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Cytotoxic withanolides from Vietnamese ethno-medicinal plant *Physalis angulata*

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***Abstract*—Withanolides are known as the main components of *Physalis* genus with broad biological activities. Using combination of various chromatographic methods, three withanolides were isolated including physagulin J (1), physagulin K (2), and physagulin N (3). Their chemical structures were determined by analysis of NMR spectra, and as well as comparison with reported literature. Compound 1 showed significant cytotoxicity toward the human lung carcinoma cell A-549 with IC_{50} of 8,27 ± 0,97 µM.**

Keywords—Physalis angulata, withanolide, physagulin J, physagulin K, physagulin N

I. INTRODUCTION

There are four species belong to genus *Physalis* wildly growing in Vietnam but not get much attention from people and scientist [1]. Oppositely, species of genus *Physalis* receive much more attractive from oversea scientist community. The main components of this genus are withanolide-type compounds [2] that possess various biological activities as antitumor, anti-inflammatory, antimicrobial, antimalarial, antinociceptive, anti-trypanosoma cruzi and anti-leishmanial activities [3]**.** Several studies have demonstrated that plants of this genus possess antitumor effects *in vivo* or *in vitro*, which was ascribed to withanolides as the effective components [4-6].

In the course of chemical investigation of *Physalis angulata* species, this work describes the isolation, chemical structure elucidation and cytotoxic evaluation of three withanolides from *P. angulata*. This is the first report on chemical investigation of this plant collected in Vietnam.

II. MATERIALS AND METHODS

A. General experiment procedures

NMR spectra were acquired on a Bruker AM500 FT-NMR spectrometer (Bruker BioSpin, Bremen, Germany) using TMS as an internal standard. Electrospray ionization mass spectra were recorded on an Agilent 1100 Mass spectrometer (Agilent technology, Santa Clara, CA, USA). Column chromatography was performed using silica gel (Merck, Whitehouse Station, NJ, USA) and reverse phase C18 resins (YMC Ltd., Kyoto, Japan). Thin layer chromatography was carried out using pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck). Spots were visualized under UV radiation (254 and 365 nm) and sprayed with aqueous solution of H_2SO_4 (10%), heating with a heat gun.

B. Plant materials

Whole plant of *Physalis angulata* were collected at Quang Tri province, Vietnam, in August 2016. Its scientific name was identified by MSc Le Tuan Anh, Mientrung Institute for Scientific Research. A voucher specimen (QT14.2016) is deposited at the Herbarium of Mientrung Institute for Scientific Research, VAST.

C. Extraction and Isolation

The dried and powdered of whole plants of *Physalis angulata* (2.0 kg) were sonically extracted with methanol at 50 °C for three times (4.0 L each). After removal of the solvent, the methanol extract (90 g) was suspended in distilled water (1.5 L) and successively partitioned with dichloromethane and ethyl acetate (three time, 1.5 L each) to give corresponding soluble extracts, dichloromethane (PMD, 24.5 g), ethyl acetate (PAE, 4.6 g), and water-soluble layer.

The PMD residue was separated on a silica gel column, eluting with gradient solvents of dichloromethane and methanol (0 to 100% volume of methanol to give 5 main fractions (PMD1 - PMD5). Fraction PMD2 (3.0 g) was continuously separated on a silica gel column, eluting with solvent system of dichloromethane/methanol (20/1, v/v) to yield 3 sub-fractions PMD2A-PMD2C. Compound **1** (7 mg) was obtained from sub-fraction PMD2C using silica gel column eluted with dichloromethane/methanol (5/1, v/v). Fraction PMD5 (4,5 g) was divided into three smaller fractions (PMD5A - PMD5C) using reverse phase RP-18 resins column and methanol/water (2/1, v/v) as an eluent. Compound **2** (11 mg) and **3** (9 mg) were obtained from fraction PMD5C using reverse phase RP-18 resins column and acetone/water (1/1, v/v) as an eluent.

- Physagulin J (**1**): Molecular formula: $C_{30}H_{42}O_8$; White amorphous powder. ^1H and ^{13}C-NMR data are given in the Table I.
- Physagulin K (**2**): Molecular formula: $C_{30}H_{42}O_9$; White amorphous powder. ^1H and ^{13}C-NMR data are given in the Table I.
- Physagulin N (**3**): Molecular formula: $C_{28}H_{40}O7$; white amorphous. ^1H and ^{13}C-NMR data are given in the Table I.

TABLE I. NMR DATA FOR COMPOUNDS **1-3**

C	1			2			3		
	δ_C [d]	δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)	δ_C [d]	δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)	δ_C [d]	δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)
1	204.9	207.65	-	204.7	207.59	-	205.4	207.16	-
2	129.0	129.02	5.80 (dd, 2.5, 10.0)	128.0	129.18	5.80 (dd, 3.0, 10.5)	127.4	129.05	5.80 (dd, 2.0, 10.0)
3	142.0	144.02	6.66 (ddd, 2.5, 5.0, 10.0)	141.9	143.71	6.68 (ddd, 2.5, 5.0, 10.0)	142.0	143.91	6.68 (m)
4	34.8	36.73	2.00* 3.26 (dt, 2.5, 20.0)	36.8	36.85	2. 02 (dd, 1.0, 5.0) 3.29 (dt, 3.0, 15.0)	34.6	36.52	2.10 (m) 2.67 (m)
5	77.2	77.49	-	77.3	77.72	-	76.6	78.45	-
6	74.9	75.46	3.60 (dd, 2.5, 5.5)	74.8	75.57	3.61 (t, 3.5)	73.5	75.52	3.65 (m)
7	29.6	29.23	1.86*	27.9	27.70	1.82 (t, 3.0) 1.88 (d, 3.0)	35.9	37.61	1.60 (m) 1.88*
8	38.1	38.18	1.97 (m)	36.2	36.37	2.10 (dd, 5.0, 12.5)	35.7	38.04	2.09 (m)
9	37.0	37.56	2.55 (m)	35.3	36.08	2.92 (ddd, 3.5, 3.5, 12.5)	36.0	37.81	2.46 (m)
10	52.7	53.15	-	52.6	53.37	-	51.7	53.35	-
11	24.7	24.98	1.22 * 2.05 (m)	23.2	23.44	1.38 (m) 2.29 (dd, 3.5, 9.5)	23.2	24.96	1.25 * 2.00*
12	44.2	44.64	1.97 (m) 2.03 (m)	42.6	48.48*	1.72 (ddd, 3.0, 3.5, 13.0) 1.63 (dt, 3.5, 13.5)	42.7	44.69	1.70 (m) 2.10 (m)
13	47.1	47.58	-	51.3	52.05	-	44.8	46.55	-
14	84.9	85.82	-	87.7	88.14	-	84.3	86.16	-
15	78.4	81.04	5.03 (d, 4.5)	79.2	80.49	5.20 (dd, 4.0, 9.0)	76.7	78.41	3.98 (d, 3.5)
16	35.1	35.32	1.65 (m) 1.99 (m)	32.3	32.23	1.94 (d, 8.5) 2.64 (dd, 8.5, 15.5)	29.3	29.30	1.95 (m) 2.15 (m)
17	53.3	53.84	1.78*	86.2	87.22		51.4	53.47	1.83*
18	16.6	18.31	1.18 (s)	15.9	15.84	1.19 (s)	14.6	18.16	1.12 (s)
19	15.0	15.39	1.26 (s)	15.1	15.19	1.28 (s)	14.0	15.90	1.29 (s)
20	38.1	38.98	2.10 (m)	42.4	43.22	2.25 (dd, 4.0, 7.0)	36.9	38.49	2.07 (m)
21	15.3	15.31	1.08 (d, 6.5)	10.0	9.58	1.09 (d, 7.0)	16.6	15.11	1.07 (d, 6.0)
22	78.6	80.34	4.46 (ddd, 3.5, 7.5, 8.5)	77.3	78.84	4.82 (ddd, 3.0, 6.0, 10.5)	78.8	80.61	4.44 (ddd, 3.5, 7.5, 13.5)
23	30.5	31.26	2.11 (m) 2.58 (m)	32.3	33.16	2.55 (m)	27.4	30.75	2.57 (brt, 16.0) 2.25 (dd, 2.5, 16.0)
24	149.6	152.79	-	150.3	153.12	-	150.7	152.91	-
25	121.7	122.15	-	121.6	122.11	-	120.8	122.18	-
26	166.5	169.36	-	166.6	169.21	-	167.9	169.52	-
27	12.6	12.42	1.86 (s)	12.5	12.26	1.88 (s)	11.4	12.44	1.87 (s)
28	19.9	20.41	2.00 (s)	20.1	20.38	1.97 (s)	19.5	20.45	2.02 (s)
29	170.4	172.22	-	170.3	172.11	-			
30	21.6	21.59	2.07 (s)	21.4	21.39	2.06 (s)			

*Measured in [a] MeOD, [b]125 MHz, [c]500 MHz, [d] $CDCl_3$, * overlapped signals*

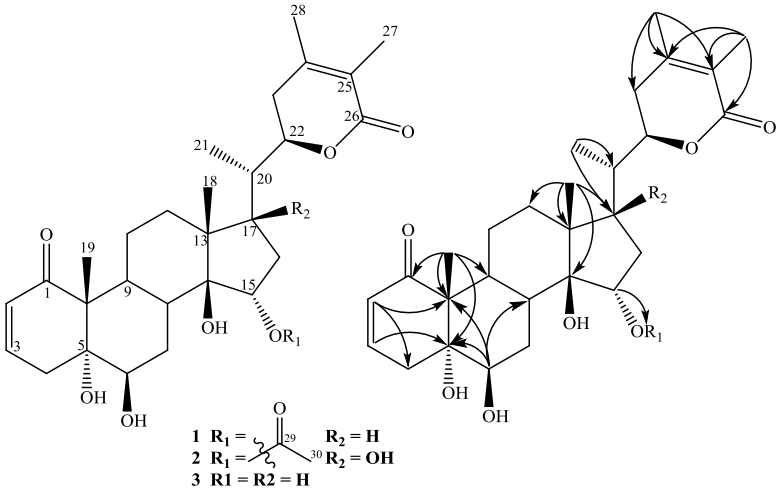

Fig. 1. *Chemical structures and main HMBC correlations of compounds **1-3***

III. Results and Discussion

The dried and powdered of whole plants of *Physalis angulata* (2.0 kg) were sonically extracted with methanol at 50 °C and separated with dichloromethane and ethyl acetate to give dichloromethane, ethyl acetate residues and water-soluble layer. Using various chromatographic technique, three withanolides (**1**-3) were isolated from dichloromethane residue (Fig. 1).

Compound **1** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **1** showed 2 olefinic proton signals at δ_H 6.66 (1H, m), 5.80 (1H, d, *J* = 2.5, 10.0), and six methyl group at δ_H 1.86 (6H, s), 1.26 (3H, s), 2.00 (3H, s), 2.07 (3H, s) and 1.08 (3H, d, *J* = 6.5 Hz). Analysis of ^{13}C-NMR spectrum of **1** revealed signals of 30 carbons which were divided by HSQC spectrum into 9 non-protonated carbons (3 carbonyl carbons at 207.65, 172.22 and 169.36), 9 methines (4 carbons of double bond at 129.02, 144.02, 152.79 and 121.7), 6 methylenes, and six methyl carbons. A couple of carbon signals including δ_C 172.22 and 21.39 were charactesized for an acetyl group. Remaining 28 carbons were belonged an steroid skeleton, withanolide-type compound. Among them, carbon signals at δ_C 169.5 was assigned for a carboxylic carbon. Assignments of 1H and 13C NMR data of **1** were completed with the aid of HSQC and HMBC (Table I). The HMBC correlations from proton signal δ_H 1.26 (19-Me) to carbons δ_C 207.65 (C-1)/ 77.49 (C-5)/ 37.56 (C-9)/ 53.15 (C-10); from 2 olefinic protons δ_H 5.80 (H-2) and 6.66 (H-3) to carbons δ_C 53.15 (C-10) and 77.49 (C-5); from oxymethine proton δ_H 3.60 (H-6) to carbons δ_C 77.49 (C-5)/ 38.18 (C-8)/ 53.15 (C-10) confirmed the position of a double bond at C-2/C-3 and the position of 2 hydroxyl groups at C-5 and C-6. The position of acetyl group at C-15 was assigned with the aid of HMBC correlation between protons δ_H 5.03 (H-15) and 2.07 (30-Me) to carbonyl carbon δ_C 172.22 (C-29). The NOE cross-peaks between methyl groups 18-Me/ 19-Me and proton H-8; between proton H-8 and proton H-15 suggested a withanolide skeleton, with *β*-orientation of H-16 and *α*-orientation of H-6. The NMR data of **1** was well agreed with those reported of physagulin J in the literature [7], the difference of chemical shifts due to NMR solvent. Based on the above evidence, compound **1** was elucidated as physagulin J.

Compound **2** was isolated as a white amorphous powder. The NMR data of **2** were similar to those of **1** except the presence of a non-protonated carbon at 87.22 and two shielded methyl groups at δ_C 15.84 (18-Me) and 9.58 (21-Me) (Table I). The position of non-protonated carbon was assigned at C-17 with the aid of HMBC correlation between methyl groups δ_H 1.19 (18-Me) and 1.09 (19-Me) and carbon δ_C 87.22 (C-17). Based the NMR evidence and comparison with the previous reported data, chemical structure of **2** was determined to be physagulin K [7].

Compound **3** was also obtained as a white amorphous powder. ^{1}H, 13C-NMR spectra of **3** were characterized for a withanolide-type skeleton as compounds **1** and **2** (Table 1). Comparison of its NMR data to those of **1** showed the only difference was that the acetyl group at carbon C-15 (δ_C 172.22 and 21.59) of **1** was replaced by a hydroxyl group in **3**. The ^{1}H and ^{13}C-NMR were fully assigned with the aid of HSQC and HMBC (Table I). Thus, the structure of **3** was identified as physagulin N, which corresponds to previous reported results [7].

All of compounds were tested against three human cancer cell lines (A-549, Hela and PANC-1) according to MTT method, using etoposide as positive control. Compound **1** only showed significant cytotoxicity toward A-549 cell lines with IC_{50} value 8,27 ± 0,97 μM while remaining compounds were inactive.

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