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WITHANGULATIN A, A NEW WITHANOLIDE FROM *PHYSALIS ANGULATA*[1]

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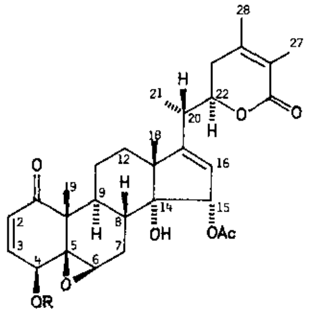
Abstract — A new withanolide as a topoisomerase II inhibitor, withangulatin A was isolated from the whole herb of *Physalis angulata* L. (Solanaceae). The structure of withangulatin A was established as (20*S*,22*R*)-15α-acetoxy-5β,6β-epoxy-4β,14α-dihydroxy-1-oxowitha-2,16,24-trienolide (I) on the basis of spectroscopic and chemical evidence.

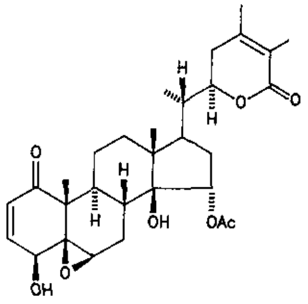
Physalis angulata L., known as an antipyretic, diuretic and antitumor folk-medicine,[2] is one of the common solanaceous plants in Taiwan.[3] Various physalins[4] and withanolides[5] together with acetylcholine[6] and chlorogenic acid[7] had previously been isolated from this species. A thorough search for the bioactive constituents of the whole herb of this plant has now led to the isolation of a new withanolide, named withangulatin A. This compound was found to act on topoisomerase II to induce topoisomerase II-mediated DNA damage *in vitro*.[8] The present paper describes the structural elucidation of withangulatin A.

Withangulatin A (I), colorless amorphous powder, mp 152-153°C, $[\alpha]_D^{20}$ +23.9° (c=0.018 in $CHCl_3$), $C_{30}H_{38}O_8$ (*m/z* M^+ found 526.2562, calcd 526.2567) was considered to be a sort of withanolides. Its ir spectrum showed the presence of a hydroxyl (3450 cm^{-1}), an acetoxyl (1735 cm^{-1}), an α,β-unsaturated δ-lactone (1710 cm^{-1}) and an α,β-unsaturated ketone (1690 cm^{-1}) functions, while the uv spectrum showed absorption at λ_{max}^{MeOH} 215 nm (ε=16500). The mass spectrum displayed characteristic peaks at *m/z* 526 (5%) M^+, 466 (16) $[M\text{-}AcOH]^+$, 451 (30) $[M\text{-}Me\text{-}AcOH]^+$, 433 (7) $[M\text{-}Me\text{-}H_2O\text{-}AcOH]^+$, 430 (3) $[M\text{-}AcOH\text{-}2H_2O]^+$ and the base peak at *m/z* 125 corresponding to the δ-lactone moiety.[9] The ^1H-nmr spectrum (400 MHz, $CDCl_3$) exhibited not only signals due to two tertiary methyls (δ 1.06, 1.40), a secondary methyl (δ 1.07, *d*, *J* = 7.0 Hz) and two vinyl methyls (δ 1.81, 1.90) but also signals at δ 6.14 (1H, *d*, *J* = 10.0 Hz, -CO-$\underline{CH}$=CH-, H-2) and δ 6.97 (1H, *dd*, *J* = 10.0, 5.8 Hz, -CH=$\underline{CH}$-CH-OH, H-3), which is regarded as a withanolide fingerprint.[10] Moreover, signals at δ 1.91 (3H, *s*) due to an acetoxy group, and at δ 3.76 (1H, *dd*, *J* = 5.8, 2.0 Hz, H-4), 3.34 (1H, *br s*, H-6), 4.21 (1H, *ddd*, *J* = 12.0, 7.0, 3.0 Hz, H-22) and δ 5.19 (1H, *d*, *J* = 2.7 Hz, H-15) ascribed to four oxymethines were observed, in which the strong deshielding doublet (δ 5.19) due to a proton geminal to a secondary acetoxyl revealed the adjacency of an olefinic proton (δ 5.63, *d*, *J* = 2.7 Hz, H-16) (see Table 1). Acetylation of I with acetic anhydride in pyridine afforded an acetate (II), mp 134-136°C, ms *m/z* 568 (32) M^+, ir ν_{max}^{KBr} 3450, 1735 cm^{-1}, which remained a tertiary hydroxy group. Its ^{1}H-nmr spectrum (Table 1) showed a singlet at δ 2.04 due to a new acetoxy group and the expected downfield shift of the signal assigned to 4α-proton from δ 3.76 (*dd*, *J* = 5.8, 2.0 Hz) in I to δ 4.68 (*d*, *J* = 6.0 Hz) which indicated the existence of an acetoxy group with β-orientation at C-4 and no hydrogen on C-5.[9] Withangulatin A, therefore, is unequivocally characterized by the presence of 5,6-epoxy-4β-hydroxy-2-en-1-one functionality in rings A and B, a typical α,β-unsaturated δ-lactone in C_9-side chain, the same as in usual withanolides,[11] and further contains a trisubstituted double bond, an acetoxy and a tertiary hydroxy groups. In agreement with this statement, the ^{13}C-nmr spectrum (see Table 2) confirmed the presence of 30 carbons and these substitution patterns,[12] and suggested that withangulatin A (I) may possess a structural resemblance to physapubenolide (III)[13] and withaminimin (IV).[14] According to two dimensional ^{1}H-^{1}H COSY spectrum of I, the allylic proton signal at δ 2.44 (1H, quintet, *J* = 7.0 Hz, H-20) coupling to an oxymethine proton at δ 4.21 (H-22) and methyl protons at δ 1.07 (H-21) was observed. The COSY spectrum also confirmed that an olefinic proton at δ 5.63 (H-16) and an oxymethine proton at δ 5.19 (H-15) were only coupled to each other with a small coupling constant (*J* = 2.7 Hz), indicating H-15 to be a quasi-axial β-proton.[14] Moreover, the ^{1}H-^{1}H NOESY spectrum showed a cross-peak due to H-4 and H-6, and spatial proximity of H-15 with H-16, H-18 and H-8. These observations along with the above data provided powerful support for β-configuration of 5,6-epoxide, the location and α-configuration of the acetoxy group at C-15, and the location of the tertiary hydroxy group at C-14 and a double bond at C-16(17). These assignments were further confirmed by comparing the ^{13}C-nmr spectral data of I with those of III and IV as shown in Table 2. Since the 14α-hydroxy group deshielded C-8 through a β-effect, shielded C-7, C-9 and C-12 through γ-effects, and the 14β-hydroxy group did not cause the shielding of C-12 in III,[13] the tertiary hydroxy group at C-14 was oriented α-configuration as the only reasonable assignment.[12]

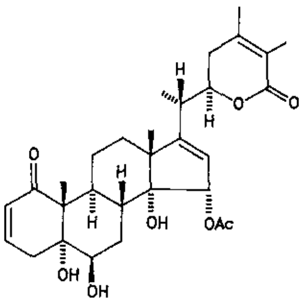


I R=H

II R=Ac



III



IV

Table 1. ^{1}H-Nmr Data for Withangulatin A (I) and Withangulatin A Acetate (II) (in CDCl$_3$)a)

Proton	I	II
2	6.14 (*d*, 10.0)	6.22 (*d*, 10.0)
3	6.97 (*dd*, 10.0, 5.8)	7.04 (*dd*, 10.0, 6.0)
4α	3.76 (*dd*, 5.8, 2.0)	4.68 (*d*, 6.0)
6α	3.34 (*br s*)	3.32 (*br s*)
7α	2.53 (*dt*, 14.0, 3.0)	2.54 (*ddd*, 14.0, 12.0, 2.0)
7β	1.66 (*ddd*, 14.0, 12.0, 2.0)	1.66 (*ddd*, 14.0, 13.0, 2.0)
8	1.84 (*m*)	1.82 (*m*)
9	1.80 (*m*)	1.80 (*m*)
15	5.19 (*d*, 2.7)	5.21 (*d*, 2.7)
16	5.63 (*d*, 2.7)	5.67 (*d*, 2.7)
18*	1.06 (*s*)	1.07 (*s*)
19*	1.40 (*s*)	1.38 (*s*)
20	2.44 (quintet, 7.0)	2.47 (quintet, 7.0)
21*	1.07 (*d*, 7.0)	1.10 (*d*, 7.0)
22	4.21 (*ddd*, 12.0, 7.0, 3.0)	4.22 (*ddd*, 12.0, 7.0, 3.0)
23α	2.17 (*dd*, 17.2, 3.0)	2.16 (*dd*, 17.2, 3.0)
23β	2.37 (*br dd*, 17.2, 12.0)	2.37 (*br dd*, 17.2, 12.0)
27*	1.81 (*s*)	1.83 (*s*)
28*	1.90 (*s*)	1.91 (*s*)
4-OH	3.23 (*br s*)	
4-OAc*		2.04 (*s*)
15-OAc*	1.91 (*s*)	1.91 (*s*)

a) Chemical shifts are in δ units. Coupling constants (in Hz) are given in parentheses.
*Intensity three protons.

Table 2. ^{13}C-Nmr Data for Withangulatin A (I), Physapubenolide (III) and Withaminimin (IV) (δ ppm)

Carbon	I a)		III b)	IV c)
1	202.44 (C)	[202.44 (*s*)]	202.6	204.06
2	131.42 (CH)	[131.37 (*d*)]	131.5	128.71
3	142.75 (CH)	[143.85 (*d*)]	143.5	141.33
4	69.55 (CH)	[69.82 (*d*)]	69.6	36.03
5	63.23 (C)	[63.80 (*s*)]	63.4	77.22
6	63.23 (CH)	[62.84 (*d*)]	62.7	74.26
7	24.66 (CH$_2$)	[25.05 (*t*)]	26.1	26.47
8	34.73 (CH)	[35.55 (*d*)]	40.3	35.43
9	39.32 (CH)	[40.06 (*d*)]	36.1	35.43
10	47.61 (C)	[48.05 (*s*)]	47.9	52.20*
11	21.85 (CH$_2$)	[22.29 (*t*)]	21.8	23.18
12	37.54 (CH$_2$)	[38.26 (*t*)]	41.4	38.82
13	52.05 (C)	[52.36 (*s*)]	46.1	52.23*
14	81.48 (C)	[81.92 (*s*)]	84.1	82.28
15	83.59 (CH)	[84.17 (*d*)]	80.8	83.36
16	120.80 (CH)	[120.95 (*d*)]	33.8	120.37
17	162.41 (C)	[163.04 (*s*)]	52.3	161.30
18	17.69 (CH$_3$)	[18.07 (*q*)]	15.6	16.80
19	15.88 (CH$_3$)	[16.18 (*q*)]	17.2	15.10
20	35.39 (CH)	[35.70 (*d*)]	37.5	36.11
21	17.32 (CH$_3$)	[17.60 (*q*)]	17.2	17.21
22	79.38 (CH)	[79.55 (*d*)]	78.5	78.47
23	33.11 (CH$_2$)	[33.40 (*t*)]	31.2	32.35
24	148.40 (C)	[148.40 (*s*)]	149.5	150.25
25	122.19 (C)	[122.23 (*s*)]	121.9	121.44
26	166.38 (C)	[166.31 (*s*)]	166.8	167.52
27	12.35 (CH$_3$)	[12.40 (*q*)]	12.4	12.37
28	20.33 (CH$_3$)	[19.83 (*q*)]	20.5	20.64
$\underline{\mathrm{CH_3}}\mathrm{CO}$	21.28 (CH$_3$)	[20.95 (*q*)]	21.5	21.36
$\mathrm{CH_3}\underline{\mathrm{C}}\mathrm{O}$	169.65 (C)	[169.57 (*s*)]	169.8	170.63

a) Measured at 100 MHz, in CDCl$_3$, and benzene-$\underline{d}_6$ in brackets. Assignments were made with the aid of DEPT and OFR (in brackets) experiments.
b) Solvent CDCl$_3$. Data are from ref. 13.
c) Solvent CDCl$_3$. Data are from ref. 14. *Values are interchangeable.

The cd spectrum of I showed the positive Cotton effect at 250 ($[\theta]$+12600 in MeOH) and 335 ($[\theta]$+4200 in MeOH) nm, indicating I to have a *22R* configuration and A/B *cis*-fused ring junction.[15] Additionally, the aforementioned quintet centered at δ 2.44 with $J_{20-21} = J_{20-22} = 7.0$ Hz of 20β-proton signal[14] indicated a *20S* configuration in I, the same as in III, IV and related withanolides. In conclusion, the structure including absolute configuration of withangulatin A was established as (*20S*,*22R*)-15α-acetoxy-5β,6β-epoxy-4β,14α-dihydroxy-1-oxowitha-2,16,24-trienolide (I).

EXPERIMENTAL

Melting points were determined on a Yanaco micro-melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. Ir spectra were taken on a Perkin Elmer 781 infrared spectrophotometer. Uv spectra were recorded on a Perkin Elmer Lambda 5 uv/vis spectrophotometer. Mass spectra were recorded on either JEOL JMS-D-100 or JMS-HX-110 spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-400 nmr spectrometer. Preparative tlc was performed on precoated Kieselgel 60 F_{254} plates (1.0 mm, Merck). Hplc was carried out on a Waters model ALC/GPC-244/M-6000A system with a model 440 absorbance detector (set at 254 nm).

Extraction and Isolation

Dried powdered whole herbs of *Physalis angulata* L. (Solanaceae) (1.0 kg), collected from Nantou (Nantou County, Taiwan) in summer, were extracted with MeOH (9 l) at room temperature for one week and the extract was evaporated *in vacuo* to leave a dark greenish residue (95.0 g). The residue was chromatographed repeatedly on silica gel column with $CHCl_3$ and an increasing content of acetone as eluents, each fraction being monitored by tlc. One of the fractions eluted with $CHCl_3$-acetone (5:3) was evaporated to give a residue (1.8 g), which was fractionated sequentially by preparative tlc ($CHCl_3$-MeOH = 12:1) and by hplc on a μBondapak C_{18} column (EtOAc) to give withangulatin A (I, 58 mg).

Withangulatin A (I)

Colorless amorphous powder, mp 152-153°C, $[\alpha]_D^{20}$+23.9° (c=0.018 in $CHCl_3$), hrms *m/z*: 526.2562 (M^+ calcd for $C_{30}H_{38}O_8$: 526.2567), 511.2310 (calcd for $C_{29}H_{35}O_8$: 511.2332); ir ν_{max}^{KBr}: 3450, 1735, 1710, 1690, 1250, 1235, 1140, 1020, 750 cm^{-1}; uv λ_{max}^{MeOH}: 215 nm (ε=16500); eims *m/z* (75 eV, rel. int. %): 526 (5), 511 (7), 484 (2), 466 (16), 451 (30), 433 (7), 430 (3), 273 (15), 260 (75), 125 (100); cd: $[\theta]_{250}$ +12600, $[\theta]_{335}$ +4200 (c=1.88 x 10^{-5}, MeOH); ^{1}H-nmr: see Table 1; ^{13}C-nmr: see Table 2.

Acetylation of Withangulatin A (I)

A mixture of withangulatin A (I, 12 mg), pyridine (1.0 ml) and acetic anhydride (1.0 ml) was kept at room temperature for 10 h, and treated as usual. The reaction product was purified by preparative tlc (*n*-hexane-EtOAc = 4:1) to give colorless amorphous powder (II, 8 mg), mp 134-136°C, ir ν_{max}^{KBr}: 3450, 1735, 1710, 1690, 1240, 1110, 1090, 1020, 960 cm^{-1}; eims *m/z* (75 eV, rel. int. %): 568 (32) M^+, 553 (17), 508 (11), 448 (14), 320 (18), 260 (35), 125 (100); ^{1}H-nmr: see Table 1.

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