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Physangulidines A, B, and C: Three New Antiproliferative Withanolides from *Physalis angulata* L.

Zhuang Jin,[†] Mark S. Mashuta,[†] Neal J. Stolowich,[†] Abraham J. Vaisberg,[‡] Nicole S. Stivers,[§] Paula J. Bates,[§] Walter H. Lewis,[∥] and Gerald B. Hammond*[,†]

Department of Chemistry, University of Louisville, Louisville, Kentucky 40292, United States, Departamento de Microbiología y Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú, James Graham Brown Cancer Center, University of Louisville, Louisville, Kentucky 40202, United States, and Department of Biology, Washington University, St. Louis, Missouri 63130, United States

gb.hammond@louisville.edu

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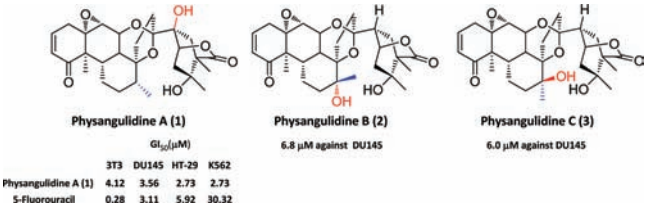
ABSTRACT



Bioassay-directed fractionation of the whole plant of *Physalis angulata* L. afforded three new antiproliferative withanolides with an unusual carbon framework: physangulidines A (1), B (2), and C (3). Structures of the three isomeric withanolides were determined by a combination of HRMS, NMR spectroscopic, and X-ray crystallographic methods. Each has shown significant antiproliferative activity against DU145 prostate cancer cells. Physangulidine A (1) was further tested against a wide range of additional cancer cell lines and found to exhibit significant antiproliferative activity.

The genus *Physalis* (Solanaceae) is represented by almost 90 species distributed throughout the tropical and subtropical regions of the world where it has been widely used in folk medicine by developing countries.[1] As a result of its medicinal value, there has been significant interest in evaluating the phytochemical and pharmaceutical properties of *Physalis angulata*. Previously, physanolide,[2] withangulatin,[3] physangulin,[4] and physalin,[2,5] among other constituents[6] isolated from *P. angulata,* were found to show significant biological activity.[7]

As part of our program to search for bioactive natural products from Amazonian rainforest plants,[8] we report now the discovery of three antiproliferative withanolides with an unusual carbon framework, namely, physangulidines A (**1**), B (**2**), and C (**3**), isolated from *P. angulata* L. using a bioassay-directed isolation technique. The ethanol extract of the dried plant was partitioned between water and dichloromethane, the active organic layer was subjected to silica gel column chromatography, and the most

[†] Department of Chemistry, University of Louisville.
[‡] Universidad Peruana Cayetano Heredia.
[§] James Graham Brown Cancer Center, University of Louisville.
[∥] Washington University.

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active fractions were further purified via HPLC to afford the three active components.

Physangulidine A (**1**), mp 213.0−215.0 °C (MeOH), was isolated as white crystalline needles, whose molecular formula was determined to be $C_{28}H_{36}O_8$ by HRMS (m/z 501.2493 $[M+H]^+$, 523.2311 $[M+Na]^+$). Simple analysis of ^1H, ^{13}C, and HSQC spectra revealed four methyls, seven methylenes, eight methines, and nine quarternary carbons and suggested two hydroxyl groups, consistent with this formula. Subsequent 2D analysis (gCOSY, ROESY, and HMBC; see Supporting Information (SI)) led to the structure **1** shown in Figure 1. The complete ^{13}C and ^1H NMR assignment is summarized in Table 1. Important structural features included two olefenic protons, H-2 (δ 6.08, dd, J = 9.8, 2.8 Hz, 1H) and H-3 (δ 6.86, ddd, J = 9.8, 6.3, 2.8 Hz, 1H), conjugated to carbonyl carbon C-1 (δ 201.83), consistent with an α,β-unsaturated ketone.[3] This moiety was also supported by IR (1714 cm^{-1}) and UV (220 nm)[3a] (IR and UV spectra are available in the SI). In addition, the presence of a C-5,6 epoxide, and ketal carbon C-17 (δ 109.71) were surmised from the NMR data. Another critical structural feature was a carbonyl carbon C-26 (δ 176.96) within an *isolated* bridged δ-lactone moiety, containing two methyls and two hydroxyl groups. The structure and stereochemistry of **1** were conclusively determined by X-ray crystallogryaphy (Figure 2) and ROESY 2D NMR analysis (see SI). The δ-lactone on C-22 and C-26[9] in physangulidine A puts it in the class of steroids known as withanolides.

Although, to date, about 650 withanolides have been isolated from different plant sources,[10] physangulidine A is the first withanolide having a disconnection between C-13 and C-17, which otherwise would have formed ring D of the ergostane skeleton.

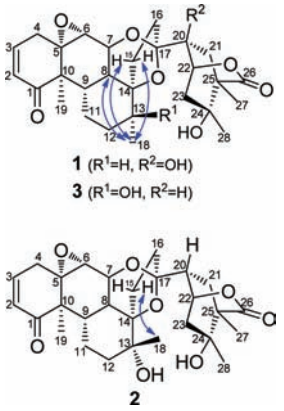

Figure 1. Structures and crucial ROESY interactions of physangulidines A (**1**), B (**2**), and C (**3**).

Physangulidine B (**2**) (mp above 260 °C) and physangulidine C (**3**) were also isolated as white needles, and both were found to have the molecular formula $C_{28}H_{36}O_8$ by HRMS (m/z 523.2311 $[M+Na]^+$ for **2**; m/z 523.2313 $[M+Na]^+$ for **3**), indicating both are isomers of physangulidine A (**1**). After careful analysis of the NMR data (Table 1), we found that, in the structures of **2** and **3**, C-13 is hydroxylated and that **2** and **3** differed from each other only in the stereochemistry at C-13 (Figure 1).

As with **1**, compounds **2** and **3** also contained the bridged δ-lactone moiety and the disconnection between C-13 and C-17. X-ray crystallographic analysis of physangulidine B (**2**) confirmed the aforementioned C-20 vs C-13 hydroxyl "migration" (Figure 3) and further demonstrated that, with the exception of the orientation of the C-13 methyl group, the overall conformations of **1** and **2** were very similar (Figures 2 and 3). Crystals of physangulidine **B** (**2**) adequate for X-ray diffraction studies were grown from methylene chloride/hexane. $C_{28}H_{36}O_8$: colorless needle, 0.42 × 0.02 × 0.02 mm^3, orthorhombic, space group $P2_12_12_1$, a = 7.6354(9) Å, b = 12.043(2) Å, c = 27.411(5) Å, $\alpha = \beta = \gamma = 90°$, V = 2520.6(7) $Å^3$, D_{calc} = 1.319 Mg/m^3, Z = 4.[11] For 4132 reflections $I > 2\sigma(I)$ [R(int) 0.049] the final anisotropic full matrix least-squares refinement on F^2 for 334 variables converged at R1 = 0.055 and wR2 = 0.085 with a GOF of 1.03. The absolute structure was determined by refinement of the Hooft parameter 0.0(4).

Crystals of physangulidine **A** (**1**), $C_{28}H_{36}O_8$: colorless prism, 0.42 × 0.22 × 0.09 mm^3, orthorhombic, space group $P2_12_12_1$, a = 10.0880(4) Å, b = 10.5957(3) Å, c = 24.5357(11) Å, $\alpha = \beta = \gamma = 90°$, V = 2622.60(18) $Å^3$, D_{calc} = 1.270 Mg/m^3, Z = 4. For 5195 reflections $I > 2\sigma(I)$ [R(int) 0.038] the final anisotropic full matrix least-squares refinement on F^2 for 425 variables converged at R1 = 0.052 and wR2 = 0.118 with a GOF of 1.03 and Hooft parameter of 0.11(6).

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(9) Carbon numbering followed steroid numbering system since we assumed that left part of physangulidine A was derived from steroid and D ring of the steroid is disconnected by C-13 and C-17. Similar numbering was applied to physalin, in which steroid is disconnected by C-13 and C-14; see refs 2b, 5. As for the lactone numbering, please see Withajardins numbering: Luis, J. G.; Echeverri, F.; Quiñones, W.; González, A. G.; Torres, F.; Cardona, G.; Archbold, R.; Perales, A. *Tetrahedron* **1994**, *50*, 1217.

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(11) Data were collected on an Agilent Technologies/Oxford Diffraction Gemini CCD diffractometer at 293 K using Cu Kα radiation (1.54184 Å).

Table 1. ^{13}CNMR and ^{1}HNMR data for compounds **1**, **2**, and **3**[a]

	1		**2**		**3**	
Position	δ_C	δ_H, (J in Hz), (number of proton)	δ_C	δ_H, (J in Hz), (number of proton)	δ_C	δ_H, (J in Hz), (number of proton)
1	201.83		201.97		201.81	
2	129.26	6.08, dd (9.8, 2.8), (1H)	129.29	6.10, dd (9.8, 2.8), (1H)	129.31	6.09, dd (9.8, 3.5), (1H)
3	143.75	6.86, ddd (9.8, 6.3, 2.8), (1H)	143.86	6.88, ddd (9.8, 6.3, 2.1), (1H)	143.86	6.89, ddd (9.8, 6.3, 2.1), (1H)
4	32.06	2.95, ax,dt (18.2, 2.8), (1H) 1.93, eq,dd (18.2, 6.3), (1H)	31.98	2.96, ax,dt (18.2, 2.8), (1H) 1.95, eq, m, (1H)	31.90	2.95, ax,dt (18.2, 2.8), (1H) 1.95, eq, m, (1H)
5	62.60		62.71		62.91	
6	63.01	3.14, d (2.1), (1H)	62.94	3.11, d (2.8), (1H)	62.96	3.12, d (2.1), (1H)
7	67.07	4.32, t (2.8), (1H)	67.06	4.39, dd (3.5, 2.8), (1H)	67.97	4.34, t (2.8), (1H)
8	33.97	1.45, m, (1H)	35.28	1.68, m, (1H)	36.50	1.40, dd (11.9, 3.5), (1H)
9	34.11	1.82, m, (1H)	33.34	1.83, dt (11.9, 3.8), (1H)	33.39	1.88, dt (12.6, 2.8), (1H)
10	47.46		47.60		47.36	
11	20.49	1.35, ax, dq (14.0, 2.8), (1H) 2.10, eq, m, (1H)	21.63	1.53, ax, m, (1H) 2.17, eq, m, (1H)	23.90	1.20, ax, m, (1H) 2.27, eq, m, (1H)
12	28.54	1.48, ax, m, (1H) 1.73, eq, m, (1H)	36.77	1.65, ax, m, (1H) 1.70, eq, m, (1H)	37.61	1.69, m, (2H)
13	37.35	1.76, m, (1H)	71.22		71.07	
14	90.03		88.32		89.00	
15	32.96	1.71, α (left), m, (1H) 1.85, β (right), m, (1H)	27.99	1.79, α (left), m, (1H) 2.17, β (right), m, (1H)	27.20	1.63, α (left), m, (1H) 2.31, β (right), m, (1H)
16	31.46	2.17, m, (2H)	33.69	2.07, α (left), m, (1H) 1.94, β (right), m, (1H)	33.38	2.03, α (left), m, (1H) 1.97, β (right), m, (1H)
17	109.71		108.11		107.84	
18	14.47	0.95, d (7.0), (3H)	23.92	1.21, s, (3H)	21.87	1.53, s, (3H)
19	14.23	1.21, s, (3H)	14.29	1.24, s, (3H)	14.21	1.19, s, (3H)
20	75.07		40.59	2.61, m, (1H)	41.31	2.54, m, (1H)
21	36.93	2.40, ax, d (15.4), (1H) 1.45, eq, m, (1H)	26.45	2.00, ax, dd (14.0, 5.6), (1H) 1.59, eq, m, (1H)	26.65	2.16, ax, dd (14.0, 6.3), (1H) 1.55, eq, dd (14.0, 11.9), (1H)
22	78.74	4.62, dd (3.5, 1.4), (1H)	74.15	4.76, dt (3.5, 2.1), (1H)	74.42	4.73, dt (3.5, 1.4), (1H)
23	40.50	2.41, ax, dd (16.1, 1.4) (1H) 2.09, eq, m, (1H)	40.93	2.03, eq, ddd (15.4, 3.5, 2.1), (1H) 2.23, ax, dd (15.4, 2.1), (1H)	40.58	2.40, ax, dd (15.4, 1.4), (1H) 1.99, eq, m, (1H)
24	69.50		70.65		70.84	
25	48.46		47.35		47.61	
26	176.96		177.63		177.57	
27	14.04	1.14, s, (3H)	14.28	1.11, s, (3H)	14.27	1.14, s, (3H)
28	27.04	1.17, s, (3H)	27.40	1.14, s, (3H)	28.04	1.19, s, (3H)

[a] All NMR data were collected at 25.0 °C in $CDCl_3$ at 699.81 MHz in a 5 mm $^{1}H\{^{13}C/^{15}N\}$ (^{13}C enhanced) cold probe on a VNMRS700 Varian (Agilent) spectrometer.

Unfortunately, a single crystal of **3** suitable for X-ray crystallography failed to be isolated, owing to its instability at rt. However, the ROESY 2D NMR comparison between **2** and **3** did confirm that the C-18 methyl of physangulidine C is axial and the hydroxyl group is equatorial on C-13 of **3**, while in physangulidine B (**2**) the C-18 methyl is equatorial on C-13 and the hydroxyl is axial (Figure 1). Accordingly, CH_3-18 shows a strong correlation with H-8 and H-15 (both α and *β*) in **3**; however, CH_3-18 of physangulidine B (**2**) has only a single

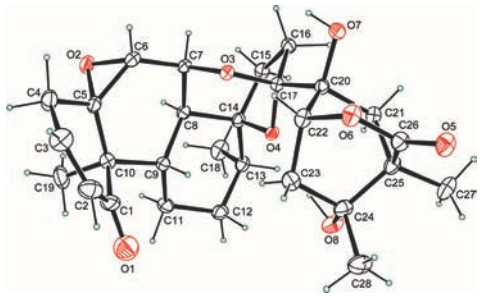

Figure 2. *ORTEP-3* diagram of **1** showing 40% ellipsoids. H-atoms are shown as small spheres of arbitrary radii. Selected bond lengths (Å) and angles (deg): O1−C1, 1.178(5); O5−C26, 1.204(4); O7−C20, 1.418(3); O8−C24, 1.437(4); C13−C18, 1.535(4); C12−C13−C18, 112.5(3). The absolute structure configuration for physangulidine A (**1**) was determined using Cu radiation including 13 stereocenters which are as follow: (chirality at C5) S, (C6) R, (C7) S, (C8) S, (C9) S, (C10) R, (C13) R, (C14) S, (C17) R, (C20) S, (C22) R, (C24) R, (C25) R.

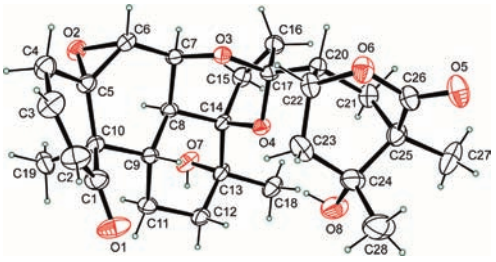

Figure 3. *ORTEP-3* diagram of **2** showing 40% ellipsoids. H-atoms are shown as small spheres of arbitrary radii. Selected bond lengths (Å) and angles (deg): O1−C1, 1.188(5); O5−C26, 1.218(7); O7−C13, 1.433 (5); C13−C18, 1.527(6); O7−C13−C18, 110.4(4); C12−C13−C18, 109.4(4). The absolute structure configuration for physangulidine B (**2**) was determined using Cu radiation including 13 stereocenters which are as follow: (chirality at C5) S, (C6) R, (C7) S, (C8) S, (C9) S, (C10) R, (C13) R, (C14) R, (C17) R, (C20) R, (C22) R, (C24) R, (C25) R.

interaction with H-15β observed in the ROESY spectra. A similar interaction was observed in physangulidine A: CH_3-18 has an interaction with H-8 and H-15 (α and β) on ROESY, indicating the preferred axial orientation of the methyl group in both **1** and **3**.

Physangulidine A (**1**) was found to exhibit significant *in vitro* antiproliferative activity against DU145 cancer cells in the bioassay (GI_{50} estimated to be 3.0 μM) and also RWPE-1 prostate epithelial cells (GI_{50} = 2.4 μM). GI_{50} values of physangulidine B (**2**) and physangulidine C (**3**) were found to be very similar to each other and comparatively lower than that of physangulidine A. The GI_{50} of physangulidine B was 6.0 and 6.8 μM against RWPE-1 and DU145, respectively, while in physangulidine C it was 6.6 and 6.0 μM against RWPE-1 and DU145, respectively. Their antiproliferative activities are comparable to, or higher than, those of related withanolides isolated from *P. angulata*.[3b] Because physangulidine A was the most active and abundant of the three bioactive components isolated from *P. angulata*, it was tested on additional cancer cell lines, using 5-fluorouracil as a positive control. The results are summarized in Table 2. Compared to 5-fluorouracil, physangulidine A had less antiproliferative activity against nonmalignant 3T3 cells and more antiproliferative activity against HT-29 and K562 cells.

Table 2. GI_{50} (μM) Values of Physangulidine A[a] and 5-Fluorouracil on Different Cell Lines

	3T3	H460	HuTu 80	DU145	MCF-7	M-14	HT-29	K562
Physangulidine A	4.12	3.59	4.18	3.56	5.26	4.66	2.73	2.73
5-Fluorouracil	0.28	1.67	4.92	3.11	2.18	5.03	5.92	30.32

[a] GI_{50} values of physangulidine A in Table 2 are average values of three tests. 3T3: Nontumorigenic, BALB/c mouse embryo cells. H460: human lung large cell carcinoma. HuTu 80: human duodenum adenocarcinoma. DU145: human prostate carcinoma. MCF-7: human breast adenocarcinoma. M-14: human amelanotic melanoma. HT-29: human colon adenocarcinoma. K562: human chronic myelogenous leukemia.

In conclusion, we have isolated three new bioactive withanolides, physangulidines A, B, and C, from *P. angulata* L. using a bioassay-guided isolation technique. Their structures, determined by NMR and X-ray crystallography, have an unusual disconnection between C-13 and C-17, a structural feature observed for the first time in the withanolides family.

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Supporting Information Available. CCDC-858987 and 858988 contain the supplementary crystallographic information for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Experimental details, including bioassay data, NMR, UV, and IR spectra of compounds **1**, **2**, and **3** are also available as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.