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Withaphysanolide A, a novel C-27 norwithanolide skeleton, and other cytotoxic compounds from *Physalis divericata*

Lei Ma,[a] Mumtaz Ali,[b] Mohammad Arfan,[b] Li-Guang Lou[a,*] and Li-Hong Hu[a,*]

[a]*Shanghai Institute of Materia Medica, Shanghai 201203, PR China*
[b]*Institute of Chemical Sciences, University of Peshawar, Peshawar, Pakistan*

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Abstract—A novel C-27 norwithasteroid, withaphysanolide A (**1**) containing a pyran ring was isolated from the aerial parts of *Physalis divericata*. Four known withaphysalins (**2**–**5**) and five physalins (**6**–**10**) were also isolated. The structural assignment for **1** was done based on spectroscopic and single-crystal X-ray diffraction data. Logical biosynthetic pathways were postulated. Compounds **6**, **7**, and **10** displayed potent cytotoxic activity against HCT-116 and H460 human cancer cell lines, with IC_{50} values less than 2.0 μM.

Withanolides are structurally diverse steroids with an ergostane skeleton in which C(22) and C(26) are oxidized to form a δ-lactone. These compounds are generally polyoxygenated, and this profusion of oxygen 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, withaphysalins, physalins, ixocarpalactones, perulactones, and acnistins.[1] Plants belonging to the family Solanaceae generally bear these compounds, in particular the genera *Withania*, *Acnistus*, *Dunalia*, *Physalis*, *Datura*, *Lycium*, and *Jaborosa*.[1] The biological activities of withanolides have been studied extensively during the past 20 years. Notable activities reported for these compounds include anti-inflammatory, anticonvulsive, antitumor, immunosuppressive, and antioxidant properties.[1]

Physalis divericata D.Don is a common field weed found throughout Pakistan. The taxonomic identification of the collected plant was done by Prof. Mehboob Ahmad. A voucher specimen was deposited in the herbarium at the Department of Botany, Jahan Zeb College, Swat, Pakistan. To our knowledge, this plant has never been studied.

A novel withanolide, withaphysanolide A (**1**), with an unprecedented skeleton, together with four known withaphysalins and five physalins were isolated from the titled plant. The details of the isolation,[2] structural elucidation, postulated biogenetic formation, and cytotoxicity of these compounds are presented below.

Withaphysanolide A (**1**) was isolated as colorless cubic crystals, with a mp of 221–222 °C, $[\alpha]_D^{20}$ +103 (*c* 0.30, $CHCl_3$), whose molecular formula with 11° of unsaturation was established as $C_{27}H_{34}O_5$ from its HREIMS ($[M]^+$ at *m*/*z* 438.2405, calcd 438.2406). UV (MeOH) λ_{max} (log ε) 224 (4.10) nm. The IR spectrum showed the presence of carbonyl (1706 cm^{-1} and 1683 cm^{-1}) groups consistent with the presence of δ-lactone and α,β-unsaturated ketone moieties. On the basis of the interpretation of ^{13}C and DEPT spectral data, the compound contained four methyl groups, seven methylenes, and eight methines (including three olefinic at δ_C 127.8, 145.4, and 125.3, and one oxygenated methine at δ_C 78.2). Eight quaternary carbon atoms of which three were sp^2 carbons (δ_C 134.1, 147.4, and 122.6) and two carbonyl carbons revealed the presence of an α,β-unsaturated lactone (δ_C 165.8), and an α,β-unsaturated ketone (δ_C 204.1). The ^1H NMR spectrum (Table 1) exhibited signals for two coupled olefinic protons at δ_H 5.85 (d, $J = 10.0$ Hz) and 6.77 (ddd, $J = 10.0$, 5.0, and 2.4 Hz) assignable to the H-2 and H-3 vicinal protons, respectively. In addition to these signals, the ^1H NMR spectrum displayed a methylene hydrogen signal

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* Corresponding authors. Tel./fax: +86 21 50272221 (L.-H.); e-mail: simmhulh@mail.shcnc.ac.cn

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Table 1. ^{13}C and ^{1}H NMR spectral data of **1** ($CDCl_3$, TMS)

No.	δ_C	δ_H
1	204.1	
2	127.8	5.85 (1H, d, $J = 10.0$ Hz)
3	145.4	6.77 (1H, ddd, $J = 10.0$, 5.0, 2.4 Hz)
4	33.4	3.26 (1H, dd, $J = 21.3$, 2.4 Hz), 2.81 (1H, dd, $J = 21.3$, 5.0 Hz)
5	134.1	
6	125.3	5.58 (1H, dd, $J = 5.2$, 2.4 Hz)
7	23.0	2.18–2.14 (1H, m), 1.66–1.59 (1H, m)
8	43.4	1.53–1.47 (1H, m)
9	38.5	2.14–2.06 (1H, m)
10	50.4	
11	28.3	2.59–2.91 (1H, m), 1.27–1.21 (1H, m)
12	22.9	2.06–2.00 (1H, m), 1.89–1.83 (1H, m)
13	46.1	2.06–1.58 (1H, m)
14	104.9	
15	59.3	3.89–3.81 (2H, m)
16	21.4	2.04–1.98 (1H, m), 1.41–1.34 (1H, m)
17	42.1	2.06–1.99 (1H, m)
19	18.7	1.12 (3H, s)
20	83.9	
21	20.6	1.37 (3H, s)
22	78.2	4.83 (1H, dd, $J = 13.2$, 3.1 Hz)
23	32.6	2.54–2.48 (1H, m), 2.23–2.18 (1H, m)
24	147.4	
25	122.6	
26	165.8	
27	12.5	1.88 (3H, s)
28	20.3	1.94 (3H, s)

at δ_H 3.26 (dd, $J = 21.3$ and 2.4 Hz) and 2.81 (dd, $J = 21.3$ and 5.0 Hz), which showed connectivity in the COSY spectrum with the hydrogen signal at δ_H 6.77. The last olefinic proton at δ_H 5.58 (dd, $J = 5.2$ and 2.4 Hz) assignable to the H-6 supported by HMBC correlations from H-3 to C-5, and from H-4 to C-5 and C-6, which indicated the structure of **1** possessing the 2,5-dien-1-one structure at the A/B ring moiety. The appearance of a signal as a double doublet at δ_H 4.83 ($J = 13.2$ and 3.1 Hz) and two vinylic methyl signals at δ_H 1.94 and 1.88 in the ^{1}H NMR spectrum together with the chemical shift at δ_C 165.8 in the ^{13}C NMR spectrum indicated the presence of an α,β-unsaturated lactone side chain of the withasteroids.

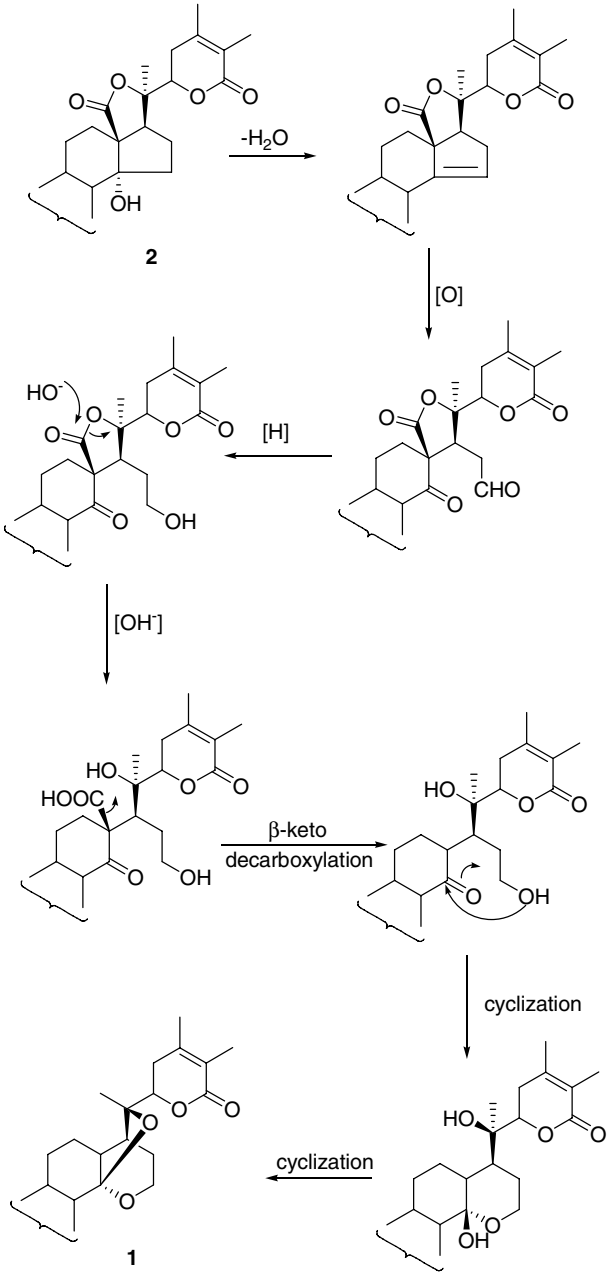
Compared to the typical withanolide skeleton, the obvious differences were attributed to rings C and D. The C(18) methyl could not be found in the skeleton which appears to have been decarboxylated during biosynthesis from a withaphysalin structure (see Scheme 1). The C-21 methyl signal, which appeared as a singlet (δ_H 1.37), showed a HMBC correlation with the resonance at δ_C 83.9 (C-20), indicating their direct connectivity. Further, the HMBC correlation of δ_H 3.85 (H-15) with δ_C 104.9 (C-14) indicated a novel ring D possessing the C(14)–O–C(15) moiety. The existence of ring D as an unprecedented 4*H*-pyran ring instead of a typical five-membered ring found in withaphysalin skeleton was further validated through the appearance of the HMBC correlations between H-15 and C-16, between H-21 and C-17, and between H-17 and C-12. This structural skeletal assignment was fully supported by X-ray diffraction analysis (see Fig. 1).

The relative stereochemistry of **1** was established on the basis of X-ray diffraction analysis, a perspective view of the structure is shown in Figure 2. Biogenetically, the chiral center at C(10) of withanolides was *R* configuration,[3] so the molecule has several asymmetric centers with the following configurations: C-8 *R*, C-9 *S*, C-10 *R*, C-13 *R*, C-14 *S*, C-17 *S*, C-20 *R*, C-22 *R* (see Fig. 3).

Further evidences for unambiguous structural assignment came through proposing a plausible biogenetic pathway for withaphysanolide A (**1**) as shown in Scheme 1.

Withaphysanolide A might be derived by sequential cleavage via C(14)–C(15) and C(18)–O–C(20), decarboxylation of C(18) and cyclization via C(14)–O–C(20) bridge and C(14)–O–C(15) bridge formation from the biogenetically acceptable withaphysalin A (**2**), which was isolated from the same plant. Consequently, the structure of **1** was unambiguously established based on evidences from spectroscopic data, X-ray crystallographic analysis and the proposed biogenetic pathway, and the novel compound was named as withaphysanolide A.

In addition to **1**, nine known compounds including four withaphysalins: withaphysalin A (**2**),[4] withaphysalin C (**3**),[4] withaphysalin E (**4**),[5] and withaphysalin D (**5**)[6] and five physalins: physalin A (**6**),[7] physalin B (**7**),[8] physalin D (**8**),[9] physalin F (**9**),[4] and physalin H (**10**),[10] were also isolated and identified by comparison of their physical and spectral data with those reported in the literature.



Scheme 1. Possible biosynthetic pathway of **1**.

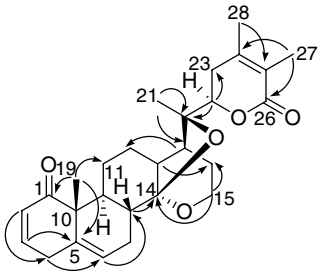


Figure 1. Key HMBC correlations of **1**.

Compounds **1**–**10** were screened for in vitro cytotoxicity against human colorectal carcinoma HCT-116 cells and human non-small cell lung cancer NCI-H460 cells

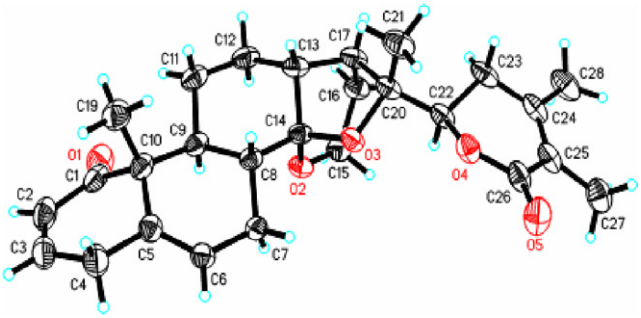


Figure 2. X-ray structure of **1** showing relative configuration.

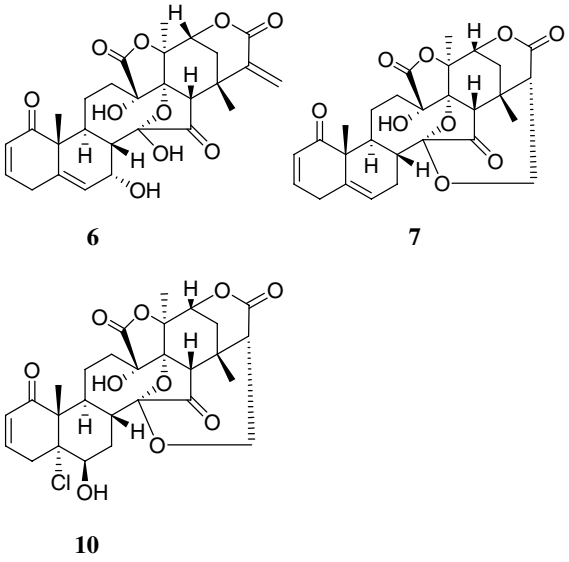


Figure 3. Structures of the most potent compounds **6**, **7**, and **10**.

(Table 2). For HCT-116 cell line, compounds **6**–**8** and **10** exhibited a strong cytotoxicity, with IC_{50} values of 1.4, 1.2, 3.7, and 0.3 μM, respectively. Physalin H (**10**) shows the strongest cytotoxicity with IC_{50} value 0.3 ± 0.04 μM, while the new compound withaphysanolide A (**1**) and the known compounds **2**, **3**, **5**, and **9** showed a moderate cytotoxicity; for NCI-H460 cell line, compounds **6**, **7**, and **10** exhibited strong cytotoxicity, with IC_{50} values of 1.4, 1.9, and 1.8 μM, respectively.

Table 2. Cytotoxicities of **1**–**10** toward HCT-116 and NCI-H460 cells

Compd	IC_{50}[a] (μM)	
	HCT116	NCI-H460
1	30.8 ± 0.5	>100
2	17.5 ± 0.2	24.4 ± 0.2
3	14.2 ± 0.3	15.3 ± 0.2
4	>100	>100
5	27.0 ± 0.6	32.1 ± 0.8
6	1.4 ± 0.05	1.4 ± 0.08
7	1.2 ± 0.04	1.9 ± 0.06
8	3.7 ± 0.06	10.2 ± 0.3
9	17.4 ± 0.3	20.8 ± 0.4
10	0.3 ± 0.04	1.8 ± 0.07
Topotecan[b]	0.026 ± 0.004	0.07 ± 0.005

[a] Mean ± SEM $n = 3$.
[b] Positive control.

Compounds **2**, **3**, **5**, **8**, and **9** showed a moderate cytotoxicity. As shown in Table 2, compounds with withaphysalin structure (**1**–**5**) have only shown a moderate cytotoxicity against the two tumor cell lines, while physalins (**6**–**10**) have a stronger cytotoxicity than withaphysalins.

Acknowledgments

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Supplementary data

One- and two-dimensional NMR spectra and crystallographic data of withaphysanolide A. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.11.081.

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2. The air-dried and powdered aerial parts (5.0 kg) of *P. divericata* were extracted with EtOH at rt, which afforded a dark residue (1.8 kg) after evaporation under reduced pressure. The residue was partitioned between $CHCl_3$ and H_2O. The organic layer (197 g, obtained after solvent evaporation) was subjected to CC (D-101 porous resin; EtOH/H_2O 25:75, 75:25, 95:5) affording three fractions (Fr. A–C). Subsequent CC (MCI gel CHP 20P H_2O/Me_2CO 1:1) of Fr. B (24.4 g) resulted in four subfractions (Fr. B.1–B.4). Fr. B.1 was re-subjected to CC (RP-18; MeOH/H_2O 40:60) to afford physalin D (**8**) (37 mg), physalin F (**9**) (14 mg), and physalin H (**10**) (40 mg). Fr. B.2 was purified similarly (RP-18; MeOH/H_2O 50:50) to yield withaphysalin C (**3**) (42 mg), withaphysalin E (**4**) (27 mg), and physalin A (**6**) (19 mg). Fr. B.3 was purified similarly (RP-18; MeOH/H_2O 60:40) to afford withaphysalin D (**5**) (24 mg), withaphysalin A (**2**) (104 mg), and withaphysanolide A (**1**) (9 mg). Fr. B.4 was subjected to CC (Sephadex LH-20; MeOH) to yield withaphysalin D (**5**) (14 mg) and physalin B (**7**) (120 mg).
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