δ* 76.2 in **5**] suggested the presence of a methoxy group at the C-3 positions in **4** and **5**. This was confirmed by the presence of ^{1}H-^{1}H COSY fragment of -CH_2–CH(OCH_3)–CH(OH)- in ring A and HMBC correlation of OCH_3/C-3 in both **4** and **5**. The structures of **4** (withalongolide D) and **5** (withalongolide E) were determined by spectroscopic methods and complete assignments of their NMR data are listed in Tables 2 and 3.

Compound **6** was isolated as colorless cube crystals obtained from a CH_2Cl_2–CH_3CN mixture. Its molecular formula, $C_{27}H_{34}O_4$, was ascertained by HRESIMS and NMR experiments (Tables 2 and 3). Similar to withaferin A (**16**), it showed signals for four methyl groups [^{13}C: *δ* 20.2 (C-28), 19.9 (C-19), 13.6 (C-21), 12.1 (C-18); ^{1}H: *δ* 2.01 (H_3-28, s), 1.15 (H_3-19, s), 1.01 (H_3-21, d, *J* = 6.6 Hz), 0.75 (H_3-18, s)], an *α*,*β*-unsaturated ketone in ring A [^{13}C: *δ* 211.6 (C-1), 129.4 (C-2), 155.2 (C-3); ^{1}H: *δ* 5.93 (H-2, d, *J* = 6.4 Hz), 7.57 (H-3, d, *J* = 6.4 Hz)], and a nine-carbon side chain with an *α*,*β*-unsaturated *δ*-lactone group ([^{13}C: *δ* 57.7 (C-27), 79.0 (C-22), 153.1 (C-24), 125.9 (C-25), 167.3 (C-26); ^{1}H: *δ* 4.42 (H-22, dt, *J* = 13.3, 3.4 Hz), 4.37 (H-27, d, *J* = 12.5 Hz), 4.32 (H-27, d, *J* = 12.5)]. A detailed comparison of the NMR data of **6** to those of **16** indicated that both compounds share identical ring C, D and side chain moieties, but are different in their A and B rings. A five-membered ring A for **6** was proposed on the basis of the following evidence: (1) the unusual chemical shift value of the conjugated ketone carbon (C-1, *δ* 211.6); (2) the coupling pattern of H-3 (*δ* 7.57, d, *J* = 6.4 Hz) and the small coupling constant of 6.4 Hz between the olefinic protons H-2 and H-3 when compared to those of H-3 (6.91, dd, *J* = 10.0, 5.9 Hz) in **16**, showing C-3 to be linked with a quaternary carbon in **6**. This five-membered ring A and a C-5,6 double bond in ring B were supported by the HMBC correlations of H_3-19/C-1, C-5 (quaternary carbon, *δ* 147.4), C-9 (*δ* 42.8), and C-10 (*δ* 47.6); of H-2/C-1, C-3, C-5, and C-10; and of H-3/C-1, C-2, C-5, C-6 (methine, *δ* 123.8), and C-10. Finally, the observation was confirmed through a single-crystal X-ray diffraction experiment (Figure 5). Thus, **6** (withalongolide F) was deduced to be A-nor-27-hydroxy-1-oxowitha-2,5,24-trienolide. This 4-norwithanolide with a 2,5-dien-1-one system was reported previously as a semi-synthetic product derived from withaferin A by an acid-catalyzed rearrangement.[16]

Compound **7** was a major compound isolated from the BuOH-soluble fraction. Its molecular formula, $C_{28}H_{40}O_{11}S$, was determined by HRESIMS and NMR experiments. The NMR data of **7** (Tables 2 and 3) were similar to those of 2,3-dihydro-3*β*-*O*-sulfate-withaferin A (**19**) (Tables 2 and 3),[17] another major withanolide isolated from the BuOH-soluble fraction during this study (Figure 1). The obvious differences between **7** and **19** were the presence of an oxygenated methylene [C-19, ^{13}C: *δ* 59.9; ^{1}H: *δ* 4.80 (1H, d, *J* = 9.4 Hz), 4.01 (1H, d, *J* = 9.4 Hz)] in **7** and a methyl carbon [C-19, ^{13}C: *δ* 15.9; ^{1}H: *δ* 1.69 (3H, s)] in the latter, suggesting that **7** is a 19-hydroxy derivative of **19**. This observation was supported by the high-frequency shift of C-10 (*δ* 55.7 in **7** and *δ* 49.9 in **19**), the low-frequency shift of C-1 (*δ* 208.0 in **7** and *δ* 209.7 in **19**), C-5 (*δ* 63.0 in **7** and *δ* 65.1 in **19**), and C-9 (*δ* 42.3 in **7** and *δ* 43.1 in **19**) in the ^{13}C NMR spectrum, and the HMBC correlations of H_2-19 [*δ* 4.01 (1H, d, *J* = 9.4 Hz), 4.80 (1H, d, *J* = 9.4 Hz]/C-1, C-5, C-9, and C-10 in **7**. Thus, **7** (withalongolide G) was determined as 2,3-dihydro-19-hydroxy-3*β*-*O*-sulfate-withaferin A.

Compound **8** was isolated as a major component from the BuOH-soluble fraction. Its molecular formula, $C_{40}H_{58}O_{15}$, was ascertained by HRESIMS and NMR experiments. The NMR data of **8** (Tables 4 and 5) showed similarities to those of 27-*O*-*β*-D-glucopyranosyl-withaferin A (**15**) (sitoindoside IX)[18] (Tables 4 and 5) isolated during this study, suggesting **8** to be a withanolide saponin. The aglycone of **8** was determined to be withaferin A as both **8** and **15**, possess the superimposable ^{1}H and ^{13}C NMR signals of the steroid aglycone moieties and both showed the same main LC-MS/MS fragments of *m/z* 471 and 281 due to the presence of a withaferin A moiety. Differing in the presence of only one glucose residue in **15**, two sugar residues were observed in **8** on the basis of the signals of two anomeric carbons [methines, *δ* 105.3 (C-1′) and 103.1 (C-1″)] and their corresponding anomeric protons [*δ* 4.97 (H-1′, 1H, d, *J* = 7.8) and 5.94 (H-1″, 1H, s)]. Furthermore, the data for **8** suggested that the compound had, besides a glucose unit, an additional five oxygenated carbons (five methines) and one low frequency methyl group [^{13}C: *δ* 19.0; ^{1}H: *δ* 1.74 (3H, d, *J* = 6.1 Hz)], corresponding to a rhamnose in the pyranose form. The rhamnose moiety was deduced by the detailed comparison the NMR data of **8** with those of rutin **22** {3-*O*-[*α*-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranosyl-quercetin} also isolated in this study and confirmed from the ^{1}H-^{1}H COSY, HSQC and HMBC spectra when starting with the characteristic methyl group [C-6″, ^{13}C: *δ* 19.0; ^{1}H: *δ* 1.74 (3H, d, *J* = 6.1 Hz)]. The *α*-anomeric configuration of the rhamnose unit was assigned from the small coupling constant between H-1″ (1H, *δ* 5.94, s) and H-2″ (1H, *δ* 4.73, s). Furthermore, the rhamnose was confirmed to be attached at C-4′ (*δ* 78.5) on the basis of HMBC correlations of H-1″/C-4′ and H-4′/C-1″, also supported by the glycosylation shifts of C-4′ (*δ* 78.5 in **8** and *δ* 72.1 in **15**), C-3′ (*δ* 77.1 in **8** and *δ* 79.0 in **15**) and C-5′ (*δ* 77.8 in **8** and *δ* 79.1 in **15**). Thus, the structure of **8** (withalongolide H) was determined as 27-*O*-[*α*-L-rhamnopyranosyl(1→4)]-*β*-D-glucopyranosyl-withaferin A.

Compounds **9-14** were isolated as the minor components from the BuOH-soluble fraction. Withanolide **9** was assigned a molecular formula of $C_{34}H_{50}O_{11}$ by HRESIMS and NMR experiments. Its NMR data (Tables 4 and 5) exhibited a close resemblance to those of withalongolide A (**1**), possessing the same nine-carbon side chain with an *α*,*β*-unsaturated *δ*-lactone, identical rings C and D, and an oxygenated C-19 methylene group. In addition, the remaining rings A and B showed similarities to those of 3-*O*-*β*-D-glucopyranosyl-20,27-dihydroxy-1-oxowitha-5,24-dienolide, a withanolide saponin reported from *Physalis peruviana*,[19] with the following signals: (1) the occurrence of a ketone (*δ* 208.9, C-1) and a double bond [^{13}C: C-5 quaternary carbon, *δ* 132.8, and C-6 methine, *δ* 129.1; ^{1}H: *δ* 5.75 (1H, brd, *J* = 5.3 Hz, H-6)]; (2) a glucose moiety attached to C-3 in ring A [^{13}C: *δ* 75.9 (CH, C-3); characteristic signals for glucose: *δ* 103.5 (CH, C-1′), 79.1 (CH, C-5′), 79.1 (CH, C-3′), 75.6 (CH, C-2′), 71.9 (CH, C-4′), and 63.0 (CH_2, C-6′)]; (3) a fragment of - CH_2–CH(O)–CH_2- in ring A deduced from the ^{1}H–^{1}H COSY and HSQC spectra; (4) HMBC correlations of H-3/C-1′ and H-1′/C-3; of H2-19/C-1, C-5, C-9 and C-10; of H_2-2/C-1, C-3, C-4, and C-10; and of H*β*-4/C-2,C-3, C-5, C-6, and C-10. Thus, the structure of **9** (withalongolide I) was determined as 3-*O*-*β*-D-glucopyranosyl-19,27-dihydroxyl-1-oxo-witha-5,24-dienolide.

Compound **10** was assigned a molecular formula of $C_{34}H_{52}O_{11}$ by HRESIMS and NMR experiments. Similar to those of **9**, the NMR data of **10** (Tables 4 and 5) displayed the presence of an oxygenated C-19 methylene group [^{13}C: δ 63.9 CH_2; ^{1}H: δ 4.32 (1H, d, J = 11.0 Hz), 4.11 (1H, d, J = 11.0 Hz)] and three methyl groups [^{13}C: δ 12.6, 13.0, 14.1; ^{1}H: δ 0.80 (s), 0.99 (d, J = 6.6 Hz), 2.04 (s)]. The obvious differences between **10** and **9** were the presence of an oxygenated methine (δ 69.5) in **10** instead of the keto carbon (C-1, δ 208.9) in **9**, implying that a hydroxy group is attached to C-1. This was supported by the presence of a ^{1}H-^{1}H COSY fragment of -CH(O)–CH_2–CH(O)–CH_2-assigned as a C-1 to C-4 moiety in ring A and confirmed by HMBC correlations of H_2-19/C-1, C-5, C-9, and C-10, of H_2-4/C-2, C-3, C-5, C-6, and C-10. The orientation of the hydroxy group at C-1 was assigned as *α*, based on the small coupling constant of H-1 (δ 4.61, s)/H_2-2. Furthermore, it was determined that the glucose was attached to C-28 by the HMBC correlations of H_2-28/C-23, 24, 25, 1′, of H-1′/C-28, as well as the chemical shifts of C-23 (δ 25.2), C-24 (δ 148.8), C-25 (δ 125.2), C-26 (δ 167.1), C-27 (δ 13.0), and C-28 (δ 67.1) and detailed comparison to those of withanolides with a 28-*O*-glucoside moiety.[20] Thus, the structure of **10** (withalongolide J) was determined as 28-*O*-*β*-D-glucopyranosyl-1*α*,3*β*,19-trihydroxywitha-5,24-dienolide.

Similar to withalongolide J (**10**), compounds **11-14** were shown to possess the same nine-carbon side chain with an *α*,*β*-unsaturated *δ*-lactone and a glucose moiety at C-28, based on their superimposable NMR signals assigned to the side chain (Tables 4 and 5).

Saponin **11** was assigned a molecular formula of $C_{33}H_{46}O_{10}$ by HRESIMS and NMR experiments. Excluding the six carbons corresponding to the glucose moiety, the 27-carbon-aglycon implied that one carbon in the C_{28} withanolide scaffold must be lost. The NMR data of its aglycon were similar to those of 1,6,27-trihydroxy-19-norwitha-1,3,5(10),24-tetraenolide (a 19-norwithanolide with an aromatic ring A).[21] A trisubstituted aromatic ring A in **11** was observed from the ^{1}H NMR (H-2: δ 7.14, 1H, d, J = 7.7 Hz; H-3: δ 7.34, 1H, t, J = 7.7 Hz; and H-4: δ 7.87, 1H, d, J = 7.7 Hz) and ^{13}C NMR (C-1: δ 157.8 C; C-2: δ 115.1, CH; C-3: δ 127.4 CH; C-4: δ 119.3 CH; C-5: δ 146.2 C; and C-10: δ 127.8, C) experiments. This was confirmed by ^{1}H-^{1}H COSY, HSQC experiments and HMBC correlations of H-2/C-4 and C-10, H-3/C-1 and C-5, and of H-4/C-2 and C-10. Moreover, the HMBC correlations of H-4/C-6 (δ 70.7 CH), of OH-6 (δ 6.81, d, J = 6.5 Hz)/C-5 and C-6, of H-7*β*/C-5, and the ^{1}H-^{1}H COSY fragment -CH(OH)–CH_2–CH–CH–CH_2–CH_2- (corresponding to -C_6–C_7–C_8–C_9–C_{11}–C_{12}-) showed that both the aglycone of **16** and 1,6,27-trihydroxy-19-norwitha-1,3,5(10),24-tetraenolide have the same planar structural moieties in rings A and B. However, the orientation of the hydroxy group at C-6 was assigned as *α* because of the large coupling constants (11.0 Hz) between H-6 (δ 5.26, dt, J = 11.0, 6.5 Hz) and H-7*α* (δ 1.63, q, J = 11.0 Hz), and (6.5 Hz) between H-6 and H-7*β* (δ 2.32, dd, J = 11.0, 6.5 Hz). Thus, the structure of **11** (withalongolide K) was determined as 28-*O*-*β*-D-glucopyranosyl-1,6*α*-dihydroxy-19-norwitha-1,3,5(10),24-tetraenolide.

Compound **12** was assigned a molecular formula of $C_{33}H_{48}O_{11}$ by HRESIMS and NMR experiments. Its NMR data (Tables 4 and 5) were similar to those of withalongolide K (**11**), containing the same rings B, C, and D because of their superimposable ^{1}H and ^{13}C NMR signals. The differences observed between **12** and **11** were caused by changes in the ring A moiety. Unlike the aromatic ring A in **11**, a conjugated 5(10)-ene-1-one system in **12** was revealed by the chemical shifts of quaternary carbons at δ 199.4 (C-1), 156.4 (C-5), 136.5 (C-10). A ^{1}H-^{1}H COSY fragment of -CH_2–CH(OH)–CH_2- was assigned as -C_2–C_3–C_4- in ring A, and confirmed by the HMBC correlations of H_2-2/C-1, C-3, and C-4, and of H_2-4/C-2, C-5, and C-10. Thus, the structure of **12** (withalongolide L) was determined as 28-*O*-*β*-D-glucopyranosyl-3*β*,6*α*-dihydroxy-1-oxo-19-norwitha-5(10),24-dienolide.

Compound **13** was assigned a molecular formula of $C_{33}H_{48}O_{10}$ by HRESIMS and NMR experiments. Its NMR data were similar to those observed for withalongolide L (**12**), containing a conjugated 5(10)-ene-1-one system [^{13}C: *δ* 198.3 (C-1), 153.6 (C-5), 136.1 (C-10)] in ring A. The obvious differences between **13** and **12** were the presence of a methylene (^{13}C: *δ* 33.8; ^{1}H: *δ* 2.30, 2H, m) instead of an oxygenated methine (^{13}C: *δ* 71.2; ^{1}H: *δ* 4.69), suggesting that **13** is a 6-deoxy derivative of **12**. This observation was supported by the ^{13}C NMR high-frequency shift of C-4 (*δ* 41.9 in **13** and *δ* 36.9 in **12**) and C-8 (*δ* 39.9 in **13** and *δ* 37.5 in **12**), the low-frequency shift of C-7 (*δ* 26.4 in **13** and *δ* 37.3 in **12**), and HMBC correlations of OH-3 (*δ* 6.81, 1H, d, *J* = 4.1 Hz)/C-2 (*δ* 49.7), C-3 (*δ* 66.1), and C-4 (*δ* 41.9), of H_2-4 (*δ* 2.68, dd, *J* = 5.9, 16.8 Hz and *δ* 2.58, dd, *J* = 2.7, 16.8 Hz)/C-2, C-3, C-5 (*δ* 153.6), C-6 (*δ* 33.8), and C-10 (*δ* 136.1), and of H-6*β* (*δ* 2.09, m)/C-4, C-5, C-7 (*δ* 26.4), C-8 (39.9), and C-10 in **13**. Thus, the structure of **13** (withalongolide M) was determined as 28-*O*-*β*-D-glucopyranosyl-3*β*-hydroxy-1-oxo-19-norwitha-5(10),24-dienolide.

Compound **14** was assigned a molecular formula of $C_{33}H_{48}O_{10}$ by HRESIMS and NMR experiments, and as an isomer of withalongolide M (**13**). The NMR data of these two compounds (Tables 4 and 5) were similar to each other, having the same functional groups and the same multiplicities for all other carbons present. A conjugated 5(10)-ene-6-one system [^{13}C: *δ* 198.6 (C-6), 158.7 (C-10), 130.4 (C-5)] in **14** was proposed instead of the 5(10)-ene-1-one one in **13** on the basis of the following observations: (1) a ^{1}H-^{1}H COSY fragment of -CH_2–CH_2–CH(OH)–CH_2- (from C-1 to C-4) in ring A of **14** replaced the ring A fragment -CH_2–CH(OH)–CH_2- (from C-2 to C-4) in **13**; (2) a ^{1}H-^{1}H COSY fragment of -CH_2–CH–CH- (from C-7 to C-9) in ring B of **14** replaced the ring B fragment -CH_2–CH_2–CH–CH- (from C-6 to C-9) in **13**; (3) HMBC correlations of H-2/C-1 (*δ* 25.6), C-3 (*δ* 64.7), and C-4 (*δ* 32.9); of H_2-7/C-5 (*δ* 130.4), C-6 (*δ* 198.6), C-8 (*δ* 40.0), and C-9 (*δ* 46.6); H-1/C-3, C-5, and C-10 (*δ* 158.7). Furthermore, the orientation of the hydroxyl group at C-3 was determined as *α* due to the small coupling constant (*J* = 2.2 Hz) between H-3 (*δ* 4.33, brs)/H-4*β* (*δ* 2.81, dd, *J* = 2.2, 15.8 Hz) and the NOESY correlations of H-3/H-1*β*, H-2*β*, H-4*β*. Thus, the structure of **14** (withalongolide N) was assigned as 28-*O*-*β*-D-glucopyranosyl-3*α*-hydroxy-6-oxo-19-norwitha-5(10),24-dienolide.

Eight known compounds were identified by comparison of their data with those published in the literature, as seven withanolides, sitoindoside IX (**15**),[18] withaferin A (**16**),[1,13] 2,3-dihydro-3*β*-methoxywithaferin A (**17**),15 viscosalactone B (**18**),[22] 2,3-dihydro-3*β*-*O*-sulfate withaferin A (**19**),[17] 2,3-dihydrowithaferin A (**20**),[15] and 3*α*,6*α*-epoxy-4*β*,5*β*,27-trihydroxy-1-oxowitha-24-enolide (**21**),[23] and a flavonoid glucoside, rutin (**22**).[24] The full assignments of the NMR data of **15, 16, 17, 18**, and **19** are listed in Tables 2-5 as these data were either unavailable or incomplete or in need of revision within the published literature.

The classically defined withanolide-type steroids (**1-21**) isolated from the title plant showed a diversity of oxygenation patterns that may be summarized as follows: (1) Six withanolides (**1, 2, 4, 7, 9** and **10**) have an oxygenated C-19 group, which is rare in Nature. A literature investigation showed that from the approximately 520 unmodified withanolides only nine C-19 oxygenated withanolides have been reported so far. They are as follows: jaborosalactones O,[25] V, W, X,[26] 46, 47, 48,[27] cinerolide [28] and bracteosin B.[29] (2) Compounds **3** and **5** are rare examples of unmodified withanolides having an oxygenated C-11 although withasomniferanolide (with 11*β*-OH group),[30] somniferanolide (with 11*β*-OH group),[30] and witharifeen (with 11*α*-OH group)[31] have been previously reported. (3)

Saponins **10-14** have a sugar constituent attached at the C-28. Only two previously published withaholide saponins (physagulins E and G)[20] were shown to have a sugar moiety at C-28 thus far. (4) Compounds **11-14** are the first reported examples of C-19 nor withanolide saponins. It should be noted, however, that there are only four C-19 nor withanolides (jaborosalactone Q,[21] 7,[32] 45, 12-*O*-methyl-jaborosalactone 45 [27]) reported in the literature. (5) Most withanolides have an oxygenated C-1 in ring A, but **15** is the exception by not being oxidized at C-1. In addition, the presence of a 3-*O*-sulfate group in naturally occurring withanolides is extremely rare. Besides withanolides **7** and **19**, there are only five other 3-*O*-sulfate withanolides previously reported from *Datura metel*,[33] *Solanum cilistum*,[34] and *Withania somnifera*.[35]

All the withanolides (**1-21**) and the four acetylated derivatives (**1a, 1b, 2a** and **2b**) were tested against the HNSCC (JMAR, MDA-1986), melanoma (B16F10 and SKMEL-28), or/and normal fetal fibroblast (MRC-5) cells for their cytotoxicity. As summarized in Table 6, withanolides **1-5, 7, 8, 15, 16**, and **19** and the four derivatives (**1a, 1b, 2a**, and **2b**) showed cytotoxic effects against the cells tested with IC_{50} values in the range 0.067–9.3 μM, while the other withanolides were inactive. Similar to withaferin A **16**, withanolides **1-3** containing the functional groups of a 2-en-1-one in ring A, a 5β,6β-epoxy in ring B, and a lactone ring in the side chain, were active, showing the importance of these three groups. The activity of the 3-*O*-sulfate withanolides **7** and **19** was due to their transformations to **1** and **16**, respectively. Withanolide glycosides **8** and **15** displayed less cytotoxicity relative to their aglycone withaferin A (**16**). However, the esterification of the hydroxy groups at C-4, C-19, and C-27 increased the resultant cytotoxicity, as shown for the acetylated derivatives **1a** and **2a** with IC_{50} values less than 1 μM against all the cells tested. These results are in agreement with previous structure-activity relationship reports.[10,36,37] In addition, it should be noted that withalongolide A (**1**), withalongolide B (**2**), and withaferin A (**16**) are most likely responsible for the cytotoxic activities of the extract prepared from the title plant due to their relative high abundance levels (0.16% for **1**, 0.10% for **16**, and 0.03% for **2**).

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were obtained using an MPA100 melting point apparatus. Optical rotations were measured with a Rudolph RS Autopol IV automatic polarimeter. IR data were obtained with a Thermo Nicolet Avatar 360 FT-IR spectrometer. NMR spectra were recorded with a Bruker AV-400 or AV-500 instrument with a cryoprobe for ^{1}H, APT, COSY/DQF-COSY, HSQC, HMBC, and NOESY/ROESY. Chemical shift values are given in δ (ppm) using the peak signals of the solvent C_5D_5N (δ_H 8.74, 7.58, and 7.22; and δ_C 150.35, 135.91, and 123.87) or $CDCl_3$ (δ_H 7.24 and δ_C 77.23) as references and coupling constants were reported in Hz. ESIMS data were measured with an Agilent 1200 Series LC-MS/MS ion trap 6300 mass spectrometer. HRESIMS data were collected with a LCT Premier time of flight mass spectrometer (Waters Corp., Milford, MA). Column chromatography was performed on silica gel (particle size 12–25 μm) (Sorbent Technologies, Atlanta, GA), or MCI CHP20P (particle size 75–150 μm) (Sigma-Aldrich, Saint Louis, MO), or Sephadex LH-20 (GE Healthcare, Piscataway, NJ), or C_{18} reversed-phase silica gel (particle size 40–65 μm) (Sigma-Aldrich, Saint Louis, MO). Normal-phase silica gel G TLC plates (w/UV 254) and reversed-phase C_{18} TLC plates (w/UV 254) (Sorbent Technologies, Atlanta, GA) were used for fraction detection. The spots were visualized using UV light at 254 nm and spraying with 10% EtOH-sulfuric acid reagent. Semi-preparative HPLC was performed on an Agilent 1200 unit equipped with a DAD detector, utilizing a Lichrospher RP-18 column (250 × 10 mm, 5 μm).

Plant Material

Fresh aerial parts of the plant *P. longifolia* were collected from the Kanopolis wildlife area (latitude: 38.74206°; longitude: 97.98467°), Ellsworth County, Kansas, in August, 2009. It was identified by plant taxonomist Dr. Kelly Kindscher at the Kansas Biological Survey, University of Kansas. A voucher specimen (Hillary Loring 3583) was deposited in the R.L. McGregor Herbarium of the University of Kansas.

Extraction and Isolation

The collected biomass was air dried at room temperature. The dried material was then ground to a coarse powder (930 g), and extracted three times with CH_2Cl_2–MeOH (50:50, 4.0 L) at room temperature. After removing the solvents under vacuum, the extract (107 g) was suspended in 400 mL H_2O, followed by partitions with *n*-hexane, EtOAc, and *n*-butanol (3 × 500 mL). The resulting ethyl acetate fraction (22 g) collected was applied to silica gel flash CC (column chromatography), and eluted subsequently with hexane–acetone mixtures of increasing polarities. The fraction obtained on elution with hexane–acetone (80:20) (1.0 g), was again subjected to silica gel CC [eluted with CH_2Cl_2–CH_3COCH_3 (90:10)] to afford compound **2** (280 mg). The fraction obtained on elution with hexane–acetone (70:30) (3.0 g), was subjected to silica gel CC [eluted with CH_2Cl_2–CH_3COCH_3 (80:20)] to yield compounds **16** (730 mg), **17** (7 mg) and **20** (10 mg). The fraction obtained on elution with hexane–acetone (65:35) (700 mg), was subjected further to silica gel CC [eluted with hexane–acetone (65:35)] to afford compounds **3** (30 mg) and **5** (5 mg). The fraction acquired on elution with hexane–acetone (60:40) (2.2 g), was applied to silica gel CC [eluted with hexane–acetone (60:40)] to afford compounds **1** (1200 mg) and **4** (15 mg). The *n*-butanol fraction (29 g) obtained was subjected to a MCI CHP20P gel CC (500 g), eluted with a mixture of H_2O–MeOH (100:0, 80:20, 60:40, 40:60, 85:15, 0:100), in order of increasing concentrations of methanol. The 85% MeOH fraction (3.5 g) was subjected to silica gel CC, eluted with CH_2Cl_2-CH_3COCH_3 with increasing amounts of acetone to afford compounds **1** (240 mg), **6** (40 mg), **16** (200 mg), **18** (250 mg) and **21** (220 mg). The 60% MeOH fraction (4.2 g) was subjected to silica gel CC, eluted with CH_2Cl_2-MeOH-H_2O (7:1:0.1) with increasing amounts of MeOH-H_2O (10:1), then the fractions were further subjected to reverse-phase C_{18} Si gel column chromatography (200 g, particle size 40-63 μm), eluted by MeOH-H_2O (40:60, 50:50, 60:40, 65:35). The fractions obtained were subjected to semi-preparative HPLC, with the mobile phase CH_3CN-H_2O (26:74; 28:72), to afford compounds **7** (40 mg), **8** (35 mg), **9** (9 mg), **10** (12 mg), **11** (6 mg), **12** (5 mg), **13** (6 mg), **14** (7 mg), **15** (22 mg), **19** (35 mg), and **22** (80 mg).

Withalongolide A (1)—colorless cuboid crystals (CH_3CN); mp 216-217 °C; $[\alpha]^{25}_{D}$ +14.2 (*c* 0.16, $CHCl_3$); IR (neat) υ_{max} 3431 (br), 3233 (br), 2922, 1706, 1671, 1400, 1037, 962 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 487 (M +H, 6), 469 (M–H_2O+H, 100), 451 (M–2 H_2O+H, 6); HRESIMS *m/z* 509.2489 $[M+Na]^+$ (calcd for $C_{28}H_{38}O_7Na$, 509.2471), *m/z* 487.2674 $[M+H]^+$ (calcd for $C_{28}H_{39}O_7$, 487.2696), *m/z* 469.2585 $[M–H_2O+H]^+$ (calcd for $C_{28}H_{37}O_6$, 469.2590).

Single-Crystal X-ray Structure Determination of Withalongolide A (1)—Crystal analysis was performed with a colorless plate crystal (dimensions 0.42 × 0.35 × 0.21 mm^3) obtained from CH_2Cl_2–CH_3CN (1:1) using Mo Kα radiation (λ = 0.71073 Å) on a Bruker SMART APEX diffractometer equipped with a sealed-tube x-ray source and a graphite monochromator. Crystal data for **1**: $C_{28}H_{38}O_7$ (formula weight 486.58), monoclinic, space group $P2_1$, T = 100(2) K, crystal cell parameters a = 8.370(2) Å, b = 10.523(3) Å, c = 14.280(3) Å, β = 104.552(4)°, V = 1217.4 (5) $Å^3$, D_c = 1.327 Mg/m^3, Z = 2, F(000) = 524, absorption coefficient μ = 0.094 mm^{-1}. A total of 11335 reflections were collected in the range 2.43 < θ < 29.21°, with 5809 independent reflections [$R_{(int)}$ = 0.050] and 5478 with I

> $2\sigma(I)$, completeness to θ_{max} was 93.1%. Multi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters were 5809/1/468; goodness-of-fit on F^2 = 1.015; final R indices [$I > 2\sigma(I)$], R_1 = 0.045, ωR_2 = 0.098; R indices (all data), R_1 = 0.048, ωR_2 = 0.099; largest difference peak and hole, 0.37 and -0.21 e/Å^{-3}.

Acetylation of Withalongolide A (1)

A solution of **1** (50 mg) in pyridine (8 mL) and acetic anhydride (2 mL), was stirred for 24 hrs at room temperature. Then 50 mL water were added to the solution. The precipitate (70 mg) was subjected to semi-preparative HPLC, eluted with the mobile phase CH_3CN–H_2O (45:55), to afford triacetate **1a** (40 mg) and diacetate **1b** (10 mg).

Withalongolide A 4, 19,27-triacetae (1a)—IR (neat) υ_{max} 2953 (br), 1731, 1702, 1674, 1366, 1208, 1023, 966 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 630 (M+H_2O, 30), 613 (M+H, 100); HRESIMS *m/z* 635.2829 [M+Na]$^+$ (calcd for $C_{34}H_{44}O_{10}Na$, 635.2832).

Withalongolide A 4,27-diacetae (1b)—IR (neat) υ_{max} 3536 (br), 2922, 1736, 1701, 1674, 1376, 1215, 1021, 967 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 1163 (2 M+Na, 6), 588 (M+H_2O, 10), 571 (M+H, 100); HRESIMS *m/z* 593.2740 [M+Na]$^+$ (calcd for $C_{32}H_{42}O_7Na$, 593.2727).

Withalongolide B (2)—colorless plate crystals (toluene); mp 197-198 °C; $[\alpha]^{25}_D$ +12.3 (*c* 0.15, $CHCl_3$); IR (neat) υ_{max} 3260 (br), 3006, 2946, 2887, 1693, 706, 1675, 1383, 1082, 763 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 963 (2 M +Na, 40), 941 (2 M+H, 70), 493 (M+Na, 10), 471 (M+H, 4), 453 (M–H_2O+H, 100), 435 (M–2 H_2O+H, 45%); HRESIMS *m/z* 493.2554 [M+Na]$^+$ (calcd for $C_{28}H_{38}O_6Na$, 493.2566).

Single-Crystal X-ray Structure Determination of Withalongolide B (2)—Crystal analysis was performed with a colorless plate (dimensions 0.21 × 0.16 × 0.15 mm^3) obtained from CH_2Cl_2–CH_3COCH_3–toluene (1:1:1) using Cu Kα radiation (λ = 1.54178 Å) on a Bruker APEX2 diffractometer equipped with a Bruker MicroStar microfocus rotating anode x-ray source and Helios multilayer optics. Crystal data for **2**: $C_{28}H_{38}O_6 \cdot C_7H_8$ (formula weight 562.72), Orthorhombic, space group $P2_12_12_1$, T = 100(2) K, crystal cell parameters a = 7.1844(3) Å, b = 26.0678(10) Å, c = 49.0852(18) Å, V = 9192.8 (6) Å^3, D_c = 1.220 Mg/m^3, Z = 12, F(000) = 3648, absorption coefficient μ = 0.65 mm^{-1}. A total of 32032 reflections were collected in the range $1.80 < \theta < 69.15°$, with 13806 independent reflections [$R_{(int)}$ = 0.035] and 12653 with $I > 2\sigma(I)$, completeness to θ_{max} was 90.8%. Multi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters were 13806/0/1108; goodness-of-fit on F^2 = 1.083; final R indices [$I > 2\sigma(I)$], R_1 = 0.096, ωR_2 = 0.233; R indices (all data), R_1 = 0.102, ωR_2 = 0.238; largest difference peak and hole, 0.84 and -0.32 e/Å^{-3}.

Acetylation of Withalongolide B (2)

A solution of **2** (50 mg) in pyridine (8 mL) and acetic anhydride (2 mL), was stirred for 24 hrs at room temperature. Then 50 mL water were added to the solution. The precipitate (65 mg) was subjected to semi-preparative HPLC, eluted with the mobile phase CH_3CN–H_2O (43:57), to afford diacetate **2a** (41 mg) and monoacetate **2b** (11 mg).

Withalongolide B 4,19-diacetate(2a)—IR (neat) υ_{max} 2943 (br), 1738, 1698, 1368, 1220, 1127, 1043, 1019, 762 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 1109 (2 M+H, 60), 577 (M+Na, 40), 555 (M+H, 30), 495 (M–HOAC+H, 100), 435 (M–2 HOAC+H, 30), 296 (70); HRESIMS *m/z* 577.2764 $[M+Na]^+$ (calcd for $C_{32}H_{42}O_8Na$, 577.2727).

Withalongolide B 4-acetate (2b)—IR (neat) ν_{max} 3536 (br), 2922, 1736, 1701, 1674, 1376, 1215, 1021, 967 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 1047 (2 M+Na, 30), 1025 (2 M+H, 75), 513 (M+H, 100), 453 (M–HOAc+H, 6); HRESIMS *m/z* 535.2670 $[M+Na]^+$ (calcd for $C_{30}H_{40}O_7Na$, 535.2672).

Withalongolide C (3)—colorless cuboid crystals, mp 197-198 °C; $[\alpha]^{25}_D$ +10.3 (*c* 0.12, $CHCl_3$); IR (neat) ν_{max} 3550 (br), 3419 (br), 2952, 2879, 1686, 1663, 1394, 1227, 1026, 957 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 469 (M–H_2O+H, 100); HRESIMS *m/z* 509.2481 $[M+Na]^+$ (calcd for $C_{28}H_{38}O_7Na$, 509.2471).

Single-Crystal X-ray Structure Determination of Withalongolide C (3)—Crystal analysis was performed with a colorless irregular chunk (dimensions 0.45 × 0.32 × 0.25 mm^3) obtained from CH_2Cl_2–CH_3CN (1:1) and measured using Mo Kα radiation (λ = 0.71073 Å) on a Bruker APEX diffractometer equipped with a sealed-tube x-ray source and a graphite monochromator. Crystal data for **3**: $C_{28}H_{38}O_7$ (formula weight 486.58), Orthorhombic, space group $P2_12_12_1$, T = 100(2) K, crystal cell parameters a = 10.679(4) Å, b = 12.245(5) Å, c = 18.674(7) Å, V = 2442 (2) $Å^3$, D_c = 1.324 Mg/m^3, Z = 4, F(000) = 1048, absorption coefficient μ = 0.094 mm^{-1}. A total of 22352 reflections were collected in the range 2.53 < θ < 29.12°, with 6145 independent reflections [$R_{(int)}$ = 0.055] and 5925 with $I > 2\sigma(I)$, completeness to ν_{max} was 95.6%. Multi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters were 6145/0/468; goodness-of-fit on F^2 = 1.080; final R indices [$I > 2\sigma(I)$], R_1 = 0.045, ωR_2 = 0.109; R indices (all data), R_1 = 0.046, ωR_2 = 0.113; largest difference peak and hole, 0.63 and -0.21 e/$Å^{-3}$.

Withalongolide D (4)—$[\alpha]^{25}_D$ +2.7 (*c* 0.08, $CHCl_3$); IR (neat) ν_{max} 3411 (br), 2944 (br), 1693, 1393, 1211, 1023 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 2 and 3; ESIMS (positive-ion mode) *m/z* 1059 (2 M+Na, 6), 519 (M+H, 25), 501 (M–H_2O+H, 100), 483 (M–2 H_2O +H, 4); HRESIMS *m/z* 541.2798 $[M+Na]^+$ (calcd for $C_{28}H_{38}O_7Na$, 541.2777).

Withalongolide E (5)—$[\alpha]^{25}_D$ +2.2 (*c* 0.12, $CHCl_3$); IR (neat) ν_{max} 3550 (br), 2940 (br), 1690, 1390, 1200, 1020 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 2 and 3; ESIMS (positive-ion mode) *m/z* 1059 (2 M+Na, 20), 541 (M+Na, 18), 501 (M–H_2O+H, 100); HRESIMS *m/z* 541.2777 $[M+Na]^+$ (calcd for $C_{28}H_{38}O_7Na$, 541.2777).

Withalongolide F (6)—colorless cuboid crystals, mp 190-191 °C; $[\alpha]^{25}_D$ +8.9 (*c* 0.16, $CHCl_3$); IR (neat) ν_{max} 2887, 1683, 1659, 1393, 1002, 851 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 2 and 3; ESIMS (positive-ion mode) *m/z* 871 (2 M+Na, 25), 425 (M+H, 100); HRESIMS *m/z* 447.2503 $[M+Na]^+$ (calcd for $C_{27}H_{38}O_4Na$, 447.2511).

Single-Crystal X-ray Structure Determination of Withalongolide F (6)—Crystal analysis was performed with a colorless rectangular parallelepiped (dimensions 0.39 × 0.37 × 0.20 mm^3) obtained from CH_2Cl_2–CH_3CN (1:1) and measured using Mo Kα radiation (λ = 0.71073 Å) on a Bruker APEX diffractometer equipped with a sealed-tube x-ray source and graphite monochromator. Crystal data for **6**: $C_{27}H_{36}O_4$ (formula weight 424.56), monoclinic, space group $P2_1$, T = 100(2) K, crystal cell parameters a = 10.873(5) Å, b = 9.233(4) Å, c = 12.271(6) Å, β = 113.273°(7), V = 1132 (9) $Å^3$, D_c = 1.246 Mg/m^3, Z = 2, F(000) = 460, absorption coefficient μ = 0.082 mm^{-1}. A total of 10347 reflections were collected in the range 2.85 < θ < 29.06°, with 5283 independent reflections [$R_{(int)}$ = 0.041] and 5133 with $I > 2\sigma(I)$, completeness to θ_{max} was 93.8%. Multi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters were 5283/1/424; goodness-of-fit on F^2 = 1.055; final R indices [$I > 2\sigma(I)$], R_1 = 0.045, ωR_2 = 0.107; R indices (all data), R_1 = 0.046, ωR_2 = 0.108; largest difference peak and hole, 0.43 and -0.21 e/Å^{-3}.

Withalongolide G (7)—$[\alpha]^{25}_D$ -2.3 (*c* 0.11, MeOH); IR (neat) ν_{max} 3385 (br), 2950 (br), 1686, 1399, 1234, 992 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 3; ESIMS (positive-ion mode) *m/z* 1031 [2 (M–SO_3)+Na, 20], 585 (M+H, 50), 505 (M–SO_3+H, 100); (negative-ion mode) *m/z* 583 (M–H, 100); HRESIMS *m/z* 607.2169 $[M+Na]^+$ (calcd for $C_{28}H_{40}O_{11}SNa$, 607.2189).

Withalongolide H (8)—$[\alpha]^{25}_D$ -0.9 (*c* 0.12, MeOH); IR (neat) ν_{max} 3369 (br), 2929 (br), 1686, 1398, 1018, 916 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 4 and 5; ESIMS (positive-ion mode) *m/z* 796 [M+H_2O, 40], 471 (M–rha–glc+H, 100); LC-MS/MS fragments of *m/z* 796 peak: *m/z* 471 (M–rha–glc+H, 100), 281 (10); LC–MS/MS fragments of *m/z* 471 peak: *m/z* 281 (100); HRESIMS *m/z* 801.3683 $[M+Na]^+$ (calcd for $C_{40}H_{58}O_{15}Na$, 801.3673).

Withalongolide I (9)—$[\alpha]^{25}_D$ -7.1 (*c* 0.08, MeOH); IR (neat) ν_{max} 3381 (br), 2921 (br), 1686, 1398, 1007 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 4 and 5; ESIMS (positive-ion mode) *m/z* 652 (M+H_2O, 6), 635 (M+H, 5), 473 (M–glc+H, 100); HRESIMS *m/z* 657.3267 $[M+Na]^+$ (calcd for $C_{34}H_{50}O_{11}Na$, 657.3251).

Withalongolide J (10)—$[\alpha]^{25}_D$ -1.0 (*c* 0.13, MeOH); IR (neat) ν_{max} 3370 (br), 2931 (br), 1687, 1396, 1017 (br) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 4 and 5; ESIMS (positive-ion mode) *m/z* 1273 (2 M+H, 100), 619 (M–H_2O+H, 20), 601 (M–2 H_2O+H, 15), 583 (M–3 H_2O+H, 10), 475 (M–glc+H, 6); HRESIMS *m/z* 659.3411 $[M+Na]^+$ (calcd for $C_{34}H_{52}O_{11}Na$, 657.3407).

Withalongolide K (11)—$[\alpha]^{25}_D$ +3.1 (*c* 0.17, MeOH); IR (neat) ν_{max} 3381 (br), 2921 (br), 1686, 1398, 1007 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 4 and 5; ESIMS (positive-ion mode) *m/z* 460 (M–glc+H, 40) 603 (M+H, 35), 1205 (2 M+H, 100); HRESIMS *m/z* 625.33007 $[M+Na]^+$ (calcd for $C_{33}H_{46}O_{10}Na$, 625.2989).

Withalongolide L (12)—$[\alpha]^{25}_D$ -3.1 (*c* 0.09, MeOH); IR (neat) ν_{max} 3350 (br), 2931 (br), 1687, 1396, 1018 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 4 and 5; ESIMS (positive-ion mode) *m/z* 1241 (2 M+H, 15), 1079 (2 M–glc+H, 30), 638 (M+H_2O, 80). 459 (M–glc+H, 100); LC-MS/MS fragments of the *m/z* 638 peak: *m/z* 621 (M+H, 80), 459 (M–glc+H, 100); HRESIMS *m/z* 643.3101 $[M+Na]^+$ (calcd for $C_{33}H_{48}O_{11}Na$, 643.3094).

Withalongolide M (13)—$[\alpha]^{25}_D$ -4.4 (*c* 0.08, MeOH); IR (neat) ν_{max} 3380 (br), 2932 (br), 1687, 1650, 1388, 1070 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 4 and 5; ESIMS (positive-ion mode) *m/z* 1209 (2 M+H, 20), 1048 (2 M–glc+H, 30), 622 (M+H_2O, 30), 605 (M+H, 10), 443 (M–glc+H, 100); HRESIMS *m/z* 627.3156 $[M+Na]^+$ (calcd for $C_{33}H_{48}O_{10}Na$, 627.3145).

Withalongolide N (14)—$[\alpha]^{25}_D$ -2.0 (*c* 0.14, MeOH); IR (neat) ν_{max} 3368 (br), 2931 (br), 1688, 1650, 1384, 1072, 1017 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 4 and 5; ESIMS (positive-ion mode) *m/z* 1209 (2 M+H, 35), 605 (M+H, 100); LC-MS/MS fragments of *m/z* 1209 peak: *m/z* 605 (M+H, 100%), 443 (M–glc+H, 100); LC-MS/MS fragments of *m/z* 605 peak: *m/z* 443 (M–glc+H, 100); HRESIMS *m/z* 627.3146 $[M+Na]^+$ (calcd for $C_{33}H_{48}O_{10}Na$, 627.3145).

Cytotoxicity Bioassay

The cytotoxicity assays were performed as previously described.[11] In general, five concentrations ranging from 0.1 to 100 μg/mL were tested for the extracts, and ten concentrations ranging from 50 *n*M to 20 μM were tested for pure compounds. Statistical analysis was carried out by one-way ANOVA on ranks test using GraphPad Prism 5 (GraphPad Software, San Diego, CA). IC_{50} values were obtained from cell viability plots fitted with a sigmoidal dose-response function with variable slope using GraphPad Prism 5 software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported, in part, by grant IND 0061464 (awarded to B.N.T. and K.K.) from the Kansas Bioscience Authority (KBA) and Center for Heartland Plant Innovations (HPI). The authors also acknowledge partial financial assistance from grant NFP0066367 from the Institute for Advancing Medical Innovation (IAMI) (awarded to M.S.C. and to B.N.T.). Partial support of the in vitro experiments was provided by the University of Kansas Center for Cancer Experimental Therapeutics NIH-COBRE P20 RR015563 (PI: B.N.T., project award PI: M.S.C.). The authors are grateful to NSF grant CHE-0923449 that was used to purchase the new Bruker APEX2 X-ray diffractometer. The authors thank Q. Long, H. Loring and M. Ferreira, botanists at the University of Kansas or at the Kansas Biological Survey at the University of Kansas for assistance in plant collections and identifications.

References

1. Lavie D, Glotter E, Shvo Y. J Org Chem. 1965; 30:1774–1778.
2. Misico RI, Nicotra VE, Oberti JC, Barboza G, Gil RR, Burton G. Prog Chem Org Nat Prod. 2011; 94:127–229.
3. Chen LX, Hao H, Qiu F. Nat Prod Rep. 2011; 28:705–740. [PubMed: 21344104]
4. Eich, E. Solanaceae and Convolvulaceae: Secondary Metabolites. Spring-Verlag; Berlin: 2008. p. 466-483.
5. Veleiro, AS.; Oberti, JC.; Burton, G. Studies in Natural Products Chemistry; Atta-ur- Rahman. Elsevier Science, BV., editor. Vol. 32. Amsterdam: 2005. p. 1019-1051.
6. Budhiraja RD, Krishan P, Sudhir S. J Sci Ind Res. 2000; 59:904–911.
7. Anjaneyulu, ASR.; Rao, DS.; Lequesne, PW. Studies in Natural Products Chemistry; Atta-ur-Rahman. Elsevier Science, BV., editor. Vol. 20. Amsterdam: 1998. p. 135-261.
8. Ray AB, Gupta M. Prog Chem Org Nat Prod. 1994; 63:1–106.
9. Glotter E. Nat Prod Rep. 1991:415–440. [PubMed: 1787922]
10. Yoshida M, Hoshi A, Kuretani K, Ishiguro M, Ikekawa N. J Pharm Dyn. 1979; 2:92–97.
11. Samadi AK, Tong XQ, Mukerji R, Zhang HP, Timmermann BN, Cohen MS. J Nat Prod. 2010; 73:1476–1481. [PubMed: 20726569]
12. Tong XQ, Zhang HP, Timmermann BN. Phytochemistry Lett. 2011 in press. 10.1016/j.phytol. 2011.04.016
13. Fuska J, Prousek J, Rosazza J, Budesinsky M. Steroids. 1982; 40:157–170. [PubMed: 7157453]
14. Pramanick S, Roy A, Ghosh S, Majumder HK, Mukhopadhyay S. Planta Med. 2008; 74:1745–1748. [PubMed: 18988152]
15. Pelletier SW, Mody NV, Nowacki J, Bhattacharyya J. J Nat Prod. 1979; 42:512–521.
16. Lavie D, Kashman Y, Glotter E, Danieli N. J Chem Soc (C). 1966:1757–1764.
17. Xu YM, Marron MT, Seddon E, McLaughlin SP, Ray DT, Whitesell L, Gunatilaka AAL. Bioorg Med Chem. 2009; 17:2210–2214. [PubMed: 19056281]

18. Ghosal S, Kaur R, Srivastava RS. Indian J Nat Prod. 1988; 4:12–13.
19. Ahmad S, Yasmmin R, Malik A. Chem Pharm Bull. 1999; 47:477–480.
20. Shingu K, Yahara S, Okabe H, Nohara T. Chem Pharm Bull. 1992; 40:2448–2451.
21. Veleiro AS, Trocca CE, Burton G, Oberti JC. Phytochemistry. 1992; 31:2550–2551.
22. Pelletier SW, Gebeyehu G, Nowacki J, Mody NV. Heterocycles. 1981; 15:317–320.
23. Zhao J, Nakamura N, Hattori M, Kuboyama T, Tohda C, Komatsu K. Chem Pharm Bull. 2002; 50:760–765. [PubMed: 12045329]
24. Li L, Henry GE, Seeram NP. J Agric Food Chem. 2009; 57:7282–7287. [PubMed: 19627089]
25. Monteagudo ES, Burton G, Gros EG, Gonzalez CM, Oberti JC. Phytochemistry. 1989; 28:2514–2515.
26. Misico RI, Oberti JC, Veleiro AS, Burton G. J Nat Prod. 1996; 59:66–68.
27. Cirigliano AM, Veleiro AS, Misico RI, Tettamanzi MG, Oberti JC, Burton G. J Nat Prod. 2007; 70:1644–1646. [PubMed: 17883258]
28. Maldonado E, Alvarado VE, Torres FR, Martínez M, Pérez-Castorena AL. Planta Med. 2005; 71:548–553. [PubMed: 15971127]
29. Riaz N, Malik A, Azia-ur-Rehman, Nawaz SA, Muhammad P, Choudhary MI. Chem Biodivers. 2004; 1:1289–1295. [PubMed: 17191906]
30. Ali M, Shuaib M, Ansari SH. Phytochemistry. 1997; 44:1163–1168.
31. Siddiqui BS, Arfeen S, Afshan F, Begum S. Heterocycles. 2005; 65:857–863.
32. Misico R, Veleiro AS, Burton G, Oberti JC. Phytochemistry. 1997; 45:1045–1048.
33. Shingu K, Furusawa Y, Nohara T. Chem Pharm Bull. 1989; 37:2132–2135.
34. Zhu XH, Ando J, Takagi M, Ikeda T, Nohara T. Chem Pharm Bull. 2001; 49:161–164. [PubMed: 11217102]
35. Misra L, Lal P, Sangwan RS, Sangwan NS, Uniyal GC, Tuli R. Phytochemistry. 2005; 66:2702–2707. [PubMed: 16293277]
36. Llanos GG, Araujo LM, Jiménez IA, Moujir LM, Vázquez JT, Bazzocchi IL. Steroids. 2010; 75:974–8. [PubMed: 20542049]
37. Machin RP, Veleiro AS, Nicotra VE, Oberti JC, Padròn JM. J Nat Prod. 2010; 73:966–968. [PubMed: 20438092]

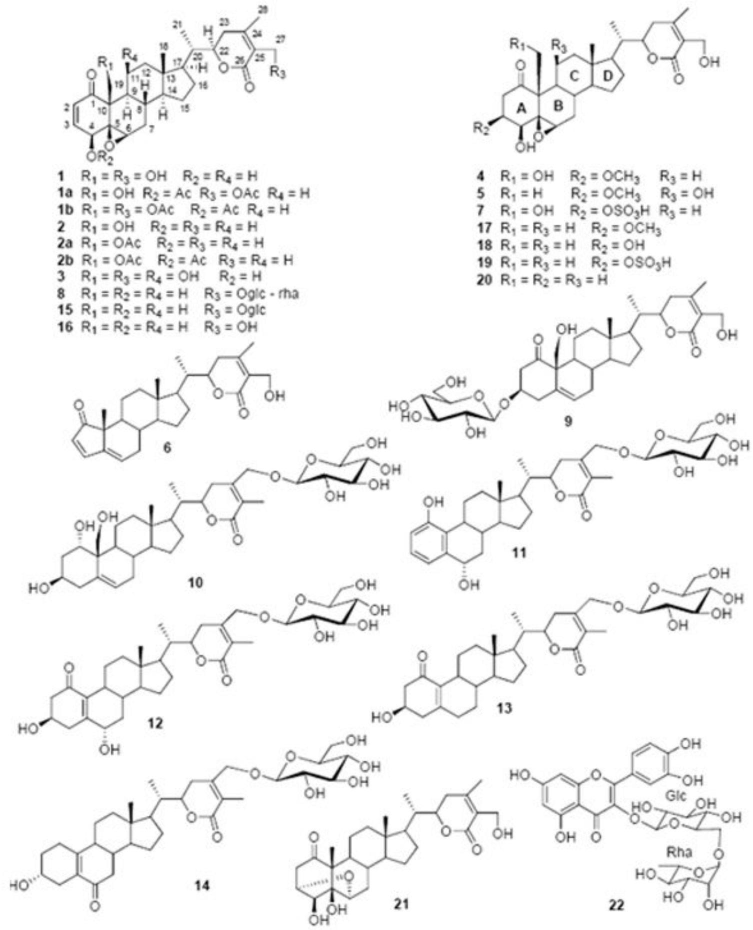

Figure 1.

Withanolides **1-21** and rutin (**22**) isolated from *Physalis longifolia*

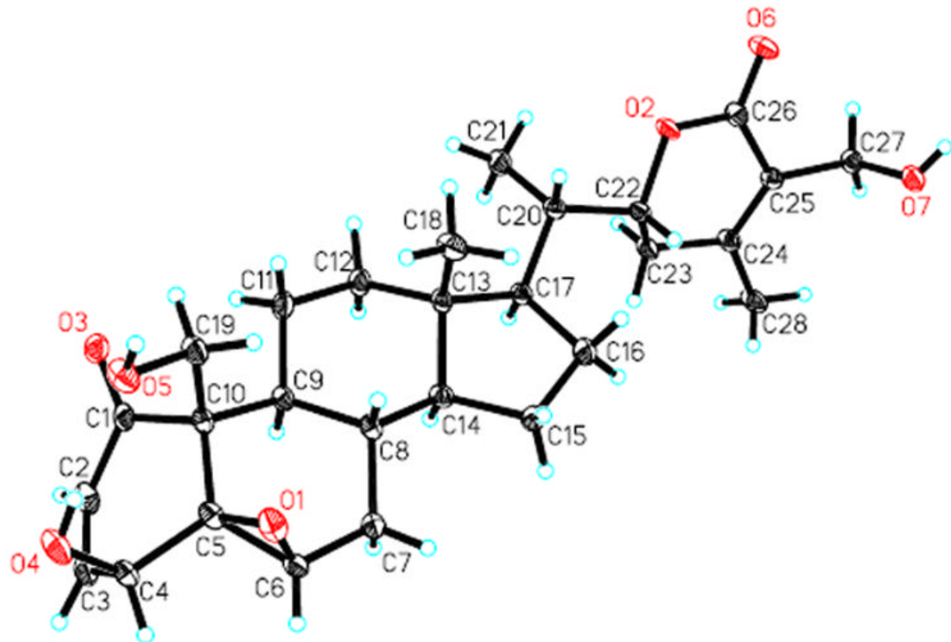

Figure 2.
X-ray ORTEP drawing of withalongolide A (**1**)

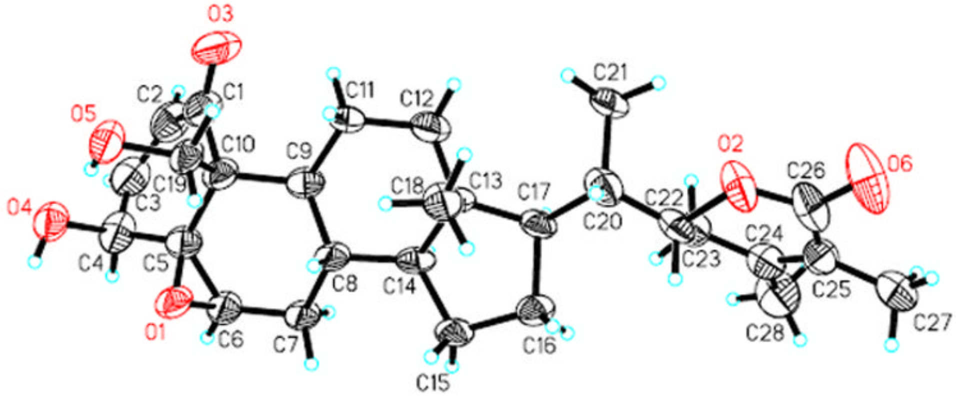

Figure 3.
X-ray ORTEP drawing of withalongolide B (**2**)

NIH-PA Author Manuscript

NIH-PA Author Manuscript

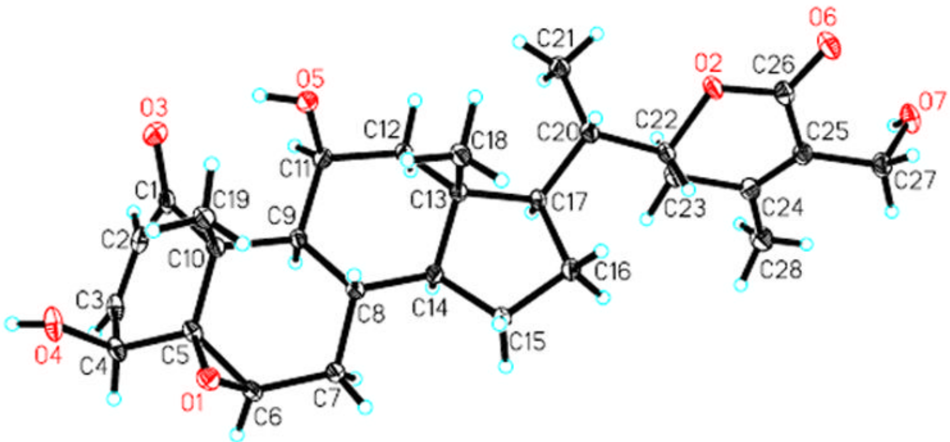

Figure 4.
X-ray ORTEP drawing of withalongolide C (**3**)

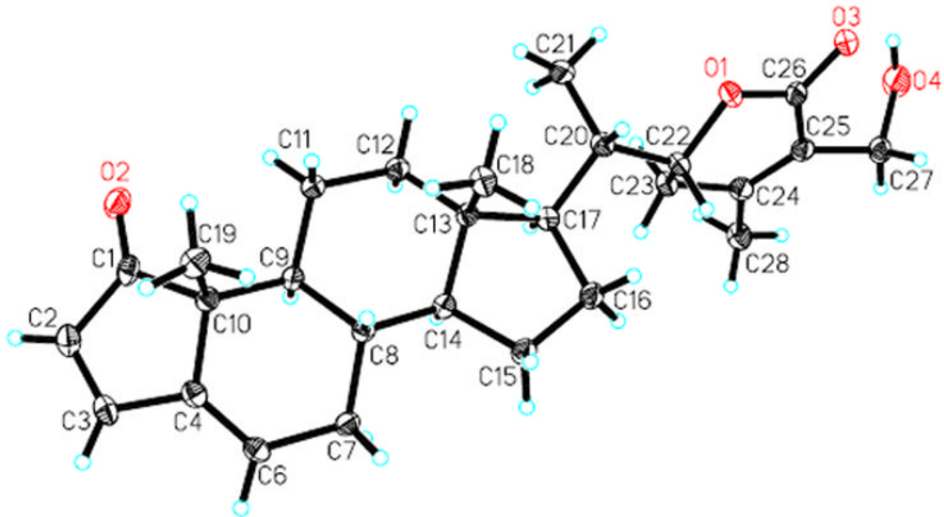

Figure 5.
X-ray ORTEP drawing of withalongolide F (**6**)

Table 1

^{1}H NMR Data for Withanolides **1, 1a, 1b, 2, 2a, 2b** and **3** (400 or 500 MHz)

Pos.	**1**[a]	**1**[b]	**1a**[c]	**1b**[c]	**2**[c]	**2a**[c]	**2b**[c]	**3**[c]
2	6.16 d (10.0)	6.46 d (10.0)	6.23 d (10.0)	6.21 d (10.0)	6.23 d (10.4)	6.23 d (9.9)	6.23 d (9.9)	6.22 d (10.0)
3	6.95 dd (10.0, 6.1)	7.26 dd (10.0, 6.1)	7.00 dd (10.0, 6.1)	7.00 dd (10.0, 6.1)	7.01 dd (10.4, 6.4)	7.00 dd (9.9, 6.0)	7.01 dd (9.9, 6.0)	6.96 dd (10.0, 6.5)
4	3.52 d (6.1)	4.01 d (6.1)	4.79 d (6.1)	4.73 d (6.1)	3.63 d (6.4)	4.79 d (6.0)	4.74 d (6.0)	3.78 d (6.5)
6	3.18 brs	3.42 brs	3.12 brs	3.20 brs	3.25 brs	3.16 brs	3.20 brs	3.23 brs
7	2.14 m, 1.25 m	2.15 ddd (2.2, 4.0, 14.5)	2.15 m	2.01 m, 1.96 m	2.19 ddd (2.2, 4.2, 15.0)	2.15 m	2.17 m	2.26 ddd (2.4, 4.7, 15.1)
		1.37 dd (11.1, 14.5)	1.25 m		1.30 m	1.25 ddd (1.4, 11.3, 15.0)	1.28 m	1.29 m
8	1.44 m	1.64 dq (4.0, 11.1)	1.77 dq (4.2, 11.1)	1.85 m	1.51 dq (4.2, 11.1)	1.76 dq (4.2, 11.3)	1.85 m	1.87 dq (4.5, 10.8)
9	0.85 m	1.15 m	1.00 m	0.91 m	0.94 m	1.00 m	0.92 m	1.25 dd (3.3, 10.8)
11	1.90 m, 1.32 m	2.31 m, 1.55 m	1.96 m, 1.62 m	1.94 m, 1.85 m	1.94 m, 1.39 m	1.96 m, 1.62 m	1.94 m, 1.84 m	4.15 (brs)
12	1.86 m, 0.95 m	1.82 m, 0.99 m	1.97 m, 1.08 m	1.96 m, 1.05 m	1.93 m, 1.01 m	1.97 m, 1.08 m	1.98 m, 1.05 m	2.17 dd (13.8, 2.8), 1.38 m
14	0.81 m	0.83 m	0.87 m	0.83 m	0.85 m	0.86 m	0.85 m	0.92 m
15	1.57 m, 1.05 m	1.49 m, 0.92 m	1.62 m, 1.14 m	1.58 m, 1.13 m	1.61 m, 1.12 m	1.61 m, 1.12 m	1.61 m, 1.12 m	1.64 m, 1.22 m
16	1.59 m, 1.29 m	1.49 m, 1.12 m	1.67 m, 1.36 m	1.65 m, 1.36 m	1.65 m, 1.35 m	1.66 m, 1.36 m	1.65 m, 1.36 m	1.63 m, 1.37 m
17	0.99 m	0.99 m	1.07 m	1.05 m	1.04 m	1.07 m	1.06 m	1.04 m
18	0.60 s	0.53 s	0.73 s	0.73 s	0.66 s	0.72 s	0.73 s	0.90 s
19	4.17 d, 3.65 d (9.6)	4.79 d, 4.21 d (9.4)	5.07 d, 4.32 d (11.5)	4.36 d, 4.07 d (11.5)	4.32 d, 3.77 d (9.7)	5.07 d, 4.32 d (11.5)	4.37 d, 4.09 d (11.6)	1.63 s
20	1.91 m	1.88 m	2.00 m	1.98 m	1.95 m	1.96 m	1.96 m	1.99 m
21	0.90 d (6.6)	0.95 d (6.7)	0.98 d (6.7)	0.97 d (6.6)	0.95 d (6.6)	0.97 d (6.6)	0.97 (6.6)	1.00 d (6.6)
22	4.33 dt (13.3, 3.4)	4.37 dt (13.3, 3.5)	4.38 dt (13.3, 3.4)	4.36 dt (13.3, 3.4)	4.31 dt (13.3, 3.4)	4.31 dt (13.3, 3.4)	4.32 dt (13.3, 3.4)	4.39 dt (13.2, 3.5)
23	2.42 dd (17.0, 13.3)	2.49 dd (18.0, 13.3)	2.50 dd (17.9, 13.3)	2.48 dd (18.2, 13.3)	2.40 dd (18.0, 13.3)	2.41 dd (18.0, 13.3)	2.41 dd (18.0, 13.3)	2.47 dd (13.2, 17.0)
	1.91 m	2.17 m	1.99 m	2.16 m	1.87 m	1.88 m	1.88 m	1.94 dd (3.5, 17.0)
27	4.28 d, 4.23 d (12.5)	4.86 d, 4.76 d (11.8)	4.88 d, 4.84 d (11.8)	4.88 d, 4.85 d (11.9)	1.85 s	1.85 s	1.85 s	4.34 br
28	1.97 s	2.11 s	2.05 s	2.04 s	1.90 s	1.91 s	1.90 s	2.01 s
4-OAc			2.09 s	2.07 s		2.09 s	2.08 s	
19-OAc			2.06 s			2.06 s		

Pos.	1[a]	1[b]	1a[c]	1b[c]	2[c]	2a[c]	2b[c]	3[c]
27-OAc			2.03 s	2.02 s				

[a] In $CDCl_3$ with trace amount of CD_3OD.

[b] In C_5D_5N.

[c] In $CDCl_3$.

NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 2

^{13}C NMR Data for Withanolides **1-7** and **16-19** (125 MHz)

Pos.	1[c]	1[b]	1a[a]	1b[a]	2[a]	2a[a]	2b[a]	3[a]	4[a]	5[a]	6[a]	7[a]	16[a]	17[a]	18[b]	19[b]	20[b]
1	200.7	201.4	198.3	198.9	200.2	198.3	198.9	204.0	208.1	213.3	211.6	208.0	202.5	210.1	210.4	209.7	210.5
2	132.9	133.2	133.7	134.4	133.1	133.8	134.5	131.6	40.5	39.5	129.4	42.0	132.5	39.8	44.4	42.0	32.2
3	145.7	147.5	141.0	140.2	145.4	140.9	140.1	143.2	77.7	76.2	155.2	73.6	142.1	77.7	67.1	73.1	27.1
4	68.0	69.0	71.2	71.7	68.5	71.2	71.8	69.7	72.8	74.4	---	75.4	70.1	75.1	78.7	76.9	72.4
5	61.5	61.7	59.4	61.1	61.7	59.4	61.1	64.5	62.5	66.0	147.4	63.0	64.1	65.2	65.5	65.1	65.9
6	61.8	62.0	59.2	59.9	62.1	59.2	59.9	63.0	57.6	59.5	123.8	57.2	62.7	60.5	57.4	57.2	58.5
7	31.1	31.9	31.0	30.4	31.2	30.9	30.4	31.7	31.1	32.2	31.9	31.4	31.3	31.3	32.3	31.9	29.4
8	30.5	30.3	30.9	31.4	30.6	31.0	31.4	27.0	30.1	27.2	32.8	30.7	29.9	29.5	30.5	30.4	31.0
9	43.9	44.8	44.8	44.6	44.0	44.9	44.7	48.1	42.1	47.1	42.8	42.3	44.3	42.9	43.7	43.1	42.7
10	54.3	55.6	51.2	52.2	54.3	51.2	52.3	49.0	56.1	51.3	47.6	55.7	47.9	50.6	49.7	49.9	50.2
11	22.1	23.0	22.3	21.9	22.3	22.3	21.9	69.5	21.6	69.1	22.0	22.1	22.3	21.8	21.8	21.7	21.3
12	39.7	40.3	39.6	39.7	39.7	39.6	39.7	47.8	39.3	47.4	39.5	39.8	39.5	39.3	39.7	39.5	38.9
13	42.5	42.9	42.9	43.1	42.6	42.8	43.0	42.1	42.8	42.0	43.6	43.1	42.8	42.9	43.2	43.2	42.5
14	56.7	57.1	56.7	57.0	56.9	56.7	57.1	58.2	56.7	58.0	56.5	56.7	56.2	56.2	56.6	56.4	56.1
15	24.2	24.7	24.5	24.3	24.3	24.5	24.4	24.4	24.3	24.5	24.6	24.7	24.5	24.5	24.9	24.9	24.0
16	27.3	27.6	27.5	27.5	27.4	27.5	27.5	27.2	27.5	27.2	27.4	27.7	27.5	27.5	27.7	27.7	27.5
17	51.9	52.4	52.0	52.0	52.1	52.1	52.1	52.7	52.0	52.5	52.1	52.3	52.1	52.1	52.4	52.4	51.6
18	11.7	12.1	11.8	12.0	11.8	11.8	11.8	14.2	11.7	13.6	12.1	12.0	11.8	11.7	11.9	11.9	11.5
19	61.0	61.7	64.4	65.6	62.1	64.4	65.6	21.3	60.4	19.0	19.9	59.9	17.6	15.9	16.1	15.9	15.2
20	38.8	39.5	39.0	39.0	39.0	39.0	39.0	39.0	38.9	39.0	39.1	39.5	39.0	38.9	39.5	39.6	38.3
21	13.3	13.9	13.5	13.5	13.6	13.6	13.6	13.6	13.6	13.6	13.6	14.0	13.5	13.5	14.0	14.0	13.5
22	78.8	78.7	78.4	78.4	78.4	78.4	78.4	78.9	78.8	78.9	79.0	78.9	78.9	78.9	78.8	78.9	78.4
23	29.9	30.3	30.3	30.3	29.8	29.8	29.8	30.1	30.0	30.1	30.0	30.3	30.0	30.0	30.4	30.3	29.1
24	154.3	154.4	157.2	157.1	149.2	149.2	149.1	153.0	153.0	152.9	153.1	154.6	153.1	153.1	154.3	154.6	152.2
25	125.6	127.9	122.1	122.1	122.2	122.2	122.2	125.9	125.9	125.9	125.9	127.7	125.8	125.5	127.9	127.7	125.4
26	167.4	166.8	165.5	165.5	167.3	167.2	167.2	167.2	167.2	167.2	167.3	167.0	167.3	167.2	166.8	167.1	167.2
27	56.7	56.6	58.2	58.2	12.7	12.7	12.7	57.7	57.6	57.7	57.7	56.6	57.7	57.6	56.6	56.6	57.6
28	20.2	20.6	20.8	20.8	20.7	20.7	20.7	20.2	20.2	20.2	20.2	20.5	20.3	20.3	20.6	20.5	20.2

Pos.	1[c]	1[b]	1a[a]	1b[a]	2[a]	2a[a]	2b[a]	3[a]	4[a]	5[a]	6[a]	7[a]	16[a]	17[a]	18[b]	19[b]	20[b]
$OCOCH_3$-4			170.4	170.0		170.4	170.1										
			21.1	21.1		21.0	21.1										
$OCOCH_3$-19			170.6			170.7											
			21.0			21.4											
$OCOCH_3$-27			171.1	171.1													
			21.4	21.0													
OCH_3-3									57.6	57.4				57.0			

[a] In $CDCl_3$.

[b] In C_5D_5N.

[c] In $CDCl_3$ with trace amount of CD_3OD.

Table 3

^{1}H NMR Data for Withanolides **4–7** and **16–19** (400 or 500 MHz)

Pos.	**4**[a]	**5**[a]	**6**[a]	**7**[b]	**16**[a]	**17**[a]	**18**[b,c]	**19**[b]
2	Hβ 2.86 dd (8.3, 16.4)	Hβ 2.86 dd (6.5, 17.5)	5.93 d (6.4)	Hβ 3.65 dd (8.1, 18.0)	6.18 d (10.0)	Hβ 2.99 dd (6.3, 15.0)	Hβ 3.62 dd (10.0, 17.1)	Hβ 3.62 dd (9.7, 17.6)
	Hα 2.56 dd (2.6, 16.4)	Hα 2.56 dd (2.2, 17.5)		Hα 3.22 dd (6.2, 18.0)		Hα 2.56 dd (2.2, 15.0)	Hα 3.02 dd (5.5, 17.1)	Hα 3.30 dd (2.0, 17.6)
3	3.70 ddd (8.3, 3.4, 2.6)	3.75 ddd (6.5, 4.0, 2.2)	7.57 d (6.4)	5.61 brt (8.1)	6.91 dd (10.0, 5.9)	3.68 ddd (6.3, 3.2, 2.2)	4.67 br	5.62 ddd (9.7, 6.5, 2.0)
4	3.42 d (3.4)	3.56 d (4.0)	---	4.54 br	3.74 d (5.9)	3.46 d (3.2)	4.05 br	4.51 br
6	3.22 brs	3.15 brs	5.76 t (3.5)	3.37 brs	3.21 s	3.19 s	3.30 br	3.62 br
7	2.25 m	2.31 dd (5.2, 14.8)	2.37 ddd (3.5, 8.8, 15.0)	2.35 m	2.12 ddd (2.2, 3.7, 14.6)	2.14 ddd (2.2, 3.7, 14.6)	2.22 ddd (1.7, 4.3, 15.0)	2.07 m
	1.42 m	1.34 m	1.70 m	2.11 m	1.30 m	1.31 m	1.40 ddd (2.0, 10.2, 15.0)	1.23 m
8	1.41 m	1.70 dq (4.5, 10.8)	1.75 m	1.50 m	1.39 m	1.39 m	1.50 m	1.41 m
9	1.14 m	1.28 m	1.17 m	1.16 m	1.14 m	1.16 m	1.17 m	1.04 m
11	1.27 m, 1.18 m	3.88 m	2.24 m, 1.58 m	1.57 m, 1.34 m	1.27 m, 1.18 m	1.35 m	1.52 m	1.44 m
12	1.90 m, 1.02 m	2.15 dd (13.9, 2.4), 1.38 m	1.99 m, 1.18 m	1.76 m, 1.02 m	1.88 m, 1.08 m	1.88 m, 1.08 m	1.85 m, 1.05 m	1.80 m, 1.05 m
14	0.95 m	0.94 m	1.03 m	0.91 m	0.95 m	0.94 m	0.93 m	0.92 m
15	1.64 m, 1.13 m	1.66 m, 1.21 m	1.61 m, 1.19 m	1.52 m, 0.90 m	1.64 m, 1.13 m	1.61 m, 1.12 m	1.54 m, 0.96 m	1.54 m, 0.92 m
16	1.66 m, 1.35 m	1.64 m, 1.38 m	1.64 m, 1.35 m	1.57 m, 1.13 m	1.66 m, 1.35 m	1.64 m, 1.31 m	1.54 m, 1.15 m	1.57 m, 1.15 m
17	1.07 m	1.04 m	1.10 m	1.13 m	1.07 m	1.05 m	1.07 m	1.14 m
18	0.62 s	0.85 s	0.75 s	0.47 s	0.67 s	0.64 s	0.55 s	0.52 s
19	4.38 d, 3.61 d (10.2)	1.49 s	1.15 s	4.80 d, 4.01 d (9.4)	1.38 s	1.28 s	1.76 s	1.69 s
20	1.96 m	1.99 m	1.99 m	1.86 m	1.97 m	1.97 m	1.90 m	1.90 m
21	0.95 d (6.7)	1.00 d (6.7)	1.01 d (6.6)	0.95 d (6.6)	0.97 d (6.6)	0.96 d (6.6)	0.97 d (6.6)	0.97 d (6.6)
22	4.39 dt (13.2, 3.5)	4.39 dt (13.2, 3.5)	4.42 dt (13.3, 3.4)	4.38 dt (13.2, 3.3)	4.39 dt (13.2, 3.5)	4.39 dt (13.2, 3.5)	4.39 dt (13.2, 3.3)	4.40 dt (13.2, 3.3)
23	2.46 dd (13.2, 17.0)	2.47 dd (13.7, 17.8)	2.48 dd (13.6, 17.6)	2.35 dd (13.9, 18.0)	2.47 dd (13.6, 17.4)	2.47 dd (13.6, 17.4)	2.35 dd (13.4, 17.7)	2.36 dd (13.7, 17.8)
	1.93 dd (3.5, 17.0)	1.94 dd (3.2, 17.8)	1.98 dd (3.2, 17.6)	2.07 m	1.94 dd (3.2, 17.4)	1.94 dd (3.2, 17.4)	2.01 dd (3.3, 17.7)	2.07 m
27	4.36 d, 4.32 d (12.5)	4.37 d, 4.31 d (12.6)	4.37 d, 4.32 d (12.5)	4.85 d, 4.75 d (11.7)	4.36 d, 4.31 d (12.6)	4.36 d, 4.31 d (12.6)	4.80 d, 4.78 d (11.8)	4.85 d, 4.75 d (11.7)
28	2.02 s	2.01 s	2.01 s	2.09 s	2.01 s	2.01 s	2.10 s	2.09 s

Pos.	4[a]	5[a]	6[a]	7[b]	16[a]	17[a]	18[b,c]	19[b]
3-OMe	3.35 s	3.34 s				3.32 s		

[a] In $CDCl_3$.

[b] In C_5D_5N.

[c] OH-4 signal 7.75 s, OH-3 signal 6.91 brs, OH-27 signal 6.61 brs.

Table 4

^{1}H NMR Data for Withanolides **8-15** in C_5D_5N (500 MHz)

Pos.	8[a]	9[b]	10[c]	11[d]	12[e]	13[f]	14[g]	15[h]
1	---	---	4.61 m	---	---	---	Hα 2.74 m, Hβ 2.10 m	---
2	6.43 d (10.0)	Hβ 3.48 dd (11.0, 12.4)	Hα 2.73 d (18.8)	7.14 d (7.7)	3.01 dd (7.1, 15.5)	2.94 dd (8.1, 15.2)	Hα 1.96 m, Hβ 1.80 m	6.43 d (10.0)
		Hα 3.26 ddd (1.5, 5.3, 12.4)	Hβ 2.35 dd (10.2, 18.8)		2.91 dd (3.7, 15.5)	2.91 dd (4.1, 15.2)		
3	7.25 dd (10.0, 6.3)	4.29 m	4.84 m	7.34 t (7.7)	4.58 m	4.47 m	4.33 br	7.25 dd (10.0, 6.3)
4	4.05 dd (6.3, 3.8)	Hβ 3.07 dt (2.0, 12.2)	Hβ 2.95 t (12.5)	7.87 d (7.7)	3.63 td (3.2, 18.0)	2.68 dd (5.9, 16.8)	Hα 2.90 d (15.8)	4.05 dd (6.3, 3.7)
		Hα 2.99 ddd (1.5, 5.5, 12.2)	Hα 2.88 dd (12.5, 5.0)		2.92 m	2.58 td (2.7, 16.8)	Hβ 2.81 dd (2.2, 15.8)	
6	3.28 s	5.75 brd (5.3)	5.92 brd (4.5)	5.26 dt (11.0, 6.5)	4.69 m	Hα 2.26 m, Hβ 2.09 m	---	3.28 s
7	2.13 dt (13.0, 1.8)	1.92 m, 1.53 m	Hα 1.66 dd (11.0, 16.0)	1.63 q (11.0)	2.23 m	1.98 m, 1.53 m	2.47 dd (3.6, 16.0)	2.13 dt (13.0, 1.8)
	1.30 dd (11.5, 13.0)		Hβ 2.02 m	2.32 dd (6.5, 11.0)			2.00 m	1.30 dd (11.5, 13.0)
8	1.58 m	1.74 dt (5.3, 10.7)	2.20 m	1.73 q (11.0)	1.48 m	1.24 m	1.62 m	1.57 m
9	1.06 m	1.94 m	2.32 m	2.71 t (11.0)	2.20 m	2.04 m	1.86 m	1.06 m
11	2.02 m, 1.57 m	2.37 m, 1.96 m	2.15 m, 1,98 m	3.68 m, 1.46 m	2.96 m, 1.17 m	2.80 m, 2.34 m	1.84 m, 1.24 m	2.02 m, 1.57 m
12	1.83 m, 1.03 m	1.95 m, 1.26 m	1.95 m, 1.02 m	1.95 m, 1.30 m	1.83 m, 1.17 m	1.82 m, 1.13 m	1.85 m, 1.03 m	1.85 m, 1.02 m
14	0.86 m	0.97 m	0.86 m	1.10 m	0.95 m	0.90 m	0.88 m	0.86 m
15	1.50 m, 0.96 m	1.50 m, 1.02 m	1.49 m, 1.06 m	1.52 m, 1.12 m	1.45 m, 1.02 m	1.44 m, 1.02 m	1.33 m, 0.88 m	1.50 m, 0.95 m
16	1.49 m, 1.11 m	1.50 m, 1.14 m	1.91 m, 1.24 m	1.98 m, 1.27 m	1.95 m, 1.23 m	1.95 m, 1.23 m	1.91 m, 1.21 m	1.49 m, 1.11 m
17	0.99 m	1.05 m	1.04 m	1.12 m	1.07 m	1.07 m	1.06 m	0.99 m
18	0.57 s	0.67 s	0.80 s	0.70 m	0.59 s	0.58 s	0.50 s	0.57 s
19	1.87 s	4.30 m, 4.23 dd (3.9, 12.3)	4.32 d, 4.11d (11.0)	---	---	---	---	1.86 s
20	1.88 m	1.90 m	1.98 m	2.01 m	1.96 m	1.96 m	1.96 m	1.88 m
21	0.96 d (6.6)	0.97 d (6.6)	0.99 d (6.6)	1.01 d (6.6)	0.97 d (6.6)	0.96 d (6.6)	0.99 d (6.6)	0.95 d (6.6)
22	4.34 m	4.40 dt (13.2, 3.4)	4.25 m	4.55 dt (13.2, 3.4)	4.53 dt (13.2, 3.4)	4.53 dt (13.2, 3.4)	4.53 dt (13.2, 3.4)	4.34 dt (13.2, 3.4)
23	2.27 dd (13.3, 18.0)	2.34 dd (18.0, 14.0)	2.78 m, 2.33 m	2.83 dd (18.0, 2.2)	2.81 dd (18.0, 2.2)	2.81 dd (18.0, 2.2)	2.81 dd (18.0, 2.2)	2.28 dd (13.3, 18.0)
	1.98 dd (3.1, 18.0)	2.03 dd (3.1, 18.0)		2.34 dd (18.0, 14.0)	2.35 dd (18.0, 14.0)	2.35 dd (18.0, 14.0)	2.37 dd (18.0, 14.0)	1.98 dd (3.1, 18.0)

Pos.	8[a]	9[b]	10[c]	11[d]	12[e]	13[f]	14[g]	15[h]
27	5.02 d, 4.80 d (10.8)	4.88 d, 4.78 d (11.0)	2.04 s	2.05 s	2.05 s	2.05 s	2.06 s	5.07 d, 4.86 d (10.8)
28	2.07 s	2.14 s	4.87 d, 4.49 d (13.5)	4.89 d, 4.47 d (13.5)	4.89 d, 4.49 d (13.5)	4.89 d, 4.49 d (13.5)	4.90 d, 4.50 d (13.5)	2.10 s
1′	4.97 d (7.8)	5.08 d (7.8)	4.84 d (7.8)	4.84 d (7.8)	4.84 d (7.8)	4.84 d (7.8)	4.85 d (7.8)	5.06 d (7.8)
2′	4.01 td (7.8, 3.6)	4.05 t (7.8)	4.10 m	4.10 m	4.10 m	4.10 m	4.11 dd (7.8, 7.0)	4.08 t (7.8)
3′	4.23 m	4.30 m	4.25 m	4.25 m	4.25 m	4.25 m	4.25 m	4.31 m
4′	4.51 t (9.0)	4.31 m	4.24 m	4.24 m	4.24 m	4.24 m	4.24 m	4.30 m
5′	3.75 ddd (2.5, 5.0, 9.0)	3.97 ddd (2.5, 5.0, 9.0)	3.99 m	4.00 m	4.00 m	4.00 m	4.00 m	4.00 ddd (2.5, 5.6, 9.0)
6′	4.31 m, 4.16 m	4.54 ddd (2.5, 5.0, 11.6)	4.62 m	4.62 ddd (2.0, 5.6, 11.6)	4.62 ddd (2.0, 5.6, 11.6)	4.62 ddd (2.0, 5.6, 11.6)	4.62 ddd (2.0, 5.6, 11.6)	4.60 ddd (2.5, 5.6,11.5)
		4.42 td (5.0, 11.6)	4.42 m	4.42 td (5.6, 11.6)	4.42 td (5.6, 11.6)	4.42 td (5.6, 11.6)	4.42 td (5.6, 11.6)	4.44 td (5.6, 11.5)

Note:

[a]H-1″5.94 s, H-2″ 4.73 s, H-3″ 4.60 br, H-4″ 4.38 dd (4.1, 9.9), H-5″ 5.06 m, H-6″ 1.74 d (6.1), OH-4 7.87 d (3.8), OH-2′ 7.36 d (3.5), OH-2″ 6.84 s, OH-4″ 6.83 s, OH-3′ 6.83 s, OH-6″ 6.82 m, OH-3″ 6.55 d (5.5).

[b]OH-2′ 7.27 s, OH-19 7.27 d (3.9), OH-6′, 6.56 t (5.0), OH-27 6.49 brs.

[c]OH-2′ 7.51, OH-3′ 7.36 s, OH-4′, 7.24 s, OH-6′, 6.52 t (5.0), OH-3, 6.20 d (5.0), OH-1 5.97 d (5.0), OH-19 5.96 brs.

[d]OH-1 11.33 s, OH-2′, 7.45 d (4.5), OH-3′, 7.35 brs, OH-4′, 7.25 brs, OH-6, 6.81 d (6.5), OH-6′, 6.53 t (5.6).

[e]OH-2′ 7.54 d (4.3), OH-3′, 7.34 brs, OH-4′ 7.26 brs, OH-6 6.93 d (6.5), OH-3 6.84 (4.0), OH-6′ 6.53 t (5.6).

[f]OH-2′, 7.54 d (4.2), OH-3′, 7.34 brs, OH-4′ 7.26 brs, OH-3 6.81 d (4.1), OH-6′, 6.53 t (5.5).

[g]OH-2′ 7.49 brs, OH-3′, 7.38 brs, OH-4′ 7.28 brs, OH-6′ 6.53 t (5.6), OH-3 6.28 brs.

[h]OH-4 7.86 d (3.7), OH-3′, 7.22 brs, OH-4′ 7.28 brs, OH-2′ 7.21 brs, OH-6′ 6.47 t (5.6), OH-3 6.28 brs.

Table 5

^{13}C NMR Data for Withanolides **8-15** in C_5D_5N (125 MHz)

Pos.	8[a]	9	10	11	12	13	14	15
1	203.1	208.9	69.5	157.8	199.4	198.3	25.6	203.1
2	132.8	49.6	41.7	115.1	49.4	49.7	30.6	132.8
3	145.6	75.9	66.6	127.4	66.3	66.1	64.7	145.6
4	70.8	39.9	44.4	119.3	36.9	41.9	32.9	70.8
5	65.0	132.8	137.8	146.2	156.4	153.6	130.4	65.0
6	60.6	129.1	127.0	70.7	71.2	33.8	198.6	60.6
7	32.2	31.9	32.6	38.3	37.3	26.4	44.0	32.2
8	30.7	33.5	33.8	39.0	37.5	39.9	40.0	30.7
9	45.1	43.6	42.4	46.6	44.3	43.6	46.6	45.1
10	49.0	60.2	48.2	127.8	136.5	136.1	158.7	49.0
11	22.2	24.1	22.3	27.1	26.4	26.6	25.1	22.2
12	39.9	40.6	40.7	41.5	41.0	41.0	40.2	39.9
13	43.1	43.5	43.6	44.2	43.9	44.1	43.3	43.1
14	56.5	57.0	57.5	54.9	54.2	54.9	54.8	56.5
15	24.9	25.0	25.2	24.7	24.5	24.5	24.0	24.9
16	27.6	27.6	27.8	27.7	27.6	27.7	27.5	27.6
17	52.4	52.5	52.6	52.9	52.7	52.7	52.3	52.4
18	12.0	12.5	12.6	12.9	12.6	12.7	12.2	12.0
19	17.7	63.7	63.9	---	---	---	---	17.7
20	39.5	39.6	39.9	40.0	39.9	39.9	39.8	39.5
21	13.9	13.9	14.1	13.9	13.9	13.9	14.0	13.9
22	78.6	78.9	79.7	79.6	79.6	79.6	79.5	78.7
23	30.4	30.3	25.2	25.2	25.2	25.2	25.2	30.4
24	157.4	154.5	148.8	148.7	148.8	148.7	148.7	157.4
25	124.3	127.8	125.2	125.3	125.2	125.2	125.2	124.4
26	166.3	166.9	167.1	167.1	167.1	167.1	167.1	166.4
27	64.0	56.6	13.0	12.9	12.9	12.9	12.9	63.9
28	21.0	20.6	67.1	66.9	67.0	67.0	67.0	21.0

Pos.	8[a]	9	10	11	12	13	14	15
1′	105.3	103.5	103.5	103.4	103.5	103.5	103.5	105.4
2′	75.9	75.6	75.4	75.4	75.4	75.4	75.4	75.7
3′	77.1	79.1	79.0	79.0	79.0	79.0	79.1	79.0
4′	78.5	71.9	72.1	72.1	72.1	72.1	72.1	72.1
5′	77.8	79.1	79.3	79.3	79.3	79.3	79.3	79.1
6′	61.9	63.0	63.3	63.3	63.3	63.3	63.3	63.2

[a]1″ (103.1), 2″ (73.1), 3″(73.3), 4″ (74.5), 5″ (70.8), 6″ (19.0)

Table 6

Cytotoxicity IC_{50} of Isolated Withanolides (μM) against Five Cell Lines[a]

Compound	B16F10	SKMEL-28	JMAR	MDA-1986	MRC-5
1	>10	5.1	5.3	3.3	>10
1a	0.067	0.54	0.16	0.91	0.58
1b	0.098	0.81	0.14	2.2	0.41
2	0.20	3.9	0.17	1.3	0.40
2a	0.13	0.27	0.24	0.11	0.51
2b	0.19	0.64	0.12	0.49	0.16
3	0.49	3.0	0.77	2.6	3.6
4	3.2	9.3	4.7	>10	6.5
5	5.6	>10	>10	8.3	7.3
7	1.3	4.8	2.3	2.0	3.3
8	>10	>10	8.2	8.1	8.7
15	3.7	8.3	4.2	>10	5.2
16	0.29	4.0	2.0	0.80	0.20
19	0.18	5.1	0.48	0.27	1.4
cisplatin (positive control)	1.0	1.1	1.1	1.6	8.9

[a]For cell lines used, see text. Withanolides **6, 9-14, 17, 18, 20** and **21** were inactive for all cell lines used ($IC_{50} > 10$ μM).