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Withanolides and Sucrose Esters from *Physalis neomexicana*

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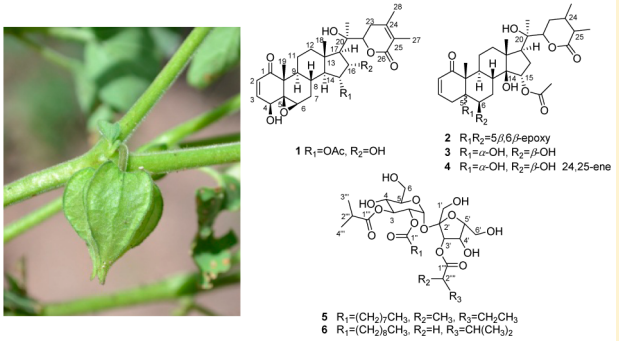
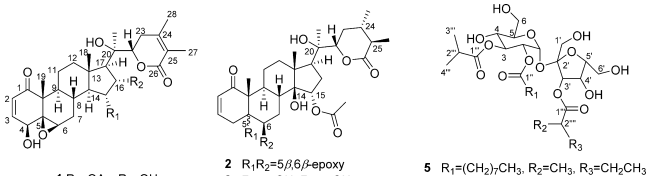
Supporting Information

ABSTRACT: Four withanolides (**1**−**4**) and two sucrose esters (**5**, **6**) were isolated from the aerial parts of *Physalis neomexicana*. The structures of **1**−**6** were elucidated through a variety of spectroscopic techniques. Cytotoxicity studies of the isolates revealed that **2** inhibited human breast cancer cell lines (MDA-MB-231 and MCF-7) with IC_{50} values of 1.7 and 6.3 μM, respectively.



Withanolides are a group of modified C_{28} ergostane-type steroids with a C-22, C-26 δ-lactone side chain. These steroids are observed predominantly in members of the Solanaceae, which include the *Datura*, *Jaborosa*, *Nicandra*, *Physalis*, *Salpichroa*, and *Withania* genera. Previous studies have demonstrated that the *Physalis* genus is an abundant source of withanolides,[1] from which our group reported the isolation of a series of such compounds from *P. coztomatl*,[2] *P. hispida*,[3] and *P. longifolia*.[4] In continuing this work, we examined *P. neomexicana* Rydb., an annual species endemic to the United States including the states of Colorado, New Mexico, Arizona, and Texas. The fruit, more commonly referred to as the New Mexico ground-cherry, was used as food by the Acoma, Chiricahua, Laguna, Mescalero, San Felipe, and Rio Grande pueblos of New Mexico.[5,6] The fruits were eaten raw, boiled, and used to make a green sauce.[7] We initiated the first phytochemical investigation of this edible species. This study resulted in the isolation and identification of four new withanolides (**1**−**4**), three sucrose esters (**5**−**7**), and a known labdane terpenoid. The cytotoxicity of the isolates (**1**−**7**) against two human breast cancer cell lines (MDA-MB-231 and MCF-7) was also investigated.



RESULTS AND DISCUSSION

Compound **1** was isolated as an amorphous solid with a $C_{30}H_{40}O_9$ molecular formula, which was determined based on the HRESIMS and NMR data (Table 1). The ^{1}H NMR, ^{13}C NMR, and HSQC spectra of **1** revealed the presence of six methyls [δ_H 0.95 (3H, s), δ_C 16.4; δ_H 1.29 (3H, s), δ_C 20.8; δ_H 1.41 (3H, s), δ_C 17.9; δ_H 1.88 (3H, s), δ_C 12.7; δ_H 1.95 (3H, s), δ_C 20.9; δ_H 1.99 (3H, s), δ_C 21.2], four methylenes [δ_H 2.05 (1H, m), 1.45 (1H, m), δ_C 30.0; δ_H 1.89 (1H, m), 1.42 (1H, m), δ_C 22.1; δ_H 1.94 (1H, m), 1.43 (1H, m), δ_C 40.2; δ_H 2.51 (1H, m), 2.23 (1H, dd, J = 17.0, 3.1 Hz), δ_C 31.1], 11 methines [including two olefinic at δ_H 6.19 (1H, d, J = 10.0 Hz), δ_C 132.1; δ_H 6.93 (1H, ddd, J = 10.0, 5.8 Hz), δ_C 142.2 and five oxygenated at δ_H 3.21 (1H, br s), δ_C 62.8; δ_H 3.76 (1H, dd, J = 5.7, 1.9 Hz), δ_C 69.8; δ_H 4.13 (1H, dd, J = 13.3, 3.5 Hz), δ_C 80.4; δ_H 5.16 (1H, dd, J = 11.4, 7.8 Hz), δ_C 71.6; δ_H 5.60 (1H, t, J = 7.6 Hz), δ_C 71.7], and nine quaternary carbons [including three carbonyl (δ_C 202.3, 169.8, and 165.8), two olefins (δ_C 148.9 and 122.3)], which corresponds to $C_{30}H_{37}$ with five degrees of unsaturation. Therefore, the three remaining hydrogen atoms were assigned as three hydroxy groups, which indicated a six-ringed structure in **1**.

The NMR data of **1** exhibited similarities to withanolide D (**8**), a known withanolide previously isolated from *Acnistus arborescens*.[8] Through ^{1}H−^{1}H COSY and HMBC experiments, compound **1** was found to contain three identical features also observed in **8**: (1) an α,β-unsaturated carbonyl [δ_H 6.19 (d, J = 10.0 Hz, H-2), 6.93 (dd, J = 10.0, 5.8 Hz, H-3); δ_C 202.3 (C-1), 132.1 (C-2), 142.2 (C-3)] functionality in ring A; (2) a 5β,6β-epoxide [δ_H 3.21 (br s, H-6); δ_C 63.6 (C-5), 62.8 (C-6)] moiety in ring B; and (3) a nine-carbon side chain with a hydroxy group at C-20 [δ_C 74.7 (C-20), 20.8 (C-21); δ_H 1.29 (s, Me-21)] and an α,β-unsaturated-δ-lactone system [δ_C 80.4 (C-22), 31.1 (C-23), 148.9 (C-24), 122.3 (C-25), 165.8 (C-26);

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Table 1. ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) Data of Withanolides 1−4 in $CDCl_3$

	1			2			3			4		
position	δ_C	type	δ_H (J in Hz)	δ_C	type	δ_H (J in Hz)	δ_C	type	δ_H (J in Hz)	δ_C	type	δ_H (J in Hz)
1	202.3	C		203.9	C		204.6	C		204.5	C	
2	132.1	CH	6.19 d (10.0)	128.9	CH	5.96 dd (10.0, 2.5)	129.0	CH	5.88 dd (10.2, 2.4)	129.0	CH	5.89 dd (10.1, 2.4)
3	142.2	CH	6.93 dd (10.0, 5.8)	145.2	CH	6.83 ddd (10.0, 6.1, 2.3)	141.5	CH	6.61 ddd (10.2, 5.0, 2.2)	141.3	CH	6.62 ddd (10.1, 5.0, 2.2)
4	69.8	CH	3.76 dd (5.7, 1.9)	33.1	CH_2	2.93 dt (19.1, 2.6)	36.5	CH_2	3.37 dt (20.1, 2.5)	36.5	CH_2	3.37 dt (22.5, 2.6)
						1.91, m			2.03, ddd (20.1, 5.2, 0.2)			2.04 m
5	63.6	C		61.5	C		77.4	C		77.3	C	
6	62.8	CH	3.21 br s	63.9	CH	3.17 d (2.5)	74.8	CH	3.70 br t (1.9)	75.0	CH	3.70 br s
7	30.0	CH_2	2.05 m	26.3	CH_2	2.43 dt (14.0, 2.5)	29.0	CH_2	1.96 dt (14.0, 3.1)	29.1	CH_2	1.96 m
			1.45 m			1.38 dt (14.0, 3.5)			1.80 m			1.81, dd (14.0, 2.7)
8	28.8	CH	1.81 qd (11.1, 4.2)	33.4	CH	1.90 m	33.7	CH	2.09 m	33.9	CH	2.10 m
9	43.5	CH	1.13 m	38.9	CH	1.90 m	35.3	CH	2.64 td (12.5, 3.8)	35.4	CH	2.64 td (12.4, 3.6)
10	47.6	C		48.7	C		52.5	C		52.5	C	
11	22.1	CH_2	1.89 m	22.8	CH_2	1.91 m	22.3	CH_2	2.18 m	22.4	CH_2	2.18 m
			1.42 m			1.45 br dt (12.7, 2.5)			1.34 dd (12.9, 3.0)			1.34 dd (13.0, 2.8)
12	40.2	CH_2	1.94 m	39.9	CH_2	1.91 m	40.6	CH_2	2.08 dt (12.9, 2.7)	41.1	CH_2	2.08 m
			1.43 m			1.46 m			1.53 dt (13.2, 3.0)			1.53 m
13	40.0	C		48.4	C		49.2	C		49.0	C	
14	56.6	CH	1.45 m	83.0	C		83.9	C		83.7	C	
15	71.6	CH	5.16 dd (11.4, 7.8)	80.8	CH	4.96 d (6.3)	80.3	CH	5.13 d (6.6)	80.4	CH	5.09 d (6.4)
16	71.7	CH	5.60 t (7.6)	31.2	CH_2	2.36, ddd (15.0, 8.0, 6.9)	31.4	CH_2	2.43 q (7.4)	31.8	CH_2	2.39 m
						1.54 dd (15.0, 9.5)			1.63 m			1.62 dd (15.0, 9.5)
17	58.3	CH	2.02 m	54.1	CH	1.78 m	54.3	CH	1.87 br t (8.6)	54.4	CH	1.87 m
18	16.4	CH_3	0.95 s	19.2	CH_3	1.29 s	19.8	CH_3	1.38, s	19.8	CH_3	1.39 s
19	17.9	CH_3	1.41 s	15.0	CH_3	1.20 s	15.0	CH_3	1.30 s	15.0	CH_3	1.30 s
20	74.7	C		75.6	C		75.7	C		75.6	C	
21	20.8	CH_3	1.29 s	20.7	CH_3	1.35 s	20.8	CH_3	1.41 s	21.5	CH_3	1.30 s
22	80.4	CH	4.13 dd (13.3, 3.5)	80.5	CH	4.17 dd (11.5, 3.3)	81.0	CH	4.24 dd (11.7, 3.5)	81.3	CH	4.27 dd (13.5, 3.4)
23	31.1	CH_2	2.51 m	31.1	CH_2	1.78 m	31.2	CH_2	1.83 m	31.5	CH_2	2.41 m
			2.23 dd (17.0, 3.1)			1.39 dt			1.45 dt (13.9, 3.5)			2.19 m
24	148.9	C		31.5	CH	1.69 m	31.6	CH	1.74 m	148.9	C	
25	122.3	C		40.5	CH	2.15 m	40.7	CH	2.19 m	122.3	C	
26	165.8	C		175.8	C		176.4	C		165.9	C	
27	12.7	CH_3	1.88 s	14.2	CH_3	1.16 d (6.7)	14.3	CH_3	1.22 d (6.7)	12.7	CH_3	1.89 s
28	20.9	CH_3	1.95 s	21.3	CH_3	1.09 d (6.7)	21.4	CH_3	1.15 d (6.6)	20.8	CH_3	1.97 s
29	169.8	C		169.8	C		170.6	C		170.5	C	
30	21.2	CH_3	1.99 s	21.8	CH_3	1.94 s	21.9	CH_3	2.05 s	21.9	CH_3	2.05 s
OH			2.31 s			3.81 br s			4.27 br s			3.75 br s
						3.45 br s			3.76 br s			3.19 br s
									2.71 br s			

δ_H 4.13 (dd, J = 13.3, 3.5 Hz, H-22), 2.51 (m, H-23a), 2.23 (dd, J = 17.0, 3.1 Hz, H-23b), 5.01 (d, J = 10.1 Hz, H-26), 3.46 (d, J = 10.4 Hz, OH-26)] with α,β-dimethyl [δ_H 1.88 (s, Me-27), 1.95 (s, Me-28); δ_C 12.7 (C-27), 20.9 (C-28)] groups.

Differences observed between **1** and **8** related to the ring D signals, where the two methylenes observed in **8** [δ_C 23.8 (C-15), 21.9 (C-16)] were absent in **1**, whereas two methines were present in **1** [δ_C 71.6, 71.7; δ_H 5.16 (dd, J = 11.4, 7.8 Hz), 5.60 (t, J = 7.6 Hz)]. This suggested that a substituted ring D functionality (with 15,16-dioxygenation) is present in the structure of **1** and was confirmed subsequently by the $^{1}H-^{1}H$ COSY and HSQC correlated structural fragment [−C(8)H−C(14)H−C(15)H−C(16)H−C(17)H at δ_H 1.81 (qd, J = 11.1, 4.2 Hz, H-8), 1.45 (m, H-14), 5.16 (dd, J = 11.4, 7.8 Hz, H-15), 5.60 (t, J = 7.6 Hz, H-16), 2.02 (m, H-17)] and HMBC correlations {H-16 to C-20 (δ_C 74.7) and C-14 (δ_C 56.6) and H-15 to a carbonyl (δ_C 169.8) in an acetoxy group [at δ_H 1.99 (3H, s); δ_C 21.2, 169.8]}. When combined, the data showed that a 15-acetoxy-16-hydroxy functionality is present in ring D of **1**. Furthermore, a H-15β and H-16β configuration in **1** was determined based on coupling constants ($J_{14,15}$ = 11.1 Hz, $J_{15,16}$ = 7.6 Hz, $J_{16,17}$ = 7.6 Hz) in conjunction with NOE correlations (H-15/Me-18; H-16/Me-18). Finally, the structure of **1** was determined as 15α-acetoxy-16α-hydroxywithanolide D

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(15α-acetoxy-5β,6β-epoxy-4β,16α,20-trihydroxy-1-oxowitha-2,24-dienolide) and subsequently named withaneomexolide A.

Compound **2** was isolated as an amorphous solid. The HRESIMS and NMR data revealed the molecular formula of **2** as $C_{30}H_{42}O_8$. The ^{1}H NMR, ^{13}C NMR, and HSQC spectra of **2** showed the presence of six methyls [δ_H 1.09 (3H, d, J = 6.7 Hz), δ_C 21.3; δ_H 1.16 (3H, d, J = 6.7 Hz), δ_C 14.2; δ_H 1.20 (3H, s), δ_C 15.0; δ_H 1.29 (3H, s), δ_C 19.2; δ_H 1.35 (3H, s), δ_C 20.7; δ_H 1.94 (3H, s), δ_C 21.8], six methylenes [δ_C 22.8, 26.3, 31.1, 31.2, 33.1, 39.9], 10 methines [including two olefinic at δ_H 5.96 (1H, dd, J = 10.0, 2.5 Hz), δ_C 128.9 and δ_H 6.83 (1H, ddd, J = 10.0, 6.1, 2.3 Hz), δ_C 145.2; with three oxygenated at δ_H 3.17 (1H, d, J = 2.5 Hz), δ_C 63.9; δ_H 4.96 (1H, d, J = 6.3 Hz), δ_C 80.8; δ_H 4.17 (1H, dd, J = 11.5, 3.3 Hz), δ_C 80.5], and eight quaternary carbons (including three carbonyl groups at δ_C 203.9, 175.8, 169.8 and three oxygenated carbons at δ_C 83.0, 75.6, 61.5), which indicated a six-ringed structure where the remaining hydrogen atoms were assigned as two hydroxy groups.

Similar to **1**, the NMR data of **2** revealed an α,β-unsaturated ketone in ring A, a 5β,6β-epoxy in ring B, and 15-acetoxy and 20-hydroxy moieties. However, three features [C-24(25) double bond; 4- and 16-hydroxy groups] observed in **1** were absent in **2**. Instead, **2** was observed to contain two methylene [C-4: δ_C 33.1; δ_H 2.93 (1H, dt, J = 19.1, 2.6 Hz), 1.91, (1H, m); C-16: δ_C 31.2; δ_H 2.36 (1H, ddd, J = 15.0, 8.0, 6.9 Hz), 1.54 (1H, dd, J = 15.0, 9.5 Hz)] and two methine [C-24: δ_C 31.5; δ_H 1.69 (1H, m); C-25: δ_C 40.5; δ_H 2.15 (1H, m)] groups. These were supported by ^{1}H−^{1}H COSY [=C(3)H−C(4)H$_2$−, −C(15)H−C(16)H$_2$−, −C(22)H−C(23)H$_2$−C(24)H−C(25)H−, −C-(24)H−C(28)H$_3$−, −C(25)H−C(27)H$_3$−] and HMBC correlations [H$_2$-4/C-2, C-3, C-5, and C-6; H-16/C-17 and C-20; C-24/H-22 and Me-27; C-25/H$_2$-23 and Me-28]. Since the NMR data of **2** presented chemical shifts [C-14 (δ_C 83.0)] and HMBC correlations [C-14/Me-18, C-14/H$_2$-12, C-14/H-15, and C-14/H-16] that were indicative of 14-hydroxy and 15-acetoxy moieties, an additional hydroxy group (at C-14) was deduced as being present in **2**. Furthermore, the elucidation of **2** was confirmed by NMR comparisons, where superimposable ring D signals were observed with other 15-acetoxy-14-hydroxy withanolides, such as physapubenolide.[9]

Analysis of the γ-effect of OH-14 to C-12 and C-18[9] allowed a determination of the configuration of this group in **2**. It has been shown that when compared to 14-unsubstituted withanolides [such as withalongolide H ($\delta_{C\text{-}12}$ 39.9, $\delta_{C\text{-}18}$ 12.0)], the presence of a 14α-hydroxy group results in the shielding of C-12, whereas a 14β-hydroxy substituent deshields C-18.[9] Therefore, the absence of the shielding at C-12 and the presence of deshielding at C-18 ($\delta_{C\text{-}12}$ 39.9, $\delta_{C\text{-}18}$ 19.2) suggested that a 14β-OH group is present in **2**, which is consistent with the results obtained for other 14β-hydroxy withanolides.[9,10] The presence of peak splitting of H-15 (d, J = 6.3 Hz), which is observed only in H-15β-containing compounds,[9] suggested that a 15α-acetoxy group is present in **2**. This deduction was subsequently confirmed by related NOESY correlations and data comparison against reported 15-acetoxy-14-hydroxy withanolides, such as physapubenolide.[9,11] Similarly, the stereochemistry of the 17β-side chain of **2** was determined based on the related NOESY correlations and data comparison against 20-hydroxy withanolides, such as **8**. The β orientation of 27-methyl and α orientation of 28-methyl were deduced from the NOESY correlations between Me-27 and H-22 and between H-24 and Me-27, respectively. Accordingly, the structure of **2** was identified as 15α-acetoxy-5β,6β-epoxy-14β,20-dihydroxy-1-oxowitha-2-enolide and named withaneomexolide B.

Compound **3** was isolated as an amorphous solid and found to possess a molecular formula of $C_{30}H_{34}O_9$ based on its HRESIMS and NMR data (Table 1). Similar to **2**, the NMR spectra of **3** showed the presence of a 20-hydroxy bearing a nine-carbon side chain (an α,β-saturated-δ-lactone system with α,β-dimethyl) as well as a 14-hydroxy-15-acetoxy ring D moiety. In contrast to ring B of **2**, the NMR spectra revealed the absence of a 5β,6β-epoxy functionality and the presence of a 5,6-dihydroxy unit in **3**. This deduction was based on chemical shifts [δ_C 77.4 (C-5), 74.8 (C-6); δ_H 3.70 (1H, br t, J = 1.9 Hz, H-6)], HMBC correlations [Me-19/C-5; H-6/C-10, C-5, C-8], and ^{1}H−^{1}H COSY correlations [−C(6)H−C(7)H$_2$−C(8)H− at δ_H 3.70 (H-6), 1.96 (1H, dt, J = 14.0, 3.1 Hz, H-7β), 1.80 (1H, m, H-7α), 2.09 (m, H-8)]. Moreover, the 5α,6β-dihydroxy orientation of **3** was determined based on coupling constants [H-6 (1.9 Hz)] and ^{13}C NMR data comparison against other published 5α,6β-dihydroxy withanolides [jaborosalactone D,[12] 16-oxojaborosalactone D,[13] acnistin J,[13] withajardin G[13]]. Therefore, compound **3** was identified as 15α-acetoxy-5α,6β,14β,20-tetrahydroxy-1-oxowitha-2-enolide and named withaneomexolide C.

Compound **4** was isolated as a colorless, amorphous solid and on the basis of HRESIMS and NMR data was assigned a molecular formula of $C_{30}H_{42}O_9$. The NMR data (^{1}H−^{1}H COSY, HSQC, and HMBC) of **3** and **4** were superimposable except for their side chain signals. Specifically, instead of the two methines observed in **3**, two olefinic signals were observed in **4** [δ_C 148.9 (C-24), 122.3(C-25)]. The presence of an α,β-dimethyl moiety [δ_H 1.89 (s, Me-27), 1.97 (s, Me-28)] supported the presence of an α,β-unsaturated-δ-lactone system in **4**. Furthermore, the NMR signals of this functional group matched those of **1**. Therefore, the structure of **4** (withaneomexolide D) was assigned as 15α-acetoxy-5α,6β,14β,20-tetrahydroxy-1-oxowitha-2,24-dienolide.

Through HRESIMS and NMR experiments, the molecular formula of compound **5** was found to be $C_{30}H_{52}O_{14}$ and could be assigned as an isomer of a sucrose ester also isolated in this study, namely, nicandrose D (**7**).[14] The NMR data of **5** and **7** exhibited signals typical of saccharides, such as for hydroxy groups, along with a terminal carbon [δ_C 104.1 and 90.1], and connected structural rings [−OC(1)H(O)−C(2)H(O)−CH(3)(O)−CH(4)(O)−CH(5)(O)−C(6)H$_2$(O)− and −CH(3′)(O)−CH(4′)(O)−CH(5′)(O)−C(6′)H$_2$(O)−, with HMBC correlations observed between H-1 (δ_H 5.53,1H, d, J = 3.7 Hz) and C-2′ (δ_C 104.1)]. The NMR signals of **5** and **7** were superimposable except for their C-2 and C-3′ substitutions. Specifically, a decanoyl (C-2) and an isobutyryl (C-3′) were found in **7**, whereas **5** was observed to contain nonanoyl (C-2) and 2-methylbutanoyl (C-3′) moieties. The presence of a nonanoyl ($CH_3(CH_2)_7CO$−) moiety in **5** was deduced on the basis of HRESIMS and the integration of proton signals at δ_H 1.25. In turn, the 2-methylbutanyl moiety [δ_C 16.4, 40.9, 27.1, 11.6; δ_H 1.21 (3H, d, J = 7.0 Hz), 2.57 (1H, tq, J = 7.0, 6.8 Hz), 1.76 dqd (J = 21.2, 7.0, 7.0 Hz), 1.58 dqd (J = 21.2, 7.3, 6.7 Hz), 0.95 (3H, t, J = 7.5 Hz s)] connected to C-3′ in **5** was revealed from ^{1}H−^{1}H COSY correlations and confirmed by HMBC correlations {H-3′ [δ_H 5.24 (1H, d, J = 8.5 Hz)], H-5′′′′ (δ_H 1.21), and H$_2$-3′′′′ (δ_H 1.76, 1.58) to the carbonyl at δ_C 177.8 (C-1′′′′)}. The stereochemistry of C-2′′′′ could not be determined, as no NOESY correlation of H$_2$-3′′′′ or Me-5′′′′ was observed. Therefore, compound **5** was identified as an isomer of **7** and named nicandrose E.

On the basis of HRESIMS and NMR data, the molecular formula of the amorphous solid **6** was found to be $C_{31}H_{54}O_{14}$. As was the case with **5**, the ^{1}H NMR spectrum of **6** was also

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Table 2. ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) Data of Sucrose Esters 5−7 in $CDCl_3$

	5			6			7		
position	δ_C	type	δ_H (J in Hz)	δ_C	type	δ_H (J in Hz)	δ_C	type	δ_H (J in Hz)
1	89.9	CH	5.53 d (3.7)	90.1	CH	5.53 d (3.7)	90.0	CH	5.49 s
2	70.0	CH	4.87 dd (10.3, 3.7)	70.2		4.83 d (10.3, 3.6)	70.3	CH	4.79 d (10.3)
3	72.7	CH	5.21 t (9.8)	72.5	CH	5.22 m	72.1	CH	5.23 t (10.2)
4	69.8	CH	3.54 m	69.7	CH	3.52 m	69.2	CH	3.55 t (9.8)
5	73.8	CH	4.01 ddd (9.9, 5.7, 2.2)	73.6	CH	3.99 m	73.2	CH	3.94 m
6	62.1	CH_2	3.95 dd (11.6, 2.7)	62.0	CH_2	3.93 m	61.6	CH_2	3.86 m
			3.76 m						
1′	63.9	CH_2	3.53 m	63.8	CH_2	3.53 m	63.3	CH_2	3.49 m
			3.46 dd (12.6, 4.6)						
2′	104.0	C		104.1	C		104.0	C	
3′	78.0	CH	5.24 d (8.5)	78.2	CH	5.23 m	77.9	CH	5.21 d (8.6)
4′	71.3	CH	4.46 t (8.6)	71.7	CH	4.42 br t (8.0)	72.1	CH	4.33 t (7.6)
5′	82.3	CH	3.95 m	82.3	CH	3.928 m	82.3	CH	3.93 m
6′	60.9	CH_2	3.90 br d (11.1)	61.1	CH_2	3.87 m	61.7	CH_2	3.74 m
			3.77 m			3.75 m			
1″	173.3	C		173.4	C		173.4	C	
2″	34.2	CH_2	2.24 dt (16.2, 7.6)	34.2	CH_2	2.24 dt (16.2, 7.7)	34.2	CH_2	2.21 dt (16.5, 7.5)
			2.22 dt (16.2, 7.5)			2.22 dt (16.2, 7.7)			2.19 dt (16.5, 7.5)
3″	24.8	CH_2	1.53 m	24.8	CH_2	1.53 m	24.8	CH_2	1.49 m
4″	29.3	CH_2	1.25 m	29.3	CH_2		29.3	CH_2	1.21 m
5″	29.5	CH_2	1.25 m	29.5	CH_2	1.24 br m	29.5	CH_2	
6″	29.6	CH_2	1.25 m	29.5	CH_2	1.24 br m		CH_2	
7″	32.1	CH_2	1.24 m	29.6	CH_2			CH_2	
8″	22.9	CH_2	1.27 m	32.1	CH_2	1.240 m	32.0	CH_2	1.21 m
9″	14.3	CH_3	0.87 to (6.9)	22.8	CH_2	1.27 m	22.8	CH_3	1.24 m
10″				14.3	CH_3	0.87 d (6.9)	14.2		0.85 t (6.9)
1‴	177.9	C		177.8	C		177.4	C	
2‴	34.3	CH	2.55 hept[a] (7.0)	34.2	CH_2	2.55 hept (7.0)	34.2	CH	2.51 hept (7.0)
3‴	19.2	CH_3	1.12 d (7.0)	19.2	CH_3	1.14 d (7.0)	18.8	CH_3	1.08 d (7.0)
4‴	19.0	CH_3	1.13 d (7.0)	19	CH_3	1.12 d (7.0)	19.1	CH_3	1.10 d (7.0)
1⁗	177.8	C		174.2	C		178.0	C	
2⁗	40.9	CH	2.57 tq (7.0, 6.8)	43.1	CH_2	2.39 dd (15.1, 7.0)	34.0	CH	2.69 hept (7.0)
						2.32 dd (15.1, 7.4)			
3⁗	27.1	CH_2	1.76 dqd (21.2, 7.0, 7.0)	25.9	CH	2.14 non[b] (6.8)	18.9	CH_2	1.18 m
			1.58 dqd (21.2, 7.3, 6.7)						
4⁗	11.6	CH_3	0.95 t (7.5)	22.4	CH_3	0.99 d (6.8)	19.4	CH_3	1.22 m
5⁗	16.4	CH_3	1.21 d (7.0)	22.6	CH_3	1.00 d (6.6)			
OH			4.57 br s			4.74 br s			5.10 br s
			4.35 br s			4.44 br s			4.66 br s
			4.22 br s			4.39 br s			4.27 br s
			3.75 br s			4.01 br s			3.93 br s
			3.37 br s			3.56 br s			3.48 br s

[a]hept = heptet. [b]non = nonet.

almost superimposable with that of **7**. The differences observed pertained to the side chain [δ_H 1.40 to 2.60, C-3 and C-3′] of **6**, where the 3-methylbutanyl moiety [δ_C 22.4, 22.6, 25.9, 43.1; δ_H 0.99 (3H, d, J = 6.8 Hz), 1.00 (3H, d, J = 6.6 Hz), 2.14 (1H, non, J = 6.8 Hz), 2.32 (dd, J = 15.1, 7.4 Hz), 2.39 (dd, J = 15.1, 7.0 Hz)] at C-3″ was determined on the basis of $^{1}H-^{1}H$ COSY and HMBC correlations [H-3′ (δ_H 5.23, 1H, m) and H-3⁗ (δ_H 2.14) to the carbonyl at δ_C 174.2 (C-1⁗)]. Therefore, compound **6** was identified as a 3′-(3-methylbutanyl)oxy derivative of **7** and named nicandrose F.

In addition to **1**−**6**, the present phytochemical investigation on *P. neomexicana* also resulted in the isolation of two known compounds, which were identified by spectroscopic comparisons with values reported in the literature, namely, nicandrose D (**7**)[14] and the labdane diterpenoid 12-*O*-acetylphysacoztomatin.[15]

Compounds **1**−**7** were examined for their cytotoxicity against human breast cancer cell (MDA-MB-231 and MCF-7) lines, where two known bioactive withanolides were utilized as positive controls, namely, withaferin A (IC_{50} 0.5 and 1.3 μM) and withalonglide B (IC_{50} 0.2 and 0.8 μM). Compound **2** exhibited cytotoxicity in both cell lines tested, with IC_{50} values of 1.7 and 6.3 μM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Rudolph RS Autopol IV automatic polarimeter. UV spectra were scanned on a Varian Cary 50 UV−visible spectrophotometer. IR data were obtained with a Thermo Nicolet Avatar 380 FT-IR spectrometer. NMR spectra were recorded with a Bruker AV-400 or AV-500 instrument with a cryoprobe used for ^{1}H NMR, APT, COSY,

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HSQC, HMBC, and NOESY/ROESY experiments. Chemical shift values are given in δ (ppm) using the peak signals of the solvent $CDCl_3$ (δ_H 7.26 and δ_C 77.23) as references, and coupling constants are reported in Hz. All ESIMS data were measured with an Agilent 1200 Series LC coupling with an ion-trap 6310 mass spectrometer, while HRESIMS data were collected with an LCT Premier time-of-flight mass spectrometer (Waters Corp., Milford, MA, USA). Column chromatography was performed on CombiFlash columns (Teledyne Isco, Lincoln, NE, USA) or Sephadex LH-20 (GE Healthcare, Piscataway, NJ, USA) columns. Normal-phase silica gel G TLC plates (w/UV 254) and reversed-phase C_{18} TLC plates (w/UV 254) (Sorbent Technologies, Atlanta, GA, USA) were used for fraction and compound detection. The spots were visualized using UV light at 254 nm and 10% EtOH−sulfuric acid spray reagent. Semipreparative HPLC was performed on an Agilent 1200 unit equipped with a DAD detector, utilizing a Phenomenex Luna RP-18 column (250 × 10 mm, 5 μm).

Plant Material. The above-ground biomass (stems, leaves, and flowers) of *Physalis neomexicana* was collected in a pinyon-juniper grassland habitat (lat 35.2318°, long 105.92197°) in September, 2013, by K.K., Hillary Loring, and Leanne Martin in Santa Fe County, New Mexico. A botanical specimen of this species (Kindscher 4115) was deposited in the R.L. McGregor Herbarium of the University of Kansas. Botanical identification was performed by K.K. at the Kansas Biological Survey, University of Kansas.

It should be noted that *P. neomexicana* is a distinct species when observed in its natural habitat. Several scientific names have been used for this plant in the literature including *P. subulata* Rydb. var. *neomexicana* (Rydb.) Waterf. ex Kartesz & Gandhi and *P. foetens* Poir. var. *neomexicana* (Rydb.) Waterf.[16] The name *P. neomexicana* was recognized in the 2011 update to the Solanaceae of North America database;[17] therefore this name is used in this study.

Extraction and Isolation. The collected biomass was air-dried, ground to a coarse powder (1100 g), and extracted three times with CH_2Cl_2−MeOH (50:50, 8.0 L) at room temperature. After removing the solvents under vacuum, the extract (174.8 g) was suspended in 2.0 L of H_2O, followed by successive partitions with equal volumes of ethyl acetate (EtOAc) to yield the EtOAc fraction (85.0 g), which was applied subsequently to silica gel (30 × 400 mm) MPLC (gradient hexane 80:20 to 20:80) to afford fractions fr1 to fr3. Subjecting fr1 (0.4 g) to CombiFlash CC (12 g of silica gel; eluted with CH_2Cl_2−EtOAc, 6:1 to 2:1) resulted in fr1-3, which was subjected subsequently to semipreparative HPLC, eluted by isocratic 60% CH_3CN to afford **8** (7.8 mg). Applying fr2 (2.5 g) to CombiFlash CC (50 g RP-C18; CH_3CN−H_2O elution) yielded two fractions, which were subjected to semipreparative HPLC (isocratic 40% CH_3CN) to afford **2** (54.7 mg) and **3** (4.7 mg), respectively. Subjecting fr3 (1.2 g) to Sephadex LH-20 CC (CH_2Cl_2−MeOH, 3:2) afforded 3-1 (0.7 g) and 3-2 (0.3 g). Reversed-phase CombiFlash separation with a gradient of 30−65% CH_3CN of fr 3-1 and subsequent semipreparative HPLC (isocratic 50% CH_3CN) yielded **1** (1.4 mg), **5** (18.5 mg), **6** (17.7 mg), and **7** (73.9 mg). Applying fr3-2 (0.3 g) to CombiFlash CC (40 g of ODS; gradient 30−50% CH_3CN) followed by semipreparative HPLC (isocratic 30% CH_3CN) purification yielded **4** (1.2 mg), **6** (18.3 mg), and **7** (43.1 mg).

*Withaneomexolide A (**1**):* solid; $[\alpha]^{25}_D$ +22.5 (*c* 0.01, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 228 (4.24) nm; IR (neat) ν_{max} 3422 (br), 2927, 1653, 1021 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 567.2549 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_9Na$, 567.2570).

*Withaneomexolide B (**2**):* solid; $[\alpha]^{25}_D$ −3.2 (*c* 0.37, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 226 (4.07) nm; IR (neat) ν_{max} 3382 (br), 2945, 1724, 1679, 1380, 1021 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 553.2756 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_8Na$, 553.2777).

*Withaneomexolide C (**3**):* solid; $[\alpha]^{25}_D$ −8.6 (*c* 0.03, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 225 (4.55) nm; IR (neat) ν_{max} 3361 (br), 2952, 1731, 1669, 1453, 1375, 1025 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 571.2855 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_9Na$, 571.2883).

*Withaneomexolide D (**4**):* solid; $[\alpha]^{25}_D$ −15.0 (*c* 0.01, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 223 (4.40) nm; IR (neat) ν_{max} 3534 (br), 3142(br), 2862, 1653, 1021 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 569.2702 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_9Na$, 569.2727).

*Nicandrose E (**5**):* solid; $[\alpha]^{25}_D$ +30.0 (*c* 0.13, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 200 (4.09) nm; IR (neat) ν_{max} 3393 (br), 2926, 1732, 1461, 1045, 1001 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 659.3273 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_{14}Na$, 659.3255).

*Nicandrose F (**6**):* solid; $[\alpha]^{25}_D$ +34.7 (*c* 0.13, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 229 (3.98) nm; IR (neat) ν_{max} 3346 (br), 2929, 1735, 1463, 1023 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 673.3373 $[M + Na]^+$ (calcd for $C_{31}H_{54}O_{14}Na$, 673.3411).

Cytotoxicity Bioassays. The MTT-based cytotoxicity assays were performed as previously reported.[18] The human breast cancer cell (MCF-7 and MDA-MB-231) lines utilized in this study were purchased from American Type Culture Collection (ATCC). In general, six concentrations ranging from 10 nM to 100 μM were tested for compounds. The IC_{50} values were calculated via sigmoid curve fitting using GraphPad Prism 5.0 software. Withaferin A (IC_{50} 0.5 and 1.3 μM) and withalongolide B (IC_{50} 0.2 and 0.8 μM) were used as positive controls in this test.

Table 3. Cytotoxicity of Compounds against Two Human Breast Cancer Cell Lines[a]

compound	MDA-MB-231 (mean ± SD, μM)	MCF-7 (mean ± SD, μM)
2	1.7 ± 0.2	6.3 ± 1.2
withaferin A (positive control 1)	0.5 ± 0.04	1.3 ± 0.2
withalongolide B (positive control 2)	0.2 ± 0.02	0.8 ± 0.2

[a] IC_{50} values averaged with three independent repeats.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00698.

^1H NMR and ^{13}C NMR (APT) of withaneomexolides A−D (**1**−**4**) and nicandroses E (**5**) and F (**6**), together with 2D NMR spectra of **1**−**3**, **5**, and **6** (PDF)

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Notes

The authors declare no competing financial interest.

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