

## Withanolides from *Physalis hispida*

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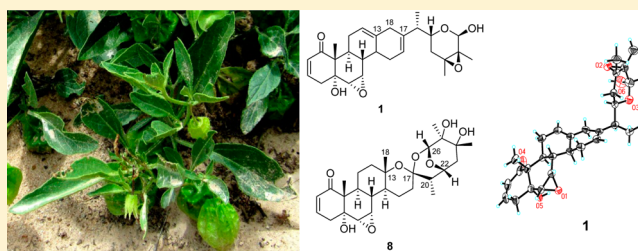
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### Supporting Information

**ABSTRACT:** Nine new withanolides (1–9), withahisolides A–I, were isolated along with nine known compounds (10–18) from the aerial parts of *Physalis hispida*. The structures of 1–9 were elucidated through a variety of spectroscopic techniques, while the structures of 1 and 2 were confirmed by X-ray crystallographic analysis. Compounds 1–3 are the first withanolides with nonaromatic six-membered ring D moieties. In addition, withanolide 8 represents a novel withanolide skeleton due to the absence of a C-13–C-17 bond within the steroidal nucleus.



Withanolides are a group of modified C<sub>28</sub> ergostane-type steroids with a C-22, C-26  $\delta$ -lactone side chain. They are distributed primarily within 25 genera of the Solanaceae, including *Datura*, *Jaborosa*, *Nicandra*, *Physalis*, *Salpichroa*, and *Withania*. The genus *Physalis* has provided an abundant source of withanolides,<sup>1</sup> where our group reported the isolation of a series of these compounds from *Physalis longifolia*.<sup>2</sup> As part of continuing research related to the discovery of novel withanolides from the Solanaceae, a phytochemical study on *Physalis hispida* (Waterf.) Cronquist was initiated. The species is commonly known as “prairie groundcherry”, distributed throughout the central and midwestern United States. Reported herein are the isolation and structure elucidation of eight new withanolides with unusual six-membered ring D moieties (1–7, 9), a novel withanolide with a disconnected C13–C17 bond (8), and nine known compounds (10–18) from the aerial parts of *P. hispida*. This study represents the first phytochemical study of this species.

## RESULTS AND DISCUSSION

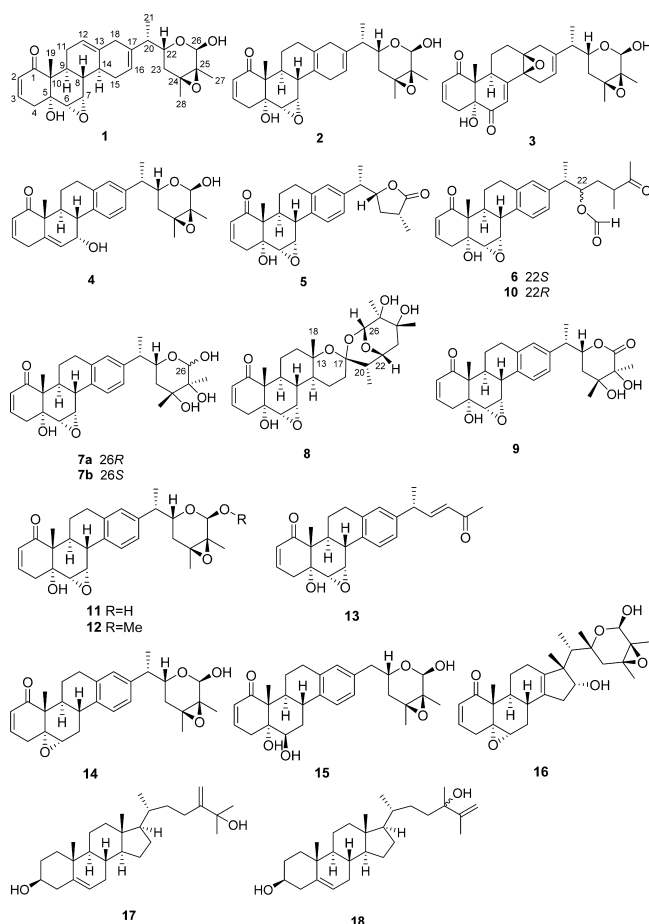
Compound 1 was isolated as colorless needles from acetone–heptane. Its molecular formula was determined as C<sub>28</sub>H<sub>36</sub>O<sub>6</sub> with 11 degrees of unsaturation based on HRESIMS and NMR data (Tables 1 and 2). The <sup>1</sup>H NMR, <sup>13</sup>C NMR, and HSQC spectra for 1 showed the presence of four methyl groups [ $\delta_{\text{H}}$  1.00 (3H, d,  $J = 7.1$  Hz),  $\delta_{\text{C}}$  15.7;  $\delta_{\text{H}}$  1.13 (3H, s),  $\delta_{\text{C}}$  14.4;  $\delta_{\text{H}}$  1.40 (3H, s),  $\delta_{\text{C}}$  19.1; and  $\delta_{\text{H}}$  1.41 (3H, s),  $\delta_{\text{C}}$  16.8], five methylenes, 12 methines [including four olefinic at  $\delta_{\text{H}}$  5.45 (1H, d,  $J = 4.6$  Hz),  $\delta_{\text{C}}$  120.0;  $\delta_{\text{H}}$  5.58 (1H, d,  $J = 5.5$  Hz),  $\delta_{\text{C}}$  121.0;  $\delta_{\text{H}}$  5.88 (1H, ddd,  $J = 10.1, 2.2, 0.9$  Hz),  $\delta_{\text{C}}$  129.1; and  $\delta_{\text{H}}$  6.63 (1H, ddd,  $J = 10.1, 5.0, 2.3$  Hz),  $\delta_{\text{C}}$  140.3 and four

oxygenated at  $\delta_{\text{H}}$  3.16 (1H, d,  $J = 3.9$  Hz),  $\delta_{\text{C}}$  57.6;  $\delta_{\text{H}}$  3.55 (1H, dd,  $J = 3.4, 2.8$  Hz),  $\delta_{\text{C}}$  56.3;  $\delta_{\text{H}}$  3.63 (1H, ddd,  $J = 10.7, 7.6, 2.5$  Hz),  $\delta_{\text{C}}$  65.8; and  $\delta_{\text{H}}$  5.01 (1H, d,  $J = 10.1$  Hz),  $\delta_{\text{C}}$  91.8], and seven quaternary carbons (including a keto at  $\delta_{\text{C}}$  203.2, two olefins at  $\delta_{\text{C}}$  139.2 and 135.0), which corresponds to C<sub>28</sub>H<sub>34</sub> with four degrees of unsaturation. The two remaining hydrogen atoms were therefore assigned as two hydroxy groups, indicating a seven-ring structure.

The NMR data of 1 exhibited similarities to a major withanolide isolated in this study, namely, nicandrenone (11).<sup>3</sup> Through <sup>1</sup>H–<sup>1</sup>H COSY and HMBC experiments, compound 1 was found to contain three moieties also observed in 11: (1) an  $\alpha,\beta$ -unsaturated carbonyl [ $\delta_{\text{H}}$  5.88 (ddd,  $J = 10.1, 2.2, 0.9$  Hz, H-2), 6.63 (ddd,  $J = 10.1, 5.0, 2.3$  Hz, H-3),  $\delta_{\text{C}}$  203.2 (C-1), 129.1 (C-2), and 140.3 (C-3)] functionality in ring A; (2) a 5 $\alpha$ -hydroxy-6 $\alpha,7\alpha$ -epoxide [ $\delta_{\text{H}}$  3.16 (d,  $J = 3.9$  Hz, H-6), 3.55 (dd,  $J = 3.4, 2.8$  Hz, H-7);  $\delta_{\text{C}}$  57.6 (C-6) and 56.3 (C-7)] moiety in ring B; (3) a nine-carbon side chain with an epoxy- $\delta$ -lactone system [ $\delta_{\text{C}}$  45.1 (C-20), 15.7 (C-21), 65.8 (C-22), 34.6 (C-23), 65.2 (C-24), 64.0 (C-25), and 91.8 (C-26);  $\delta_{\text{H}}$  2.17 (m, H-20), 1.00 (d,  $J = 7.1$  Hz, Me-21), 3.63 (ddd,  $J = 10.7, 7.6, 2.5$  Hz, H-22), 1.89 (m, H-23a), 1.59 (m, H-23b), 5.01 (d,  $J = 10.1$  Hz, H-26), and 3.46 (d,  $J = 10.4$  Hz, OH-26)] and  $\beta,\gamma$ -dimethyl [ $\delta_{\text{H}}$  1.40 (s, Me-28), 1.41 (s, Me-27);  $\delta_{\text{C}}$  19.1 (C-28), 16.8 (C-27)] groups. The differences observed between 1 and 11

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corresponded to the signals of rings C and D, where the aromatic ring observed in **11** was absent in **1**.

The  $^1\text{H}$ – $^1\text{H}$  COSY and HSQC spectra of **1** revealed the structural fragments of  $-\text{CH}-\text{CH}-\text{CH}-\text{CH}_2-\text{CH}=\text{}$  [ $\delta_{\text{H}}$  3.55 (dd,  $J = 3.4, 2.8$  Hz, H-7), 1.62 (m, H-8), 1.88 (m, H-9), 3.10 (m, H-11 $\alpha$ ), 1.87 (m, H-11 $\beta$ ), and 5.58 (d,  $J = 5.5$  Hz, H-12)] assigned as  $-\text{C}(7)\text{H}-\text{C}(8)\text{H}-\text{C}(9)\text{H}-\text{C}(11)\text{H}_2-\text{C}(12)\text{H}=\text{}$ ;  $-\text{CH}-\text{CH}-\text{CH}_2-\text{CH}=\text{}$  [ $\delta_{\text{H}}$  1.62 (H-8), 2.46 (q,  $J = 8.4$  Hz, H-14), 2.70 (m, H-15 $\alpha$ ), 1.81 (m, H-15 $\beta$ ), and 5.45 (d,  $J = 4.6$  Hz, H-16)] assigned as  $-\text{C}(8)\text{H}-\text{C}(14)\text{H}-\text{C}(15)\text{H}_2-\text{C}(16)-\text{H}=\text{}$ ; and an isolated  $-\text{CH}_2-$  [ $\delta_{\text{H}}$  2.83 (d,  $J = 19.4$  Hz, H-18 $\alpha$ ), 2.61 (m, H-18 $\beta$ );  $\delta_{\text{C}}$  32.0] assigned as  $-\text{C}(18)\text{H}_2-$ . This suggested that a 12(13),16(17)-diene functionality [a C-12(13) double bond in ring C and a C-16(17) double bond in six-membered ring D] was present in the structure of **1**, which was subsequently confirmed by the HMBC correlations of H-7/C-9 ( $\delta_{\text{C}}$  30.5); H-8/C-11 ( $\delta_{\text{C}}$  26.9); H-11 $\alpha$ /C-10 ( $\delta_{\text{C}}$  51.2); H-12/C-9, C-14 ( $\delta_{\text{C}}$  37.3), and C-18 ( $\delta_{\text{C}}$  32.9); H-15 $\alpha$ /C-13 ( $\delta_{\text{C}}$  135.0) and C-17 ( $\delta_{\text{C}}$  139.2); H-16/C-14 and C-18; H-20/C-16 ( $\delta_{\text{C}}$  120.0) and C-18; Me-21/C-17; and H-22/C-17.

Finally, through single-crystal X-ray diffraction experiments, the structure of **1** was confirmed as (5*R*,6*S*,7*S*,8*S*,9*S*,10*R*,14*S*,20*S*,22*R*,24*S*,25*S*,26*R*)-6 $\alpha$ ,7 $\alpha$ :22,26:24,25-triepoxy-5 $\alpha$ ,26-dihydroxy-17(13 $\rightarrow$ 18)-abeo-5 $\alpha$ -ergost-2,12,16-trien-1-one (Figure 1), and this compound has been named withahisolide A.

To date, withanolides containing a six-membered ring D have been confined within *Nicandra physalodes* and *Salpichroa origanifolia*, where they contain ubiquitously an aromatic ring D.<sup>1</sup> As such, compound **1** is not only the first withanolide of this rare type to have been found outside of these two genera

**Table 1.**  $^1\text{H}$  NMR Data of Withanolides 1–4 in  $\text{CDCl}_3$  (500 MHz)

position	1	2	3	4
	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{H}}$ (J in Hz)
2	5.88 ddd (10.1, 2.2, 0.9)	5.87 dd (10.1, 2.3)	5.97 dd (10.1, 2.2)	5.95 ddd (10.0, 3.1, 1.0)
3	6.63 ddd (10.1, 5.0, 2.3)	6.60 ddd (10.1, 5.1, 2.2)	6.70 ddd (10.1, 5.2, 2.3)	6.84 ddd (10.0, 4.9, 2.5)
4	2.71 m	2.68 dt (18.8, 2.6)	2.88 dt (20.8, 2.9)	3.34 brd (21.1)
	2.62 m	2.56 dd (18.8, 5.1)	2.57 dd (20.6, 5.2)	2.99 dd (21.0, 4.7)
6	3.16 d (3.9)	3.10 d (4.0)		5.92 dd (5.9, 1.9)
7	3.55 dd (3.4, 2.8)	3.60 dd (3.7, 2.2)	6.32 d (2.8)	4.66 dd (5.9, 3.3)
8	1.62 m	2.54 m		2.91 brd (12.6)
9	1.88 m	1.87 m	3.00 dt (12.5, 3.0)	2.44 td (12.2, 2.1)
11	3.10 m	2.89 dd (12.4, 6.5)	2.21 m	2.68 brdt (12.1, 2.2)
	1.87 m	1.33 m	1.45 m	1.47 m
12	5.58 d (5.5)	2.23 m	2.15 m	2.99 m
		1.95 dd (18.0, 4.5)	2.06 td (12.4, 4.0)	2.80 dt (16.1, 2.8)
14	2.46 q (8.4)			
15	2.70 m	2.98 m	2.90 ddd (18.7, 5.0, 2.5)	7.25 d (8.2)
	1.81 m	2.74 m	2.51 dd (18.7, 2.4)	
16	5.45 d (4.6)	5.50 brs	5.23 brd (2.3)	7.06 dd (8.2, 1.5)
18	2.83 d (19.4)	2.59 m	2.43 brs	6.98 brs
	2.61 m	2.43 dt (21.3, 6.5)		
19	1.13 s	1.16 s	1.09 s	1.27 s
20	2.17 m	2.18 m	2.18 m	2.75 dd (13.2, 6.8)
21	1.00 d (7.1)	1.01 d (7.1)	0.98 d (7.1)	1.25 d (7.12)
22	3.63 ddd (10.7, 7.6, 2.5)	3.67 ddd (10.7, 7.2, 2.5)	3.56 ddd (10.9, 8.4, 2.5)	3.86 dd (11.3, 5.7, 2.5)
23	1.89 m	1.88 dd (14.5, 2.5)	1.94 dd (14.5, 2.6)	1.86 dd (14.4, 2.6)
	1.59 m	1.61 dd (14.5, 11.3)	1.54 dd (14.5, 11.2)	1.59 m
26	5.01 d (10.1)	5.00 d (8.4)	4.99 d (9.7)	4.99 d (9.5)
27	1.41 s	1.41 s	1.41 s	1.40 s
28	1.40 s	1.40 s	1.39 s	1.37 s
OH-26	3.46 d (10.4)	3.47 d (9.6)	3.62 d (9.3)	3.44 d (10.5)
OH-5	3.11 brs	3.14 brs		

but also the first withanolide with a nonaromatic six-membered ring D.

Compound **2** was isolated as colorless needles from acetone–heptane. Similar to **1**, the HRESIMS and NMR data revealed the molecular formula of **2** as  $\text{C}_{28}\text{H}_{36}\text{O}_6$ . The  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and HSQC spectra of **2** showed the presence of four methyl groups [ $\delta_{\text{H}}$  1.01 (3H, d,  $J = 7.1$  Hz),  $\delta_{\text{C}}$  15.4;  $\delta_{\text{H}}$  1.16 (3H, s),  $\delta_{\text{C}}$  14.5;  $\delta_{\text{H}}$  1.40 (3H, s),  $\delta_{\text{C}}$  19.0; and  $\delta_{\text{H}}$  1.41 (3H, s),  $\delta_{\text{C}}$  16.8], six methylenes, 10 methines [including three olefinic at  $\delta_{\text{H}}$  5.50 (brs),  $\delta_{\text{C}}$  119.2;  $\delta_{\text{H}}$  5.87 (1H, dd,  $J = 10.1, 2.3$  Hz),  $\delta_{\text{C}}$  129.1; and  $\delta_{\text{H}}$  6.60 (1H, ddd,  $J = 10.1, 5.1, 2.2$  Hz),  $\delta_{\text{C}}$  139.9; together with four oxygenated at  $\delta_{\text{H}}$  3.10 (1H, d,  $J = 4.0$  Hz),  $\delta_{\text{C}}$  57.0;  $\delta_{\text{H}}$  3.60 (1H, dd,  $J = 3.7, 2.2$  Hz),  $\delta_{\text{C}}$  56.2;  $\delta_{\text{H}}$  3.67 (1H, ddd,  $J = 10.7, 7.2, 2.5$  Hz),  $\delta_{\text{C}}$  66.1; and  $\delta_{\text{H}}$  5.00 (1H, d,  $J = 8.4$  Hz),  $\delta_{\text{C}}$  91.8], and eight quaternary carbons (including a keto at  $\delta_{\text{C}}$  203.3; three olefins at  $\delta_{\text{C}}$  136.8, 128.5, and 123.8; together with three oxygenated at  $\delta_{\text{C}}$  73.2, 65.1, and 63.9),

Table 2. <sup>13</sup>C NMR Data of Withanolides 1–10 in CDCl<sub>3</sub> (125 MHz)

position	1		2		3		4		5		6		7 <sup>a</sup>		8		9		10	
	$\delta_C$	type	$\delta_C$	type	$\delta_C$	type	$\delta_C$	type	$\delta_C$	type	$\delta_C$	type	$\delta_C$	type	$\delta_C$	type	$\delta_C$	type	$\delta_C$	type
1	203.2	C	203.3	C	201.8	C	203.1	C	202.9	C	202.9	C	203.1	C	203.3	C	202.9	C	203.0	C
2	129.1	CH	129.1	CH	127.9	CH	128.3	CH	129.0	CH	129.0	CH	129.0	CH	129.1	CH	129.0	CH	129.1	CH
3	140.3	CH	139.9	CH	141.8	CH	145.0	CH	140.3	CH	140.2	CH	140.3	CH	139.9	CH	140.3	CH	140.2	CH
4	37.3	CH <sub>2</sub>	36.8	CH <sub>2</sub>	31.8	CH <sub>2</sub>	33.2	CH <sub>2</sub>	37.1	CH <sub>2</sub>	37.1	CH <sub>2</sub>	37.1	CH <sub>2</sub>	37.1	CH <sub>2</sub>	37.1	CH <sub>2</sub>	37.1	CH <sub>2</sub>
5	72.8	C	73.2	C	78.7	C	142.0	C	73.0	C	73.1	C	73.1	C	72.8	C	73.0	C	73.1	C
6	57.6	CH	57.0	CH	196.0	C	125.5	CH	57.2	CH	57.1	CH	57.1	CH	58.1	CH	57.1	CH	57.1	CH
7	56.3	CH	56.2	CH	124.2	CH	65.2	CH	55.9	CH	55.9	CH	55.9	CH	56.6	CH	55.8	CH	55.9	CH
8	41.3	CH	39.6	CH	160.7	C	41.7	CH	38.9	CH	38.9	CH	38.9	CH	40.8	CH	38.8	CH	38.8	CH
9	30.5	CH	32.2	CH	38.1	CH	34.5	CH	32.0	CH	32.0	CH	32.0	CH	35.2	CH	32.0	CH	31.9	CH
10	51.2	C	51.0	C	54.3	C	51.7	C	51.9	C	51.9	C	51.9	C	51.6	C	51.9	C	51.9	C
11	26.9	CH <sub>2</sub>	24.2	CH <sub>2</sub>	19.1	CH <sub>2</sub>	25.4	CH <sub>2</sub>	24.6	CH <sub>2</sub>	24.6	CH <sub>2</sub>	24.7	CH <sub>2</sub>	24.3	CH <sub>2</sub>	24.6	CH <sub>2</sub>	24.6	CH <sub>2</sub>
12	121.0	CH	31.4	CH <sub>2</sub>	30.5	CH <sub>2</sub>	30.7	CH <sub>2</sub>	29.6	CH <sub>2</sub>	29.6	CH <sub>2</sub>	29.7	CH <sub>2</sub>	42.9	CH <sub>2</sub>	29.6	CH <sub>2</sub>	29.6	CH <sub>2</sub>
13	135.0	C	128.5	C	61.6	C	139.6	C	137.7	C	137.6	C	137.4	(137.3), C	73.5	C	137.7	C	137.4	C
14	37.3	CH	123.8	C	59.1	C	133.8	C	136.1	C	135.9	C	135.4	(135.5), C	50.5	CH	136.5	C	135.8	C
15	31.8	CH <sub>2</sub>	28.8	CH <sub>2</sub>	28.6	CH <sub>2</sub>	126.3	CH	124.7	CH	124.5	CH	124.4	(124.3), CH	19.5	CH <sub>2</sub>	124.6	CH	124.4	CH
16	120.0	CH	119.2	CH	117.3	CH	126.3	CH	125.1	CH	125.4	CH	125.5	(125.9), CH	36.5	CH <sub>2</sub>	126.0	CH	125.5	CH
17	139.2	C	136.8	C	134.9	C	141.2	C	140.4	C	140.3	C	142.6	(142.0), C	100.0	C	138.9	C	140.5	C
18	32.9	CH <sub>2</sub>	32.2	CH <sub>2</sub>	30.2	CH <sub>2</sub>	129.9	CH	129.0	CH	129.1	CH	128.6	(128.7), CH	21.0	C	129.5	CH	129.1	CH
19	14.4	CH <sub>3</sub>	14.5	CH <sub>3</sub>	14.8	CH <sub>3</sub>	17.5	CH <sub>3</sub>	14.3	CH <sub>3</sub>	14.3	CH <sub>3</sub>	14.3	CH <sub>3</sub>	14.6	C	14.4	CH <sub>3</sub>	14.3	CH <sub>3</sub>
20	45.1	CH	44.9	CH	45.5	CH	43.3	CH	43.8	CH	43.4	CH	44.3	(44.1), CH	38.0	CH	43.9	CH	43.7	CH
21	15.7	CH <sub>3</sub>	15.4	CH <sub>3</sub>	15.4	CH <sub>3</sub>	17.3	CH <sub>3</sub>	16.6	CH <sub>3</sub>	16.8	CH <sub>3</sub>	18.3	(17.6), CH <sub>3</sub>	14.1	C	17.1	CH <sub>3</sub>	18.1	CH <sub>3</sub>
22	65.8	CH	66.1	CH	66.0	CH	67.6	CH	82.3	CH	75.6	CH	71.9	(76.1), CH	72.8	CH	81.6	CH	76.1	CH
23	34.6	CH <sub>2</sub>	34.4	CH <sub>2</sub>	34.8	CH <sub>2</sub>	33.9	CH <sub>2</sub>	35.0	CH <sub>2</sub>	33.8	CH <sub>2</sub>	40.4	(40.0), CH <sub>2</sub>	36.2	CH <sub>2</sub>	37.0	CH <sub>2</sub>	35.2	CH <sub>2</sub>
24	65.2	C	65.1	C	65.0	C	65.2	C	36.1	CH	43.8	CH	74.4	(73.8), C	75.3	C	77.3	C	43.3	CH
25	64.0	C	63.9	C	64.0	C	63.9	C	179.5	C	211.9	C	77.5	(76.7), C	67.9	C	72.3	C	212.0	C
26	91.8	CH	91.8	CH	91.8	CH	91.9	CH	15.2	CH <sub>3</sub>	28.2	CH <sub>3</sub>	97.4	(96.8), CH	98.5	CH	177.4	C	28.9	CH <sub>3</sub>
27	16.8	CH <sub>3</sub>	16.8	CH <sub>3</sub>	16.8	CH <sub>3</sub>	16.8	CH <sub>3</sub>	16.3	CH <sub>3</sub>	16.3	CH <sub>3</sub>	21.1	(15.2), CH <sub>3</sub>	18.7	CH <sub>3</sub>	23.2	CH <sub>3</sub>	18.0	CH <sub>3</sub>
28	19.1	CH <sub>3</sub>	19.0	CH <sub>3</sub>	19.0	CH <sub>3</sub>	19.0	CH <sub>3</sub>	160.9	CH	160.9	CH	24.4	(22.9), CH <sub>3</sub>	21.3	CH <sub>3</sub>	23.9	CH <sub>3</sub>	161.1	CH

<sup>a</sup>Shift data correspond to the 26R epimer (7a); distinct resonances for the 26S epimer (7b) are shown in parentheses.

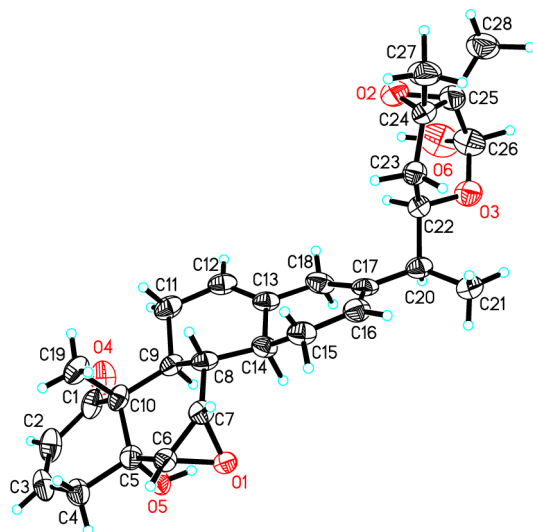


Figure 1. X-ray ORTEP drawing of withahisolide A (1).

corresponding to  $C_{28}H_{34}$ . The two remaining hydrogen atoms were therefore assigned as two hydroxy groups, indicating a seven-ring structure.

The NMR data of the isomers **1** and **2** were very similar, where the main differences observed related to the olefinic signals of ring C, with specifically the H-12 olefinic signal in **1** being absent in **2**. Moreover, a  $-CH=C-$  subunit [the C-12(13) double bond] was observed in **1**, whereas a  $-C=C-$  subunit ( $\delta_C$  128.5 and 123.8) was present in **2**. This suggested the occurrence of a C-13(14) double bond in the molecule **2**. This deduction was confirmed subsequently by the  $^1H-^1H$  COSY fragment of  $-C(8)H-C(9)H-C(11)H_2-C(12)H_2-$  in **2** [ $\delta_H$  2.54 (m, H-8), 1.87 (m, H-9), 2.89 (dd,  $J = 12.4, 6.5$  Hz, H-11 $\alpha$ ), 1.33 (m, H-11 $\beta$ ), 2.23 (m, H-12 $\alpha$ ), and 1.95 (dd,  $J = 18.0, 4.5$  Hz, H-12 $\beta$ )] and HMBC correlations including H-9/C-11 ( $\delta_C$  24.2) and C-14 ( $\delta_C$  123.8); H-11 $\alpha$ /C-8 ( $\delta_C$  39.6), C-10 ( $\delta_C$  51.0), and C-13 ( $\delta_C$  128.5); H-12 $\beta$ /C-9 ( $\delta_C$  32.2) and C-14; and Me-19/C-9.

Finally, the structure of **2** was confirmed through single-crystal X-ray diffraction experiments (Figure 2). Thus,

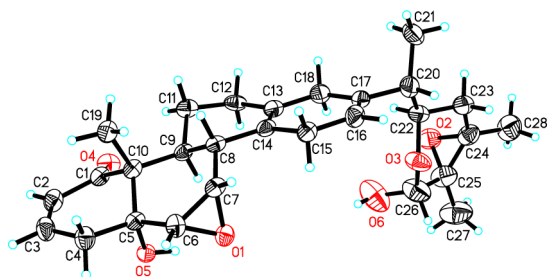


Figure 2. X-ray ORTEP drawing of withahisolide B (2).

compound **2** was identified as (5*R*,6*S*,7*S*,8*S*,9*S*,10*R*,20*S*,22*R*,24*S*,25*S*,26*R*)-6 $\alpha$ ,7 $\alpha$ :22,26:24,25-triepoxy-5 $\alpha$ ,26-dihydroxy-17(13 $\rightarrow$ 18)-abeo-5 $\alpha$ -ergost-2,13,16-trien-1-one and named withahisolide B.

Compound **3** was isolated as an amorphous solid and found to possess a molecular formula of  $C_{28}H_{34}O_7$  based on its HRESIMS and NMR data (Tables 1 and 2). Similar to **1** and **2**, the NMR spectra of **3** showed the presence of a nine-carbon side chain with an epoxy- $\delta$ -lactol system; a ring A with an  $\alpha,\beta$ -

unsaturated carbonyl and a methyl group (Me-19); and a C16(17) double bond within the six-membered ring D. In contrast to **1**, the NMR data of **3** revealed the presence of (1) an additional conjugated carbonyl moiety in ring B [ $\delta_C$  196.0 (C-6), 124.2 (C-7), and 160.7 (C-8);  $\delta_H$  6.32 (1H, d,  $J = 2.8$  Hz, H-7)], which was based on HMBC correlations of Me-19/C-9 ( $\delta_C$  38.1); H-9 ( $\delta_H$  3.00, dt,  $J = 12.5, 3.0$  Hz)/C-1 ( $\delta_C$  201.8), C-6, C-7, C-8, C-10 ( $\delta_C$  54.3), C-11 ( $\delta_C$  19.1), C-12 ( $\delta_C$  30.5), and C-19 ( $\delta_C$  14.8); H-7/C-5 ( $\delta_C$  78.7), C-8, and C-9; and H-11 $\beta$  ( $\delta_H$  1.45, m)/C-8 as well as a  $^1H-^1H$  COSY fragment of  $-C(9)H-C(11)H_2-C(12)H_2-$  [ $\delta_H$  3.00 (dt,  $J = 12.5, 3.0$  Hz, H-9), 2.21 (m, H-11 $\alpha$ ), 1.45 (m, H-11 $\beta$ ), 2.06 (td,  $J = 12.4, 4.0$  Hz, H-12 $\alpha$ ), and 2.15 (m, H-12 $\beta$ )], and (2) a C-13,14 epoxide [ $\delta_C$  61.6 (C-13) and 59.1 (C-14)] in ring C, which were assigned on the basis of the chemical shifts of C-11 ( $\delta_C$  19.1, low-frequency shift due to a  $\gamma$ -gauche effect), C-13 ( $\delta_C$  61.6), and C-14 ( $\delta_C$  59.1) together with the HMBC correlations of H-7/C-14; H-11 $\alpha$ /C-13;  $H_2$ -12/C-13 and C-14;  $H_2$ -15/C-13 and C-14; H-20 ( $\delta_H$  2.18, m)/C-18 ( $\delta_C$  30.2); and  $H_2$ -18/C-13 and C-14. Furthermore, the orientation of the epoxy was determined as 13 $\beta$ ,14 $\beta$ -epoxy based on the NOE correlations of H-12 $\alpha$ /H-20. Therefore, compound **3** (withahisolide C) was identified as (20*S*,22*R*,24*S*,25*S*,26*R*)-13 $\beta$ ,14 $\beta$ :22,26:24,25-triepoxy-5 $\alpha$ ,26-dihydroxy-17(13 $\rightarrow$ 18)-abeo-5 $\alpha$ -ergost-2,7-diene-1,6-dione.

In conjunction with the nonaromatic six-membered ring D, **3** also exhibited a C-13, C-14 epoxide. These two features are reported in withanolides for the first time.

Compound **4** was isolated as a colorless, amorphous solid and assigned a molecular formula of  $C_{28}H_{34}O_5$  based on the HRESIMS and NMR data. Through  $^1H-^1H$  COSY, HSQC, and HMBC experiments, compound **4** was found to contain moieties also observed in nicanrenone (**11**), which include an  $\alpha,\beta$ -unsaturated carbonyl in ring A; an aromatic ring D with an ABX coupling system [ $\delta_H$  7.25 (d,  $J = 8.2$  Hz, H-15), 7.06 (dd,  $J = 8.2, 1.5$  Hz, H-16), and 6.98 (brs, H-18)];  $\delta_C$  139.6 (C-13), 133.8 (C-14), 126.3 (C-15), 126.3 (C-16), 141.2 (C-17), and 129.9 (C-18)]; and an epoxy- $\delta$ -lactol system with a  $\beta,\gamma$ -dimethyl moiety in the side chain. In contrast to **11**, the 5 $\alpha$ -hydroxy-6 $\alpha$ ,7 $\alpha$ -epoxide moiety in ring B was absent in **4**, and instead a double bond and an oxygenated methine [ $\delta_H$  5.92 (1H, dd,  $J = 5.9, 1.9$  Hz), 4.66 (1H, dd,  $J = 5.9, 3.3$  Hz);  $\delta_C$  142.0, 125.5, 65.2] were observed. This partial structure in **4** was determined to be a 7-hydroxy-5,6-ene moiety in ring B based on the HMBC correlations of Me-19 ( $\delta_H$  1.27)/C-5 ( $\delta_C$  142.0); H-4 $\beta$  [ $\delta_H$  2.99 (d,  $J = 21.0, 4.7$  Hz)]/C-5 and C-6 ( $\delta_C$  125.5); and H-6 ( $\delta_H$  5.92)/C-4 and C-10, together with a  $^1H-^1H$  COSY fragment of  $-C(6)H-C(7)H-C(8)H-C(9)H-C(11)H_2-C(12)H_2-$  in **4** [ $\delta_H$  5.92 (H-6), 4.66 (H-7), 2.91 (brd,  $J = 12.6$  Hz, H-8), 2.44 (td,  $J = 12.2, 2.1$  Hz, H-9), 2.68 (brdt,  $J = 12.1, 2.2$  Hz, H-11 $\alpha$ ), 1.47 (m, H-11 $\beta$ ), 2.99 (m, H-12 $\alpha$ ), and 2.80 (dt,  $J = 16.1, 2.8$  Hz, H-12 $\beta$ )].

The 7-hydroxy group in **4** was deduced to be in an  $\alpha$ -orientation based on the small proton-proton coupling constant ( $J = 3.3$  Hz) between H-7 [ $\delta_H$  4.66 (dd,  $J = 5.9, 3.3$  Hz)] and H-8 $\beta$ . This value agreed with that observed for the 7 $\alpha$ -hydroxy-5,6-ene moiety in daturaturin A<sup>4</sup> and a 7 $\alpha$ -methoxy-5,6-ene moiety in (22*R*)-27-hydroxy-7 $\alpha$ -methoxy-1-oxowitha-3,5,24-trienolide.<sup>5</sup> Therefore, the structure of **4** (withahisolide D) was identified as (20*S*,22*R*,24*S*,25*S*,26*R*)-22,26:24,25-diepoxy-7 $\alpha$ ,26-dihydroxy-17(13 $\rightarrow$ 18)-abeo-ergost-2,5,13,15,17-pentaen-1-one.

Compound **5** was isolated as a colorless, amorphous solid and found to possess a molecular formula of  $C_{26}H_{30}O_5$ , as determined from the HRESIMS and NMR data. The NMR data of **5** (Tables 2 and 3) closely resembled those of **11** and

**Table 3.**  $^1H$  NMR Data of Withanolides **5**, **6**, and **10** in  $CDCl_3$  (500 MHz)

position	$\delta_H$ (J in Hz)		
	<b>5</b>	<b>6</b>	<b>10</b>
2	5.91 dd (10.1, 2.2)	5.91 dd (10.1, 2.1)	5.90 dd (10.1, 2.6)
3	6.65 ddd (10.1, 5.1, 2.2)	6.65 ddd (10.1, 5.1, 2.9)	6.64 ddd (10.1, 5.1, 2.3)
4	2.76 d (18.7)	2.77 dt (18.6, 6.5)	2.76 dt (18.8, 2.6)
4	2.64 m	2.66 dd (18.6, 5.1, 0.7)	2.65 dd (18.8, 5.1, 0.7)
6	3.27 d (3.9)	3.27 d (4.0)	3.26 d (4.0)
7	4.05 dd (3.7, 2.6)	4.05 dd (3.7, 2.6)	4.04 dd (3.8, 2.5)
8	3.08 dd (5.2, 3.9)	3.09 dd (6.3, 5.0)	3.08 d (11.6)
9	1.93 td (11.0, 3.6)	1.94 m	1.93 td (10.8, 3.5)
11	2.88 m	2.89 m	2.87 m
	1.58 m	1.58 m	1.57 m
12	2.98 m	3.00 m	2.97 m
	2.87 m	2.87 m	2.86 m
15	7.39 d (8.0)	7.37 d (8.0)	7.36 d (8.0)
16	7.08 dd (8.0, 1.6)	7.08 dd (8.0, 1.6)	7.05 dd (8.0, 1.6)
18	6.99 dd (1.6)	6.99 d (1.6)	6.98 d (1.6)
19	1.26 s	1.26 s	1.26 s
20	2.93 m	2.99 m	2.91 qd (7.2, 5.5)
21	1.31 d (7.1)	1.28 d (7.2)	1.27 d (7.2)
22	4.46 ddd (12.3, 6.9, 5.5)	5.26 dddd (10.3, 5.3, 2.6, 0.8)	5.13 ddd (10.2, 5.5, 2.0)
23	2.41 ddd (13.9, 8.6, 5.4)	1.92 ddd (14.5, 10.6, 5.3)	2.06 ddd (14.1, 10.2, 2.0)
	1.59 m	1.49 dddd (14.5, 8.8, 2.6, 0.8)	1.35 ddd (14.1, 10.2, 3.4)
24	2.65 m	2.46 dqd (10.6, 7.0, 2.0)	2.56 dqd (10.2, 7.2, 3.4)
26	1.21 d (7.1)	2.11 s	2.12 s
27		1.08 d (7.0)	1.06 d (7.2)
28		8.02 s	8.01 s

contained an  $\alpha,\beta$ -unsaturated carbonyl in ring A, as well as an ABX coupling system within the aromatic ring D. The obvious differences were found in the side chain, where an epoxy- $\delta$ -lactol system with  $\beta,\gamma$ -dimethyl groups was observed in **11**, whereas an  $\alpha$ -methyl- $\gamma$ -lactone [ $\delta_H$  4.46 (ddd,  $J = 12.3, 6.9, 5.5$  Hz, H-22), 2.41 (ddd,  $J = 13.9, 8.6, 5.4$  Hz, H-23 $\beta$ ), 1.59 (m, H-23 $\alpha$ ), and 2.65 (m, H-24);  $\delta_C$  82.3 (C-22), 35.0 (C-23), 36.1 (C-24), 179.5 (C-25), and 15.2 (C-26)] was identified in **5**. This observation was supported by the HMBC correlations of Me-21/C-22; H-20/C-23; Me-16 [ $\delta_H$  7.08 (dd,  $J = 8.0, 1.6$  Hz)]/C-23 and C-25; H-22/C-17 ( $\delta_C$  141.2); H-23 $\alpha$ /C-20 ( $\delta_C$  43.8); and H-23 $\beta$ /C-25 together with the typical chemical shifts of oxygenated methine (C-22,  $\delta_C$  82.3) and ester carbonyl (C-25,  $\delta_C$  179.5) groups from the  $^{13}C$  NMR spectrum of **5**. The 24R configuration was determined by the observed NOESY correlations of H-24/H-22, as this NOESY correlation can be observed when both of H-24 and H-22 are on the same side of the lactone ring. All withanolides containing C-22 within the lactone ring are identified as an R configuration when there is no substitution at C-23.<sup>6</sup> This observation, together with biogenetic considerations, supports a 22R configuration in **5**. The stereochemistry of 24R was determined by the observed

NOESY correlations of H-24/H-22, as this correlation can be observed only when both H-24 and H-22 are on the same side of the lactone ring. Therefore, the structure of **5** (withahisolide E) was elucidated as (20S,22R,24R)-6 $\alpha$ ,7 $\alpha$ -epoxy-5 $\alpha$ -hydroxy-27,28-dinor-17(13 $\rightarrow$ 18)-abeo-1-oxo-5 $\alpha$ -ergost-2,13,15,17-tetraen-22,25-olide.

The molecular formula of compound **6**, a colorless, amorphous solid, was determined to be  $C_{28}H_{34}O_6$  by HRESIMS, which is identical to that of nicaphysalin E (**10**),<sup>7</sup> a known withanolide also isolated in this study. Except for four proton signals, the  $^1H$  NMR spectra of **6** [ $\delta_H$  2.99 (1H, m), 5.26 (1H, dddd,  $J = 10.3, 5.3, 2.6, 0.8$  Hz), 1.92 (1H, ddd,  $J = 14.5, 10.6, 5.3$  Hz), 1.49 (1H, dddd,  $J = 14.5, 8.8, 2.6, 0.8$  Hz)] and **10** [ $\delta_H$  2.91 (1H, qd,  $J = 7.2, 5.5$ ), 5.13 (1H, ddd,  $J = 10.2, 5.5, 2.0$  Hz), 2.06 (1H, ddd,  $J = 14.1, 10.2, 2.0$  Hz), 1.35 (1H, ddd,  $J = 14.1, 10.2, 3.4$  Hz)] were almost superimposable. These signals were identified as corresponding to H-20, H-22, and H<sub>2</sub>-23, which agreed with the HSQC,  $^1H$ - $^1H$  COSY, and HMBC spectra. Additionally, the  $^{13}C$  NMR spectra of **6** and **10** (Table 2) were also superimposable except for four carbon signals of C-21, C-22, C-23, and C-27 in **6** [ $\delta_C$  16.8, 75.6, 33.8, and 16.3] and **10** [ $\delta_C$  18.1, 76.1, 35.2, and 18.0]. These NMR differences identified **6** and **10** as diastereomers bearing differences in their side chains. The chemical shifts of Me-21 in **6** ( $\delta_C$  16.8) and **10** ( $\delta_C$  18.1) suggested the diastereomers are different at C-22. Furthermore, **10** has been reported previously without identification of the configuration at C-22.<sup>7</sup> The same side chain observed in **10** was reported previously in salpichrolide E with a 22R configuration,<sup>8</sup> where it was proposed to arise from the oxidative cleavage of C-25-C-26 in the epoxy- $\delta$ -lactol system with 22R configuration. Thus, the stereochemistry at C-22 in **10** was deduced to be R. This led us to establish the C-22 configuration in **6** as S. Therefore, compound **6** (withahisolide F) was elucidated as (20S,22S)-6 $\alpha$ ,7 $\alpha$ -epoxy-22-formyloxy-5 $\alpha$ -hydroxy-27-nor-17(13 $\rightarrow$ 18)-abeo-5 $\alpha$ -ergost-2,13,15,17-tetraene.

Compound **7** was isolated as an amorphous solid. Its molecular formula was determined to be  $C_{28}H_{36}O_7$  based on HRESIMS and NMR data. The NMR chemical shifts pertaining to rings A–D in **7** and **11** were almost identical. NMR signal pairs corresponding to its side chain revealed that **7** was in fact a mixture of two unresolvable epimeric isomers, similar to salpichrolide M,<sup>9</sup> a reported withanolide mixture with a 24,25,26-trihydroxy-22,26-olide side chain. Analogous  $^1H$  and  $^{13}C$  NMR data and NOESY correlations were observed for the side chain signals of **7** and salpichrolide M, which were comparably utilized to identify the structure and stereochemistry of **7**. Therefore, compound **7** (withahisolide G) was identified as (20S,22R,24R,25S,26 $\Phi$ )-5 $\alpha$ ,24,25,26-tetrahydroxy-17(13 $\rightarrow$ 18)-abeo-ergost-2,13,15,17-tetraen-1-one.

Compound **8** was isolated as small cluster needles from acetone. Its molecular formula was determined as  $C_{28}H_{40}O_8$  from HRESIMS and NMR data. The  $^1H$  NMR,  $^{13}C$  NMR, and HSQC spectra of **8** showed the presence of five methyl groups [ $\delta_H$  1.14 (3H, s),  $\delta_C$  14.6;  $\delta_H$  1.15 (3H, s),  $\delta_C$  21.0;  $\delta_H$  1.17 (3H, d,  $J = 7.0$  Hz),  $\delta_C$  14.1;  $\delta_H$  1.17 (3H, s),  $\delta_C$  21.3; and  $\delta_H$  1.31 (3H, s),  $\delta_C$  18.7], six methylenes, 10 methines [including two olefinic methine groups at  $\delta_H$  5.85 (1H, dd,  $J = 10.1, 2.2$  Hz),  $\delta_C$  129.1; and  $\delta_H$  6.59 (1H, ddd,  $J = 10.1, 5.1, 2.2$  Hz),  $\delta_C$  139.9; together with four oxygenated at  $\delta_H$  3.16 (1H, d,  $J = 4.0$  Hz),  $\delta_C$  58.1;  $\delta_H$  3.56 (1H, dd,  $J = 3.8, 2.2$  Hz),  $\delta_C$  56.6;  $\delta_H$  3.95 (1H, dt,  $J = 4.7, 1.1$  Hz),  $\delta_C$  72.8; and  $\delta_H$  4.67 (1H, s),  $\delta_C$  98.5], and 11 quaternary carbons (including a carbonyl at  $\delta_C$  203.3

and five oxygenated at  $\delta_C$  67.9, 72.8, 73.5, 75.3, and 100.0), corresponding to  $C_{28}H_{37}$ . The remaining three hydrogen atoms were therefore assigned as three hydroxy groups.

Compared to **7**, compound **8** was also found to possess an  $\alpha,\beta$ -unsaturated carbonyl in ring A [ $\delta_H$  5.85 (dd,  $J = 10.1$ , 2.2 Hz, H-2), 6.59 (ddd,  $J = 10.1$ , 5.1, 2.2 Hz, H-3);  $\delta_C$  203.3 (C-1), 129.1 (C-2), 139.9 (C-3)], a  $6\alpha,7\alpha$ -epoxide in ring B [ $\delta_H$  3.16 (d,  $J = 4.0$  Hz, H-6), 3.56 (dd,  $J = 3.8$ , 2.2 Hz, H-7);  $\delta_C$  58.1 (C-6), 56.6 (C-7)], and a  $\beta,\gamma$ -dimethyl- $\delta$ -lactol system in the side chain [ $\delta_H$  3.95 (dt,  $J = 4.7$ , 1.1 Hz, H-22), 2.44 (dd,  $J = 13.7$ , 4.7 Hz, H-23 $\alpha$ ), 1.62 (m, H-23 $\beta$ ), 4.67 (s, H-26), 1.31 (s, Me-27), and 1.17 (s, Me-28);  $\delta_C$  72.8 (C-22), 36.2 (C-23), 75.3 (C-24), 67.9 (C-25), 98.5 (C-26), 18.7 (C-27), and 21.3 (C-28)].

Considerable differences between **7** and **8** were observed related to the ring D signals, where the aromatic ring with an ABX coupling system present in **7** was absent in **8**. The  $^1H$ - $^1H$  COSY spectrum showed two partial structures in **8**: (1)  $-C(7)H-C(8)H-C(14)H-C(15)H_2-C(16)H_2-$  in ring D [ $\delta_H$  3.56 (dd,  $J = 3.8$ , 2.2 Hz, H-7), 1.52 (m, H-8), 1.37 (m, H-14), 1.98 (m, H-15 $\alpha$ ), 1.40 (m, H-15 $\beta$ ), 1.56 (m, H-16 $\alpha$ ), and 1.95 (dd,  $J = 11.0$ , 3.6 Hz, H-16 $\beta$ )], confirmed by the HMBC correlations of H-7/C-8 ( $\delta_C$  40.8), H-14/C-16 ( $\delta_C$  36.5), and H-15/C-8, and (2)  $-C(8)H-C(9)H-C(11)H_2-C(12)H_2-$  in ring C [ $\delta_H$  1.52 (m, H-8), 1.66 (m, H-9), 2.82 (dq,  $J = 13.3$ , 3.3 Hz, H-11 $\alpha$ ), 1.11 (m, H-11 $\beta$ ), 1.84 (dt,  $J = 12.9$ , 3.3 Hz, H-12 $\alpha$ ), and 1.53 (m, H-12 $\beta$ )], confirmed by the HMBC correlations of Me-19 ( $\delta_H$  1.14)/C-9 ( $\delta_C$  35.2); H-9/C-12 ( $\delta_C$  42.9); H-11 $\alpha$ /C-8; H-12 $\alpha$ /C-9; and H-12 $\beta$ /C-14 ( $\delta_C$  50.5). Analysis of the HMBC spectrum revealed the Me-18 [ $\delta_H$  1.15 (3H, s);  $\delta_C$  21.0] was connected at C-13 [a quaternary carbon at  $\delta_C$  73.5] on the basis of the H-11 $\alpha$ /C-13; H-12 $\beta$ /C-13 and C-18 ( $\delta_C$  21.0); Me-18/C-12, C-13, and C-14; H-14/C-13 and C-18; and H-15/C-13 correlations. In addition, the HMBC correlations of H-15 $\alpha$ /C-17 ( $\delta_C$  100.0) and H-16 $\beta$ /C-20 identified C-17 as an oxygenated quaternary carbon. Furthermore, the absence of Me-18/C-17, H<sub>2</sub>-12/C-17, and H<sub>2</sub>-16/C-13 HMBC correlations suggested C-13 and C-17 were not directly linked. Instead, an oxygen atom linking C-13 to C-17 was identified based on the typical oxygenated chemical shifts of C-13 ( $\delta_C$  73.5) and C-17 ( $\delta_C$  100.0). Moreover, the HMBC correlation of H-26/C-17 revealed an unusual oxygen bridge between C-17 and C-26, supported by the H-26 ( $\delta_H$  4.67), C-26 ( $\delta_C$  98.5), and C-17 ( $\delta_C$  100.0) chemical shifts.

In the NOESY spectrum of **8**, the observed correlations of H-8/Me-18 and Me-18/H-20 implied that the Me-18 group and H-20 are in the  $\beta$ -orientation, while H-14 was deduced to be in an  $\alpha$ -orientation, based on the H-9/H-14 NOESY correlations. The observed H-16 $\beta$ /Me-21 correlation implied the C-17–C-21 is  $\beta$ -oriented, from which the 17S configuration was determined. The  $\beta$ -orientation of H-22 was deduced based on the coupling constant ( $J = 1.2$  Hz) between H-22 [ $\delta_H$  3.95 (ddd,  $J = 4.7$ , 1.2, 1.0 Hz)] and H-20. Furthermore, the observed H-22/Me-28, H-23 $\beta$ /Me-28, H-23 $\beta$ /H-26, and H-23 $\alpha$ /Me-27 NOESY correlations implied Me-27 is  $\alpha$ -oriented, while H-26 and Me-28 are in a  $\beta$ -orientation. Therefore, compound **8** (withahisolide H) was identified as (17S,20S,22R,24R,25S,26R)- $6\alpha,7\alpha$ :13,17:17,26:22,26-tetraepoxy-5 $\alpha$ ,24,25,26-tetrahydroxy-13,17-seco-5 $\alpha$ -ergost-2-en-1-one.

Compound **8** is based on a novel withanolide skeleton in which the C-13 to C-17 linkage is absent. Although three

withanolides, physangulidines A–C, were reported recently to also have a disconnected C-13 to C-17 linkage, these compounds present a different scaffold with an additional C-21 to C-25 linkage as compared to **8**.<sup>10</sup>

Compound **9** was isolated as an amorphous solid. The HRESIMS and NMR data indicated a molecular formula of  $C_{28}H_{34}O_7$ . The NMR data of rings A–D for **9** closely resembled those of **11**. The difference was observed within the side chain, where the common 24,25-epoxy-lactol in **11** was modified in **9** [ $\delta_H$  4.50 (1H, dt,  $J = 12.2$ , 4.4 Hz),  $\delta_C$  81.6;  $\delta_H$  2.03 (1H, m), 1.76 (1H, dd,  $J = 13.9$ , 4.7 Hz),  $\delta_C$  37.0;  $\delta_H$  1.16 (3H, s),  $\delta_C$  23.2;  $\delta_H$  1.37 (3H, s),  $\delta_C$  23.9; two oxygenated quaternary carbons at  $\delta_C$  77.3 and 72.3; and an ester carbonyl at  $\delta_C$  177.4]. Analysis of the HMBC spectrum of **9** revealed the side chain to be 24,25-dihydroxy-24,25-dimethyltetrahydro-22,26-olid-26-one [ $\delta_C$  81.6 (C-22), 37.0 (C-23), 77.3 (C-24), 72.3 (C-25), 177.4 (C-26), 23.2 (Me-27), and 23.9 (Me-28);  $\delta_H$  4.50 (H-22), 2.03 (H-23 $\alpha$ ), 1.76 (H-23 $\beta$ ), 1.16 (Me-27), and 1.37 (Me-28)]. The configuration of this side chain was identified as 22R,24R,25S based on the NOESY correlation of H-22/Me-28 and the absence of a H-22/Me-27 correlation. Therefore, compound **9** (withahisolide I) was elucidated as (20S,22R,24R,25S)-5 $\alpha$ ,24,25-trihydroxy-17(13 $\rightarrow$ 18)-abeo-ergost-2,13,15,17-tetraene-1,26-dione.

In addition to withahisolides A–I (**1–9**), nine known compounds were also isolated from *P. hispida* and identified by comparison of their spectroscopic data with values reported in the literature, as nicaphysalin E (**10**),<sup>7</sup> nicandrenone (Nic-1, **11**),<sup>3</sup> nicandrenone methyl ether (**12**),<sup>11</sup> nicandrenone 12 (Nic-12, **13**),<sup>12</sup> salpichrolides A (**14**),<sup>13</sup> C (**15**),<sup>14</sup> and N (**16**),<sup>9</sup> and physalindicanols A (**17**) and B (**18**).<sup>4,15</sup>

The 18 diverse withanolide-related compounds isolated in this study represent four structural types: (1) six-membered ring D withanolides, which include aromatic ring D withanolides (**4–7** and **9–15**) as well as the nonaromatic six-membered ring D withanolides (**1–3**); (2) a novel withanolide skeleton with an absent C-13 to C-17 linkage (**8**); (3) a withanolide with rearranged Me-18 (**16**); and (4) withanolide precursors (**17** and **18**).<sup>4,15</sup>

Evidence pertaining to the biosynthesis of the six-membered ring D was observed through carbon skeleton comparisons of **1**, **2**, **10**, **11**, and **16**. Previously, it was proposed that oxidation of the angular methyl group (C-18) of a five-membered ring D withanolide, via cleavage of the C-13–C-17 bond of the cyclopropyl-fused intermediate (C-13–C-18–C-17), would yield a six-membered ring D, which through subsequent aromatization would form the aromatic ring D, as present in **10** and **11**.<sup>16</sup> In addition, cleavage of the C-13–C-18 bond of the same cyclopropyl-fused intermediate would result in migration of the angular methyl group (C-18) to C-17, as observed in **16**.<sup>9</sup> Therefore, the double bonds observed in **1** [C-12(13), C-16(17)] and **2** [C-13(14), C-16(17)] would form prior to the aromatic ring D in **11**.

Previously, aromatic ring D withanolides have been isolated from two genera of the Solanaceae, where the 5 $\alpha,6\alpha$ -epoxy variety is restricted to *Salpichroa organifolia*<sup>6,8,9,13,14,17,18</sup> and *S. tristis* var. *lehmannii*,<sup>19</sup> while the 6 $\alpha,7\alpha$ -epoxy variety is confined to *Nicandra physaloides*.<sup>6,7,11,12a,b,18</sup> In our opinion, these functional groups may be considered as chemotaxonomic markers for these two genera. Since aromatic ring D withanolides carrying either 5 $\alpha,6\alpha$ -epoxy (**14** and **15**) or 6 $\alpha,7\alpha$ -epoxy (**5–7**, **9–13**) moieties were isolated from *P. hispida*, this suggests a close chemotaxonomic relationship of *P.*

*hispidia* with *N. physalodes*, *S. organifolia*, and *S. tristis* var. *lehmannii*.

## 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  $^1\text{H}$  NMR, APT, COSY, HSQC, HMBC, and NOESY/ROESY experiments. Chemical shift values are given in  $\delta$  (ppm) using the peak signals of the solvent  $\text{CDCl}_3$  ( $\delta_{\text{H}}$  7.26 and  $\delta_{\text{C}}$  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  $\text{C}_{18}$  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  $\times$  10 mm, 5  $\mu\text{m}$ ).

**Plant Material.** The aerial parts of *P. hispidia* were collected from Morton County (latitude: 37°10'5" N; longitude: 101°41'33" W), Kansas, USA, by Hillary Loring in August 2010. It was identified by plant taxonomist Dr. Kelly Kindscher at the Kansas Biological Survey, University of Kansas. A voucher specimen (Hillary Loring 4106) was deposited in the R.L. McGregor Herbarium of the University of Kansas.

**Extraction and Isolation.** The collected biomass was air-dried, ground to a coarse powder (1380 g), and extracted three times with  $\text{CH}_2\text{Cl}_2$ –MeOH (50:50, 2.0 L) at room temperature. After removing the solvents under vacuum, the extract (174.8 g) was suspended in 2.0 L of  $\text{H}_2\text{O}$ , followed by successive partitions to yield the *n*-hexane, ethyl acetate, and *n*-butanol fractions. The ethyl acetate fraction (34.8 g) was applied to silica gel (30  $\times$  400 mm) MPLC eluted with increasing polarities of hexane–acetone mixtures (90:10 to 25:75) to afford combined fractions 1 to 9. Fr1 (0.3 g) was subjected to Sephadex LH-20 CC (eluted with acetone) to afford fractions 1-1 and 1-2. Semipreparative HPLC purification of fr1-1 eluted by isocratic  $\text{CH}_3\text{CN}$  (80%) and fr1-2 eluted by isocratic  $\text{CH}_3\text{CN}$  (77%) afforded 17 (3.1 mg) and 18 (4.0 mg), respectively. Fr2 (0.3 g) was subjected to CombiFlash CC (24 g silica gel), eluted with  $\text{CH}_2\text{Cl}_2$ –acetone (95:5 to 85:15) with increasing amounts of acetone, after which the resulting fraction 2-1 was subjected to semipreparative HPLC, eluted by 60% isocratic  $\text{CH}_3\text{CN}$  (60%), to afford 12 (2.7 mg). Fr3 (0.51 g) was applied to Sephadex LH-20 CC (eluted with acetone) to yield fractions 3-1, 3-2 and 3-3. Semipreparative HPLC of fr3-1 (73.2 mg), fr3-2 (50.4 mg), and fr3-3 (68.5 mg), eluted with 60% isocratic  $\text{CH}_3\text{CN}$ , afforded 10 (8.3 mg), 12 (3.6 mg), 11 (4.4 mg), and 14 (11.7 mg). Fr4 (0.5 g) was applied to a Sephadex LH-20 column to afford fraction 4-1 (40.7 mg), which was subjected to semipreparative HPLC and eluted with gradient  $\text{CH}_3\text{CN}$  (30–60%) to yield 11 (15.2 mg) and 14 (7.4 mg). Fr5 (0.6 g) was applied to CombiFlash CC (40 g ODS), eluted with  $\text{CH}_3\text{CN}$ –water (20:80 to 60:40) with increasing amounts of  $\text{CH}_3\text{CN}$ , to afford fractions 5-1 and 5-2. Fr5-1 (23.0 mg) was subjected to semipreparative HPLC, eluted by isocratic  $\text{CH}_3\text{CN}$  (30%), to afford 3 (6.6 mg). Fr5-2 (102.2 mg) was applied to CombiFlash CC (24 g silica gel), eluted with  $\text{CH}_2\text{Cl}_2$ –EtOAc (90:10 to 80:20), with increasing amounts of EtOAc, to yield two fractions, 5-2-1 and 5-2-2. Fr5-2-1 was subjected to semipreparative HPLC, eluting with isocratic  $\text{CH}_3\text{CN}$  (38%), and yielded three compounds, 11 (11.1 mg), 1 (3.1 mg), and 2 (29.5 mg). Fr6 (0.58 g) was applied to CombiFlash CC (40 g ODS), eluted with  $\text{CH}_3\text{CN}$ –water (20:80 to

50:50) with increasing amounts of  $\text{CH}_3\text{CN}$ , to afford two fractions, 6-1 and 6-2. Compound 3 (6.9 mg) was obtained from the semipreparative HPLC of fr6-1 (21.1 mg), eluted by isocratic  $\text{CH}_3\text{CN}$  (30%). Fr6-2 (43.4 mg) was subjected to semipreparative HPLC, eluted by isocratic  $\text{CH}_3\text{CN}$  (30%), to afford 16 (2.0 mg), 11 (1.8 mg), 4 (1.7 mg), 1 (1.5 mg), and 2 (5.6 mg). Fr7 (0.70 g) was applied to CombiFlash CC (40 g silica gel), eluted with  $\text{CH}_2\text{Cl}_2$ –acetone (98:2 to 88:12) with increasing amounts of acetone, to yield fractions 7-1 and 7-2. Fr7-1 (3.7 mg) and fr7-2 (5.6 mg) were combined and separated by semipreparative HPLC, eluted with 30–50%  $\text{CH}_3\text{CN}$ , to afford 13 (0.6 mg), 5 (1.1 mg), 6 (1.2 mg), and 10 (2.9 mg). Fr8 (0.61 g) was applied to CombiFlash CC (40 g silica gel), eluted with  $\text{CH}_2\text{Cl}_2$ –acetone (98:2, 95:5, 92:8, 90:10, 88:12), with increasing amounts of acetone, to yield two fractions, 8-1 and 8-2. Compound 8 (4.4 mg) was obtained from semipreparative HPLC of fr8-1 (35.3 mg) eluted by isocratic  $\text{CH}_3\text{CN}$  (25%). Compound 15 (6.4 mg) was obtained from the semipreparative HPLC of fr8-2 (29.3 mg) eluted by isocratic  $\text{CH}_3\text{CN}$  (33%). Fr9 (2.02 g) was applied to CombiFlash CC (150 g of ODS), eluted with  $\text{CH}_3\text{CN}$ –water (10:90, 15:85, 18:82, 20:80, 22:78, 25:75), with increasing amounts of  $\text{CH}_3\text{CN}$ , to afford fraction 9-1. Fr9-1 (80.5 mg) was subjected to semipreparative HPLC, eluted by isocratic  $\text{CH}_3\text{CN}$  (30%), to yield fr9-1-1, fr9-1-2, and fr9-1-3. Fr9-1-1 (9.2 mg) and fr9-1-2 (11.2 mg) were combined and applied to CombiFlash CC (4 g silica gel), eluted with hexane–acetone (75:25 to 40:60), with increasing amounts of acetone, to yield 7 (5.8 mg). Compound 9 (2.3 mg) was obtained from the semipreparative HPLC purification of fr9-1-3, eluted by isocratic  $\text{CH}_3\text{CN}$  (30%).

**Withahisolid A** [(5R,6S,7S,8S,9S,10R,14S,20S,22R,24S,25S,26R)-6 $\alpha$ ,7 $\alpha$ :22,26:24,25-triepoxy-5 $\alpha$ ,26-dihydroxy-17(13 $\rightarrow$ 18)-abeo-5 $\alpha$ -ergost-2,12,16-trien-1-one] (1):  $[\alpha]_{\text{D}}^{25}$  +0.21 (c 0.09,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 229 (1.17) nm; IR (neat)  $\nu_{\text{max}}$  3332 (br), 2927, 1672, 1453, 1021  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Tables 1 and 2; HRESIMS  $m/z$  491.2404  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{28}\text{H}_{36}\text{O}_6\text{Na}$ , 491.2410).

**Withahisolid B** [(5R,6S,7S,8S,9S,10R,20S,22R,24S,25S,26R)-6 $\alpha$ ,7 $\alpha$ :22,26:24,25-triepoxy-5 $\alpha$ ,26-dihydroxy-17(13 $\rightarrow$ 18)-abeo-5 $\alpha$ -ergost-2,13,16-trien-1-one] (2):  $[\alpha]_{\text{D}}^{25}$  +1.3 (c 0.3,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 240 (1.69) nm; IR (neat)  $\nu_{\text{max}}$  3443 (br), 2926, 1686, 1032  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Tables 1 and 2; HRESIMS  $m/z$  491.2398  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{28}\text{H}_{36}\text{O}_6\text{Na}$ , 491.2410).

**Withahisolid C** [(20S,22R,24S,25S,26R)-13 $\beta$ ,14 $\beta$ :22,26:24,25-triepoxy-5 $\alpha$ ,26-dihydroxy-17(13 $\rightarrow$ 18)-abeo-5 $\alpha$ -ergost-2,7-diene-1,6-dione] (3):  $[\alpha]_{\text{D}}^{25}$  +0.47 (c 0.1,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 233 (3.04) nm; IR (neat)  $\nu_{\text{max}}$  3423 (br), 2926, 1679, 1029  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Tables 1 and 2; HRESIMS  $m/z$  505.2180  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{28}\text{H}_{34}\text{O}_7\text{Na}$ , 505.2202).

**Withahisolid D** [(20S,22R,24S,25S,26R)-22,26:24,25-diepoxy-7 $\alpha$ ,26-dihydroxy-17(13 $\rightarrow$ 18)-abeo-ergost-2,5,13,15,17-pentaen-1-one] (4):  $[\alpha]_{\text{D}}^{25}$  –0.85 (c 0.03,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 232 (7.05) nm; IR (neat)  $\nu_{\text{max}}$  3333 (br), 2926, 1032  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Tables 1 and 2; HRESIMS  $m/z$  473.2286  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{28}\text{H}_{34}\text{O}_5\text{Na}$ , 473.2304).

**Withahisolid E** [(20S,22R,24R)-6 $\alpha$ ,7 $\alpha$ -epoxy-5 $\alpha$ -hydroxy-27,28-dinor-17(13 $\rightarrow$ 18)-abeo-1-oxo-5 $\alpha$ -ergost-2,13,15,17-tetraen-22,25-olide] (5):  $[\alpha]_{\text{D}}^{25}$  +0.12 (c 0.1,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 225 (2.50) nm; IR (neat)  $\nu_{\text{max}}$  3340 (br), 2925, 1766, 1687  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Tables 2 and 3; HRESIMS  $m/z$  445.1972  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{26}\text{H}_{30}\text{O}_5\text{Na}$ , 445.1991).

**Withahisolid F** [(20S,22S)-6 $\alpha$ ,7 $\alpha$ -epoxy-22-formyloxy-5 $\alpha$ -hydroxy-27-nor-17(13 $\rightarrow$ 18)-abeo-5 $\alpha$ -ergost-2,13,15,17-tetraene] (6):  $[\alpha]_{\text{D}}^{25}$  +0.18 (c 0.1,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 229 (1.84) nm; IR (neat)  $\nu_{\text{max}}$  3332 (br), 2925, 1730, 1023  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Tables 2 and 3; HRESIMS  $m/z$  489.2238  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{28}\text{H}_{34}\text{O}_6\text{Na}$ , 489.2253).

**Withahisolid G** [(20S,22R,24R,25S,26F)-5 $\alpha$ ,24,25,26-tetrahydroxy-17(13 $\rightarrow$ 18)-abeo-ergost-2,13,15,17-tetraen-1-one] (7):  $[\alpha]_{\text{D}}^{25}$  +0.01 (c 0.5,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 231 (1.68) nm; IR (neat)  $\nu_{\text{max}}$  3428 (br), 2925, 1685, 1377, 1066  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Tables 2 and 4; HRESIMS  $m/z$  507.2357  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{28}\text{H}_{36}\text{O}_7\text{Na}$ , 507.2359).

**Table 4.**  $^1\text{H}$  NMR Data of Withanolides 7–9 in  $\text{CDCl}_3$  (500 MHz)

position	7a	7b	8	9
	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{H}}$ (J in Hz)
2	5.92 dd (10.2, 2.4)	5.92 dd (10.2, 2.3)	5.85 dd (10.1, 2.2)	5.91 dd (10.2, 2.5)
3	6.67 ddd (10.1, 5.1, 2.3)	6.67 ddd (10.1, 5.1, 2.3)	6.59 ddd (10.1, 5.1, 2.2)	6.65 ddd (10.1, 5.1, 2.1)
4	2.76 brs	2.76 brs	2.68 dt (18.9, 2.0)	2.77 brd (18.7, 2.0)
	2.65 dd (18.7, 5.1)	2.65 dd (18.7, 5.1)	2.56 ddd (18.8, 5.1, 0.8)	2.64 dd (18.7, 5.2)
6	3.28 d (3.91)	3.28 d (3.91)	3.16 d (4.0)	3.27 d (3.9)
7	4.07 brs	4.07 brs	3.56 dd (3.8, 2.2)	4.05 t (3.6)
8	3.1	3.1	1.52 m	3.09 m
9	1.95 m	1.95 m	1.66 m	1.91 dd
11	2.98 m	2.98 m	2.82 dq (13.3, 3.3)	2.88 m
	1.59 m	1.59 m	1.11 m	1.59 m
12	2.99 m	2.99 m	1.84 dt (12.9, 3.3)	2.97 m
	2.88 m	2.88 m	1.53 m	2.88 m
14			1.37 m	
15	7.37 d (8.0)	7.36 d (8.0)	1.98 m	7.38 d (8.0)
			1.40 m	
16	7.10 brd (7.2)	7.08 brd (7.2)	1.95 dd (11.0, 3.6)	7.09 dd (8.1, 1.5)
			1.56 m	
18	7.00 brs	7.01 brs	1.15 s	6.99 d (1.5)
19	1.26 s	1.26 s	1.14 s	1.27 s
20	2.81 t (7.0)	2.86 t (7.0)	1.62 m	2.97 m
21	1.25 d (7.0)	1.29 d (7.0)	1.17 d (7.0)	1.43 d (7.2)
22	4.16 ddd (10.8, 7.1, 3.0)	3.70 ddd (11.4, 6.0, 2.9)	3.95 ddd (4.7, 1.2, 1.0)	4.50 dt (12.2, 4.4)
23	1.76 dd (13.3, 3.1)	1.76 dd (13.3, 3.1)	2.44 dd (13.7, 4.7)	2.03 m
	1.60 m	1.60 m	1.62 m	1.76 dd (13.9, 4.7)
26	4.95 d (2.0)	4.61 s	4.67 s	
27	1.27 s	1.21 s	1.31 s	1.16 s
28	1.46 s	1.36 s	1.17 s	1.37 s
OH-5			3.14 brs	

*Withahisolide H* [(17S,20S,22R,24R,25S,26R)-6 $\alpha$ ,7 $\alpha$ :13,17:17,26:22,26-tetraepoxy-5 $\alpha$ ,24,25,26-tetrahydroxy-13,17-seco-5 $\alpha$ -ergost-2-en-1-one] (**8**):  $[\alpha]_{\text{D}}^{25}$   $-0.01$  ( $c$  0.5,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 235 (1.68) nm; IR (neat)  $\nu_{\text{max}}$  3490 (br), 2924, 1685  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Tables 2 and 4; HRESIMS  $m/z$  527.2609  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{28}\text{H}_{40}\text{O}_8\text{Na}$ , 527.2621).

*Withahisolide I* [(20S,22R,24R,25S)-5 $\alpha$ ,24,25-trihydroxy-17(13 $\rightarrow$ 18)-abeo-ergost-2,13,15,17-tetraene-1,26-dione] (**9**):  $[\alpha]_{\text{D}}^{25}$   $+0.45$  ( $c$  0.1,  $\text{CHCl}_3$ ); UV ( $\text{CHCl}_3$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 239 (2.36) nm, 270 (1.56) nm, 320 (1.46) nm; IR (neat)  $\nu_{\text{max}}$  3398 (br), 2925, 1682, 1062  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, see Tables 2 and 4; HRESIMS  $m/z$  505.2194  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{28}\text{H}_{34}\text{O}_7\text{Na}$ , 505.2202).

**X-ray Crystallographic Analysis.** *Crystal data for withahisolide A* (**1**):  $\text{C}_{28}\text{H}_{36}\text{O}_6$ , fw = 468.57, monoclinic space group  $P2_1$ ,  $C_2$  (No. 4),<sup>20</sup> crystal cell parameters  $a = 12.0702(11)$  Å,  $b = 6.6948(7)$  Å,  $c = 15.1204(12)$  Å,  $\alpha = 90^\circ$ ,  $\beta = 100.271(6)^\circ$ ,  $\gamma = 90^\circ$ ,  $V = 1202.3(2)$  Å<sup>3</sup>,  $D_c = 1.294$  g/cm<sup>3</sup>,  $Z = 2$ , crystal dimensions  $0.29 \times 0.02 \times 0.01$  mm<sup>3</sup>, absorption coefficient  $\mu_a(\text{Cu K}\alpha) = 0.726$  mm<sup>-1</sup>,  $F(000) = 504$ . A total of 9173 reflections were measured in the range  $2.97^\circ < \theta < 69.72^\circ$ , with 3381 independent reflections [ $R_{\text{int}} = 0.043$ ]; completeness to  $\theta_{\text{max}} = 66.00^\circ$  was 98.0%. The numbers of data/restraints/parameters were 3381/1/451; goodness-of-fit on  $F^2 = 1.032$ ; final  $R$  indices [ $I > 2\sigma(I)$ ],  $R_1 = 0.045$ ,  $wR_2 = 0.110$ ;  $R$  indices (all data),  $R_1 = 0.058$ ,  $wR_2 = 0.119$ ; largest difference peak and hole, 0.21 and  $-0.18$  e<sup>-</sup>/Å<sup>3</sup>.

*Crystal data for withahisolide B monohydrate* (**2**):  $\text{C}_{28}\text{H}_{36}\text{O}_6 \cdot \text{H}_2\text{O}$ , fw = 486.58, orthorhombic, space group  $P2_12_12_1$ ,  $D_2^4$  (No. 19),<sup>20</sup> crystal cell parameters  $a = 6.5559(9)$  Å,  $b = 11.7079(13)$  Å,  $c = 33.660(4)$  Å,  $V = 2583.6(5)$  Å<sup>3</sup>,  $D_c = 1.251$  g/cm<sup>3</sup>,  $Z = 4$ , crystal dimensions  $0.50 \times 0.10 \times 0.02$  mm<sup>3</sup>, absorption coefficient  $\mu_a(\text{Cu K}\alpha) = 0.723$  mm<sup>-1</sup>,  $F(000) = 1048$ . A total of 13 831 reflections were collected in the range  $2.63^\circ < \theta < 69.60^\circ$ , with 4446 independent reflections [ $R_{\text{int}} = 0.052$ ]; completeness to  $\theta_{\text{max}} = 66.00^\circ$  was 96.3%. The numbers of data/restraints/parameters were 4446/0/333; goodness-of-fit on  $F^2 = 1.064$ ; final  $R$  indices [ $I > 2\sigma(I)$ ],  $R_1 = 0.071$ ,  $wR_2 = 0.176$ ;  $R$  indices (all data),  $R_1 = 0.087$ ,  $wR_2 = 0.187$ ; largest difference peak and hole, 0.85 and  $-0.31$  e<sup>-</sup>/Å<sup>3</sup>.

All crystallographic data were collected<sup>21</sup> at 100(2) K using monochromated Cu K $\alpha$  radiation ( $\lambda = 1.54178$  Å) on a Bruker Proteum Single Crystal Diffraction System equipped with Helios multilayer optics, an APEX II CCD detector, and a Bruker MicroSTAR microfocus rotating anode X-ray source operating at 45 kV and 60 mA. Lattice constants were determined with the Bruker SAINT software package.<sup>22</sup> Colorless single crystals of both compounds were obtained from acetone–heptane. Multiscan absorption corrections were applied. Both structures were solved by direct methods with the SHELXTL software package.<sup>23</sup> All non-hydrogen atoms for both structures were refined anisotropically. The H atoms for **1** were located from a difference Fourier and included in the structural model as individual isotropic atoms whose parameters were allowed to vary in least-squares refinement cycles. All hydrogen atoms for **2** were initially located from a difference Fourier. All but two hydrogens (ethylenic hydrogen atoms H-2 and H-3) for **2** were then included in the structural model at idealized positions (sp<sup>3</sup>-hybridized geometry with O–H bond lengths of 0.84 Å and C–H bond lengths of 0.98–1.00 Å) and with isotropic thermal parameters that were fixed at values 1.20 (nonmethyl) or 1.50 (methyl) times the equivalent isotropic thermal parameter of the carbon or oxygen atom to which they were covalently bonded. Four methyl groups and two hydroxy groups were refined as idealized sp<sup>3</sup>-hybridized rigid rotors that were allowed to rotate about their C–C or C–O bonds in least-squares refinement cycles. Hydrogen atoms bonded to water oxygen atom O1w were fixed at idealized positions near their difference Fourier positions.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

$^1\text{H}$  NMR,  $^{13}\text{C}$  NMR (APT), and 2D NMR spectra of withahisolides A–I (**1**–**9**) are available. This material is available free of charge via the Internet at <http://pubs.acs.org>. Crystallographic data for the structure of **1** and **2** as reported in this paper were deposited at the Cambridge Crystallographic Data Centre, under reference numbers CCDC 970816 and 970817, 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 e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)).

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### Notes

The authors declare no competing financial interest.

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## ■ DEDICATION

Dedicated to Prof. Dr. Otto Sticher, of ETH-Zurich, Zurich, Switzerland, for his pioneering work in pharmacognosy and phytochemistry.

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