Cytotoxic Withaphysalins from *Physalis minima*

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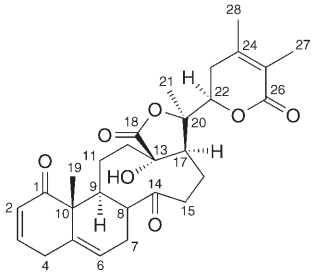
A novel withanolide, withaphysalin P (**1**) with a nine-membered ring, and six other new withaphysalins, **2**–**7**, together with the three known withaphysalins **8**–**10** were isolated from *Physalis minima* L. The structures were deduced by means of spectroscopic analyses, and that of withaphysalin P (**1**) was confirmed by a single-crystal X-ray diffraction analysis. Plausible biosynthetic pathways were postulated (*Scheme 1*). All compounds were tested for their antiproliferative activities toward the human colorectal-carcinoma HCT-116 cells and human nonsmall-cell lung-cancer NCI-H460 cells (*Table 4*). Compounds **1**–**3**, **7**–**10**, **7a**, and **7b** displayed moderate cytotoxic activity against the two human cancer cell lines.

Introduction. – Withanolides are steroidal lactones with an ergostane skeleton, *i.e.*, 22-hydroxyergostan-26-oic acid δ-lactones. These compounds are specific to the Solanaceae family, and in particular to the genera *Physalis*, *Withania*, *Datura, Acnistus*, *Dunalia*, *Lycium*, *Vassobia, Deprea, Exodeconus, Iochroma, Tacca, Jaborosa*, *Nicandra, Tubocapsicm*, and *Salpichroa* [1–3]. These compounds are generally polyoxygenated, and this profusion of O-functions has led to several natural modifications of the carbocyclic skeleton as well as of the side chain, resulting in compounds with complex structural features classified as withanolides, physalins, withaphysalins, perulactones, ixocarpalactones, and acnistins. The biological activities of withanolides had been studied extensively in the past. Some notable activities were reported for these compounds including cytotoxicity and immunosuppressive, antitumor, anti-inflammatory, anticonvulsive, and antioxidant properties [1–3]. A mechanistic study concerning the cytotoxicity and antitumor activity of the withanolides has been reported recently [4].

The withaphysalins are characterized by oxygenation at the Me(18) group to the level of an alcohol, aldehyde, or acid function, which in the latter two cases can lead to cyclization with the OH–C(20) to afford a hemiacetal or a lactone. The withaphysalins have been evaluated for several biological activities, including cytotoxicity [5] and antibacterial, antileishmanial, antitrypanocidal [6], and induction of quinone reductase activity [7]. The withaphysalins are more limited in their distribution and have, thus far, been shown to be present in the genera *Dunalia*, *Iochroma*, *Physalis*, *Vassobia*, and *Acnistus*, from which withaphysalins A–O have been isolated and elucidated [8–14].

Physalis minima, known in China as 'Xiaosuanjiang', is a branched annual herb and is widely distributed throughout tropical and subtropical regions of the world. Extracts or infusions from this plant have been used in various countries in folk medicine as a treatment for different illnesses, such as spleen disorder, or as a tonic, diuretic, purgative, anticancer or antimycobacterial agent [9][10]. In continuation of our phytochemical work on the plants of the *Physalis* genus [5][15], the seven new withaphysalins **1**–**7**, together with three known withaphysalins, withaphysalin A (**8**) [8], withaphysalin C (**9**) [14], and (5α,6α)-5,6-epoxywithaphysalin A (**10**) [9] were isolated from the aerial part of *P. minima*.



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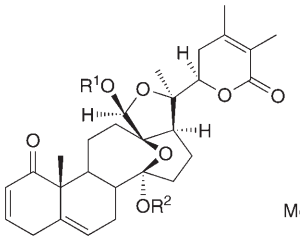

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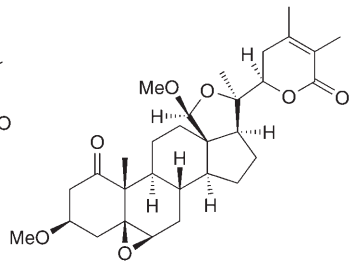
3 R^1 = Ac, R^2 = Ac



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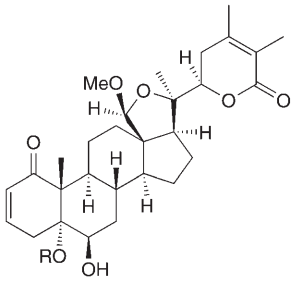

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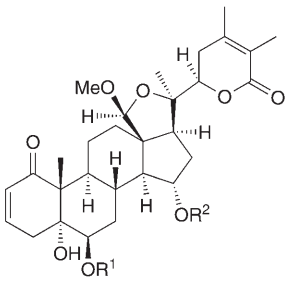
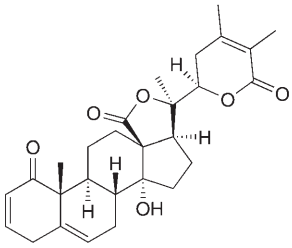
6 R = OMe



7 R^1 = H, R^2 = H
7a R^1 = H, R^2 = Ac
7b R^1 = Ac, R^2 = Ac



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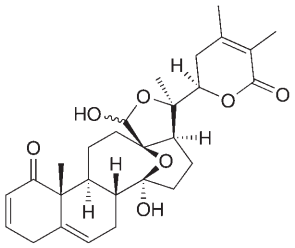

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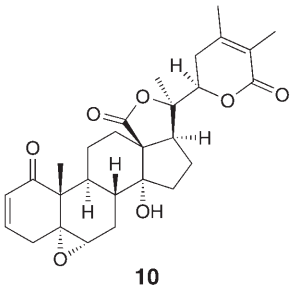

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Results and Discussion. – 1. *Structure Elucidation.* Compound **1** was isolated as colorless cubic crystals. Its molecular formula, indicating twelve degrees of unsaturation, was established as $C_{28}H_{34}O_7$ from the HR-EI-MS (M^+ at m/z 482.2296). The IR spectrum showed the presence of C=O groups at 1750, 1735, and 1676 cm^{-1}, consistent with the presence of an α,β-unsaturated-δ-lactone, a γ-lactone, and an α,β-unsaturated-ketone moiety, respectively. The structure of **1**, named withaphysalin P, was derived from the ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), COSY, and HMBC data (*Fig. 1*), and the spectacular novel nine-membered ring moiety (as compared to the typical withanolide skeleton) was confirmed by a single-crystal X-ray-analysis (*Fig. 2*).

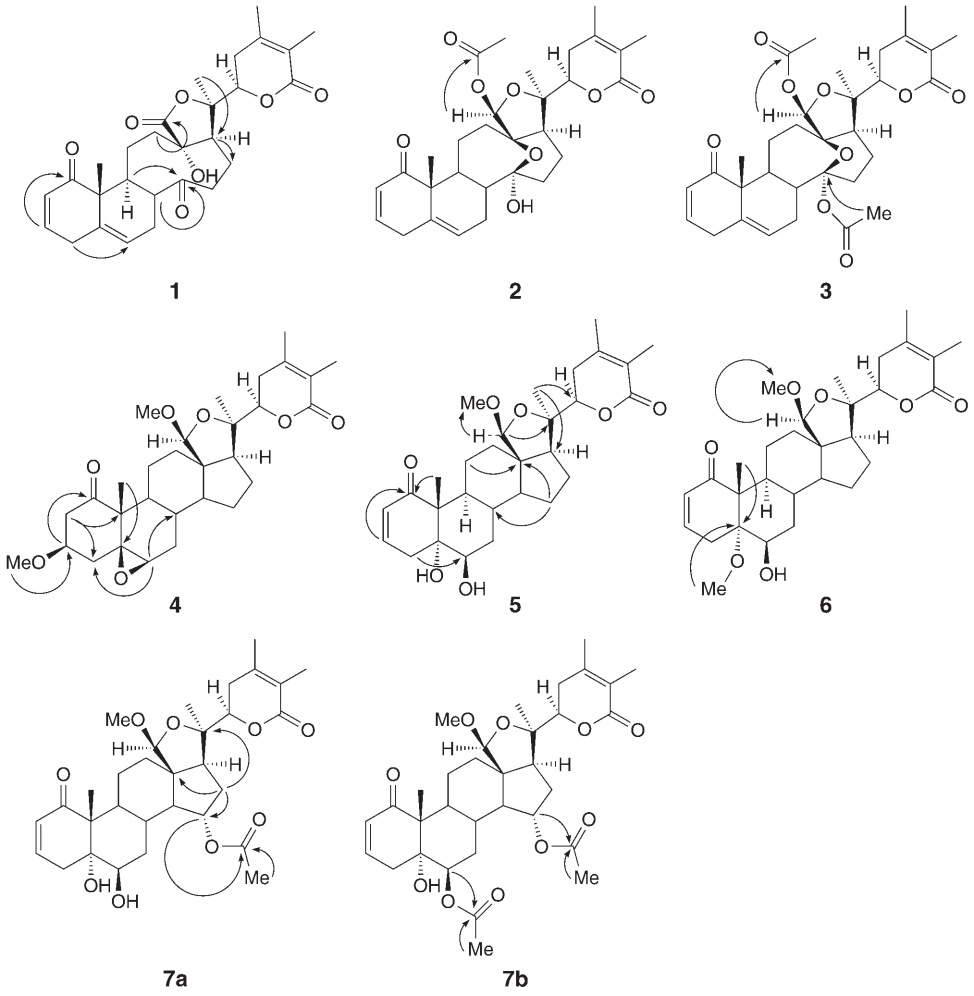


Fig. 1. *Key HMBC correlations of* **1**–**6**, **7a**, *and* **7b**

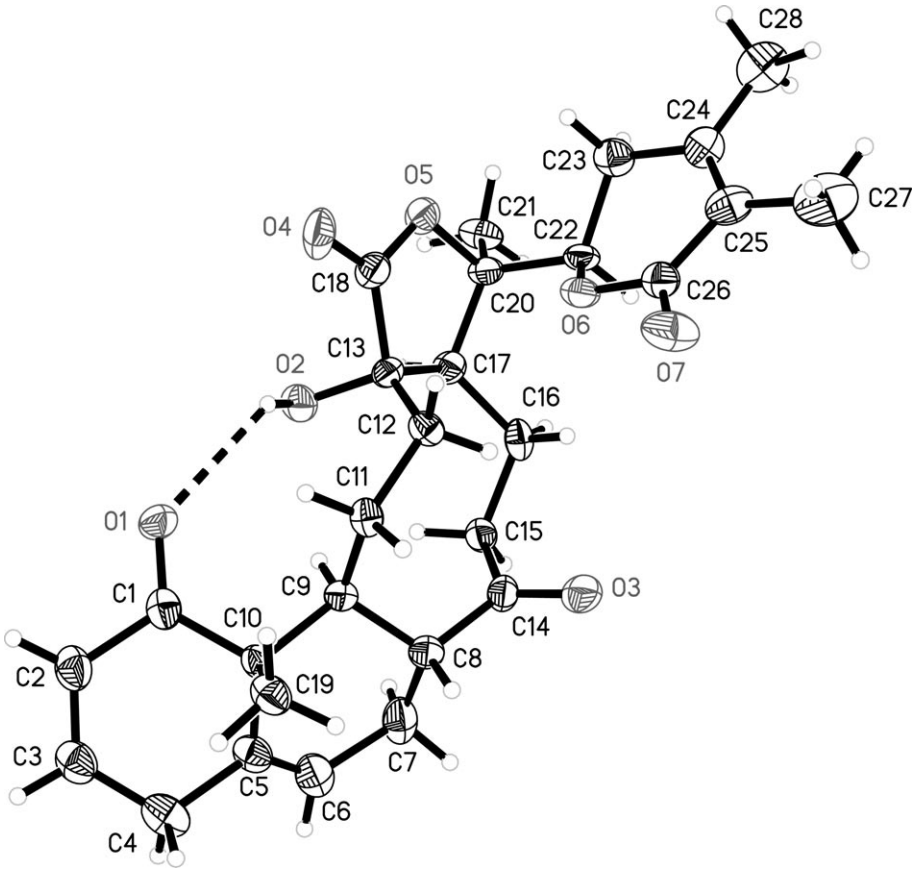


Fig. 2. *X-Ray structure of* **1** *showing relative configuration*

According to the ^{13}C-NMR DEPT data, **1** contained four Me groups, seven CH_2, and seven CH (including three olefinic CH at δ(C) 127.2, 146.0, and 122.1, and one oxygenated CH at δ(C) 75.8). Ten quaternary C-atoms, of which three were sp^2 C-atoms (δ(C) 135.6, 148.2, and 121.3) and four C=O C-atoms, revealed the presence of an α,β-unsaturated lactone (δ(C) 163.7), an α,β-unsaturated ketone (δ(C) 204.4), a saturated ketone (δ(C) 216.5), and a γ-lactone (δ(C) 179.5). The appearance of a *dd* at δ(H) 4.20 ($J = 12.8, 3.5$ Hz) and two olefinic Me signals at δ(H) 1.82 and 1.70 in the ^{1}H-NMR spectrum as well as the chemical shift at δ(C) 163.7 in the ^{13}C-NMR spectrum indicated the presence of a typical α,β-unsaturated lactone moiety of the withanolide skeleton. The ^{1}H-NMR spectrum exhibited signals for two coupled olefinic protons at δ(H) 5.87 (*dd*, $J = 10.2, 2.3$ Hz) and 6.77 (*ddd*, $J = 10.2, 5.0, 2.1$ Hz) assignable to the vicinal H–C(2) and H–C(3), respectively. In addition to these signals, the ^{1}H-NMR spectrum displayed a CH_2 signal at δ(H) 2.82–2.86 (*m*, H_α–C(4)) and 2.03–2.06 (*m*, H_β–C(4)) which showed ^{1}H,^{1}H-COSY connectivity with the signal at δ(H) 6.77 (H–C(3)). The olefinic proton at δ(H) 5.52 (*dd*, $J = 5.2, 2.4$ Hz) were assigned to H–C(6). This assignment was confirmed by the HMBC correlations H–C(3)/C(5) and CH_2(4)/C(5) and C(6), which indicated that the structure of **1** possesses a 2,5-dien-1-one structure in the *A*/*B* ring moiety. The presence of the HMBC correlations δ(H) 4.20 (H–C(22))/δ(C) 59.9 (C(17)), δ(H) 1.35 (Me(21))/δ(C) 59.9 (C(17)), δ(H) 2.40–2.44 (H–C(17))/δ(C) 21.2 (C(16)), δ(H) 3.12 (H–C(9))/δ(C) 216.5 (C(14)), δ(H) 2.36–2.40 (H–C(8))/δ(C) 216.5 (C(14)), and δ(H) 2.40–2.43 and 2.09–2.12 (CH_2(12))/δ(C) 179.5 (C(18)) indicated the presence in **1** of an

Table 1. *^{1}H-NMR Data* (400 MHz, $CDCl_3$) *of Compounds* **1**–**4**. δ in ppm; J in Hz.

	1	**2**	**3**	**4**
H–C(2) or CH_2(2)	5.87 (*dd*, *J* = 10.2, 2.3)	5.88 (*dd*, *J* = 10.4, 2.5)	5.84 (*dd*, *J* = 10.5, 2.5)	2.74 (*m*, 2 H)
H–C(3)	6.77 (*ddd*, *J* = 10.2, 5.0, 2.1)	6.73 (*dt*, *J* = 10.5, 2.2)	6.70 (*dt*, *J* = 10.5, 2.2)	2.22–2.25, 1.56–1.69 (2*m*)
CH_2(4)	2.03–2.06, 2.82–2.86 (2*m*)	3.25–3.29, 2.79–2.82 (2*m*)	3.25–3.29, 2.78–2.82 (2*m*)	3.77–3.81 (*m*)
H–C(6)	5.52 (*dd*, *J* = 5.2, 2.4)	5.51 (br. *d*, *J* = 5.8)	5.51 (br. *d*, *J* = 5.8)	3.20 (br. *s*)
CH_2(7)	2.03–2.06, 1.89–1.92 (2*m*)	1.91–1.95, 1.80–1.83 (2*m*)	1.91–1.94, 1.79–1.82 (2*m*)	1.61–1.65, 1.15–1.19 (2*m*)
H–C(8)	2.36–2.40 (*m*)	3.04–3.09 (*m*)	3.03–3.07 (*m*)	1.44–1.48 (*m*)
H–C(9)	3.12 (*t*, *J* = 11.4)	1.76–1.80 (*m*)	1.76–1.80 (*m*)	1.26–1.30 (*m*)
CH_2(11)	2.17–2.20, 1.52–1.56 (2*m*)	1.95–1.99, 1.76–1.79 (2*m*)	1.94–1.97, 1.77–1.80 (2*m*)	1.65–1.68, 1.42–1.45 (2*m*)
CH_2(12)	2.40–2.43, 2.09–2.12 (2*m*)	2.30–2.33, 1.80–1.84 (2*m*)	2.30–2.32, 1.82–1.84 (2*m*)	2.23–2.26, 1.46–1.49 (2*m*)
H–C(14)				1.17–1.21 (*m*)
CH_2(15)	3.30 (*d*, *J* = 11.7), 2.88–2.92 (*m*)	1.73–1.77, 1.63–1.66 (2*m*)	1.73–1.77, 1.63–1.67 (2*m*)	2.42–2.45, 1.36–1.39 (2*m*)
CH_2(16)	2.03–2.08, 1.93–1.97 (2*m*)	1.70–1.73 (*m*)	1.70–1.73 (*m*)	1.70–1.73, 1.41–1.45 (2*m*)
H–C(17)	2.40–2.44 (*m*)	2.01–2.05 (*m*)	2.01–2.04 (*m*)	1.96–2.01 (*m*)
H–C(18)		5.94 (*s*)	5.99 (*s*)	4.56 (*s*)
Me(19)	1.15 (*s*)	1.25 (*s*)	1.25 (*s*)	1.12 (*s*)
Me(21)	1.35 (*s*)	1.37 (*s*)	1.37 (*s*)	1.39 (*s*)
H–C(22)	4.20 (*dd*, *J* = 12.8, 3.5)	4.34 (*dd*, *J* = 13.0, 3.1)	4.34 (*dd*, *J* = 13.0, 3.1)	4.43 (*dd*, *J* = 13.3, 3.2)
CH_2(23)	2.60 (*t*, *J* = 12.8), 2.38–2.42 (*m*)	2.28–2.30 (*m*)	2.28–2.31 (*m*)	2.36–2.38, 2.00–2.03 (2*m*)
Me(27)	1.70 (*s*)	1.86 (*s*)	1.86 (*s*)	1.87 (*s*)
Me(28)	1.82 (*s*)	1.98 (*s*)	1.92 (*s*)	1.92 (*s*)
MeO–C(3)				3.29 (*s*)
MeO–C(18)				3.38 (*s*)
AcO–C(18)		1.98 (*s*)	1.98 (*s*)	
AcO–C(14)			2.00 (*s*)	

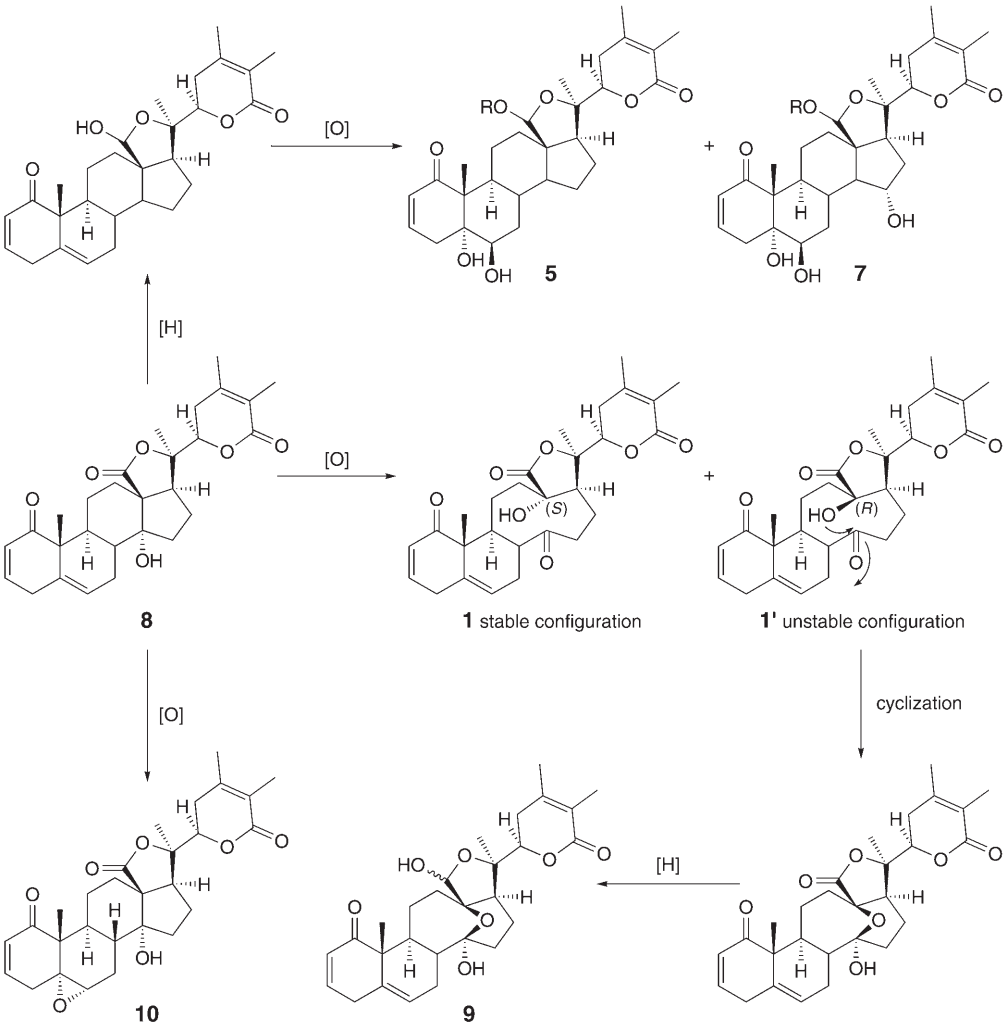
Table 2. *^{13}C-NMR Data* (100 MHz, $CDCl_3$) *of Compounds* **1**–**6**, **7a**, *and* **7b**. δ in ppm.

	1	**2**	**3**	**4**	**5**	**6**	**7a**	**7b**
C(1)	204.4	204.4	203.7	210.5	205.6	204.1	203.9	203.5
C(2)	127.2	128.0	127.8	42.6	127.9	129.2	128.7	128.9
C(3)	146.0	145.0	144.5	72.8	141.9	139.4	141.2	140.6
C(4)	32.8	33.4	33.1	36.3	35.2	27.5	35.9	35.6
C(5)	135.6	135.3	135.7	62.2	76.9	81.4	77.2	76.2
C(6)	122.1	125.3	123.9	61.3	73.5	68.4	74.0	74.7
C(7)	26.5	26.9	26.8	31.6	25.8	25.5	32.9	31.6
C(8)	52.0	47.8	41.0	30.3	31.2	31.5	30.8	29.5
C(9)	36.0	43.1	42.9	42.3	40.4	40.6	41.1	40.8
C(10)	50.9	52.9	52.8	52.0	51.6	52.4	51.9	51.8
C(11)	27.5	25.7	25.3	24.1	30.9	29.6	34.0	34.1
C(12)	27.2	44.6	45.6	34.6	34.7	35.1	36.4	36.4
C(13)	76.7	81.3	82.1	57.8	58.1	58.4	59.7	59.7
C(14)	216.5	100.5	108.7	56.3	55.7	56.0	55.7	55.6
C(15)	32.9	27.7	27.7	25.6	33.0	34.1	76.7	77.1
C(16)	21.2	15.3	16.5	26.2	25.1	26.2	25.5	25.7
C(17)	59.9	56.1	57.1	56.3	56.0	56.4	53.2	53.2
C(18)	179.5	105.1	104.7	108.0	108.4	108.6	108.7	108.8
C(19)	17.8	18.3	18.1	13.9	15.1	15.6	15.7	15.5
C(20)	83.3	87.9	87.7	84.7	84.6	84.8	84.7	84.9
C(21)	22.7	25.3	26.4	21.0	20.4	20.8	23.3	23.4
C(22)	75.8	77.5	78.1	80.7	80.7	80.8	81.1	81.2
C(23)	30.5	30.5	31.8	31.3	30.9	31.3	32.1	32.1
C(24)	148.2	149.4	148.0	147.5	148.8	147.9	147.9	148.2
C(25)	121.3	121.2	121.8	122.4	121.6	122.6	122.3	122.4
C(26)	163.7	166.0	165.3	165.9	166.5	166.0	166.0	166.2
C(27)	12.1	12.3	12.4	12.5	11.9	12.5	12.4	12.6
C(28)	20.3	20.7	20.3	20.3	20.0	20.3	20.4	20.6
MeO				55.6	55.2	55.7	55.0	55.0
MeO				56.0		49.7		
Ac		170.2, 21.4	169.9, 21.0				170.9, 21.4	171.1, 21.5
Ac			168.5, 22.5					169.9, 21.4

unprecedented nine-membered ring instead of a typical withaphysalin ring-*C* and ring-*D* skeleton. The above stated moieties and the rings *C* and *D* as a nine-membered ring accounted for the twelve degrees of unsaturation in **1**.

For biogenetic reasons, the chiral center at C(10) of withanolides has (*R*) configuration [16]. Thus, the asymmetric centres of withaphysalin P (**1**) have the following configurations: (8*R*,9*S*,10*R*,13*S*,17*S*,20*R*,22*R*). Further evidence for the established structure **1** furnished a plausible biogenetic pathway (*Scheme 1*). Withaphysalin P (**1**) and its counterpart **1′** its with (13*R*) configuration might be derived by cleavage of the C(13)–C(14) bond from the biogenetically acceptable withaphysalin A (**8**), which was also isolated from the title plant [7]. The OH–C(13) in withaphysalin P (**1**) can form an H-bond with the α,β-unsaturated ketone moiety if it has the configuration (13*S*), thus contributing to the stabilization of **1** (*Fig. 2*), while the

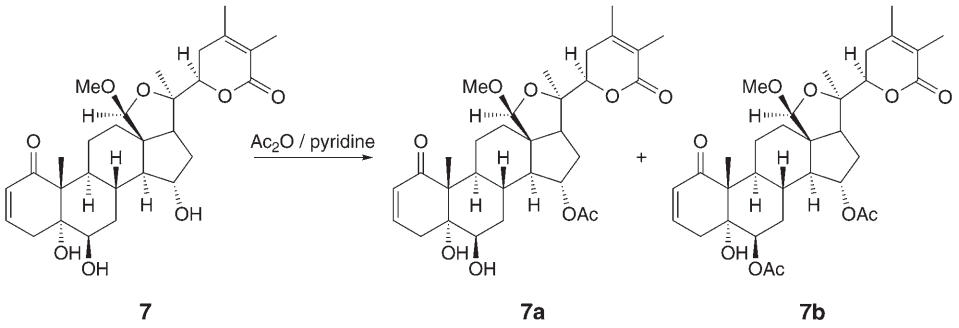
Scheme 1. *Possible Biosynthetic Pathway of Isolated Compounds*



configuration (13*R*) in the counterpart **1′** cannot form such an H-bond and thus lacks the stabilization. Therefore, **1′** may be the important biosynthesis precusor of compound **9** (*Scheme 1*).

Compound **2** was isolated as an amorphous powder. Its molecular formula was determined as $C_{30}H_{38}O_8$ by HR-EI-MS (M^+ at m/z 526.2571). The IR spectrum showed the presence of OH (3501 cm^{-1}) and C=O groups (1752 and 1684 cm^{-1}) consistent with the presence of an α,β-unsaturated-lactone and an α,β-unsaturated-ketone moiety. Its ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) were very similar to those of the known withaphysalin C (**9**). The ^{13}C-NMR spectrum indicated that the differences between **2** and **9** were the substitution pattern at C(18) and the presence of an additional *O*-acetyl

Scheme 2. *Acetylation of Compound* **7**



group in **2**. The HMBC (*Fig. 1*) and NOESY data were in accord with the structure of 18-*O*-acetylwithaphysalin C for **2**.

Instead of the signals δ(C) 106.5 (C(18)) and δ(H) 5.12 (H–C(18)) in **9**, the spectrum of **2** showed signals at δ(C) 105.1 (C(18)) and δ(H) 5.94 (H–C(18)), suggesting that the OH–C(18) of **9** was acetylated in **2**. This fact was further validated by the HMBC correlation δ(H) 5.94 (H–C(18))/δ(C)170.2 (Me*C*O) (*Fig. 1*). According to the NOESY plot, the configuration of the acetyloxy group was β which was further confirmed by the NOE δ(H) 5.94 (H–C(18))/δ(H) 1.37 (Me(21)). Thus the 18,20-hemiacetal-type withaphysalin **2**, was established as a (17*S*,18β,20*R*,22*R*)-18-(acetyloxy)-1-oxo-13,14-secowitha-2,5,24-trienolide.

Compound **3** was isolated as an amorphous powder. Its molecular formula was determined as $C_{32}H_{42}O_7$ by HR-EI-MS (M^+ at m/z 568.2674). The IR spectrum showed the presence of C=O groups (1751 and 1684 cm^{-1}) consistent with the presence of an α,β-unsaturated-lactone and an α,β-unsaturated-ketone moiety. The ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) were very similar to those of 18-*O*-acetyloxywithaphysalin C (**2**). The ^{13}C-NMR spectrum indicated that the only difference between **3** and **2** was the substitution pattern at C(14) and the presence of an additional *O*-acetyl group. The data were compatible with the structure of 14,18-di-O-acetylwithaphysalin C for **3**.

Instead of the signal δ(C) 100.5 (C(14)) in **2**, the spectrum of **3** showed this signal at δ(C) 108.7 (C(14)), establishing that OH–C(14) of **2** was acetylated in **3**. This fact was further validated by the HMBC correlation δ(H) 2.00 (MeCO/δ(C) 108.7 (C(14)) (*Fig. 1*). Thus the 18,20-hemiacetal-type withaphysalin **3** was established as a (14α,17*S*,18β,20*R*,22*R*)-14,18-bis(acetyloxy)-1-oxo-13,14-secowitha-2,5,24-trienolide.

Compound **4** was isolated as an amorphous powder. Its molecular formula was determined as $C_{30}H_{38}O_8$ by HR-EI-MS (M^+ at m/z 514.2933). The IR spectrum showed the presence of OH (3398 cm^{-1}) and C=O groups (1750 and 1710 cm^{-1}) consistent with the presence of an α,β-unsaturated-lactone and a ketone moiety. Its ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) were very similar to those of the known compound withaphysalin H = (4β,18*R*,22*R*)-18,20-epoxy-4,22-dihydroxy-18-methoxy-1-oxoergosta-2,5,24-trien-26-oic acid δ-lactone [12]. The ^{13}C-NMR spectrum indicated that the main differences between **4** and withaphysalin H were attributed to rings *A* and *B*. The

structure of **4**, named withaphysalin Q, was in accord with its ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HMBC (*Fig. 1*), and NOESY data.

The ^{1}H-NMR spectrum of **4** suggested that the MeO group at δ(H) 3.29 (*s*) was connected to C(3), which was validated by the HMBC correlations δ(H) 3.77–3.81 (H–C(3))/δ(C) 210.5 (C(1)) and δ(H) 3.29 (MeO–C(3)/δ(C) 72.8 (C(3)). Signals at δ(H) 3.20 (br. *s*, H–C(6)) together with δ(C) 62.2 (C(5)) and 61.3 (C(6)), confirmed that in **4** C(5) and C(6) are linked by an epoxy group. The NOEs H–C(3)/H–C(6), and δ(H) 3.29 (MeO–C(3)/δ(H) 1.12 (Me(19)) suggested that the MeO group and the 5,6-epoxy group were both β-oriented. The β-configuration of the MeO–C(18) group was further confirmed by the NOE δ(H) 1.96–2.01 (H–C(17))/δ(H) 4.56 (H–C(18)). Thus, the 18,20-hemiacetal-type withaphysalin **4** was established as a (3β,5β,6β,17*S*,18β,20*Rd*,22*R*)-5,6-epoxy-3,18-dimethoxy-1-oxowith-24-enolide.

Compound **5** was isolated as an amorphous powder. Its molecular formula was determined as $C_{29}H_{40}O_7$ by HR-EI-MS (M^+ at 500.2778). The IR spectrum showed the presence of OH (3402 cm^{-1}) and C=O groups (1745 and 1685 cm^{-1}) consistent with the presence of an α,β-unsaturated-lactone and an α,β-unsaturated-ketone moiety. Its ^{1}H- and ^{13}C-NMR spectra (*Tables 3* and *2*) were very similar to those of the known compound withaphysalin H [12]. Further spectroscopic data established the structure of **5**, which is named whitaphysalin R.

The ^{13}C-NMR spectrum of **5** indicated that the points of differences between **5** and withaphysalin H were in the substitution patterns at C(4), C(5), and C(6). Instead of the signals of the olefinic C-atoms at δ(C) 135.1 (C(5)) and 124.6 (C(6)) of withaphysalin H, the spectrum of **5** showed two signals at δ(C) 76.9 (C(5)) and 73.5 (C(6)) typical of a 5α,6β-diol [17]. The multiplicity and the chemical shift of H–C(6) (δ 3.41, *t*, *J* = 2.5 Hz) were in good agreement with the β-orientation of the OH group at C(6) [17]. According to the NOESY plot, the MeO group was β-oriented which was further confirmed by the NOE δ(H) 1.97–2.01 (H–C(17))/δ(H) 4.58 (H–C(18)). Thus the 18,20-hemiacetal-type withaphysalin **5**, was established as (5α,6β,17*S*,18β,20*R*,22*R*)-5,6-dihydroxy-18-methoxy-1-oxowitha-2,24-dienolide.

Compound **6** was isolated as an amorphous powder. Its molecular formula was determined as $C_{30}H_{42}O_7$ by HR-EI-MS (M^+ at *m*/*z* 514.2937). The IR spectrum showed the presence of OH (3446 cm^{-1}) and C=O groups (1747 and 1683 cm^{-1}) consistent with the presence of an α,β-unsaturated-lactone and an α,β-unsaturated-ketone moiety. Its ^{1}H- and ^{13}C-NMR spectra (*Tables 3* and *2*) were very similar to those of withaphysalin R (**5**). The NMR spectrum indicated that the only difference between **6** and **5** was an additional MeO group instead of the OH–C(5) which was validated by an HMBC correlation (δ(H) 2.96 (MeO–C(5)/δ(C) 81.4 (C(5))). Thus the 18,20-hemiacetal-type withaphysalin **6**, was established as (5α,6β,17*S*,18β,20*R*,22*R*)-6-hydroxy-5,18-dimethoxy-1-oxowitha-2,24-dienolide, and is named 5-*O*-methylwithaphysalin R.

Compound **7** was isolated as an amorphous powder. Its molecular formula was determined as $C_{29}H_{40}O_8$ by HR-EI-MS (M^+ at 516.2726). The IR spectrum showed the presence of OH (3357 cm^{-1}) and C=O groups (1751 and 1683 cm^{-1}) consistent with the presence of an α,β-unsaturated-lactone and an α,β-unsaturated-ketone moiety. However, it was difficult to deduce the structure with its NMR spectra because of the overlapped signals which may be caused by the unstable skeleton. To stabilize the skeleton, **7** was acetylated (*Scheme 2*). Two major products, **7a** and **7b**, were obtained as

Table 3. *^{1}H-NMR Data* (400 MHz, $CDCl_3$) *of Compounds* **5**, **6**, **7a**, *and* **7b**. δ in ppm; J in Hz.

	5	**6**	**7a**	**7b**
H–C(2)	5.78 (*dd*, *J* = 10.1, 2.2)	5.77 (*dd*, *J* = 10.1, 2.2)	5.88 (*dd*, *J* = 10.1, 2.5)	5.90 (*dd*, *J* = 10.0, 2.4)
H–C(3)	6.47 (*ddd*, *J* = 10.1, 4.8, 1.8)	6.50 (*ddd*, *J* = 10.1, 4.8, 1.8)	6.61 (*dt*, *J* = 10.1, 2.1)	6.59 (*dt*, *J* = 10.0, 2.1)
CH_2(4)	3.10–3.13, 1.87–1.90 (2*m*)	3.04–3.08, 2.32–2.36 (2*m*)	2.87–2.91, 2.08–2.12 (2*m*)	2.85–2.88, 2.08–2.11 (2*m*)
H–C(6)	3.41 (*t*, *J* = 2.5)	3.90 (*t*, *J* = 2.5)	3.30 (br. *s*)	4.87 (br. *s*)
CH_2(7)	2.03–2.07, 1.47–1.51 (2*m*)	2.24–2.27, 1.57–1.60 (2*m*)	1.89–1.92, 1.45–1.47 (2*m*)	1.90–1.93, 1.45–1.48 (2*m*)
H–C(8)	1.30–1.34 (*m*)	1.30–1.34 (*m*)	1.78–1.82 (*m*)	1.78–1.81 (*m*)
H–C(9)	1.60–1.63 (*m*)	1.60–1.63 (*m*)	1.88–1.92 (*m*)	1.91–1.94 (*m*)
CH_2(11)	2.27–2.30, 1.91–1.94 (2*m*)	2.24–2.68, 1.90–1.93 (2*m*)	2.48–2.51, 1.45–1.49 (2*m*)	2.50–2.54, 1.48–1.52 (2*m*)
CH_2(12)	2.45–2.49, 1.52–1.56 (2*m*)	2.44–2.48, 1.51–1.55 (2*m*)	2.10–2.13, 1.60–1.64 (2*m*)	2.10–2.14, 1.60–1.64 (2*m*)
H–C(14)	1.48–1.51 (*m*)	1.46–1.50 (*m*)	1.78–1.81 (*m*)	1.85–1.88 (*m*)
CH_2(15) or H–C(15)	1.60–1.63, 1.52–1.55 (2*m*)	1.64–1.67, 1.52–1.55 (2*m*)	5.16–5.18 (*m*)	5.10–5.14 (*m*)
CH_2(16)	2.05–2.09, 1.48–1.52 (2*m*)	2.06–2.08, 1.48–1.51 (2*m*)	2.47–2.49 (*m*)	2.57–2.60 (*m*)
H–C(17)	1.97–2.01 (*m*)	2.00–2.03 (*m*)	2.20 (*dd*, *J* = 11.0, 7.7)	2.20 (*dd*, *J* = 11.0, 7.7)
H–C(18)	4.58 (*s*)	4.56 (*s*)	4.68–4.72 (*m*)	4.70–4.73 (*m*)
Me(19)	1.21 (*s*)	1.20 (*s*)	1.28 (*s*)	1.29 (*s*)
Me(21)	1.36 (*s*)	1.33 (*s*)	1.33 (*s*)	1.35 (*s*)
H–C(22)	4.32 (*dd*, *J* = 13.3, 3.2)	4.32 (*dd*, *J* = 13.3, 3.2)	4.40 (*dd*, *J* = 13.1, 3.5)	4.38 (*dd*, *J* = 13.1, 3.5)
CH_2(23)	2.28 (*t*, *J* = 13.3), 1.90–1.93 (*m*)	2.28 (*t*, *J* = 13.3), 1.90–1.93 (*m*)	2.40–2.44, 2.03–2.06 (2*m*)	2.41–2.44, 2.03–2.07 (2*m*)
Me(27)	1.81 (*s*)	1.82 (*s*)	1.87 (*s*)	1.88 (*s*)
Me(28)	1.91 (*s*)	1.90 (*s*)	1.90 (*s*)	1.91 (*s*)
MeO–C(18)	3.33 (*s*)	3.33 (*s*)	3.30 (*s*)	3.30 (*s*)
MeO–C(5)		2.96 (*s*)		
AcO–C(15)			1.98 (*s*)	1.98 (*s*)
AcO–C(6)				2.11 (*s*)

amorphous powders, with the molecular masses of 558 and 600, respectively, consistent with mono- and diacetylation of **7**. Their structures were deduced from the ^{1}H- and ^{13}C-NMR (*Tables 3* and *2*), NOE, and HMBC (*Fig. 1*) data, and thus, the structure of **7**, named withaphysalin S, was established.

The ^{1}H- and ^{13}C-NMR spectra of **7a** were very similar to those of withaphysalin R (**5**). The NMR spectrum indicated that the only difference between **7a** and **5** was an additional acetyloxy group, and that this acetyloxy group was bound to C(15), which was validated by the HMBC correlations δ(H) 5.16–5.18 (H–C(15))/δ(C) 170.9 (Me*C*O) and δ(H) 2.47–2.49 (H–C(16))/δ(C) 76.7 (C(15)) (*Fig. 1*). The NOE δ(H) 5.16–5.18 (H–C(15))/δ(H) 1.78–1.82 (H–C(8)) suggested that AcO–C(15) was α-oriented. Thus the 18,20-hemiacetal-type withaphysalin **7a**, was established as (5α,6β,15α,17*S*,18β,20*R*,22*R*)-15-(acetyloxy)-5,6-dihydroxy-18-methoxy-1-oxowitha-2,24-dienolide, *i.e.*, as 15-*O*-acetylwithaphysalin S. The structure of **7b** was confirmed by the HMBC correlations δ(H) 5.10–5.14 (H–C(15))/δ(C) 171.1 (Me*C*O) and δ(H) 4.87 (H–C(6))/δ(C) 169.9 (Me*C*O) (*Fig. 1*). Thus **7b** was established as (5α,6β,15α,17*S*,18β,20*R*,22*R*)-6,15-bis(acetyloxy)-5-hydroxy-18-methoxy-1-oxowitha-2,24-dienolide, *i.e.*, 6,15-*O*-acetylwithaphysalin S.

2. *Biological Studies.* The compounds **1**–**10**, **7a**, and **7b** were screened for *in vitro* cytotoxicity against HCT-116 and NCI-H460 tumor cell lines (*Table 4*). Compounds **2**, **8**, and **10** exhibited moderate cytoxicities against HCT-116 and NCI-H460 cells. Withaphysalin C (**9**) showed the strongest cytotoxicity against both cancer cell lines with IC_{50} values 14.2 ± 0.5 and 15.3 ± 0.5 μM, respectively. *Table 4* also reveals that compounds **4**–**6** were inactive against the two cancer cell lines. Based on these observations, it is hypothesized that the α,β-unsaturated ketone moiety in ring *A* and a C=C bond or an epoxy at positions 5 and 6 in ring *B* are activity-enhancing structural features within this series of compounds.

Table 4. *Cytotoxicities of* **1**–**10** *toward HCT-116 and H460 Cell Lines*

	IC_{50} [μM][a])			IC_{50} [μM][a])	
	HCT116	H460		HCT116	H460
1	46.5 ± 0.9	40.3 ± 0.8	**7a**	47.3 ± 0.8	51.6 ± 0.7
2	17.9 ± 0.7	21.4 ± 0.5	**7b**	58.2 ± 0.7	60.1 ± 0.3
3	23.2 ± 0.8	27.0 ± 0.6	**8**	17.5 ± 0.7	24.4 ± 0.6
4	> 100	> 100	**9**	14.2 ± 0.5	15.3 ± 0.5
5	> 100	> 100	**10**	18.0 ± 0.6	33.9 ± 0.8
6	> 100	> 100	Topotecan[b])	0.026 ± 0.004	0.07 ± 0.005

[a]) Mean ± s.e.m. for $n = 3$. [b]) Positive control.

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Experimental Part

General. All solvents used were of analytical grade (*Shanghai Chemical Plant*). Column chromatography (CC): silica gel *H* (200–300 mesh; *Qingdao Marine Chemical Ltd.*), *Sephadex LH-20* (25–100 μm; *Pharmacia Fine Chemicals*), *MCI* gel *CHP 20P* (75–150 μm; *Mitsubishi Chemical Ind.*), *D-101* porous resin (*Chemical Factory of Tianjin University*), and *RP-18* (20–45 μm; *Fuji Silysia*

Chemical Ltd.). Thin-layer chromatography (TLC): silica gel GF_{254} (*Yantai Huiyou Inc.*). Melting points: *Leica Galen-III* apparatus; uncorrected. Optical rotations: $CHCl_3$ or MeOH solns.; *Perkin-Elmer PE-241* polarimeter. IR Spectra: *Perkin-Elmer 16-PC-FT-IR* spectrophotometer; in cm^{-1}. ^1H- (400 MHz) and ^{13}C-NMR (100 MHz) Spectra: *Bruker AMX-400* spectrometer; δ in ppm, J in Hz, with $SiMe_4$ as an internal standard. HR-EI-MS and EI-MS: *Finnigan MAT-90/95* sector-field mass spectrometer; in m/z.

Plant Material. The aerial parts of *Physalis minima* L. were collected in Nanchuan County, Chongqing city, China, and identified by *Si-Rong Yi*, The Plant Garden of Jinfoshan, P. R. China. A voucher specimen was deposited at the Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Shanghai.

Extraction and Isolation. The aerial parts (2 kg) of *P. minima* L. were extracted with MeOH at r.t., affording a dark residue (45.1 g) after evaporation. The residue was partitioned between $CHCl_3$ and H_2O. The org. layer (19.7 g after evaporation) was subjected to CC (*D-101* porous resin, EtOH/H_2O 25:75, 50:50, 75:25, and 95:5): *Factions. A–D. Fr. B* (4.7 g) was subjected to CC (*MCI* gel *CHP 20P*, H_2O/Me_2CO 1:1): *Fr. B.1–B.4*. *Fr. B.1* was resubjected to CC (*RP-18*, MeOH/H_2O 40:60): **1** (37 mg), **2** (14 mg), and **5** (27 mg). *Fr. B.2* was separated by CC (*RP-18*, MeOH/H_2O 50:50): **3** (79 mg), **4** (42 mg), **6** (27 mg), and **7** (19 mg). *Fr. B.3* was separated by CC (*RP-18*, MeOH/H_2O 60:40): **8** (92 mg) and **9** (19 mg). *Fr. B.4* was subjected to CC (*Sephadex LH-20*, MeOH): **10** (120 mg).

Withaphysalin P (=*(22*R*)-13,20,22-Trihydroxy-1,14-dioxo-13,14-secoergosta-2,5,24-trien-18,26-dioic Acid 18,20:26,22-Dilactone* = *(6a*R*,9a*S*,10*R*,12a*S*,14a*S*,14b*R*)-10-[(2*R*)-3,6-Dihydro-4,5-dimethyl-6-oxo-2*H*-pyran-2-yl]-6,8,9,9a,10,12a,13,14,14a,14b-decahydro-12a-hydroxy-10,14b-dimethyl-1*H*-naphtho[1′,2′:5,6]acyclonona[1,2-c]furan-1,7,12(4*H*,6a*H*)-triane*; **1**): Colorless cubic crystal (MeOH). $[\alpha]_D^{20} = +16$ ($c = 0.20$, $CHCl_3$). IR (KBr): 3444, 2925, 1750, 1735, 1676, 1448, 1386, 1242, 1180, 1137. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*. HR-EI-MS: 482.2296 (M^+, $C_{28}H_{34}O_7^+$; calc. 482.2305).

18-O-*Acetylwithaphysalin C* (=*(14β,22*R*)-18-(Acetyloxy)-13,14:18,20-diepoxy-14,22-dihydroxy-1-oxo-13,14-secoergosta-2,5,24-trien-26-oic Acid δ-Lactone* = *(6a*R*,7*R*,9a*R*,10*R*,12*R*,12a*R*,14a*S*,14b*R*)-12-(Acetyloxy)-10-[(2*R*)-3,6-dihydro-4,5-dimethyl-6-oxo-2*H*-pyran-2-yl]-4,6,6a,7,8,9,9a,10,13,14,14a,14b-dodecahydro-7-hydroxy-10,10b-dimethyl-12*H*-7,12a-epoxy-1*H*-naphtho[1′,2′:5,6]cyclonona[1,2-c]furan-1-one*; **2**). White amorphous powder. $[\alpha]_D^{20} = +47$ ($c = 0.20$, MeOH). IR (KBr): 3501, 2939, 1752, 1684, 1382, 1141, 1045, 918, 846. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. HR-EI-MS: 526.2571 (M^+, $C_{30}H_{38}O_8^+$; calc. 526.2567).

14,18-Di-O-*acetylwithaphysalin C* (=*(14α,22*R*)-14,18-Bis(acetyloxy)-13,14:18,20-diepoxy-22-hydroxy-1-oxo-13,14-secoergosta-2,5,24-trien-26-oic Acid δ-Lactone* = *(6a*R*,7*S*,9a*R*,10*R*,12*R*,12a*R*,14a*S*,14b*R*)-7,12-Bis(acetyloxy)-10-[(2*R*)-3,6-dihydro-4,5-dimethyl-6-oxo-2*H*-pyran-2-yl]-4,6,6a,7,8,9,9a,10,13,14,14a,14b-dodecahydro-10,14b-dimethyl-12*H*-7,12a-epoxy-1*H*-naphtho[1′,2′:5,6]cyclonona[1,2-c]furan-1-one*; **3**): Amorphous white powder. $[\alpha]_D^{20} = +49$ ($c = 0.20$, MeOH). IR (KBr): 2939, 1751, 1697, 1684, 1400, 1039, 678. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. HR-EI-MS: 568.2674 (M^+, $C_{32}H_{40}O_9^+$; calc. 568.2672).

Withaphysali Q (=*(3β,5β,6β,18*S*,22*R*)-5,6:18,20-Diepoxy-22-hydroxy-3,18-dimethoxy-1-oxoergost-24-en-26-oic Acid δ-Lactone*; **4**): Amorphous powder. $[\alpha]_D^{20} = +13$ ($c = 0.20$, MeOH). IR (KBr): 3398, 2923, 1750, 1710, 1400, 1076, 601. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. HR-EI-MS: 514.2933 (M^+, $C_{30}H_{42}O_7^+$; calc. 514.2931).

Withaphysalin R (=*(5α,6β,18*S*,22*R*)-18,20-Epoxy-5,6,22-trihydroxy-18-methoxy-1-oxoergosta-2,24-dien-26-oic Acid δ-Lactone*; **5**): Amorphous powder. $[\alpha]_D^{20} = +54$ ($c = 0.20$, MeOH). IR (KBr): 3402, 2939, 1745, 1685, 1450, 1386, 1128, 759. ^1H- and ^{13}C-NMR: *Tables 3* and *2*. HR-EI-MS: 500.2778 (M^+, $C_{29}H_{40}O_7^+$; calc. 500.2774).

5-O-*Methoxywithaphysalin R* (=*(5α,6β,18*S*,22*R*)-18,20-Epoxy-6,22-dihydroxy-5,18-dimethoxy-1-oxoergosta-2,24-dien-26-oic Acid δ-Lactone*; **6**): Amorphous powder. $[\alpha]_D^{20} = +53$ ($c = 0.20$, MeOH). IR (KBr): 3446, 2933, 1747, 1683, 1450, 1380, 1081, 977. ^1H- and ^{13}C-NMR: *Tables 3* and *2*. HR-EI-MS: 514.2937 (M^+, $C_{30}H_{42}O_7^+$; calc. 514.2931).

Withaphysalin S (=*(5α,6β,15α,18*S*,22*R*)-18,20-Epoxy-5,6,15,22-tetrahydroxy-18-methoxy-1-oxoergosta-2,24-dien-26-oic Acid δ-Lactone*; **7**): Amorphous powder. $[\alpha]_D^{20} = +64$ ($c = 0.20$, MeOH). IR (KBr): 3357, 2939, 1751, 1683, 1569, 1400, 1039, 678. HR-EI-MS: 516.2726 (M^+, $C_{29}H_{40}O_8^+$; calc. 516.2723).

15-O-Acetylwithaphysalin S (= *(5α,6β,15α,18S,22R)-15-(Acetyloxy)-18,20-epoxy-5,6,22-trihydroxy-18-methoxy-1-oxoergosta-2,24-dien-26-oic Acid α-Lactone*; **7a**): Amorphous powder. ^{1}H- and ^{13}C-NMR: *Tables 3* and *2*. EI-MS: 558.2 (M^+, $C_{31}H_{42}O_9^+$).

6,15-Di-O-acetylwithaphysalin S (= *(5α,6β,15α,18S,22R)-6,15-Bis(acetyloxy)-18,20-epoxy-5,22-dihydroxy-18-methoxy-1-oxoergosta-2,24-dien-26-oic Acid δ-Lactone*; **7b**): Amorphous powder. ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. EI-MS: 600.2 (M^+, $C_{33}H_{44}O_{10}^+$).

Bioassay. Cytotoxicity of compounds against human colorectal-carcinoma HCT-116 cells and human nonsmall-cell lung-cancer NCI-H460 cells was determined by the sulforhodamine B (SRB) assay [18]. Cells were plated in a 96-well plate 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. After compound treatment, cells were fixed and stained with SRB as described by *Monks et al.* [18].

X-Ray Crystal-Structure Analysis of **1**[1]). Colorless cubic crystals of **1** were obtained by recrystallization in MeOH. The crystal ($0.516 \times 0.475 \times 0.084$ mm) belongs to the orthorhombic system. Formula $C_{29}H_{38}O_8$ (M_r 514.59), space group $P2_12_12_1$ with $a = 11.1346(18)$, $b = 13.3118(19)$, $c = 18.222(3)$ Å; $\alpha = \beta = \gamma = 90.0°$; $V = 2700.9(7)$ Å^3; $Z = 4$; and $\rho_{calc} = 1.266$ mg m^{-3}. A total of 15879 reflections were collected to a maximum 2θ value of 54.00° by using the Φ/ω scan technique at 293(2) K. The structure was solved by direct methods and was refined by means of the full-matrix least-squares procedure. The collection data were reduced by using the Saint program [19], and the empirical absorption correction was performed by using the Sadabs program [20]. All non-H-atoms were given anisotropic thermal parameters. The H-atom positions were geometrically idealized and allowed to ride on their parent atoms. The refinement converged to the final $R = 0.0549$, $wR = 0.0999$ for 5825 observed reflections ($I > 2\sigma(I)$) and 360 variable parameters.

REFERENCES

[1] E. Glotter, *Nat. Prod. Rep.* **1991**, *8*, 415.
[2] A. S. Veleiro, J. C. Oberti, G. Burton, in 'Studies in Natural Products Chemistry, (Part L) Bioactive Natural Products', Ed. Atta-ur-Rahman, Elsevier, Amsterdam, 2005, Vol. 32, p. 1019.
[3] A. B. Ray, M. Gupta, in 'Progress in the Chemistry of Organic Natural Products', Eds. W. Hertz, G. W. Kirby, R. E. Moore, W. Steglich, and Ch. Tamm, Springer-Verlag, Vienna, 1994. Vol. 63, p. 1.
[4] R. R. Falsey, M. T. Marron, G. M. Gunaherath, N. Shirahatti, D. Mahadevan, A. A. Gunatilaka, L. Whitesell, *Nat. Chem. Biol.* **2006**, *2*, 33.
[5] L. Ma, M. Ali, M. Arfan, L. G. Lou, L. H. Hu, *Tetrahedron Lett.* **2007**, *48*, 449.
[6] J. A. Bravo, M. Sauvain, A. Gimenez, E. Balanza, L. Serani, O. Laprevote, G. Massiot, C. Lavaud, *J. Nat. Prod.* **2001**, *64*, 720.
[7] R. I. Misico, L. L. Song, A. S. Veleiro, A. M. Cirigliano, M. C. Tettamanzi, G. Burton, G. M. Bonetto, V. E. Nicotra, G. L. Silva, R. R. Gil, J. C. Oberti, A. D. Kinghorn, J. M. Pezzuto, *J. Nat. Prod.* **2002**, *65*, 677.
[8] E. Glotter, I. Kirson, A. Abraham, P. D. Sethi, S. S. Subramanian, *J. Chem. Soc., Perkin Trans. 1* **1975**, 1370.
[9] M. Sahai, I. Kirson, *J. Nat. Prod.* **1984**, *47*, 527.
[10] S. C. Sinha, A. B. Ray, Y. Oshima, A. Bagchi, H. Hikino, *Phytochemistry* **1987**, *26*, 2115.
[11] G. L. Silva, G. Burton, J. C. Oberti, *J. Nat. Prod.* **1999**, *62*, 949.
[12] R. I. Misico, R. R. Gil, J. C. Oberti, A. S. Veleiro, G. Burton, *J. Nat. Prod.* **2000**, *63*, 1329.
[13] M. L. Veras, M. Z. Bezerra, T. L. Lemos, D. E. Uchoa, R. Braz-Filho, H. B. Chai, G. A. Cordell, O. D. Pessoa, *J. Nat. Prod.* **2004**, *67*, 710.
[14] I. Kirson, Z. Zaretskii, E. Glotter, *J. Chem. Soc., Perkin Trans. 1* **1976**, 1244.

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[1]) CCDC-653033 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

[15] Q. P. He, L. Ma, J. Y. Luo, F. Y. He, L. G. Lou, L. H. Hu, *Chem. Biodiv.* **2007**, *4*, 961.
[16] J. G. Luis, F. Echeverri, W. Quinones, A. G. Torres, G. Cardona, R. A. Perales, *Tetrahedron* **1994**, *50*, 1217.
[17] A. M. Cirigliano, A. S. Veleiro, J. C. Oberti, G. Burton, *J. Nat. Prod.* **2002**, *65*, 1049.
[18] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, *J. Natl. Cancer Inst.* **1991**, *83*, 757.
[19] Bruker AXS Inc., Saint: Program to integrate and reduce raw crystallographic area detector data, Madison, USA, 1996.
[20] G. M. Sheldrick, Sadabs: Program to empirical absorption correction of area detector data, University of Göttingen, Germany, 1996.

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